DARUNAVIR/COBICISTAT

MODULE 2.6.1

NONCLINICAL INTRODUCTION

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MODULE 2.6.1

INTRODUCTION

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ABBREVIATIONS

ARV	antiretroviral
ATZ	atazanavir
COBI	cobicistat
CYP3A	cytochrome P450 3A
DRV	darunavir
EC_{50}	50% effective concentration
FDC	fixed dose combination
HIV	human immunodeficiency virus
IC_{50}	median inhibitory concentration
PI	protease inhibitor
RTV	ritonavir

1. INTRODUCTION

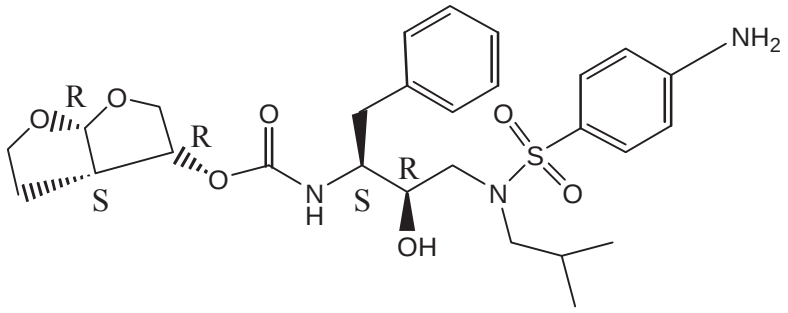
The darunavir/cobicistat (DRV/COBI) fixed dose combination (FDC) tablet contains 800 mg DRV and 150 mg COBI and is developed by the Applicant in collaboration with Gilead Sciences Inc (Gilead).

Darunavir (PREZISTA®, formerly known as TMC114) is a human immunodeficiency virus (HIV) protease inhibitor (PI). Darunavir was developed in combination with low-dose ritonavir (RTV) as a pharmacoenhancer (booster) of DRV which inhibited the metabolism of DRV via cytochrome P450 (CYP) 3A. DRV, in combination with low dose RTV and with other antiretrovirals (ARVs), is indicated for the treatment of HIV-1 infection in adults and pediatric patients aged 3 years and above. In adults, the dosing regimen of DRV/RTV 800/100 mg once daily is recommended in ARV treatment-naive patients and ARV treatment-experienced patients without DRV resistance associated mutations.

Darunavir exhibits activity against wild-type HIV in acutely infected T-cell lines, human peripheral blood mononuclear cells and human monocytes/macrophages with median 50% effective concentration in cell-based assays (EC_{50}) values ranging from 1.2 to 8.5 nM (0.7 to 5.0 ng/mL), which are well below the 50% cellular toxicity concentration range of 87 µM to > 100 µM. No significant increase in EC_{50} value was observed for the majority of recombinant clinical isolates resistant to at least 1 of the currently licensed PIs.

The chemical name of the active compound DRV is [(1S,2R)-3-[[(4-aminophenyl)sulfonyl](2-methylpropyl)amino]-2-hydroxy-1-(phenylmethyl)propyl]-carbamic acid (3R,3aS,6aR)-hexahydrofuro[2,3-b]furan-3-yl ester (Figure 1).

Figure 1: Structural Formula of DRV

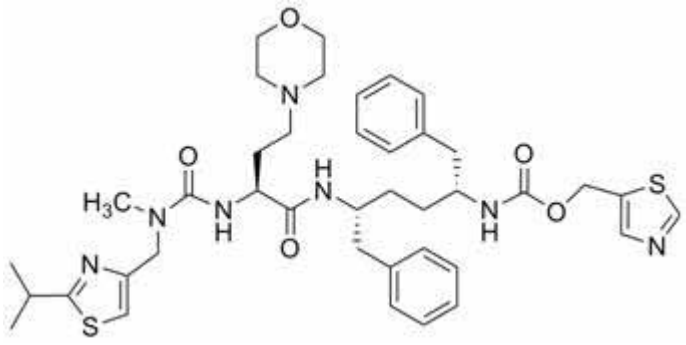


The product is isolated as the ethanolate

Cobicistat (COBI; GS-9350) is a new chemical entity and structural analogue of ritonavir (RTV) with no ARV activity. The primary pharmacodynamic effect of COBI is mechanism-based inhibition of CYP3A enzymes (eg, the in vitro median inhibitory concentration [IC50] value for inhibition of human hepatic microsomal midazolam 1'-hydroxylase activity is 0.15 μM). Cobicistat is being developed by Gilead as a pharmacoenhancer to boost systemic exposures to CYP3A substrates, including the protease inhibitors ATZ and DRV that are commonly prescribed in combination with low-dose RTV as a booster for the treatment of HIV-1 infected patients. Cobicistat has recently been registered to be coadministered as a pharmacokinetic enhancer of ATZ and DRV

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)butanoyl] amino}-1,6-diphenylhexan-2-yl)carbamate (Figure 2)

Figure 2: Structural Formula of COBI



The available nonclinical package is considered complete and in line with ICH, and CHMP guidelines and supports the proposed registration of DRV/COBI.

2. PROPOSED CLINICAL USE

The intended indication of the DRV/COBI 800/150 mg FDC is the same as the approved indication of the DRV/rtv 800/100 mg once daily regimen, ie, for the treatment of HIV-1 infection in HIV-1 infected treatment-naïve adult subjects and treatment-experienced adult subjects with no DRV resistance-associated mutations, and with plasma HIV-1 ribonucleic acid (RNA) <100,000 copies/mL and CD4+ cell count ≥100x106 cells/L. This is a so called 'substitution indication' of an already approved regimen, ie, the single agents have already been licensed for combined use at the same dose levels as in the FDC.

