SECTION 2.6
NONCLINICAL SUMMARY

SECTION 2.6.1—INTRODUCTION

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
ARV	antiretroviral
bisPOC	bis-isopropyloxycarbonylmethyl; disoproxil
COBI	cobicistat
CYP3A	cytochrome P450 3A
dATP	deoxyadenosine triphosphate
DNA	deoxyribonucleic acid
EC_{50}	concentration of compound inhibiting virus replication by 50%
EVG	elvitegravir (GS-9137; JTK-303)
EVG/COBI/FTC/TDF	elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, QUAD
FDC	fixed-dose combination
FTC	emtricitabine, Emtriva®
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate, TVD, Truvada®
FTC-TP	emtricitabine 5'-triphosphate
GSI	Gilead Sciences, Inc.
HIV-1	human immunodeficiency virus type 1
HS	human serum
HSA	human serum albumin
IC_{50}	median inhibitory concentration
INSTI	integrase strand transfer inhibitor
monoPOC PMPA	tenofovir soproxil
NDA	new drug application
N(t)RTI	nucleoside/nucleotide reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
PBMC	peripheral blood mononuclear cell
RTV	ritonavir
STR	single tablet regimen
TDF	tenofovir DF, Viread®
TFV	tenofovir, PMPA
TFVpp	tenofovir diphosphate
US	United States

1. NONCLINICAL SUMMARY

1.1. Introduction

This application is being submitted in support of a new drug application (NDA) for a 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-1 (HIV-1) infection in adults aged 18 years and over who are antiretroviral (ARV) naive or have no known resistance mutations 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 ARV activity. Cobicistat 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 (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 (NRTI/NtRTI) 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 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).

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

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

In order to simplify the review, the order of presentation in each section follows the general format: EVG, followed by COBI, EVG/COBI combination, and EVG/COBI/FTC/TDF combination studies. Results of FTC, TDF, and FTC/TDF studies are incorporated into the Nonclinical Written Summaries (Modules 2.6.2, 2.6.4, and 2.6.6) and the Nonclinical Overview (Module 2.4) when needed to describe the presence or absence of overlapping toxicities.

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

The nonclinical data discussed within this document support the proposed use of the EVG/COBI/FTC/TDF STR as a complete single tablet regimen for the treatment of HIV-1 infection in adults who are ARV treatment-naive or who have no known resistance mutations to the individual components of EVG/COBI/FTC/TDF STR. All information from nonclinical studies that is of relevance to the prescriber and patient has been included in the proposed Prescribing Information and Patient Package Insert.

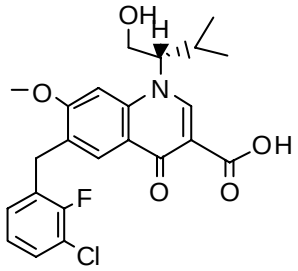
1.1.1. Elvitegravir

Elvitegravir (EVG; GS-9137; JTK-303) specifically inhibits HIV-1 integrase strand transfer activity and the integration of viral deoxyribonucleic acid (DNA) into host chromosomal DNA. Elvitegravir 150 mg is being evaluated by GSI as a treatment for HIV-1 infection in combination with other ARVs. Elvitegravir has not yet been approved for use in any country.

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

and 100 nM (44.8 ng/mL) in the presence of the HS components, human serum albumin (HSA) and α1-acid glycoprotein (AAG), in HIV-1 infected human PBMC cultures.

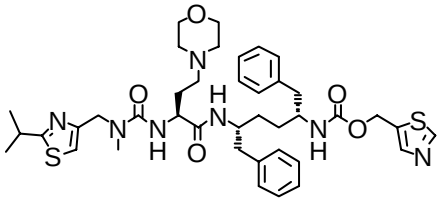
The chemical name for EVG is 3-quinolinecarboxylic acid, 6-[(3-chloro-2-fluorophenyl)-methyl]-1,4-dihydro-1-[(1S)-1-(hydroxymethyl)-2-methylpropyl]-7-methoxy-4-oxo-. The molecular formula is $C_{23}H_{23}ClFNO_5$ and its molecular weight is 447.88 Daltons. Elvitegravir has the following structural formula:



1.1.2. Cobicistat

The primary pharmacodynamic effect of COBI is mechanism-based inhibition of human CYP3A enzymes (eg, the in vitro median inhibitory concentration [IC_{50}] value for inhibition of human hepatic microsomal midazolam 1'-hydroxylase activity is 0.15 μM). Cobicistat is being developed by GSI as a pharmacoenhancer to boost systemic exposures to CYP3A substrates, including the HIV-1 integrase strand transfer inhibitor EVG, and protease inhibitors such as atazanavir and darunavir that are commonly prescribed in combination with low-dose RTV as a booster for the treatment of HIV-1 infected patients. Cobicistat has not yet been approved for use in any country.

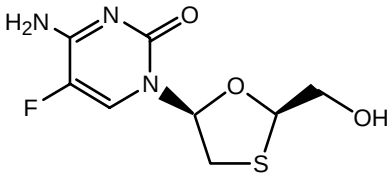
The chemical name for COBI is 1,3-Thiazol-5-ylmethyl (2R,5R)-(5-{[(2S)-2-[(methyl{[2-(propan-2-yl)-1,3-thiazol-4-yl]methyl}carbamoyl)amino]]-4-(morpholin-4-yl)butanamido}-1,6-diphenylhexan-2-yl)carbamate. The molecular formula is $C_{40}H_{53}N_7O_5S_2$ and its molecular weight is 776.02 Daltons. Cobicistat, a structural analogue of RTV, has the following chemical structure:



1.1.3. Emtricitabine

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

Emtricitabine is a synthetic analogue of the naturally occurring 2′-deoxycytidine, a pyrimidine nucleoside. The chemical name of FTC is 5-fluoro-1-[(2R,5S-2-hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Emtricitabine is the (-) enantiomer of the compound and has also been referred to as (-)-2',3'-dideoxy-5-fluoro-3'-thiacytidine. It has a molecular formula of $C_8H_{10}FN_3O_3S$ and a molecular weight of 247.24. The chemical structure of FTC is as follows:

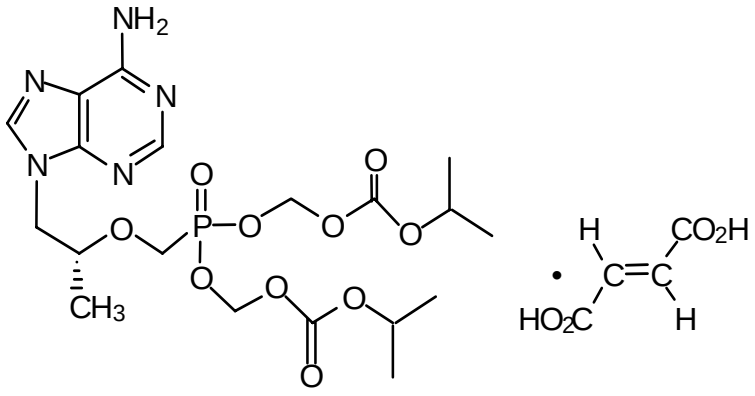


Following absorption, FTC is phosphorylated by cellular enzymes to emtricitabine 5'-triphosphate (FTC-TP), the active metabolite. Emtricitabine 5'-triphosphate inhibits the activity of HIV-1 reverse transcriptase through high affinity binding, competing with the natural substrate deoxycytidine 5'-triphosphate. Emtricitabine 5'-triphosphate is efficiently incorporated into the nascent (viral) DNA chain by HIV-1 RT resulting in termination of DNA synthesis due to the lack of a hydroxyl group in the 3'- position of the sugar moiety of FTC, which in turn inhibits viral replication. In a clinical study, the intracellular half-life of FTC-TP in peripheral blood mononuclear cells was 39 hours. Intracellular triphosphate levels increased with dose, but reached a plateau at doses of 200 mg or greater. Emtricitabine has activity against retroviruses and hepadnaviruses.

1.1.4. Tenofovir DF

Tenofovir disoproxil fumarate (tenofovir DF; TDF), the oral prodrug of tenofovir (TFV; PMPA), is a nucleotide reverse transcriptase inhibitor (NtRTI). It is the active ingredient in Viread® that has been approved in the US, the European Union, and other countries worldwide as a once a day tablet (300 mg, equivalent to 245 mg tenofovir disoproxil), in combination with other ARV agents, for the treatment of HIV-1 infection. Viread® is also approved for the treatment of chronic hepatitis B in adults in the US, the European Union, and other markets worldwide, including Australia, New Zealand, and Canada. The international birthdate for TDF is 31 October 2001.

Tenofovir DF is an alkoxycarbonyloxymethyl ester prodrug of tenofovir (TFV) with high water solubility (13.4 mg/mL) and a molecular weight of 635.52. Tenofovir is not well absorbed from the intestine because of the presence of the negative charges associated with the phosphonate group. Therefore the prodrug, TDF was developed to mask the charge and improve oral bioavailability. As the drug is absorbed through the intestinal wall, the disoproxil (bisPOC) prodrug moiety is rapidly cleaved by esterases so that free TFV along with a minor component of tenofovir soproxil (monoPOC PMPA), is detected in the circulation. The chemical structure of TDF is shown below.



Following absorption, TDF is rapidly converted to TFV, which is metabolized intracellularly to the active metabolite, tenofovir diphosphate (TFVpp). Tenofovir is converted to TFVpp by constitutively expressed cellular enzymes through 2 phosphorylation reactions. This conversion occurs in both resting and activated T cells. The active intracellular anabolite, TFVpp, exerts its antiviral effect by terminating the extension of the developing viral DNA chain. The viral DNA chain termination occurs because TFVpp can be incorporated in place of the natural substrate, deoxyadenosine triphosphate (dATP), and the DNA chain cannot be extended significantly beyond the point of its incorporation. In nonproliferating human PBMCs, the half-life of TFVpp was found to be approximately 50 hours, whereas the half-life in phytohaemagglutinin-stimulated PBMCs was found to be approximately 10 hours. Tenofovir has activity against retroviruses and hepadnaviruses.

1.1.5. Elvitegravir/Cobicistat/Emtricitabine/Tenofovir DF

The proposed STR is based on the complimentary pharmacological mechanisms of action of EVG, FTC, and TDF and the body of clinical experience with nucleoside/nucleotide reverse transcriptase inhibitors (N[t]RTIs) and INSTI in HIV-infected patients. As expected, combinations of these agents are not antagonistic and are synergistic in cell-based in vitro studies. 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. The EVG/COBI/FTC/TDF STR provides clinically equivalent exposures of EVG, COBI, FTC, and 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. Pending approval, the EVG/COBI/FTC/TDF STR would

be the first INSTI-based STR and offers once-daily administration for the treatment of HIV-1 infection.

1.1.6. References

Not applicable.