DARUNAVIR/COBICISTAT

MODULE 2.6.2

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ABBREVIATIONS

AP	action potential
APD	action potential duration
ATV	atazanavir
AUC	area under the plasma concentration-time curve
AV	atrioventricular
BACE-1	beta-site APP-cleaving enzyme 1
CC_{50}	50% cytotoxic concentration
C_{max}	maximum plasma concentration
CNS	central nervous system
COBI	cobicistat
CPP	coronary perfusion pressure
CYP	cytochrome P450
DMSO	dimethylsulfoxide
DRV	darunavir
EAD	early after depolarization
ECG	electrocardiogram
HEK	human embryonic kidney
hERG	Human ether-à-go-go
HIV	human immunodeficiency virus
HR	heart rate
Hz	Hertz
IC_{50}	50% inhibitory concentration
K^+	potassium
K_I	concentration at 50% k_{inact}
k_{inact}	inactivation constant
LV	left ventricular
LVDP	left ventricular developed pressure
MAPD	monophasic action potential duration
MDZ	midazolam
NOAEL	no observed adverse effect level
PEG400	polyethylene glycol 400
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
QTcF	QT interval corrected for heart rate using the Fridericia formula
RR	interval between the peak of R waves of 2 consecutive ECG complexes
RTV	ritonavir
T_{max}	the time (observed time point) of C_{max}
V_{max}	maximum velocity

1. BRIEF SUMMARY

The darunavir/cobicistat (**DRV/COBI**) fixed dose combination tablet contains 800 mg of the medicinal product DRV and 150 mg of the medicinal product COBI and is developed by the Applicant in collaboration with Gilead Sciences Inc (Gilead). Darunavir is a registered human immunodeficiency virus type 1 (HIV-1) protease inhibitor and COBI is a pharmacokinetic enhancer that has recently been approved as a pharmacokinetic enhancer of DRV and atazanavir (ATV). No nonclinical studies have been conducted with DRV in combination with COBI since the combined use of DRV and COBI is not expected to induce clinically relevant additive or synergistic effects. In this summary the pharmacology of DRV (also named TMC114 or Prezista®) and COBI (a structural analog of ritonavir [RTV]) is described. Both compounds have been investigated separately and therefore the individual DRV and COBI data are described in this document.

The primary and secondary pharmacodynamics, except the in vitro virology studies of DRV and COBI, are discussed, as well as a series of in vitro and in vivo safety pharmacology studies. The studies conducted are listed in Tabulated Summary 2.6.3.1 Overview. Details on the safety pharmacology studies can be found in Tabulated Summary 2.6.3.4.

Darunavir

The drug substance is a solvate (ethanolate). The dose levels used in all studies in this document are expressed as the nominal dose. Details concerning the study can be found in the Overview Table (Tabulated Summary 2.6.3.1).

Cardiovascular safety

In vitro, DRV at a concentration of 10 µM (5.9 µg/mL) in dimethylsulfoxide (DMSO) showed no significant effect on membrane potassium current in human Ether-à-go-go (hERG).T.human embryonic kidney (HEK) 293 cells and there were no effects on the electrophysiological cardiac action potential parameters in sheep isolated cardiac Purkinje fibers at the same concentration. In vivo cardio-hemodynamic and electrocardiogram (ECG) parameters did not change when dogs were dosed once up to 120 mg/kg DRV. Mean peak plasma concentration (C_{max}) and area under the curve

($AUC_{0-\infty}$) values for male and female dogs were 16.6 and 15.0 µg/mL and 69.4 and 53.4 µg.h/mL, respectively, after a single administration at 120 mg/kg at the start of the 12-month dog study.

Gastrointestinal safety

There was no effect on gastrointestinal transit time of a charcoal solution after oral administration of 20, 200 and 2000 mg/kg DRV in rats.

Neurobehaviour and motor activity

There were no effects suggestive of neurological impairment or delayed neurotoxicity in rats dosed with DRV up to a single oral dose of 2000 mg/kg.

Pulmonary safety

Oral administration of DRV had no acute effects on respiration in rats at doses levels up to 2000 mg/kg when compared with vehicle.

Throughout this summary, clinical exposure data for DRV were derived from GS-US-210-0130 PK substudy in which HIV-1 infected subjects were treated with fixed dose DRV/COBI at 800/150 mg once daily for 24 weeks. Mean DRV exposure values amounted to 7.66 !g/mL (C_{max}) and 81.6 !g.h/mL (AUC_{0-24h}).

Cobicistat

Primary and Secondary Pharmacodynamics

No remarkable cytotoxicity was observed with COBI in vitro in human MT-2 and HepG2 cells, with 50% cytotoxic concentration (CC_{50}) values of 89 and 44 !M, respectively (69.1 and 34.1 !g/mL). In vitro data indicate that COBI shows low potential for inhibition of host proteases (50% inhibitory concentration [IC_{50}] > 30 !M) and a low potential for effects on adipocyte functions (lipid accumulation and glucose uptake).

Cardiovascular safety

Electrophysiology (in vitro patch clamp) studies indicated that COBI inhibited the hERG potassium current (IC_{50} = 1.8 !M [1.40 !g/mL]) and the $hCa_v1.2$ L-type calcium channel (IC_{50} = 6 !M [4.66 !g/mL]), but was a weak inhibitor of the $hNa_v1.5$ sodium channel (IC_{50} = 86.5 !M [67.1 !g/mL]). In

rabbit Purkinje fibers (protein-free environment), COBI caused a shortening of the action potential duration (APD) at ≥ 1 μM (0.78 μg/mL); there was no evidence of triangulation, instability, or alternans predictive of prolongation of the QT interval. In Langendorff studies in isolated rabbit hearts (protein-free environment), COBI showed the potential to decrease left ventricular (LV) function and prolong the PR interval at ≥ 1 μM (0.78 μg/mL). No evidence of decreased left ventricular (LV) function was observed in clinical studies. When hearts were exposed to COBI in combination with ATV, effects on the PR interval and LV function were similar to the changes noted with COBI or ATV dosed alone.