SECTION 2.6
NONCLINICAL SUMMARY

SECTION 2.6.2—PHARMACOLOGY WRITTEN SUMMARY

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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LIST OF IN-TEXT TABLES

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

AAG	α1 acid glycoprotein
AP	action potential
APD	action potential duration
APD_{60}, APD_{90}	action potential duration at 60% or 90% repolarization
ATV	atazanavir
AV	atrioventricular
BACE-1	beta-site APP-cleaving enzyme 1
CC_{50}	drug concentration that results in a 50% reduction in cell viability
CHO	Chinese hamster ovary
CNS	central nervous system
CPP	coronary perfusion pressure
COBI	cobicistat (GS-9350)
CYP3A	cytochrome P450 3A
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DRV	darunavir
EAD	early after depolarization
EC_{50}	half maximal effective concentration or concentration of compound inhibiting virus replication by 50%
EC_{95}	concentration of compound inhibiting virus replication by 95%
ECG	electrocardiogram
EtOH	ethanol
EVG	elvitegravir, JTK-303
EVG/COBI/FTC/TDF	elvitegravir, cobicistat, emtricitabine, and tenofovir DF (coformulated); QUAD
FOB	functional observation battery
FTC, 524W91	emtricitabine (Emtriva®, Gilead Sciences)
FTC/TDF	emtricitabine/tenofovir DF (Truvada®, Gilead Sciences), TVD
GI	gastrointestinal
GLP	Good Laboratory Practice
GS-8374	Gilead's investigational HIV protease inhibitor
GS-9200	EVG metabolite M4 (JTP-65386 and JTP-71051; glucuronide conjugate of the carboxylic acid)
GS-9202	EVG metabolite M1 (JTP-71081; hydroxylation of the chlorofluorophenyl group)
HBV	hepatitis B virus
HCV	hepatitis C virus
HEK	human embryonic kidney
hERG	human ether-à-go-go related gene
HIV-1	human immunodeficiency virus type 1
HR	heart rate
HS	human serum

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS (CONTINUED)

HSA	human serum albumin
IC_{50}	median inhibitory concentration
I_{Kr}	rapidly activating rectifying potassium current
K_I	affinity constant for enzyme inactivation
k_{inact}	theoretical maximum enzyme inactivation rate
IL-2	interleukin-2
IN	integrase
INSTI	integrase strand transfer inhibitor
LV	left ventricular
LVDP	left ventricular developed pressure
LV dP/dt max	maximum positive rate of change of isovolumic left ventricular pressure
LV dP/dt min	maximum negative rate of change of isovolumic left ventricular pressure
MAP	monophasic action potential
MAPD	monophasic action potential duration
$MAPD_{xx}$	monophasic action potential duration at xx percent repolarization
MC	methylcellulose
MDZ	midazolam
mtDNA	mitochondrial DNA
NDA	new drug application
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NRTI	nucleoside/nucleotide reverse transcriptase inhibitor
NtRTI	nucleotide reverse transcriptase inhibitor
PBMC	peripheral blood mononuclear cell
PG	propylene glycol
PI	protease inhibitor
QRS	part of the ECG complex comprising the Q, R, and S-waves
QT	interval between the start of the Q wave and the end of the T wave on ECG
QTc	QT interval corrected for heart rate
RR	interval between the peak of R waves of 2 consecutive ECG complexes
RTV, r	ritonavir, GS-017415
SI	selectivity index
STR	single tablet regimen
TDF	tenofovir disoproxil fumarate, tenofovir DF
TFV	tenofovir
TVD	emtricitabine/tenofovir DF, FTC/TDF, Truvada®

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 (tenofovir DF, 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] meeting between Gilead Sciences, Inc. (Gilead) and the Food and Drug Administration (FDA; refer to the Agency's comments, dated [redacted] in Module 1.6.3), this NDA is supported [redacted].

In order to simplify the review, the order of presentation in each section follows the general format: EVG, followed by COBI, and EVG/COBI/FTC/TDF combination studies. Results of FTC, TDF, and FTC/TDF studies are incorporated when needed to describe the presence or absence of overlapping toxicities.

The following conversions are provided to aid the reviewer:

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

— COBI (GS-9350) 1 μM = 0.776 μg/mL

1. BRIEF SUMMARY

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-1 (HIV-1) infection in adults aged 18 years and over who are antiretroviral-naive or have no known resistance mutations 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 (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 (NRTI) backbone emtricitabine/tenofovir disoproxil fumarate (FTC/TDF, TVD).

The EVG/COBI/FTC/TDF (QUAD) tablet contains the same dosages of FTC and TDF that are currently approved within Viread, Emtriva, and Truvada® (FTC/TDF) 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 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).

Comprehensive programs of nonclinical pharmacology studies with EVG, COBI, FTC, and TDF have been conducted. Information from all nonclinical studies with EVG, COBI, FTC, and tenofovir (TFV)/TDF 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 and Phase 3 clinical experience with EVG administered with RTV, the Phase 2 clinical experience with COBI, and the Phase 2 and 3 experience with the EVG/COBI/FTC/TDF STR.

To facilitate the evaluation of the EVG/COBI/FTC/TDF STR, nonclinical virology studies of EVG and COBI are described in detail in the integrated 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 and COBI are described in detail in this section. The order of presentation in each section follows the general format: EVG, followed by COBI,

and EVG/COBI/FTC/TDF combination studies. Results of FTC, TDF, and FTC/TDF studies are incorporated when needed to describe the presence or absence of overlapping toxicities.

The nonclinical data discussed within this document support the proposed use of the EVG/COBI/FTC/TDF STR as a complete regimen for the treatment of HIV-1 infection in adults who are antiretroviral treatment-naive or who have no known resistance mutations to the individual components of EVG/COBI/FTC/TDF STR tablet. All information from nonclinical pharmacology studies that is of relevance to the prescriber and patient has been included in the proposed Prescribing Information and Patient Leaflet.

EVG

Elvitegravir is an investigational agent of the INSTI class being developed by Gilead for use once daily in the treatment of HIV-1 infection. Elvitegravir prevents integration of the HIV-1 genetic material into the host-cell genome.

Elvitegravir inhibited viral replication in laboratory strains and various clinical isolates of HIV-1. 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 peripheral blood mononuclear cell (PBMC) cultures. The nonclinical virology data of EVG are described in detail in Module 2.7.2, Section 4.1.

Elvitegravir did not inhibit the activity of human topoisomerase I and II enzymes at up to 50 and 150 μM, respectively.

Elvitegravir (10 μM) did not show greater than 50% inhibition (IC_{50}) on 22 receptors, 7 enzymes, and 3 cell-based assay systems, including the immune cell functions of cell adhesion (ICAM-1/VCAM-1 mediated), interleukin-2 (IL-2) secretion, and mixed lymphocyte reaction (splenic lymphocytes).

The cytotoxicity of EVG was evaluated in a number of cell lines and primary cells. In HIV-1-infected human PBMC cultures, the EVG concentration needed for 50% cytotoxicity (CC_{50}) in the presence of 50% HS was 170 μM (selectivity index [SI] of > 100,000). There was no difference in the cytotoxicity of EVG in stimulated versus unstimulated PBMCs.

Following a 14-day treatment of EVG at 10 μM in HepG2 liver cells, EVG induced no mitochondrial toxicity (changes in the content of mitochondrial deoxyribonucleic acid [mtDNA]).

Elvitegravir had little effect on vital organ systems in safety pharmacology studies, including the central nervous, cardiovascular, respiratory, gastrointestinal (GI), and renal/urinary systems. In the central nervous, renal/urinary, and GI systems, there were no adverse effects in rats at doses up to 2000 mg/kg, the highest dose tested. In the cardiovascular and respiratory systems, EVG did not show any significant effects on blood pressure, heart rate (HR), electrocardiogram (ECG), respiratory rate, or the degree of oxygen saturation in

conscious beagle dogs at doses up to 100 mg/kg, the highest dose tested. In human embryonic kidney 293 (HEK 293) cells expressing the human ether-à-go-go-related gene (hERG) channel, EVG had no effect on the hERG tail current at concentrations up to 1 μM. A slight reduction (24.3%) in tail current was observed at the maximum feasible concentration of 10 μM. No effect on action potential (AP) was observed in isolated guinea pig papillary muscle at concentrations up to 3 μM.

The 2 major metabolites of EVG in nonclinical studies are M4 (acyl glucuronide; GS-9200) and M1 (p-hydroxylated; GS-9202). In humans dosed with 150 mg EVG boosted with RTV, 3.5% of EVG was present as M4 whereas M1 was below quantification limits (see Module 2.7.2, Section 4.1). M4 and M1 did not inhibit the hERG channel in a Chinese Hamster Ovary (CHO) cell line in which M4 and M1 had IC_{50} (50% inhibitory concentration) values greater than 100 and 81 ± 8 μM, respectively.

COBI

Cobicistat is a new chemical entity that is a structural analog of RTV and is a potent mechanism-based inhibitor of CYP3A. 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 protease inhibitors (PIs) atazanavir (ATV) and darunavir (DRV).

The primary pharmacodynamic effect of COBI is mechanism-based inhibition of human CYP3A enzymes (eg, the IC_{50} value for inhibition of human hepatic microsomal midazolam 1'-hydroxylase activity is 0.15 μM). As the primary pharmacodynamic effect of COBI is inhibition of human CYP3A enzymes, the primary pharmacodynamic properties of this drug are briefly noted in this document. More specific details are provided in Module 2.6.4, Pharmacokinetics Written Summary, Section 7.2 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. The nonclinical virology data of COBI, including demonstration of a specific lack of anti-HIV-1 activity, are also described in detail in Module 2.7.2, Section 4.1. Safety pharmacology studies conducted during the early development program for Gilead's investigational HIV-1 PI, GS-8374, that utilized COBI as a pharmacoenhancer, are briefly summarized in this document.

No remarkable cytotoxicity was observed with COBI in vitro in human MT-2 and HepG2 cells, with CC_{50} values of 89 and 44 μM, respectively. In vitro data indicate that COBI shows low potential for inhibition of host proteases (IC_{50} > 30 μM) and a low potential for effects on adipocyte functions (lipid accumulation and glucose uptake).

In vitro studies (protein-free media) demonstrated binding of COBI at sodium, calcium, and potassium (hERG) binding sites. 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).

In rabbit Purkinje fibers (protein-free environment), COBI caused a shortening of the action potential duration (APD) at ≥ 1 μM; there was no evidence of triangulation, instability, or alternans predictive of prolongation of the QT interval. In Langendorff studies in rabbit hearts (protein-free environment), COBI prolonged the PR interval and produced decreases in left ventricular (LV) function at concentrations ≥ 1 μM.

In conscious telemetered dogs, there were no adverse effects on hemodynamic or ECG parameters up to 45 mg/kg (mean plasma COBI concentration 1-hour post-dose was 7.7 μM), the highest dose administered. Mild PR prolongation was noted primarily from 1 to 6 hours postdose, predominantly at 45 mg/kg and sporadically at 15 mg/kg, although mean PR intervals never exceeded the upper limits of normal for canines at any time point.

Although COBI inhibited the L-type calcium ion channel and potassium hERG current at low micromolar concentrations, data from the Purkinje fiber assay, 2 Langendorff studies, the cardiovascular dog study, and ECG evaluations in the repeat-dose toxicity studies in dogs up to 39 weeks dosing (Module 2.6.6, Section 3.2) suggest that COBI has a low potential for QT prolongation, but may have a tendency to slightly prolong the PR interval. Data from the Langendorff studies also suggest that COBI may have the potential to decrease LV function at concentrations that also prolonged the PR interval. The shortening of the APD in rabbit Purkinje fibers, the PR prolongation, and the negative inotropic effects may be a consequence of interaction with cardiac calcium channels {11873}, {11876}.

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 (see Module 2.7.2, Section 2.3.2.2.2 [GS-US-216-0116]).

Cobicistat had no effects on respiratory parameters in rats after single oral doses up to 500 mg/kg. A single oral dose of 50 mg/kg caused no effects on the central nervous system (CNS) in rats. At higher doses (≥ 150 mg/kg), decreased arousal and locomotor activity, salivation, and a decrease in body temperature and motor activity were noted 2 and 6 hours postdose. These effects at ≥ 150 mg/kg may represent a general toxicity response and are not considered a direct effect on the CNS.