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 postdose was 7.7 μM [5.98 μg/mL]). 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.

Based upon above data and ECG evaluations in the repeat-dose toxicity studies in dogs up to 39 weeks dosing (Module2.6.6/Section 3.2.2), 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[1,2].

In a thorough QT clinical study (Module2.7.4/Section 2.2.1), 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 (Module2.7.4/ Section 2.2.2) at baseline and after receiving 150 mg COBI for at least 15 days indicated no clinically significant change in LV function.

Neurobehaviour and motor activity

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.

Pulmonary safety

Cobicistat had no effects on respiratory parameters in rats after single oral doses up to 500 mg/kg.

2. PRIMARY PHARMACODYNAMICS

2.1. Darunavir

Darunavir is an inhibitor of the dimerization and of the catalytic activity of HIV-1 protease. It selectively inhibits the cleavage of HIV encoded Gag-Pol polyproteins in virus infected cells, thereby preventing the formation of mature infectious disease particles. Darunavir has potent in vitro activity against both wild type and multi-drug resistant HIV-1 strains.

For more detailed primary pharmacodynamics of DRV, please refer to Module2.7.2/Summary of Virology.

2.2. Cobicistat

2.2.1. Inhibition of Human CYP3A Activity by Cobicistat

Cobicistat is a structural analog of RTV with no antiviral activity. Its intended pharmacological effect is inhibition of human cytochrome P450 3A (CYP3A) enzyme activity.[3]

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 6#-hydroxylase, and terfenadine hydroxylase). Both COBI and RTV were potent inhibitors of all human hepatic microsomal CYP3A activities tested (Table 1). The effect of COBI on the human hepatic microsomal metabolism of several antiviral drugs is shown as well.

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

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

As the apparent inhibitory potency against both MDZ 1'-hydroxylase and testosterone 6β-hydroxylase could be increased in a preincubation-dependent and cofactor-dependent manner, COBI is suggested to be a mechanism-based inhibitor of human CYP3A enzymes, 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).

3. SECONDARY PHARMACODYNAMICS

3.1. Darunavir

No relevant findings were noted when DRV was investigated for activity in a comprehensive range of primary and secondary cell, tissue and in vivo assays in the cardiovascular, gastro-intestinal and central nervous systems and in models of allergy, inflammation and metabolism (Pharmascreen®). The only significant finding was an increase in antagonistic activity, which was observed in spontaneously beating guinea pig right atria[1].

3.2. Cobicistat

Underneath, the secondary pharmacodynamics of COBI are described, except for the vitro virology data, which are summarized in Module2.7.2/Summary of Virology.

3.2.1. Effect on Proteases and Proteasome Activity

Ritonavir inhibits some host aspartic proteases in addition to the HIV protease (IC_{50} = 0.0006 μM), and was therefore characterized against selected human enzymes (using purified enzymes and specific substrates), including cathepsin D, renin, and beta-site APP-cleaving enzyme 1 (BACE-1). 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. On the other hand, COBI did not have any effect on the HIV-1 protease enzymatic activity or Cathepsin D at concentrations up to 30 μM[4].

As RTV has been observed to inhibit proteasome activity[5,6], the inhibition by COBI of the chymotryptic-like activity of the 26S proteasome was assessed[4]. 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). This low level of proteasome inhibitory activity is

unlikely to be significant at maximal unbound clinical exposure level of 0.095 !M COBI.

3.2.2. 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[7]. In vitro, RTV has been shown to affect adipocyte functions such as differentiation-associated lipid accumulation and insulin-stimulated glucose uptake[8].

The effects of COBI and RTV on adipocyte functions were evaluated using 2 in vitro assays[9], monitoring normal lipid accumulation in cultured human adipocytes following the induction of differentiation and evaluating insulin-stimulated glucose uptake in differentiated mouse adipocytes. While RTV showed a significant effect in both assays, COBI had no or a less pronounced effect, suggesting a lower potential of COBI for metabolism-related toxicities compared to RTV.

3.2.3. In Vitro Receptor Binding Potencies

Radioligand binding assays were used for screening potential molecular targets for COBI. At 10 !M, COBI demonstrated significant binding to 3 ion channels: calcium channel L-type (benzothiazepine), potassium channel (hERG), and sodium channel (Site 2) [10,11]. Using the same screen, RTV demonstrated binding to the sodium channel and to the kappa-opiate receptor[12,13]. 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 4.2.1.

3.2.4. In Vitro Cytotoxicity

The in vitro cytotoxicity of COBI and RTV was evaluated in MT-2 lymphoblastoid T-cells following 5-day incubation and in HepG2 hepatoma cells following 3-day incubation[14]. Cobicistat was slightly less cytotoxic than RTV in MT-2 cells and showed cytotoxicity similar to RTV in HepG2 cells (Table 2).

Table 2: Cytotoxicity of COBI in MT-2 and HepG2 Cells

Compounds	CC_{50} &SD (! M)[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] 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT)-based assay

[c] CellTiter Glo-based assay

4. SAFETY PHARMACOLOGY

4.1. Darunavir

Darunavir was investigated in a series of in vitro and in vivo safety pharmacology studies. The studies conducted are listed in Overview Table 2.6.3.1. Study details are given in the Individual Study Overview table (Tabulated Summary 2.6.3.4).

4.1.1. In Vitro Studies

4.1.1.1. Membrane Potassium (K+) Current (hERG Current)

The effects of 10 µM (5.9 µg/mL) DRV in 0.1% v/v DMSO or vehicle on membrane potassium (K^+) current were investigated in hERG.T.HEK 293 cells. E-4031 was used as a positive control and inhibited hERG currents by 75 to 89%, blocking outward and tail currents, respectively.[2]

DRV did not inhibit hERG current up to 10 µM (5.9 µg/mL) (Tabulated Summary 2.6.3.4).

4.1.1.2. Cardiac Action Potential (Purkinje Fibers)

Electrophysiological cardiac action potential parameters (action potential duration and repolarisation, maximum rate of depolarisation, upstroke amplitude and resting membrane potential) were evaluated in sheep isolated cardiac Purkinje fibers treated with 0.1, 1 or 10 µM (0.059, 0.59 or 5.9 µg/mL) DRV (in 0.1% v/v DMSO). As positive control dl-sotalol hydrochloride was used.

No effects were seen of DRV on the investigated parameters up to 10 µM (5.9 µg/ml) (Tabulated Summary 2.6.3.4)[3].

4.1.2. In Vivo Studies

4.1.2.1. Cardiovascular Effects on Conscious, Telemetered Beagle Dogs

Four conscious, telemetered male beagle dogs were dosed orally via gavage at single escalating doses of 0 (vehicle), 30, 60 or 120 mg/kg of DRV dissolved in polyethylene glycol 400 (PEG400) (1mL/kg) [4]. The interval between administrations was 3 to 5 days. Arterial blood pressure, heart rate and lead II ECG (PR, RR, QRS and QT intervals and QTc) were measured continuously at least 30 minutes before dosing up to 6 hours post-dose.

No effect on cardio-hemodynamic and ECG parameters was measured up to 120 mg/kg (Tabulated Summary 2.6.3.4). Toxicokinetic data were not measured in this study, but mean C_{max} and $AUC_{0-\infty}$ values for male and female dogs were 16.6 and 15.0 µg/mL and 69.4 and 53.4 µg.h/mL, respectively, after a single administration at a similar dose level (120 mg/kg) at the start of the 12-month dog study (see Module2.6.6/Section3.1.2.1).

4.1.2.2. Gastro-Intestinal Transit Time in Rats

Darunavir was examined for its ability to induce a delay of the gastro-intestinal transit time in male Wistar rats (5/group)[5]. This was determined by measuring the maximal travel in a time period of 30 minutes of a charcoal suspension applied 30 minutes after a single oral (gavage) administration of vehicle (40%v/v PEG400), 20, 200 and 2000 mg/kg DRV in PEG400. Atropine served as positive control (= delay in gastric emptying).

No effect of DRV on gastrointestinal transit time was detected up to 2000 mg/kg (Tabulated Summary 2.6.3.4).

4.1.2.3. Neurobehaviour and Motor Activity in Rats

A single oral (gavage) dose of DRV at 0 (vehicle; 40 % v/v PEG400), 20, 200 or 2000 mg/kg was given to male Wistar rats (10/group) to assess behavioral, neurologic and autonomic parameters before treatment and 1, 6 and 24 hours post-dose. Chlorpromazine hydrochloride was also administered as a positive control.

DRV did not affect the neurofunctional integrity up to 2000 mg/kg (Tabulated Summary 2.6.3.4) [6].

4.1.2.4. Pulmonary Safety in Rats

Male Wistar rats (5/group) received a single oral (gavage) dose of DRV in PEG400 at 0 (vehicle), 20, 200 or 2000 mg/kg to assess pulmonary safety.[7] Breathing frequency and tidal volume were measured during 100 minutes after dosing. Methacholine chloride was used as positive control.

No acute effects of DRV on respiration were measured up to 2000 mg/kg (Tabulated Summary 2.6.3.4).

4.2. Cobicistat

4.2.1. In Vitro Studies

4.2.1.1. Effects on Ion Channels

The in vitro effects of COBI at 0.3, 1, 3, and 10 μM (0.23, 0.78, 2.33 and 7.76 μg/mL; in a vehicle of 0.3% DMSO in HEPES-buffered physiological saline) on membrane K^+ current were determined. (Tabulated Summary 2.6.3.4)[15]. Cobicistat inhibited hERG K^+ current by 3.2% at 0.3 μM (0.23 μg/mL), 37.1% at 1 μM (0.78 μg/mL), 64.2% at 3 μM (2.33 μg/mL), and 89.5% at 10 μM (7.76 μg/mL) (versus 0.8% in the vehicle control). The IC_{50} for the inhibitory effect of COBI on hERG K^+ current was 1.8 μM (1.40 μg/mL; 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.

In a second study, the potential effects of COBI on the steady-state block of cardiac ion channels were determined using patch clamp techniques. Cobicistat inhibited the hERG potassium current (IC_{50} = 1.9 μM [1.47 μg/mL]) and the $hCa_v1.2$ L-type calcium channel (IC_{50} = 6 μM [4.66 μg/mL]), but was a weak inhibitor of the $hNa_v1.5$ sodium channel (IC_{50} = 86.5 μM [67.1μg/mL]). (Tabulated Summary 2.6.3.4)[16]. For comparison, effects of RTV 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.