When COBI was administered in combination with Gilead's investigational HIV PI, GS-8374, findings in cardiovascular, respiratory, and CNS studies were consistent with studies conducted with COBI alone.

FTC

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

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

TDF

Tenofovir DF, the oral prodrug of TFV, is a nucleotide reverse transcriptase inhibitor (NtRTI), developed by Gilead, that is marketed as a once-daily, film-coated, 300-mg tablet. After absorption, TDF is rapidly converted to TFV, which is metabolized intracellularly to the active metabolite, tenofovir diphosphate.

A comprehensive nonclinical pharmacology 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.

EVG/COBI/FTC/TDF

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.

No remarkable cytotoxicity or mitochondrial toxicity was observed with EVG, COBI, FTC, or TFV. The potential for exacerbating cytotoxicity and 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 ≥ 1 μM, which is approximately 11-fold above the anticipated clinical exposure at the 150 mg COBI dose (maximal plasma concentrations of approximately 1.4 μM and fraction unbound of 6.3% based on in vitro equilibrium dialysis). Further, as the fraction of unbound COBI is lower in plasma samples obtained in clinical studies (2.49% to 3.23%) compared to the in vitro studies, including clinical studies in subjects with moderate hepatic impairment or severe renal impairment (Module 2.7.2, Sections 2.4.1.1 and 2.4.1.2 [Studies GS-US-183-0133 and GS-US-216-0124, respectively]), the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT/QTc clinical study (Module 2.7.2, Section 2.3.2.2.1 [Study GS-US-216-0107]), a modest, dosing-related increase in PR interval was observed, but was not considered to be clinically significant. Given the favorable safety pharmacology profiles of EVG, FTC, and TDF, combination of these 3 agents with COBI is not expected to exacerbate the minor cardiovascular findings of COBI.

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

2. PRIMARY PHARMACODYNAMICS

The primary pharmacodynamic studies evaluating the antiviral activity of EVG are described in detail in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.1.

2.1. EVG

Elvitegravir is a low molecular weight, HIV-1 INSTI that prevents integration of the HIV-1 genetic material into the host-cell genome. The primary pharmacodynamics of EVG are described in detail in the integrated virology summary contained in Module 2.7.2, Section 4.1.1.

Elvitegravir inhibited viral replication in laboratory strains and various clinical isolates of HIV-1 with mean EC_{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 PBMCs in vitro. Elvitegravir also showed activity against HIV-2. The calculated EC_{95} value for EVG was 1.25 nM (0.61 ng/mL) in the absence of HS components and 100 nM (44.8 ng/mL) in the presence of the HS components HSA and AAG in HIV-1 infected human PBMC cultures (see Module 2.7.2, Section 4.1.1).

2.2. COBI

Cobicistat is a structural analog of RTV and is a potent mechanism-based inhibitor of human CYP3A. As the primary pharmacodynamic effect of COBI is inhibition of human CYP3A enzymes, the primary pharmacodynamics of this drug is briefly noted in this document; more specific details are provided in Module 2.6.4, Pharmacokinetics Written Summary, Section 7.2, Pharmacokinetic Drug Interactions and in Module 2.7.2, Summary of Clinical Pharmacology Studies, Section 4.1 to facilitate the presentation and interpretation of this information in the context of the overall drug-drug interaction profile. Further, studies confirming that COBI lacks selective HIV-1 activity are summarized in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.2.

2.2.1. Inhibition of Human CYP3A Activity by COBI

The intended pharmacological effect of COBI is inhibition of human CYP3A enzyme activity. CYP3A inhibition studies, using an established clinical CYP3A inhibitor, RTV, as a comparator, were performed to test the specificity of CYP3A inhibition, mechanism of inhibition, and to determine the enzyme inactivation parameters of COBI (Tabulated Summary 2.6.3.1.2, AD-216-2028).

The potency of COBI as an inhibitor of human hepatic microsomal CYP3A was compared with RTV using established marker activities of CYP3A enzymes (midazolam [MDZ] 1'-hydroxylase, testosterone 6β-hydroxylase, and terfenadine hydroxylase {10575}, {11006}). The effect on the formation of the oxidative metabolite (M1) of EVG, a reaction that is catalyzed by human CYP3A, was also measured. In addition, the effects of COBI and RTV

on the human hepatic microsomal metabolism of ATV and the HCV PI, telaprevir (VX-950), were determined by monitoring the loss of the parent molecule.

Cobicistat and RTV were both potent inhibitors of all human hepatic microsomal CYP3A activities tested (Table 1).

Table 1. Effect of COBI and RTV on Various Activities Catalyzed by Human Hepatic Microsomal CYP3A Enzymes

Activity	Calculated IC_{50} (μM)	
	COBI	RTV
MDZ 1'-hydroxylase	0.15	0.11
Testosterone 6β-hydroxylase	0.15	0.12
Terfenadine *t*-butyl-hydroxylase	0.29	0.28
EVG hydroxylase	0.03	0.03
ATV oxidation	0.04	0.04
Telaprevir oxidation	0.03	0.02

ATV = atazanavir; COBI = cobicistat; EVG = elvitegravir; MDZ = midazolam; RTV= ritonavir
IC_{50} = concentration at which 50% inhibition was achieved
Source: Report AD-216-2028

Studies also showed that the apparent inhibitory potency against both MDZ 1'-hydroxylase and testosterone 6β-hydroxylase could be increased in a preincubation-dependent and cofactor-dependent manner, suggesting that COBI, like RTV, is a mechanism-based inhibitor of human CYP3A enzymes (Tabulated Summary 2.6.3.1.2, AD-216-2028).

Detailed enzyme inactivation kinetic studies were performed comparing COBI with RTV. Cobicistat was found 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; see Tabulated Summary 2.6.3.1.2, AD-216-2028).

2.2.2. In Vivo Pharmacodynamics

In vivo pharmacodynamic studies of COBI (as a pharmacoenhancer) are summarized in Module 2.7.2., Summary of Clinical Pharmacology studies.

3. 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 nonclinical virology summary contained in Module 2.7.2, Section 4.1.2.

The remaining secondary pharmacodynamic effects of EVG and COBI are described in Sections 3.1 and 3.2.

3.1. EVG

3.1.1. Activity Against Cellular Homologs

There is no functional equivalent to integrase (IN) activity in host cells. However, IN and topoisomerases display the analogous activities of deoxyribonucleic acid (DNA) binding, DNA cleavage, and transesterification reactions. The effects of EVG on human topoisomerase I and II activities were examined by measuring the conversion of supercoiled DNA to the relaxed form (Tabulated Summary 2.6.3.1.4; JTK303-PH-004). Elvitegravir did not inhibit the activity of human topoisomerase I and II enzymes at up to 50 and 150 μM, respectively (Table 2). The control compounds camptothecin and amsacrine inhibited topoisomerase I and II, with EC_{50} values of 9.8 and 105 μM, respectively.

Table 2. Effect of EVG Against Human Topoisomerase Activities in DNA Relaxation Assays

Enzyme	IC_{50} (μM)[a]		
	EVG	Camptothecin	Amsacrine
Human topoisomerase I	> 50 (−3.2 ± 2.1%)[b]	9.8 ± 2.2	ND
Human topoisomerase II	> 150 (2.4 ± 2.5%)[c]	ND	105.1 ± 8.0

EVG = elvitegravir; ND = not done

a Mean ± standard deviation from 3 separate experiments

b % inhibition at 50 μM, Mean ± standard deviation

c % inhibition at 150 μM, Mean ± standard deviation

Source: Report JTK303-PH-004

3.1.2. In Vitro Receptor Binding Potencies

The effects of EVG on 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) were evaluated. IC_{50} values were not determined because EVG did not show greater than 50% inhibition at 10 μM in any of these systems (Tabulated Summary 2.6.3.1.4, JTK303-PH-008).

3.1.3. In Vitro Cytotoxicity

3.1.3.1. Cytotoxicity in Human Cells

The cytotoxicity of EVG was evaluated in a number of human cell lines and primary cells. Elvitegravir showed weak cytotoxicity to primary PBMCs (Tabulated Summary 2.6.3.1.4; JTK303-PH-010), primary T-lymphocytes (Tabulated Summary 2.6.3.1.4; PC-186-2004), primary monocytes/macrophages, and macrophages (Tabulated Summary 2.6.3.1.4, PC-186-2004). The results are presented in Table 3.

Table 3. Cytotoxicity of EVG on Human Cells

Cell Type	Study Duration (Days)	CC_{50} (μM)[a]
Primary Human PBMC[b]	7	> 100
Primary Human T-lymphocytes[c]	5	40
Primary Human Monocytes/macrophages[c]	7	> 500
Human Macrophages[c]	5	25.6

CC_{50} = concentration at which 50% maximum cytotoxicity was achieved

a In the absence of human serum

b MTS-based assay

c XTT-based assay

In a separate study, EVG showed cytotoxicity in a dose-dependent manner after 7 days of culture with PBMCs using a [^{3}H]thymidine incorporation assay, 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 in stimulated and unstimulated PBMCs, respectively; Tabulated Summary 2.6.3.1.4, PC-183-2001). In HepG2 and Huh7 cells containing hepatitis B virus (HBV) and hepatitis C virus (HCV), respectively, the EVG CC_{50} values were 16 and >6 μM, respectively (Module 2.7.2, Section 4.1.2.2 [PC-183-2024]).

3.1.4. Mitochondrial Toxicity

A variety of clinical symptoms observed in HIV patients treated with prolonged NRTI therapy may be linked to mitochondrial toxicity. These include myopathy and cardiomyopathy, polyneuropathy, lactic acidosis, pancreatitis, lipodystrophy, and possibly others {2522}. While EVG is an INSTI and not expected to have this activity, EVG was tested in vitro for its effect on mitochondrial DNA levels in HepG2 liver cells. Following a 14-day treatment of EVG at 10 μM, EVG induced no measurable changes in the content of mtDNA as determined by a dot-blot hybridization analysis (Tabulated Summary 2.6.3.1.4, TX-183-2009).

3.2. COBI

3.2.1. Effect on Host Proteases

As RTV is known to inhibit some host aspartic proteases in addition to the HIV protease {11952}, the inhibitory activity of RTV was characterized against selected human enzymes (using purified enzymes and specific substrates), including cathepsin D, renin, and beta-site APP-cleaving enzyme 1 (BACE-1; Tabulated Summary 2.6.3.1.5, PC-216-2001). Ritonavir (up to 30 μM concentration) did not show any inhibition of renin and BACE-1, but significantly inhibited cathepsin D activity with an IC_{50} value of 0.87 μM (Table 4). Therefore, the inhibitory activity of COBI against cathepsin D was evaluated in vitro. Unlike RTV, COBI did not exhibit any activity on this enzyme, showing an IC_{50} value of > 30 μM.

Table 4. Effect of COBI and RTV on HIV-1 Protease and Cathepsin D Activity

Compounds	IC_{50} ± SD (μM)[a]	
	HIV-1 Protease	Cathepsin D
COBI	> 30	> 30
RTV	0.0006 ± 0.0001	0.87 ± 0.37

COBI = cobicistat; RTV = ritonavir; IC_{50} = concentration at which 50% maximum inhibition was achieved

a Data shown represent the mean and standard deviation from at least 2 independent experiments.