4.2.1.2. Effects 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. Effects of COBI and RTV on cardiac action potential (APs) using Purkinje fibers excised from adult female rabbit ventricles were assessed at 4 concentrations (0.03, 0.1, 1, and 10 !M [0.0023, 0.078, 0.78 and 7.76 !g/mL]), 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)[17]. As positive control dl-sotalol hydrochloride was used

Cobicistat at 1 and 10 !M (0.78 and 7.76 !g/mL) 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 !M (7.76 !g/mL) 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 !M DMSO) suggesting a low potential for QT prolongation for COBI. Ritonavir at 9.6 !M 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.

4.2.1.3. Effects on Isolated Rabbit Hearts (Langendorff Method)

Two studies[18, 19] were conducted to evaluate the effects of COBI (first study) and COBI, ATV, as well as a combination of ATV and escalating doses of COBI (second study) on cardiac hemodynamic and electrophysiologic parameters in isolated female rabbit hearts.

In the first study, COBI concentrations of 0.3, 1, 3, and 10 !M (0.23, 0.78, 2.33 and 7.76 !g/mL) were applied (Tabulated Summary 2.6.3.4)[18]. 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 heart rate (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 LV 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.

Exposure to 0.3, 1, 3, and 10 !M COBI (0.23, 0.78, 2.33 and 7.76 !g/mL) shortened the QT interval by 8.8% to 20%. The average QT interval was significantly different from baseline at 3 and 10 !M (2.33 and 7.76 !g/mL) COBI only. The QRS interval was unaffected at concentrations up to 10 !M (7.76 !g/mL) COBI.

Exposure to 0.3, 1, 3, and 10 !M COBI (0.23, 0.78, 2.33 and 7.76 !g/mL) 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 !M for $MAPD_{30}$, ≥1 !M for $MAPD_{60}$, and at ≥3 !M for $MAPD_{90}$. Triangulation was slightly reduced after exposure to 0.3 and 1 !M COBI (0.23 and 0.78 !g/mL) and slightly increased after exposure to 3 and 10 !M (2.33 and 7.76 !g/mL), 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 !M COBI (0.78, 2.33 and 7.76 !g/mL).

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 !M, respectively (0.23, 0.78, 2.33 and 7.76 !g/mL). The PR interval was statistically different from baseline after exposure to 3 and 10 !M (2.33 and 7.76 !g/mL). Also, in 3 of the 4 hearts tested, second degree AV block developed after exposure to 10 !M COBI. Cobicistat significantly increased the RR interval by 25.8% and 63.5% at 3 and 10 !M (2.33 and 7.76 !g/mL), respectively.

In conclusion, COBI was associated with negative inotropic effects and shortening of the APD on the isolated rabbit heart at concentrations ≥ 1 ! M (0.78 ! g/mL). At ≥ 3 ! M (2.33 ! g/mL), 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 ! M COBI.

In a second study, the effects of COBI alone (0.15, 0.45, 1.5, and 4.5 µM [0.12, 0.35, 1.16 and 3.49 ! g/mL]), 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 [0.035, 0.12, 0.35 and 1.16 ! g/mL]) on cardiac hemodynamic and electrophysiologic parameters of isolated rabbit hearts were evaluated (Tabulated Summary 2.6.3.4)[19]. 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 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 (1.16 ! g/mL) 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 [1.16 ! g/mL]). Significant increases in CPP were noted at ≥ 1.5 µM (37% increase at 1.5 µM [1.16 ! g/mL]), 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 [1.16 ! g/mL]) 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 (3.49 ! g/mL) 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 (3.49 ! g/mL).

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 (1.16 !g/mL) 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 (0.35 !g/mL), 4.5 µM ATV, or with the combination at 0.45 µM COBI plus 1.5 µM ATV.

4.2.2. In Vivo Studies

4.2.2.1. Neurobehaviour and Motor Activity in Rats

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) or 50, 150, or 500 mg/kg of COBI to assess CNS effects (functional observation battery and motor activity evaluation). (Tabulated Summary 2.6.3.4)[20].

No CNS effects were observed in rats dosed at 50 mg/kg. 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.

Based on these findings, the no-observed-adverse-effect-level (NOAEL) for this study was 50 mg/kg.

4.2.2.2. Cardiovascular Effects on Conscious, Telemetered Beagle Dogs

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 as a single oral gavage dose with a minimum of 2 days between each dose (Tabulated Summary 2.6.3.4)[21]. Arterial blood pressures (mean arterial pressure, systolic blood pressure, diastolic blood pressure, and pulse pressure), HR , and quantitative ECG intervals were evaluated. Plasma samples were taken from all animals at approximately T_{max} (1 hour postdose) and analyzed for levels of COBI.

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 (0.7, 4.9, and 7.7 !M, respectively) following doses of 5, 15, and 45 mg/kg, respectively.

Based on these findings, the NOAEL for COBI in this study was at least 45 mg/kg.

4.2.2.3. Pulmonary Safety in Rats

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 to evaluate its pharmacological effects on the respiratory system (Tabulated Summary 2.6.3.4)[22].

No treatment-related effects were measured on 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.

5. PHARMACODYNAMIC DRUG INTERACTIONS

For pharmacodynamic drug interactions, please refer to Module2.7.2 Summary of Virology.

6. DISCUSSION AND CONCLUSIONS

Darunavir

Darunavir has been tested in 2 in vitro safety pharmacology studies at concentrations significantly exceeding the free plasma concentration determined in patients treated with a dose of 800/150 mg DRV/COBI (human free C_{max} for DRV is 0.46 µg/mL calculated based on a human total C_{max} of 7.66 µg/mL and an estimated plasma protein binding = 94%). In vitro, DRV at a concentration of 10 µM (5.9 µg/mL) in DMSO showed no significant effect on membrane potassium current in hERG.T.HEK 293 cells and there were no effects on the electrophysiological cardiac action potential parameters in sheep isolated cardiac Purkinje fibers at the same concentration corresponding to 13-fold the clinical unbound concentration at recommended doses.

In vivo, DRV (in PEG400) administered to 4 conscious telemetered dogs had no effect on cardio-hemodynamic and ECG parameters following single oral (gavage) doses of up to 120 mg/kg. Although DRV systemic exposure was not determined in the telemetry study, mean C_{max} and $AUC_{0-\infty}$ values for male and female dogs were 16.6 and 15.0 µg/mL and 69.4 and 53.4 µg.h/mL, respectively, after a single administration at a similar dose level (120 mg/kg) at the start of the 12-month dog study (see Toxicology Tabulated Summary 2.6.7.7.F). DRV peak plasma levels in dogs are higher (2.0- to 2.2-fold) than those attained in humans (mean C_{max}= 7.66 µg/mL) at therapeutic doses, AUC values are slightly below the clinical exposure (mean AUC_{0-24h} =81.6 µg.h/mL). In addition, no treatment-related effects on heart rate or ECG morphology were noted in vivo after repeated administration in dogs (Module2.6.6/Section3.1.2).

In rats, after single oral administration of up to 2000 mg/kg DRV, there was no effect on gastrointestinal transit time of a charcoal solution, no relevant effects on neurobehaviour and motor activity and no acute effects on respiration.

Overall, DRV safety pharmacology studies did not detect any significant nonclinical safety signals. Therefore, DRV is considered to have no potential for cardiovascular, pulmonary or nervous system effects that could be of any concern for the use of DRV in combination therapy (DRV/COBI) for the treatment of HIV 1 infection at fixed dose of 800/150 mg.

Cobicistat

Cobicistat is a structural analog of RTV, which retains its potent mechanism-based inhibition of human CYP3A, but lacks anti-HIV activity. Under physiological conditions, no inhibition of HIV-1 replication was detected at concentrations of COBI as high as 90 !M. These results indicate that COBI is devoid of antiretroviral activity at concentrations exceeding the clinical exposures by over 300-fold (see Module2.7.2/Section 4.2.3). The nonclinical in vitro virology data of COBI, including demonstration of a specific lack of anti-HIV-1 activity, are described in the nonclinical virology summary contained in Module2.7.2 Summary of Virology.

Unlike RTV, COBI did not show any effects on the host protease cathepsin D and was less inhibitory against the host proteasome activity. 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[23,24,25]. 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 !M) and the hCa_v1.2 L-type calcium channel (IC_{50} 6 !M), but was a weak inhibitor of the hNa_v1.5 sodium channel (IC_{50} 86.5 !M). 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[26], 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 effects (PR interval prolongation and decreases in LV function) at concentrations ≥ 1.5 μM. When hearts were exposed to COBI in combination with ATV, effects on PR interval and LV function were similar to the decreases noted with COBI alone. Cobicistat had no notable effects alone, or in combination with ATV, on QRS and QT intervals, 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; mean of 7.7 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[27,28]. 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[29,30].

Although COBI inhibits the L-type calcium ion channel and K^+ 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 (Module2.6.6/Section 3.2.2) 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, 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[31,32].

COBI has shown the potential to decrease LV function and prolong the PR interval in the isolated rabbit heart at ≥ 1 μM, which is approximately 10-fold above the anticipated unbound clinical exposure at the 150 mg COBI dose. However, 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, the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT clinical study (Module2.7.4/Section 2.2.1), COBI demonstrated a lack of prolongation effects on the QTcF interval in healthy adult subjects at therapeutic and supratherapeutic exposures. A small but statistically significant negative association between COBI plasma concentration and QTc interval, and a modest, dose-related increase in PR interval, were observed in the QT/QTc study, which are not considered to be clinically significant. Further, echocardiograms performed in healthy subjects in Study GS-US-216-0116 at baseline and after receiving 150 mg COBI for at least 15 days indicated no clinically significant change in LV function (Module 2.7.4/Section2.2.2).

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 cardiovascular 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.

7. TABLES AND FIGURES

Tabulated Summaries are located in Module 2.6.3.

8. REFERENCES

Reports in **bold** are submitted. Reports and literature in black are available upon request.

DARUNAVIR REFERENCES

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MODULE 2.6.3

PHARMACOLOGY TABULATED SUMMARY

2.6.3.1. Pharmacology: Overview- DARUNAVIR

Test Article: Darunivir

Type of Study	Test System	Route (Vehicle/Formulation)	GLP	Testing Facility	Study No.	Location in CTD
Primary Pharmacodynamics	Please refer to Module 2.7.2 Virology Summary					
Secondary Pharmacodynamics Receptor binding (Pharmascreen®)	In vitro and in vivo systems	Various	No	[redacted]	TMC114-NC118	4.2.1.2
Safety Pharmacology Cardiovascular safety (cardiac membrane potassium current)	HERG transfected HEK 293 cells	In vitro	Yes	[redacted]	TMC114-NC103	4.2.1.3
Cardiovascular safety (cardiac action potential)	Sheep isolated Purkinje fibers	In vitro	Yes	[redacted]	TMC114-NC105	4.2.1.3
Cardiovascular safety (cardiohemodynamic parameters and ECG)	Conscious telemetered dog, Beagle	Oral/gavage	Yes	[redacted]	TMC114-NC108	4.2.1.3
Gastro-intestinal safety (GI transit time)	Rat/Wistar	Oral/gavage	Yes	[redacted]	TMC114-NC120	4.2.1.3
Neuro-behavior and motor activity	Rat/Wistar	Oral/gavage	Yes	[redacted]	TMC114-NC116	4.2.1.3
Respiratory safety	Rat/Wistar	Oral/gavage	Yes	[redacted]	TMC114-NC117	4.2.1.3
Pharmacodynamic Drug Interactions	Please refer to Module 2.7.2 Virology Summary					