Source: Report PC-216-2001

3.2.2. Effect on Proteasome Activity

As RTV has been observed to inhibit proteasome activity {11952}, {11358}, the inhibition by COBI of the chymotryptic-like activity of the 26S proteasome was assessed (Tabulated Summary 2.6.3.1.5, PC-216-2001). Compared to RTV, COBI showed slightly reduced inhibition of the proteasome chymotryptic-like activity, with an IC_{50} value of 12.8 μM (versus 7.9 μM for RTV; Table 5). This low level of proteasome inhibitory activity is unlikely to be significant at maximal unbound clinical exposure level of 0.095 μM COBI (GS-US-236-0103).

Table 5. Effect of COBI and RTV on Proteasome Activity

Compounds	Proteasome Activity IC_{50} ± SD (μM)[a]
COBI	12.8 ± 3.7
RTV	7.9 ± 1.0

COBI = cobicistat; RTV = ritonavir; IC_{50} = concentration at which 50% maximum inhibition was achieved

a Data shown represent the mean and standard deviation from 2 independent experiments.

Source: Report PC-216-2001

3.2.3. Effects on Adipocytes

Chronic treatment of HIV-infected patients with RTV is known to induce changes in body fat distribution (lipodystrophy); elevated blood levels of cholesterol (hypercholesterolemia) and triglycerides (hyperlipidemia); and insulin resistance {5117}. Some of these effects appear at least in part to be due to the direct effects of RTV on adipocytes {11024}. In vitro, RTV has been shown to affect adipocyte functions such as differentiation-associated lipid accumulation and insulin-stimulated glucose uptake {5495}.

The effects of COBI and RTV on adipocyte functions were evaluated using 2 in vitro assays (Tabulated Summary 2.6.3.1.5, PC-216-2004). The first assay monitored normal lipid accumulation in cultured human adipocytes following the induction of differentiation and the second assay evaluated insulin-stimulated glucose uptake in differentiated mouse adipocytes. In both assays, ATV was evaluated as a comparator, representing an HIV PI with the least metabolism-related adverse effects {11058}.

Ritonavir showed a significant effect on both lipid accumulation in differentiating human adipocytes and on inhibition of glucose uptake in mouse adipocytes (Table 6). In contrast, both COBI and ATV exhibited no effect on lipid accumulation and a less pronounced effect on glucose uptake at the tested concentrations, suggesting a lower potential of COBI for metabolism-related toxicities compared to RTV.

Table 6. Effects of COBI, RTV, and ATV on Adipocyte Functions

Compounds	Lipid Accumulation EC_{50} ± SD (μM) [a]	Glucose Uptake (% Inhibition at 10 μM ± SD) [a]
COBI	> 30	9.5 ± 6.4
ATV	> 30	0.4 ± 0.9
RTV	16 ± 8	55 ± 10

COBI = cobicistat; ATV = atazanavir; RTV = ritonavir; EC_{50} = half maximal effective concentration

a Data shown represent the mean and standard deviation from 3 independent experiments.

Source: Report PC-216-2004

3.2.4. In Vitro Receptor Binding Potencies

Potential molecular targets for COBI were screened, using radioligand binding assays against a panel of 67 mammalian ion channels and receptors. Responses of > 50% at a concentration of 10 μM (free COBI concentration) were considered significant. At 10 μM, COBI demonstrated significant binding to 3 ion channels (Tabulated Summary 2.6.3.1.5, TX-168-2007 and TX-168-2011). Using the same screen, RTV demonstrated binding to the sodium channel and to the kappa-opiate receptor. A summary of the data, with a comparison to RTV, is provided in Table 7 (Tabulated Summary 2.6.3.1.5, PC-137-2004 and PC-168-2005). Additional electrophysiology studies conducted to evaluate the effects of COBI and RTV on the steady-state block of cardiac ion channels (potassium, calcium, and sodium channels) using patch clamp techniques are summarized in Section 4.2.2.1.

Table 7. Molecular Target Screen with COBI and RTV

Channel or Receptor	IC_{50} or % Inhibition at 10 μM	
	COBI	RTV
Calcium Channel L-type, benzothiazepine	6.45 μM	NS
Potassium Channel, hERG	54%	NS
Sodium Channel, Site 2	0.137 μM	7.22 μM
Opiate k (OP2)	NS	61%

COBI = cobicistat; RTV = ritonavir; NS = no significant binding activity (< 35% inhibition)
Source: Reports TX-168-2007 and TX-168-2011, PC-137-2004 and PC-168-2005

3.2.5. In Vitro Cytotoxicity

The in vitro cytotoxicity of COBI and RTV was evaluated in MT-2 lymphoblastoid T-cells following a 5-day incubation and in HepG2 hepatoma cells following a 3-day incubation (Tabulated Summary 2.6.3.1.5, PC-216-2003). Cobicistat was slightly less cytotoxic than RTV in MT-2 cells and showed cytotoxicity similar to RTV in HepG2 cells (Table 8).

Table 8. Cytotoxicity of COBI in MT-2 and HepG2 Cells

Compounds	$CC_{50} \pm SD$ (μM)[a]	
	MT-2 cells[b]	HepG2 cells[c]
COBI	88.6 ± 13.2	44 ± 7
RTV	37.6 ± 18.5	64 ± 25

CC_{50} = 50% cytotoxic concentration; COBI = cobicistat; RTV, ritonavir
a Data shown represent the mean and standard deviation from at least 3 independent experiments.
b XTT-based assay
c CellTiter Glo-based assay
Source: Report PC-216-2003

3.3. EVG/COBI/FTC/TDF

Given the lack of remarkable changes in the in vitro studies with the individual agents, no additional secondary pharmacodynamic studies have been conducted for the EVG/COBI/FTC/TDF combination.

4. SAFETY PHARMACOLOGY

In vitro and in vivo safety pharmacology data for EVG, COBI, and the EVG/COBI/FTC/TDF combination are presented in Sections 4.1 to 4.3.

4.1. EVG

The effects of EVG on the CNS, cardiovascular system, and respiratory system were examined in a core battery of safety pharmacology studies. Effects on the GI and renal/urinary systems were also examined. In vivo studies were performed in rats to evaluate the effects on the central nervous system, GI, and renal/urinary system, and in beagle dogs to evaluate the effects on the cardiovascular and respiratory systems. The core battery of studies were conducted according to Japanese Good Laboratory Practice (GLP), and consistent with recommendations provided in ICH S7A (Safety Pharmacology Studies for Human Pharmaceuticals), while the GI and renal/urinary system studies were conducted following good scientific practices and established methods and protocols.

In the in vitro tests, EVG was dissolved in dimethyl sulfoxide (DMSO) and diluted with each test buffer. The maximally soluble concentration in each of the assay solutions was used as the maximum concentration of EVG.

4.1.1. Central Nervous System

Single doses of EVG vehicle control (0.5% w/v methyl cellulose [MC] solution), 100, 300, and 2000 mg/kg were administered orally to male Sprague-Dawley rats via gavage at a dose volume of 10 mL/kg (6 animals/dose group; Tabulated Summary 2.6.3.4.2, JTK303-SP-001). There were no adverse effects of EVG in the Irwin test of central nervous system general signs and behavior at doses up to 2000 mg/kg.

4.1.2. Cardiovascular System

4.1.2.1. In Vitro

Elvitegravir at concentrations of 0.1 and 1 μM had no effect on the 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. In isolated guinea pig papillary muscle, EVG at concentrations up to and including 3 μM had no effect on action potential parameters, including resting membrane potential, action potential amplitude, action potential duration at 50% and 90% of repolarization, and maximal upstroke velocity (Tabulated Summary 2.6.3.4.1, JTK303-SP-004).

4.1.2.2. In Vivo

Elvitegravir was orally administered to 4 male conscious, telemetered beagle dogs at 0 (vehicle; corn oil), 10, 30, and 100 mg/kg, in a dose volume of 2.5 mL/kg, in ascending order with an interval of 7 days between each of the doses. At doses up to 100 mg/kg, EVG

did not produce adverse effects on blood pressure, HR, or the ECG up to 24 hours after dose administration (Tabulated Summary 2.6.3.4.2; JTK303-SP-002).

4.1.3. Respiratory System

Elvitegravir was orally administered to 4 male beagle dogs at 0 (vehicle; corn oil), 10, 30, and 100 mg/kg in ascending order with an interval of 7 days between each of the doses. At doses up to 100 mg/kg, EVG did not produce any adverse effects on respiratory rate or oxygen saturation (Tabulated Summary 2.6.3.4.2; JTK303-SP-002).

4.1.4. Renal/Urinary System

The effects of EVG on the urinary system were examined in saline-loaded rats (Tabulated Summary 2.6.3.4.2; JTK303-SP-007). Fasted Sprague-Dawley rats (n=6/group) were administered vehicle (0.5% MC), EVG (100, 300, and 2,000 mg/kg), or the positive control (furosemide, 30 mg/kg) orally at a dose volume of 10 mL/kg. Immediately after administration, the animals were administered physiological saline orally in a dose volume of 25 mL/kg. The rats were then placed in metabolism cages, water and food were withheld for 5 hours, and urine was collected for 5 hours. Elvitegravir at doses up to 2000 mg/kg had no effect on the urine volume or the urinary excretion of electrolytes (Na^+, K^+, Cl^-) in saline-loaded Sprague-Dawley rats.

4.1.5. Gastrointestinal System

4.1.5.1. Ex Vivo

The effect of EVG on the autonomic nervous system and smooth muscle was examined using isolated guinea pig ileum (Tabulated Summary 2.6.3.4.1; JTK303-SP-005). Guinea pigs fasted overnight were sacrificed by exsanguination and the ileum was harvested. The effect of EVG on single contractions induced by acetylcholine (1 μM), histamine (1 μM), or barium chloride (3 mM) were measured, and the effect on resting tone was examined. Elvitegravir at 1.0 and 10 μM had no effect on single contractions by any of the contraction inducers. At 30 μM, inhibition of contractions induced by acetylcholine (9.0%) and barium chloride (19.6%) was observed. Slight inhibition (14.7%) was observed with contractions induced by histamine, but the difference was not significant. For resting tone, EVG showed no differences in the single contraction rate when compared with the addition of vehicle. In summary, EVG at a high concentration (30 μM) inhibited single contractions by each of the contraction inducers, but the inhibition was slight, indicating that EVG has no definite effect on the autonomic nervous system or smooth muscle.

4.1.5.2. In Vivo

In a GI system study, EVG did not affect the intestinal transport of a charcoal meal in male Sprague-Dawley rats (n=10/group) at doses up to 2000 mg/kg (Tabulated Summary 2.6.3.4.2; JTK303-SP-006).

4.1.6. Pharmacologic Profiles of Metabolites, Stereoisomers, and Impurities

The most prominent metabolites of EVG, GS-9200 (M4) and GS-9202 (M1) were characterized to determine their ability to inhibit the hERG channel in CHO cells (Tabulated Summary 2.6.3.4.1, TX-183-2005). GS-9200 and GS-9202 had IC_{50} values of > 100 μM and 81 μM, respectively. Of note, precipitate was evident in GS-9202 solutions at 30 and 100 μM and therefore the IC_{50} value was an estimate. Nevertheless, these concentrations are at least 250-fold above clinical exposures, and not clinically significant.

4.2. COBI

The effects of COBI on the central nervous, cardiovascular, and respiratory systems have been examined in a battery of safety pharmacology studies. The core battery of safety pharmacology studies were conducted in accordance with GLP guidelines and with recommendations provided in ICH S7A. Pilot, exploratory, and mechanistic cardiovascular studies were conducted using appropriate protocols and documentation to ensure data integrity. These in vitro and in vivo studies with COBI, and with COBI administered with the HIV PI ATV, are presented in Sections 4.2.1 to 4.2.3. Female rats were used in the in vivo CNS and respiratory studies as higher exposures could be obtained in this sex (Module 2.6.6, Section 2.2.2). In addition, studies conducted during the early development program for Gilead's investigational HIV PI, GS-8374, that used COBI as a pharmacoenhancer, are only briefly summarized as findings in these studies were consistent with studies conducted with COBI alone.

4.2.1. Central Nervous System

4.2.1.1. Effects of COBI in Rats

The purpose of this study was to evaluate the pharmacological effects of COBI on the CNS following oral administration in the albino rat (Tabulated Summary 2.6.3.4.4, TX-216-2006). Four groups of female Sprague-Dawley rats (8/group) were given a single oral gavage dose of vehicle (95% propylene glycol [PG], 5% ethanol [EtOH] with 0.005M HCl), 50, 150, or 500 mg/kg of COBI at a dose volume of 10 mL/kg. Assessment was based on mortality, clinical signs, body weight, and a Functional Observation Battery (FOB) and motor activity evaluation.

There were no deaths noted in this study. Cobicistat administered at 50 mg/kg resulted in no adverse effects on the qualitative or quantitative parameters of the FOB assessment. Decreases in arousal incidence in the observation arena were noted in rats treated with 150 and 500 mg/kg at 2 hours postdose and were still present at the 6-hour postdose assessment. Furthermore, rats administered COBI at doses of 150 and 500 mg/kg displayed slight decreases in locomotor activity level (qualitative) in the arena during the 2-hour postdose assessment. The decreases in locomotor activity in the arena were still evident during the 6-hour postdose assessment. A 4% and 8% decrease in the body temperature was noted following doses of 150 and 500 mg/kg, respectively. The decreased temperature reached a maximum at 2 hours postdose for animals dosed with 150 mg/kg COBI and returned to predose and control values by 6 hours postdose. For animals dosed with

500 mg/kg COBI, the maximum effect was seen at 6 hours postdose. Slight to moderate salivation was seen at the 30 minutes postdose assessment following doses of 150 and 500 mg/kg. Doses of 150 and 500 mg/kg COBI caused statistically significant decreases in motor activity (quantitative) at 2 hours postdose. Decreases in motor activity were still evident at the 6-hour postdose assessment at 500 mg/kg.

In conclusion, a single oral administration of COBI at 50 mg/kg caused no effects on the CNS of the albino rat. Administration of 150 and 500 mg/kg COBI resulted in decreased arousal and locomotor activity in the arena, salivation, and a decrease in body temperature and motor activity. These findings were most notable at 2 or 6 hours postdose. Based on these findings, the no-observable-adverse-effect-level (NOAEL) for this study was 50 mg/kg.

4.2.1.2. Effects of COBI and GS-8374 in Rats

Single oral gavage doses of coformulations of 50 mg/kg COBI with 100, 300, or 1000 mg/kg GS-8374; or 1000 mg/kg GS-8374 alone administered to male Crl:CD(SD) rats (6 animals/group) produced no effects as determined by a modified Irwin observational battery designed to detect potential effects on the central and peripheral nervous system (Tabulated Summary 2.6.3.4.4, PC-201-2002). The no-observed-effect-level (NOEL) was considered to be 50/1000 mg/kg COBI/GS-8374.

4.2.2. Cardiovascular System

4.2.2.1. Standard Battery Studies

4.2.2.1.1. Effects of COBI on the Cloned hERG (I_{kr}) Potassium Channel

The in vitro effects of COBI on ionic currents in voltage-clamped HEK293 cells that stably express hERG were determined in a GLP study (Tabulated Summary 2.6.3.4.3, TX-216-2009). Four concentrations of COBI (0.3, 1, 3, and 10 μM), in a vehicle of 0.3% DMSO in HEPES-buffered physiological saline, were tested. Cobicistat inhibited hERG potassium current by (mean; n = 3) 3.2% at 0.3 μM, 37.1% at 1 μM, 64.2% at 3 μM, and 89.5% at 10 μM (versus 0.8% in the vehicle control). hERG inhibition was statistically significant for all 4 concentrations ($p < 0.05$). The IC_{50} for the inhibitory effect of COBI on hERG potassium current was 1.8 μM (Hill coefficient = 1.3). Under identical conditions, the positive control (60 nM terfenadine) inhibited hERG potassium current by (mean; n = 2) 86.3% confirming the sensitivity of the test system to hERG inhibition.

4.2.2.1.2. Effect of COBI on the Cardiovascular System in Telemetered Conscious Dogs

The purpose of this GLP study was to evaluate the pharmacological effects of COBI on hemodynamic and ECG parameters in the beagle dog via telemetry following an oral gavage administration (Tabulated Summary 2.6.3.4.4, TX-216-2008). In a dose escalation design, each of 4 male dogs received vehicle (95% PG, 5% EtOH [with 0.005M HCl]), 5, 15, and 45 mg/kg COBI, at a dose volume of 2 mL/kg, as a single oral gavage dose with a minimum of 2 days between each dose. Individual body weights were measured and all animals were

examined twice daily for mortality and signs of ill health or reaction to treatment. The following parameters were evaluated: arterial blood pressures (mean arterial pressure, systolic blood pressure, diastolic blood pressure, and pulse pressure), HR, and quantitative ECG intervals. Plasma samples were taken from all animals at approximately T_{max} (1 hour postdose) and analyzed for levels of COBI.

There were no deaths in this study. Emesis was observed in 3 of 4 animals following doses of 15 and 45 mg/kg. For 2 animals, emesis was noted up to 5 and 8 times and for up to 2 hours following dosing with 45 mg/kg. There were no compound-related effects on any hemodynamic parameter, no qualitative waveform abnormalities, and no effects on HR or QRS interval parameters. After oral administration of COBI, an increase in the mean PR interval was observed, predominantly following the high dose (45 mg/kg) and sporadically at the mid dose (15 mg/kg). The magnitude of the mean PR interval prolongation was mild with PR interval increases up to 12.2 msec compared to predose values. However the range of absolute PR interval values was 91.8 to 99.6 msec, which did not exceed the upper limits of normal for canines (130 msec) at any time point. Administration of COBI caused a mild increase in the mean QTc interval only following the high (45 mg/kg) dose. The magnitude of the QTc interval prolongation was mild (< 4%), unlikely to be biologically significant, and not considered adverse. There was no effect of the low dose (5 mg/kg) on any quantitative ECG parameter. Plasma samples taken 1 hour postdose to assess exposure showed mean concentrations of 560, 3770, and 5960 ng/mL (0.72, 4.86 and 7.68 μM, respectively) following doses of 5, 15, and 45 mg/kg, respectively.

In conclusion, a single oral dose of COBI, at dose levels of 5, 15, and 45 mg/kg, had no adverse effects on hemodynamic and electrocardiographic parameters as measured in this study. Based on these findings, the NOAEL for COBI in this study was at least 45 mg/kg.

4.2.2.1.3. Effect of COBI and GS-8374 on the Cardiovascular System in Telemetered Conscious Dogs

In a GLP study, 6 male purebred beagle dogs were given a single oral gavage administration of each of the following dosages using a Latin square design: vehicle control (5% ethanol, 20% solutol HS-15, 65% propylene glycol, and 10% 0.10 M sodium phosphate in reverse osmosis water, pH 3), 15 mg/kg COBI alone, 15 mg/kg COBI coformulated with 15, 75, or 200 mg/kg GS-8374, or 200 mg/kg GS-8374 alone at a dose volume of 2 mL/kg (Tabulated Summary 2.6.3.4.4, PC-201-2004). No potentially undesirable pharmacodynamic effects attributable to the administration of COBI or GS-8374 alone or in a coformulation were noted in this study. Based on these results, the NOEL for cardiovascular parameters was 15 mg/kg COBI, and 15/200 mg/kg COBI/GS-8374.

4.2.2.2. Follow-Up Studies

4.2.2.2.1. Effects of COBI and RTV on Cardiac Ion Channels Expressed in Human Embryonic Kidney Cells

As in vitro studies demonstrated binding of COBI at sodium, calcium, and potassium (hERG) binding sites (Section 3.2.4 and Tabulated Summary 2.6.3.1.5, TX-168-2007 and

TX-168-2011), the potential effects of COBI on the steady-state block of cardiac ion channels were determined using patch clamp techniques in a non-GLP study. Cobicistat inhibited the hERG potassium current (IC_{50} 1.9 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-216-2015).

For comparison, effects of RTV (GS-017415) on the steady-state block of cardiac ion channels were also determined. The IC_{50} for the hERG channel was 8.75 μM, with a Hill coefficient of 1.11. However, the presence of precipitates were noted in all physiological saline solutions at concentrations > 7.7 μM RTV. Owing to the low solubility limit of RTV in the sodium and calcium channel physiological solutions, the IC_{50} and Hill coefficients could not be determined for RTV inhibition of the cardiac sodium and calcium channels.

4.2.2.2.2. Effect of COBI on Action Potentials in Isolated Rabbit Cardiac Purkinje Fibers

Since COBI was inhibitory at the I_{Kr} (hERG), $Ca_v1.2$, and $Na_v1.5$ ion channels, further testing was conducted with rabbit Purkinje fibers. Purkinje fibers provide a tool to study the effect of a compound in a more complex system than a binding assay, and the resultant AP depicts the sum effect of all ion channels and allows a prediction of the resultant ECG. Intracellular recordings in Purkinje fibers have been recommended as an important component of nonclinical screening for potentially proarrhythmic side effects of drug-induced AP prolongation {11877}.

In a non-GLP study, effects of COBI and RTV on cardiac APs using Purkinje fibers excised from adult female rabbit ventricles were assessed at 4 concentrations (0.03, 0.1, 1, and 10 μM), with increasing concentrations added sequentially to 4 fiber preparations at 2 stimulus frequencies (1 and 2 Hz); AP parameters were compared to time-matched vehicle controls (Tabulated Summary 2.6.3.4.3, TX-168-2012).

Cobicistat at 1 and 10 μM caused a shortening of the action potential duration (APD_{60} and APD_{90}) that was statistically significant over the effect produced by the vehicle at a stimulus frequency of 1 Hz. At a stimulus frequency of 2 Hz, only the shortening of APD_{60} and APD_{90} observed at 10 μM was statistically significant. Changes in other AP parameters were not statistically different compared to those produced by the vehicle (rabbit Purkinje fiber Tyrode's solution + 0.3 μM DMSO) suggesting a low potential for QT prolongation for COBI. Ritonavir at 9.6 μM produced a decrease in the rate of conduction, V_{max} (dv/dt_{max}), which was statistically significant over that produced by the vehicle at stimulus frequencies of 1 and 2 Hz. The other AP parameters were not affected in a statistically significant manner over those observed with the vehicle. The positive control agent, *dl*-Sotalol, significantly prolonged action potential duration (APD_{60} and APD_{90}) validating the performance of the assay system.

4.2.2.2.3. Effects on Isolated Rabbit Hearts (Langendorff Method)

The isolated heart acts as a physiologically relevant bridge between purely in vitro assays and whole animal studies. It allows for simultaneous observation of a test article's hemodynamic, electrocardiographic, and electrophysiologic effects. The rabbit has been used extensively for cardiovascular studies and is an appropriate species to model potential effects on the human heart, since rabbit cardiac APs (similar to human cardiac APs) appear to be strongly driven by I_{Kr} {17595}, {17597}. Female rabbit hearts were used due to their increased susceptibility to develop Torsades de pointes compared to male rabbit hearts {17596}.