ECG = electrocardiogram; GLP = good laboratory practice

2.6.3.1. Pharmacology: Overview- COBICISTAT

Test Article: Cobicistat

Type of Study/Description	GLP	Test System	Method of Administration	Testing Facility	Study No. Location in CTD
Primary pharmacodynamics					
Inhibition of Human CYP3A Activity	No	CYP3A enzyme	In Vitro	Gilead Sciences, Foster City, CA USA	AD-216-2028 4.2.2.4
Secondary pharmacodynamics					
Activity Against HIV-1 and Host Proteases	No	HIV-1 protease enzyme, cathepsin D enzyme, 26S proteasome	In Vitro	Gilead Sciences, Foster City, CA USA	PC-216-2001 4.2.1.2
Cytotoxic Effect on Adipocytes	No	Human primary adipocytes; mouse adipocyte cell line (OP9)	In Vitro	Gilead Sciences, Foster City, CA USA	PC-216-2004 4.2.1.2
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 67 mammalian receptors (human, rat and mouse) with COBI	In Vitro	[redacted], Taiwan	TX-168-2007 4.2.1.2
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 2 mammalian ion channels with COBI (rat)	In Vitro	[redacted] Taiwan	TX-168-2011 4.2.1.2
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 67 mammalian receptors (human, rat and mouse) with RTV	In Vitro	[redacted] Taiwan	PC-137-2004 4.2.1.2
Mammalian Receptor Binding Assay	No	Radioligand binding assay to 1 mammalian ion channel with RTV (rat)	In Vitro	[redacted] Taiwan	PC-168-2005 4.2.1.2
General Cytotoxicity	No	MT-2 cells, HepG2 cells	In Vitro	Gilead Sciences, Foster City, CA USA	PC-216-2003 4.2.1.2

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2.6.3.1. Pharmacology: Overview- COBICISTAT (Continued)

Test Article: Cobicistat

Type of Study/Description	GLP	Test System	Method of Administration	Testing Facility	Study No. Location in CTD
Safety Pharmacology					
Cardiovascular Safety (Effects on hERG Channel)	Yes	Human embryonic kidney cells (HEK293)	In Vitro	[redacted] USA	TX-216-2009 4.2.1.3
Cardiovascular Safety (Effects on Cardiac Ion Channels)	No	Human embryonic kidney cells (HEK293)	In Vitro	[redacted] USA	TX-216-2015 4.2.1.3
Cardiovascular Safety (Effects on Action Potential)	No	Isolated rabbit cardiac purkinje fibers	Ex Vivo	[redacted] USA	TX-168-2012 4.2.1.3
Cardiovascular Safety (Effects on Isolated Rabbit Hearts)	No	Isolated rabbit hearts/ New Zealand White	Ex Vivo	[redacted] USA	PC-216-2007 4.2.1.3
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 4.2.1.3
Effects on Central Nervous System	Yes	Rat/Crl:CD[1] (Sprague Dawley)	Oral Gavage	[redacted] Canada	TX-216-2006 4.2.1.3
Effects on Cardiovascular System	Yes	Dog/Beagle	Oral Gavage	[redacted] Canada	TX-216-2008 4.2.1.3
Effects on Respiratory System	Yes	Rat/Crl:CD[1] (Sprague Dawley)	Oral Gavage	[redacted] Canada	TX-216-2007 4.2.1.3
Pharmacodynamic Drug Interactions	Please refer to Module 2.7.2 Virology Summary				

COBI = cobicistat; CYP = cytochrome P450; GLP = good laboratory practice; RTV = ritonavir

2.6.3.4. Safety Pharmacology- DARUNAVIR

Test Article: Darunavir

Organ Systems Evaluated	Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Doses (mg/kg)	Noteworthy Findings	GLP Compliance	Study No./ Location in CTD
Cardiovascular safety (cardiac membrane potassium current)	HERG transfected HEK 293 cells n=4 [a] (reference, n=5)	In vitro	10 !M (5.9 !g/mL) (9.4 !M [5.6 !g/mL]) [b] (vehicle, 0.1% DMSO) (reference, 100nM E-4031)	No effect of TMC114 or vehicle. Reference inhibited HERG current.	Yes	TMC114-NC103/ 4.2.1.3
Cardiovascular safety (cardiac action potential)	Sheep isolated Purkinje fibers n=4 [a]	In vitro	0.1, 1, 10 !M (0.059, 0.59, 5.9 !g/mL) (0.1, 0.9, 9.4 !M [0.056, 0.56, 5.6 !g/mL]) [b] (vehicle, 0.1% DMSO) (reference, 50 !M dl-sotalol HCl)	No effect of TMC114 or vehicle on electrophysiologic parameters. Reference increased action potential duration.	Yes	TMC114-NC105/ 4.2.1.3
Cardiovascular safety (cardio-hemodynamic parameters and ECG)	Conscious telemetered dog / Beagle M/4	Oral/gavage	30, 60, 120 mg/kg (28, 56, 112 mg/kg) (vehicle, 1 mL/kg PEG400)	No effect of TMC114 or vehicle on cardio-hemodynamic (BP and HR) and ECG (PR, RR, QRS and QT intervals and QTc) parameters.	Yes	TMC114-NC108/ 4.2.1.3
Gastro-intestinal safety (GI transit time)	Rat / Wistar M/5	Oral/gavage	20, 200, 2000 mg/kg (19, 187, 1866 mg/kg) (vehicle, 10 mL/kg 40% PEG400) (reference, 50 !g/kg atropine)	No effect on gastrointestinal (GI) transit time of a charcoal solution. Positive control delayed GI transit time.	Yes	TMC114-NC120/ 4.2.1.3
Neurobehavior and motor activity	Rat / Wistar M/10	Oral/gavage	20, 200, 2000 mg/kg (19, 187, 1866 mg/kg) (vehicle, 10 mL/kg 40% PEG400) (reference, 20 mg/kg chlorpromazine HCl)	No effects on neurobehavior and motor activity over 24 hours. Positive control changed behavior.	Yes	TMC114-NC116/ 4.2.1.3