4.2.2.2.3.1. Effects of COBI on Isolated Rabbit Hearts

The objective of this non-GLP study was to evaluate the effects of COBI on isolated rabbit hearts at nominal concentrations of 0.3, 1, 3, and 10 μM (Tabulated Summary 2.6.3.4.4, PC-216-2007). In one group of hearts (n = 4 per vehicle and test group), the atrioventricular (AV) node was ablated and the heart paced to circumvent QT effects secondary to drug-induced changes in HR and to measure rate dependent effects of COBI. Changes in the QT interval; QRS duration of the ECG together with the monophasic action potential duration at 30, 60, and 90 percent repolarization ($MAPD_{30}$, $MAPD_{60}$, and $MAPD_{90}$); triangulation ($MAPD_{90}$-$MAPD_{30}$); stability of the MAP; and left ventricular contractility (LVDP, dP/dt_{min} and dP/dt_{max}) were evaluated. In another group of hearts (n = 4 per vehicle and test group), the ECG was measured from free running hearts and the effects of COBI on the RR and PR intervals were determined. At each concentration, mean values were compared to baseline values.

Exposure to 0.3, 1, 3, and 10 μM COBI shortened the QT interval by 8.8% to 20%. The average QT interval was significantly different from baseline at 3 and 10 μM COBI only. The QRS interval was unaffected at concentrations up to 10 μM COBI.

Exposure to 0.3, 1, 3, and 10 μM COBI caused a dose-dependent shortening of the $MAPD_{30, 60, 90}$ by 3.8% to 43.9%. Compared to baseline, statistically significant decreases were noted at ≥3 μM for $MAPD_{30}$, ≥1 μM for $MAPD_{60}$, and at ≥3 μM for $MAPD_{90}$. Triangulation was slightly reduced after exposure to 0.3 and 1 μM COBI and slightly increased after exposure to 3 and 10 μM, although none of the triangulation values were statistically different to baseline values. In addition, stability of the MAP (beat-to-beat variability) and reverse use-dependency of $MAPD_{60}$ were not significantly affected by COBI.

Cobicistat reduced left ventricular contractility of the isolated heart in a dose-dependent manner. LVDP was reduced by 7% to 61%; dP/dt_{min} by 7.5% to 91%; and dP/dt_{max} by 10.7% to 88.6%. Decreases in LVDP, dP/dt_{min}, and dP/dt_{max} were statistically different from baseline at 1, 3, and 10 μM COBI.

In free running hearts, COBI increased the PR interval by 4.3%, 16.6%, 41.3%, and 95.5% at concentrations of 0.3, 1, 3, and 10 μM, respectively. The PR interval was statistically different from baseline after exposure to 3 and 10 μM. Also, in 3 of the 4 hearts tested,

second degree AV block developed after exposure to 10 μM COBI. Cobicistat significantly increased the RR interval by 25.8% and 63.5% at 3 and 10 μM, respectively.

In conclusion, COBI was associated with negative inotropic effects and shortening of the APD on the isolated rabbit heart at concentrations ≥ 1 μM. At ≥ 3 μM, decreases in the QT interval and increases in the PR and RR intervals were noted. There were no notable effects on the QRS interval, triangulation, and stability. There were no remarkable effects noted on hemodynamic, electrophysiologic, and electrocardiographic parameters at a concentration of 0.3 μM COBI.

4.2.2.2.3.2. Effects of COBI, ATV, and COBI Plus ATV on Isolated Rabbit Hearts

The isolated rabbit heart was utilized in this non-GLP study to determine the effects of COBI alone (0.15, 0.45, 1.5, and 4.5 μM), ATV alone (1.5, 4.5, 15, and 45 μM), as well as a combination of ATV (fixed, 1.5 μM) and escalating concentrations of COBI (0.045, 0.15, 0.45, and 1.5 μM) on cardiac hemodynamic and electrophysiologic parameters (Tabulated Summary 2.6.3.4.3, PC-216-2009).

Hearts (4 per test group) from female New Zealand White rabbits were perfused in Langendorff constant-flow mode with Krebs-Henseleit buffer and exposed to increasing concentrations of test articles for 15 minutes at each concentration. Left ventricular (LV) function, including reduced developed pressure (LVDP), contractility (LV dP/dt_{max}), relaxation (LV dP/dt_{min}), and coronary perfusion pressure (CPP) were determined to assess the effects of the test articles on hemodynamic function. Heart rate; QT, QRS, and PR intervals; $MAPD_{30}$, $MAPD_{50}$ and $MAPD_{90}$; and action potential triangulation ($MAPD_{90}$–$MAPD_{30}$) were determined to assess the effects of the test articles on cardiovascular electrophysiology. At each concentration of test article, mean values were compared to baseline values. In addition, the appearance of early after depolarizations (EADs) on the monophasic action potential waveform was also quantified.

The concentrations of COBI and ATV were measured in DMSO stock solutions and post-perfusion medium. As initial analyses showed low concentrations of COBI in post-perfusion samples compared to target values (35%-48% of target); additional research was conducted to investigate whether these low values were reflective of low stability of COBI in perfusion buffer, or were due to problems encountered in extraction of COBI from perfusion buffer prior to analysis. These results suggested that inadequate extraction of COBI from perfusion buffer was responsible for the low measured COBI concentrations in post perfusion samples. As a result, measured concentrations of COBI and ATV in perfusion samples are not considered reflective of probable exposures achieved in the Langendorff study, and nominal concentrations are noted below.

Exposure to COBI at concentrations ≥ 1.5 μM was associated with significant decreases in LV function, including LVDP (–50% decrease at 1.5 μM), contractility (–50% decrease at 1.5 μM), and impairment of relaxation (–54% decrease at 1.5 μM). Significant increases in CPP were noted at ≥ 1.5 μM (37% increase at 1.5 μM), suggesting a possible vasoconstrictive effect of the compound.

Exposure to ATV was associated with slight, but not statistically significant, decreases in LV function (LVDP, LV dP/dt_{max}, LV dP/dt_{min}) at concentrations ≥ 15 µM. There were no notable effects of ATV on CPP at concentrations up to 15 µM.

When hearts were exposed to escalating concentrations of COBI (≤ 1.5 µM) in combination with 1.5 µM ATV, the negative inotropic effects of 1.5 µM COBI appeared slightly reduced, with LV developed pressure decreasing approximately 30% compared to baseline (versus 50% with 1.5 µM COBI alone). In addition, there were no notable effects of the combination on CPP, suggesting a reversal of the putative COBI-induced vasoconstriction.

The highest concentration of the combination (1.5 µM COBI/1.5 µM ATV) significantly decreased heart rate by 26% compared to baseline, as compared to an 8% decrease with 1.5 µM COBI alone, and to a 12% decrease with 1.5 µM ATV alone.

Exposure to 4.5 µM COBI or 15 µM ATV significantly increased the PR interval by 62% and 45% (versus baseline), respectively. Trends toward increased PR interval were noted with both compounds alone at concentrations of 1.5 µM COBI (23% increase) and 1.5 µM ATV (10% increase). When tested in combination, a significant increase in the PR interval was noted at the highest concentration of 1.5 µM COBI/1.5 µM ATV (37% increase versus baseline) only.

There were no notable changes in QRS duration, QT interval, MAPD, or triangulation at concentrations up to 4.5 µM COBI. Atazanavir, when tested alone, was associated with significant increases in $MADP_{90}$ at 45 µM, and a trend towards increases in triangulation at 4.5 µM that reached statistical significance at 45 µM. In addition, 1 of 4 hearts developed ventricular tachycardia, and 2 hearts developed AV dissociation at 45 µM ATV. There were no notable changes in QRS duration, QT interval, MAPD, or triangulation with combinations of COBI and ATV. Further, COBI, ATV, and combinations of COBI and ATV, were not associated with the development of EADs.

In summary, all 3 regimens (COBI, ATV, and COBI/ATV in combination) were associated with negative inotropic effects on the isolated rabbit heart. When 1.5 µM COBI was coadministered with 1.5 µM ATV, effects on LV function were similar to the decreases noted at 1.5 µM COBI alone. Decreases in HR and increases in the PR interval were noted with both compounds alone, and with the highest concentration of the combination (1.5 µM COBI/1.5 µM ATV). There were no notable effects of the combination on QRS, QT interval, MAPD, and triangulation, and there was no association with EADs. There were no remarkable effects noted on hemodynamic, electrophysiologic, or electrocardiographic parameters at concentrations of 0.45 µM COBI, 4.5 µM ATV, or with the combination at 0.45 µM COBI plus 1.5 µM ATV.

4.2.3. Respiratory System

4.2.3.1. Effects of COBI in Rats

The purpose of this GLP study was to evaluate the pharmacological effects of COBI on the respiratory system following oral gavage administration in the albino rat using a ‘head out’

plethysmography system (Tabulated Summary 2.6.3.4.4, TX-216-2007). Groups of 6 female Sprague-Dawley rats received a single oral gavage administration of vehicle (95% PG, 5% EtOH with 0.005 M HCl), 50, 150, or 500 mg/kg COBI at a dose volume of 10 mL/kg. Assessment was based on body weight, mortality, clinical signs, and ventilatory parameters (tidal volume, respiratory rate, and derived minute volume).

Treatment-related clinical signs were noted in all COBI-treated groups. Erected fur, decreased activity, and salivation were noted in all groups dosed with COBI. A single oral gavage of COBI at doses up to 500 mg/kg resulted in no treatment-related effects on respiratory rate, tidal volume, or derived minute volume in the female albino rat. Mean respiratory rates of control- and COBI-treated animals were decreased 12% to 36% at the 30- to 45-minute interval after dosing, when compared to the respective predose values; the rates remained below the predose values up to 6 hours postdose in both control- and COBI-treated groups. Although these decreases were slightly larger in treated animals compared to controls, there was no apparent dose-response relationship, the changes were not considered related to COBI administration, nor were the changes considered adverse in nature as they did not translate into any effects in the derived minute volume or into any compensatory increases in the tidal volume.

In conclusion, COBI administered orally to female rats at dose levels up to 500 mg/kg had no adverse effects on the respiratory rate, tidal volume, or derived minute volume. Based on these results, the NOAEL for respiratory effects in the rat was considered to be 500 mg/kg COBI, the highest dose administered.

4.2.3.2. Effects of COBI and GS-8374 in Rats

No potentially undesirable pharmacodynamic effects on respiratory function in male rats (8 animals/group) were observed following a single oral gavage dose of coformulations of 50 mg/kg COBI with 100, 300, or 1000 mg/kg GS-8374, or 1000 mg/kg GS-8374 alone (Tabulated Summary 2.6.3.4.4, PC-201-2003). Based on these results, the NOEL on respiratory function was considered to be 50/1000 mg/kg COBI/GS-8374.

4.3. EVG/COBI/FTC/TDF

Elvitegravir, FTC, and TDF had little effect on vital organ systems in safety pharmacology studies as determined in a variety of in vitro and in vivo safety pharmacology studies. While COBI has a 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, the minimal effects observed with high-dose administration of the individual agents (EVG, FTC, or TDF) alone indicate that additional safety pharmacology studies on the combination product are considered unwarranted as they would be unlikely to reveal new pharmacologic activities.

5. PHARMACODYNAMIC DRUG INTERACTIONS

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

6. DISCUSSION AND CONCLUSIONS

6.1. EVG

Elvitegravir specifically inhibits HIV-1 integrase strand transfer activity and the integration of viral DNA into host chromosomal DNA. Elvitegravir inhibited viral replication in laboratory strains and various clinical isolates of HIV-1 with mean EC_{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 PBMCs in vitro. Elvitegravir also showed activity against HIV-2. A discussion of the nonclinical virology data of EVG is provided in Module 2.7.2, Section 4.1.

Although there is no functional equivalent to IN activity in host cells, IN and 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 μM and 150 μM, respectively. The effects of EVG on 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), were evaluated and did not show greater than 50% inhibition at 10 μM in any of these systems.

The CC_{50} for EVG in human PBMCs in vitro was 9.7 μM (SI of > 48,000). There was no difference in the cytotoxicity of EVG in stimulated versus unstimulated PBMCs. Elvitegravir showed decreased cytotoxicity in PBMC cultures in the presence of 50% HS (CC_{50} value of 170 μM with 50% HS [SI of >100,000]).

In a battery of safety pharmacology studies, the effects of EVG on the central nervous, cardiovascular, respiratory, GI, and renal/urinary systems were examined. There were no adverse effects of EVG in Sprague-Dawley rats in the Irwin test of CNS general signs and behavior at doses up to 2000 mg/kg. In the studies of the cardiovascular and respiratory systems in conscious beagle dogs at doses up to 100 mg/kg, EVG did not produce adverse effects on blood pressure, HR, ECG, respiratory rate, or oxygen saturation. Elvitegravir at concentrations of 0.1 and 1 μM had no effect on the hERG tail current in vitro. A slight reduction (24.3%) in the hERG tail current was observed at the highest feasible concentration of 10 μM. No effect on AP was observed in isolated guinea pig papillary muscle at concentrations up to 1.0 μM. The cardiovascular risk of EVG is considered minimal. In gastrointestinal system studies, EVG did not affect the intestinal transport of a charcoal meal in Sprague-Dawley rats at doses up to 2000 mg/kg. In renal/urinary system studies, EVG at doses up to 2000 mg/kg had no effect on the urine volume or the urinary excretion of electrolytes in saline-loaded Sprague-Dawley rats.

Overall, the pharmacodynamic and safety pharmacology assessments of EVG support the effective use of this agent in therapy for HIV-1 disease.

6.2. COBI

Cobicistat is a structural analog of RTV, which retains its potent mechanism-based inhibition of human CYP3A, but completely lacks anti-HIV activity. Under physiological conditions, no inhibition of HIV-1 replication was detected at concentrations of COBI as high as 90 μM, contrasting the potent antiretroviral activity of RTV. These results indicate that COBI is devoid of antiretroviral activity at concentrations exceeding the clinical exposures by over 300-fold (see Module 2.7.2, Section 4.1). The nonclinical virology data of COBI, including demonstration of a specific lack of anti-HIV-1 activity, are described in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.

Unlike RTV, COBI did not show any effects on the host protease cathepsin D and was less inhibitory against the host proteasome activity (see Sections 3.2.1 and 3.2.2 and Module 2.7.2, Section 4.1). Compared with RTV, COBI also showed similar or lower cytotoxicity in human lymphoid and hepatic cell lines. In vitro data from studies with differentiated adipocytes suggest that COBI may have reduced effects on lipid metabolism and adipocyte functions compared to RTV.

Safety pharmacology studies were conducted to determine the potential effects of COBI on the central nervous, cardiovascular, and respiratory systems. In the rat CNS study, there were no significant neurotoxic effects; 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. 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. No adverse effects were observed in the rat respiratory study (NOAEL 500 mg/kg).

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). In rabbit Purkinje fibers (protein-free environment), which are considered more sensitive to drug-induced APD prolongation and 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.

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. In a second Langendorff study in rabbit hearts, COBI produced similar negative inotropic effects (PR interval prolongation, and produced decreases in left ventricular function) at concentrations ≥ 1.5 μM. When hearts were exposed to COBI in combination with ATV, effects on PR interval and LV function were similar to the decreases noted with COBI alone. Cobicistat had no notable effects alone, or in combination with ATV, on QRS and QT intervals, MAPD, or triangulation; and there were no EADs.

In conscious telemetered dogs, there were no adverse effects on hemodynamic and ECG parameters up to 45 mg/kg, the highest dose administered. 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). 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 {11874}, {11872}. 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, Toxicology Written Summary, 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. Of note, in the 39-week dog toxicity study (TX-216-2016), there were no notable 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 were between 7090 to 8405 ng/mL (9.1 to 10.8 μM). The shortening of the APD in rabbit Purkinje fibers and the mild delay in the PR interval in dogs may be a consequence of interaction with cardiac calcium channels {11876}, {11873}. 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 left ventricular function (Module 2.7.2, Section 2.3.2.2.2).

In summary, safety pharmacology studies with COBI did not reveal any significant safety findings, with the exception of the Langendorff studies. However, no clinically significant changes have been observed at clinical exposures up to 4-fold higher than those achieved at the clinical dose of 150 mg COBI.

Overall, the pharmacodynamic and pharmacological assessment of COBI supports the effective and safe use of this agent in combination therapy for the treatment of HIV-1 infection.

6.3. EVG/COBI/FTC/TDF

A discussion of the nonclinical virology data of the EVG/COBI/FTC/TDF combination is included in 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 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 exacerbating 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 ≥ 1 μM, which is approximately 11-fold above the anticipated clinical exposure at the 150 mg COBI dose (maximal plasma concentrations of approximately 1.4 μM and fraction unbound of 6.3% based on in vitro equilibrium dialysis). Further, as the fraction of unbound COBI is lower in plasma samples obtained in clinical studies (2.49% to 3.23%) compared to the in vitro studies, including clinical studies in subjects with moderate hepatic impairment or severe renal impairment (Module 2.7.2, Sections 2.4.1.1 and 2.4.1.2 [Studies GS-US-183-0133 and GS-US-216-0124, respectively]), the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT/QTc clinical study (Module 2.7.2, Section 2.3.2.2.1 [Study GS-US-216-0107]), a modest, dosing-related increase in PR interval was observed, but was not considered to be clinically significant. Given the favorable safety pharmacology profiles of EVG, FTC, and TDF, combination of these 3 agents with COBI is not expected exacerbate the minor findings of COBI. Thus, additional safety pharmacology studies on the EVG/COBI/FTC/TDF combination are considered unwarranted.

Overall, the pharmacodynamic and pharmacological 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.

7. TABLES AND FIGURES

Tables and figures have been integrated within the textual summary.

8. REFERENCES

References are available upon request.

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SECTION 2.6
NONCLINICAL SUMMARY

SECTION 2.6.3—PHARMACOLOGY TABULATED SUMMARY

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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NOTE TO REVIEWER

This application is being submitted in support of a new drug application (NDA) for a film-coated single tablet regimen (STR) that contains the active substances elvitegravir (EVG), cobicistat (COBI), emtricitabine (FTC), and tenofovir disoproxil fumarate (tenofovir DF, 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. (GSI) and the Food and Drug Administration (FDA; refer to the Agency's comments, dated [redacted] 20[redacted] in Module 1.6.3), [redacted].

2.6.3.1. Pharmacology Overview

2.6.3.1.1. Primary Pharmacodynamics of EVG

The primary pharmacodynamics of EVG are described in detail in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.

2.6.3.1.2. Primary Pharmacodynamics of COBI

For additional information, refer to Module 2.6.5, Pharmacokinetics Tabulated Summary.

Test Article: COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Inhibition of Human CYP3A Activity	No	CYP3A enzyme	In Vitro	[redacted], [redacted], [redacted] USA	AD-216-2028

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

2.6.3.1.3. Primary Pharmacodynamics of EVG/COBI/FTC/TDF

The primary pharmacodynamics of EVG/COBI/FTC/TDF are described in detail in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.

2.6.3.1.4. Secondary Pharmacodynamics of EVG

Test Article: EVG

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Effect of EVG on Human Topoisomerase I and II Activities	No	In Vitro	In Vitro	Japan Tobacco Inc. Osaka, Japan	JTK303-PH-004
In Vitro Receptor Binding of EVG	No	In Vitro	In Vitro	[redacted], France	JTK303-PH-008
Anti-HIV Activity and Cytotoxicity of One Coded Compound (EVG) Tested Against a Panel of HIV Viruses in Fresh Human PBMC and Macrophage Cultures	No	PBMC and Macrophage cells	In Vitro	[redacted], USA	JTK303-PH-010
Antiviral Activity of GS-9160 in Primary Human T-Lymphocytes and Macrophages	No	T-cells and Macrophage cells	In Vitro	[redacted], USA	PC-186-2004
Anti-Human Immunodeficiency Virus Type 1 Activity and Cytotoxicity of EVG in the Absence or Presence of Human Serum in Human PBMC Culture	No	PBMC	In Vitro	Japan Tobacco Inc. Osaka, Japan and [redacted], Japan	JTK303-PH-006
Cytotoxicity of EVG in Stimulated and Unstimulated PBMCs	No	PBMC	In Vitro	[redacted], USA	PC-183-2001
In Vitro Evaluation of the Effect of EVG on Mitochondrial DNA Synthesis in HepG2 Liver Cells	No	HepG2 Liver cells	In Vitro	[redacted], USA	TX-183-2009

DNA = deoxyribonucleic acid; EVG = elvitegravir; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV = human immunodeficiency virus; PBMC = peripheral blood mononuclear cell

Note: GS-9160 is an investigational integrase inhibitor.

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

2.6.3.1.5. Secondary Pharmacodynamics of COBI

Test Article: COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Activity Against HIV-1 and Host Proteases	No	HIV-1 protease enzyme, cathepsin D enzyme, 26S proteasome	In Vitro	[redacted], [redacted], [redacted] USA	PC-216-2001
Cytotoxic Effect on Adipocytes	No	Human primary adipocytes; mouse adipocyte cell line (OP9)	In Vitro	[redacted], [redacted], [redacted] USA	PC-216-2004
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 67 mammalian receptors (human, rat and mouse) with COBI	In Vitro	[redacted], [redacted], Taiwan	TX-168-2007
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 2 mammalian ion channels with COBI (rat)	In Vitro	[redacted], [redacted], Taiwan	TX-168-2011
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 67 mammalian receptors (human, rat and mouse) with RTV	In Vitro	[redacted], [redacted], Taiwan	PC-137-2004
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 1 mammalian ion channel with RTV (rat)	In Vitro	[redacted], [redacted], Taiwan	PC-168-2005

2.6.3.1.5. Secondary Pharmacodynamics of COBI (Continued)

Test Article: COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
General Cytotoxicity	No	MT-2 cells, HepG2 cells	In Vitro	[redacted], [redacted], [redacted] USA	PC-216-2003

COBI = cobicistat; RTV = ritonavir

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

2.6.3.1.6. Safety Pharmacology: EVG In Vitro

Test Article: EVG

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Cardiovascular (Effects on hERG Current)	Yes	Human embryonic kidney cells (HEK293)	In Vitro	[redacted], Japan	JTK303-SP-003
Cardiovascular (Effects on Action Potential Parameters)	Yes	Isolated guinea pig papillary muscle	Ex Vivo	[redacted], Japan	JTK303-SP-004
Effects on Autonomic Nervous System and Smooth Muscle	No	Isolated guinea pig ileum	Ex Vivo	Japan Tobacco Inc. Osaka, Japan	JTK303-SP-005
Cardiovascular/ hERG Assay of GS-9200 and GS-9202, Metabolites of GS-9137	No	Chinese hamster ovary cells (CHO)	In Vitro	[redacted], USA	TX-183-2005