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2.6.3.4. Safety Pharmacology- DARUNAVIR (Continued)

Test Article: Darunavir

Organ Systems Evaluated	Species/Strain Sex/No. Per Group	Route (Vehicle/ Formulation)	Doses (mg/kg)	Noteworthy Findings	GLP Compliance	Study No./ Location in CTD
Respiratory safety	Rat / Wistar M/5	Oral/gavage	20, 200, 2000 mg/kg (19, 187, 1866 mg/kg) (vehicle, 10 mL/kg 40% PEG400) (reference, 6-8 mg/L methacholine chloride, by inhalation)	No acute effects on respiration. Positive control reduced breathing frequency.	Yes	TMC114-NC117/ 4.2.1.3

[a] n is the number of tested cells; [b] TMC114 is an ethanolate, therefore the actual doses expressed as TMC114 base were 5.8% lower than the nominal doses.
BP = blood pressure, DMSO = dimethyl sulfoxide, ECG = electrocardiogram, GI = gastro-intestinal, HCl = hydrochloride, HEK = human embryonic kidney, hERG = human-ether-à-go-go-gene, HR = heart rate, N/A = not applicable, PEG400 = polyethylene glycol 400

2.6.3.4. Safety Pharmacology- COBICISTAT

Test Article: Cobicistat

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (!M)	No. per Group	Noteworthy Findings	GLP	Study No./ Location in CTD
Cardiovascular (hERG Inhibition)	HEK293 Cells	In Vitro	0.3, 1, 3 and 10 !M	3 Cells/ Conc-entration	hERG IC_{50} = 1.8 µM	Yes	TX-216-2009 4.2.1.3
Cardiovascular (Ion Channels)	HEK293 Cells	In Vitro	COBI: hERG: 0.1-10 !M $hCa_v1.2$: 1-30 !M $hNa_v1.5$: 10-100 !M RTV: hERG: 0.5-23.2 !M $hCa_v1.2$: 7.7-23.2 !M $hNa_v1.5$: 7.7-23.2 !M	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 4.2.1.3
Cardiovascular (Action Potential)	Isolated Rabbit Cardiac Purkinje Fibers	Ex Vivo	0.03, 0.1, 1 and 10 !M	4 Fiber Prepar-ations at 2 Freq-uencies	COBI: ↓ APD_{60} and APD_{90} at 1 and 10 !M RTV: ↓ in maximum rate of depolarization (V_{max}) at 9.6 !M	No	TX-168-2012 4.2.1.3
Cardiovascular (Langendorff)	Rabbit/ New Zealand White	Ex Vivo	0.3, 1, 3 and 10 !M	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 4.2.1.3

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2.6.3.4. Safety Pharmacology- COBICISTAT (Continued)

Test Article: Cobicistat

Organ Systems Evaluated	Species / Strain	Method of Administration	Dose (!M or mg/kg)	No. per Group	Noteworthy Findings	GLP	Study No./ Location in CTD
Cardiovascular (Langendorff)	Rabbit/ New Zealand White	Ex Vivo	COBI: 0.15, 0.45, 1.5, 4.5 !M ATV: 1.5, 4.5, 15, 45 !M, COBI + ATV: COBI: 0.045, 0.15, 0.45, 1.5 !M + ATV: 1.5 !M (fixed)	4 Hearts/ Group	COBI: ≥1.5 !M = significant ↓ LV function (↓LVDP, ↓ contractility, impaired relaxation), ↑ CPP 4.5 !M = ↑ PR interval ATV: ≥15 !M = ↑ PR interval COBI+ATV: 1.5 !M/1.5 !M: negative inotropic effects of 1.5 □M 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 !M COBI, 4.5 !M ATV or 0.45/1.5 !M COBI/ATV.	No	PC-216-2009 4.2.1.3
CNS (Modified Irwin Screen)	Rat/ Crl:CD(SD)	Oral Gavage	0, 50, 150, 500 mg/kg	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 4.2.1.3
Cardiovascular	Dog/Beagle	Oral Gavage	0, 5, 15, 45 mg/kg	4 M (Dose Escalation Design)	NOAEL = 45 mg/kg	Yes	TX-216-2008 4.2.1.3
Respiratory	Rat/ Crl:CD(SD)	Oral Gavage	0, 50, 150, 500 mg/kg	6 F	NOAEL = 500 mg/kg	Yes	TX-216-2007 4.2.1.3

APD = action potential duration ; ATV = atazanavir ; CNS = central nervous system; CPP = coronary perfusion pressure ; EADs = early after depolarizations ; F = female; GS-017415 = ritonavir (RTV); HR = heart rate ; LVDP = left ventricular developed pressure ; M = male; MAPD = monophasic action potential duration ; NOAEL = no-observed-adverse-effect level; NOEL = no-observed-effect level