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

2.6.3.1.7. Safety Pharmacology: COBI In Vitro

Test Article: COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Cardiovascular Safety (Effects on hERG Channel)	Yes	Human embryonic kidney cells (HEK293)	In Vitro	[redacted], USA	TX-216-2009
Cardiovascular Safety (Effects on Cardiac Ion Channels)	No	Human embryonic kidney cells (HEK293)	In Vitro	[redacted], USA	TX-216-2015
Cardiovascular Safety (Effects on Action Potential)	No	Isolated rabbit cardiac purkinje fibers	Ex Vivo	[redacted], USA	TX-168-2012
Cardiovascular Safety (Effects on Isolated Rabbit Hearts)	No	Isolated rabbit hearts/ New Zealand White	Ex Vivo	[redacted], USA	PC-216-2007
Cardiovascular Safety (Effects on Isolated Rabbit Hearts with COBI and Atazanavir)	No	Isolated rabbit hearts/ New Zealand White	Ex Vivo	[redacted], USA	PC-216-2009

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

2.6.3.1.8. Safety Pharmacology: EVG In Vivo

Test Article: EVG

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Effects on Central Nervous System	Yes	Rat/Sprague -Dawley	Oral Gavage	[redacted], Japan	JTK303-SP-001
Effects on Cardiovascular and Respiratory Systems	Yes	Dog/Beagle	Oral Gavage	[redacted], Japan	JTK303-SP-002
Effects on Urine Volume and Urinary Electrolytes Excretion	No	Rat/Sprague -Dawley	Oral Gavage	Japan Tobacco Inc. Osaka, Japan	JTK303-SP-007
Effects on Gastrointestinal Transport of Charcoal	No	Rat/Sprague -Dawley	Oral Gavage	Japan Tobacco Inc. Osaka, Japan	JTK303-SP-006

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

2.6.3.1.9. Safety Pharmacology: COBI In Vivo

Test Article: COBI

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Study No.
Effects on Central Nervous System	Yes	Rat/Crl:CD®(SD)	Oral Gavage	[redacted], [redacted] Canada	TX-216-2006
Effects on Central Nervous System[b]	Yes	Rat/Crl:CD®(SD)	Oral Gavage	[redacted], [redacted] USA	PC-201-2002
Effects on Cardiovascular System	Yes	Dog/Beagle	Oral Gavage	[redacted], [redacted] Canada	TX-216-2008
Effects on Cardiovascular System[b]	Yes	Dog/Beagle	Oral Gavage	[redacted], [redacted] USA	PC-201-2004
Effects on Respiratory System	Yes	Rat/Crl:CD®(SD)	Oral Gavage	[redacted], [redacted] Canada	TX-216-2007
Effects on Respiratory System[b]	Yes	Rat/Crl:CD®(SD)	Oral Gavage	[redacted], [redacted] USA	PC-201-2003

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

b Studies conducted with COBI alone, or in combination with an experimental HIV protease inhibitor, GS-8374.

2.6.3.1.10. Pharmacodynamic Drug Interactions of EVG

The pharmacodynamic drug interactions of EVG are described in detail in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.

2.6.3.1.11. Pharmacodynamic Drug Interactions of COBI

The pharmacodynamic drug interactions of COBI are described in detail in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.

2.6.3.2. Primary Pharmacodynamics

Studies of the primary pharmacodynamics of EVG, COBI, and EVG/COBI/FTC/TDF are presented in the nonclinical virology summary contained in Module 2.7.2, Section 4.1. A study of the primary pharmacodynamics of COBI is listed in Section 2.6.3.1.2.

2.6.3.3. Secondary Pharmacodynamics

Studies of the secondary pharmacodynamics of EVG and COBI are listed in Sections 2.6.3.1.4 and 2.6.3.1.5.

2.6.3.4. Safety Pharmacology

2.6.3.4.1. In Vitro Studies with EVG

Test Article: EVG

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP[a]	Study No.
Cardiovascular (hERG Inhibition)	Human embryonic kidney cells (HEK293)	In Vitro	0.1, 1, and 10 μM	5 Cells per concentration	3.4% and 24.3% inhibition at 1 and 10 μM, respectively. IC_{50} > 10 μM	Yes	JTK303-SP-003
Cardiovascular (Action Potential Parameters)	Isolated guinea pig papillary muscle	Ex Vivo	0.1, 1, and 3 μM	6 Preparations per concentration	No effects on action potential of guinea pig myocardial cells.	Yes	JTK303-SP-004
ANS and Smooth Muscle	Isolated Guinea Pig Ileum	Ex Vivo	1, 10 and 30 μM	N=5 (male) per concentration	1, 10 μM: No effect on ANS or smooth muscle. 30 μM: Slight inhibition of single contractions by inducers.	No	JTK303-SP-005
Cardiovascular (hERG Inhibition of Metabolites)	CHO cells	In Vitro	3, 10, 30 and 100 μM	3 Cells per concentration	M1 (GS-9200) IC_{50} > 100 μM M4 (GS-9202) IC_{50} = 81μM	No	TX-183-2005

ANS = autonomic nervous system; CHO = Chinese hamster ovary cells; hERG = human ether-à-go-go related gene

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

2.6.3.4.2. In Vivo Studies with EVG

Test Article: EVG

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP[a]	Study No.
Central Nervous System	Rat/Sprague -Dawley	Oral Gavage	0, 100, 300, 2000	6 M	None. NOEL = 2000 mg/kg	Yes	JTK303-SP-001
Cardiovascular and Respiratory Systems	Dog/Beagle	Oral Gavage	0, 10, 30, 100	4 M, Latin Square Design	None. NOEL= 100 mg/kg	Yes	JTK303-SP-002
Renal (Urine Volume and Electrolyte Excretion)	Rat/Sprague -Dawley	Oral Gavage	0, 100, 300, 2000	6 M	None. NOEL = 2000 mg/kg	No	JTK303-SP-007
Gastrointestinal (Transport of Charcoal)	Rat/Sprague -Dawley	Oral Gavage	0, 100, 300, 2000	10 M	None. NOEL = 2000 mg/kg	No	JTK303-SP-006

NOEL = no-observed-effect level; M = male

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

2.6.3.4.3. In Vitro Studies with COBI

Test Article: COBI

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (μM)	No. per Group	Noteworthy Findings	GLP[a]	Study No.
Cardiovascular (hERG Inhibition)	HEK293 Cells	In Vitro	0.3, 1, 3 and 10 μM	3 Cells/ Conc-entration	hERG IC_{50} = 1.8 μM	Yes	TX-216-2009
Cardiovascular (Ion Channels)	HEK293 Cells	In Vitro	COBI: hERG: 0.1-10 μM $hCa_v1.2$: 1-30 μM $hNa_v1.5$: 10-100 μM RTV: hERG: 0.5-23.2 μM $hCa_v1.2$: 7.7-23.2 μM $hNa_v1.5$: 7.7-23.2 μM	3-4 Cells/ Conc-entration	COBI: hERG IC_{50} = 1.85 μM $hCa_v1.2$ IC_{50} = 6 μM $hNa_v1.5$ IC_{50} = 86.5 μM RTV: hERG IC_{50} = 8.75 μM Precipitate observed at concentrations > 7.7 μM	No	TX-216-2015
Cardiovascular (Action Potential)	Isolated Rabbit Cardiac Purkinje Fibers	Ex Vivo	0.03, 0.1, 1 and 10 μM	4 Fiber Prepar-ations at 2 Freq-uencies	COBI: ↓ APD_{60} and APD_{90} at 1 and 10 μM RTV: ↓ in maximum rate of depolarization (V_{max}) at 9.6 μM	No	TX-168-2012

2.6.3.4.3. In Vitro Studies with COBI (Continued)

Test Article: COBI

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (μM)	No. per Group	Noteworthy Findings	GLP[a]	Study No.
Cardiovascular (Langendorff)	Rabbit/ New Zealand White	Ex Vivo	0.3, 1, 3 and 10 μM	4 Hearts/ Group	No effects on QRS interval, triangulation or stability. 0.3 μM: No effects ≥ 1 μM: Negative inotropic effects; shortening of MAPD ≥ 3 μM: decreases in QT interval and increases in PR and RR intervals.	No	PC-216-2007
Cardiovascular (Langendorff)	Rabbit/ New Zealand White	Ex Vivo	COBI: 0.15, 0.45, 1.5, 4.5 μM ATV: 1.5, 4.5, 15, 45 μM COBI + ATV: COBI: 0.045, 0.15, 0.45, 1.5 μM + ATV: 1.5 μM (fixed)	4 Hearts/ Group	COBI: ≥1.5 μM = significant ↓ LV function (↓LVDP, ↓ contractility, impaired relaxation), ↑ CPP 4.5 μM = ↑ PR interval ATV: ≥15 μM = ↑ PR interval COBI + ATV: 1.5 μM/1.5 μM: negative inotropic effects of 1.5 μM COBI alone were reduced; HR ↓ by 26% compared to baseline; ↑ PR interval. No notable effects on QRS and QT intervals, MAPD, triangulation; no EADs. No remarkable effects at 0.45 μM COBI, 4.5 μM ATV or 0.45/1.5 μM COBI/ATV.	No	PC-216-2009

GS-017415 = ritonavir (RTV); ATV = atazanavir; APD = action potential duration; LVDP = left ventricular developed pressure; CPP = coronary perfusion pressure; EADs = early after depolarizations; MAPD = monophasic action potential duration; HR = heart rate

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

2.6.3.4.4. In Vivo Studies with COBI

Test Article: COBI

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (mg/kg)	No. per Group and Gender	Noteworthy Findings	GLP[a]	Study No.
CNS (Modified Irwin Screen)	Rat/ Crl:CD(SD)	Oral Gavage	0, 50, 150, 500	8 F	Decreased arousal and locomotor activity, salivation and decreased body temperature and motor activity at 150 and 500 mg/kg. NOAEL = 50 mg/kg	Yes	TX-216-2006
CNS (Modified Irwin Screen)[b]	Rat/ Crl:CD(SD)	Oral Gavage	COBI/GS-8374: 0, 0/1000, 50/100, 50/300, 50/1000	6 M	NOEL = 50/1000 mg/kg COBI/ GS-8374 in combination	Yes	PC-201-2002
Cardiovascular	Dog/Beagle	Oral Gavage	0, 5, 15, 45	4 M (Dose escalation design)	NOAEL = 45 mg/kg	Yes	TX-216-2008
Cardiovascular[b]	Dog/Beagle	Oral Gavage	COBI/GS-8374: 0, 15/0, 15/15, 15/75, 15/200, 0/200	6 M (Cross-over design)	NOEL = 15 mg/kg COBI, or 15/200 mg/kg COBI/GS-8374 in combination	Yes	PC-201-2004
Respiratory	Rat/ Crl:CD(SD)	Oral Gavage	0, 50, 150, 500	6 F	NOAEL = 500 mg/kg	Yes	TX-216-2007
Respiratory[b]	Rat/ Crl:CD(SD)	Oral Gavage	COBI/GS-8374: 0, 50/100, 50/300, 50/1000, 0/1000	8 M	NOEL = 50/1000 mg/kg COBI/GS-8374 in combination	Yes	PC-201-2003

CNS = central nervous system; M = male; F = female; NOAEL = no-observed-adverse-effect level; NOEL = no-observed-effect level

a An entry of "Yes" indicates that the study includes a GLP compliance statement.
b Studies conducted with COBI alone, or in combination with an experimental HIV protease inhibitor, GS-8374.

2.6.3.5. Pharmacodynamic Drug Interactions

Studies of the pharmacodynamic drug interactions of EVG, COBI, and EVG/COBI/FTC/TDF are presented in the nonclinical virology summary contained in Module 2.7.2, Section 4.1.