# DARUNAVIR/COBICISTAT

# MODULE 2.4

# NONCLINICAL OVERVIEW

**Issue/Report Date:** [redacted]

**Department:** Drug Safety Sciences

**Document No.:** EDMS-ERI-68858077

---

# TABLE OF CONTENTS

## IN-TEXT FIGURES

## IN-TEXT TABLES

## RESPONSIBLE INVESTIGATORS:

[redacted]

[redacted]

Date

Project Leader/Scientific Director

## ABBREVIATIONS

| | |
|---|---|
| ALT | alanine aminotransferase |
| APD | action potential duration |
| APTT | activated partial thromboplastin time |
| ARV | antiretroviral |
| AST | asparate aminotransferase |
| ATV | atazanavir |
| AUC | area under the plasma concentration-time curve |
| BCRP | breast cancer resistance protein |
| BUN | blood urea nitrogen |
| $CC_{50}$ | 50% cytotoxic concentration |
| $C_{max}$ | maximum plasma concentration |
| CNS | central nervous system |
| COBI | cobicistat |
| CYP | cytochrome P450 |
| DMSO | demethylsulfoxide |
| DRV | darunavir |
| EAD | early after depolarization |
| ECG | electrocardiogram |
| EVG | elvitegravir |
| FDC | fixed dose combination |
| HEK293 | human embryonic kidney |
| hERG | human-ether-à-go-go-gene |
| HIV | human immunodeficiency virus |
| $IC_{50}$ | concentration at which 50% maximum inhibition is achieved |
| Ig | immunoglobulin |
| $k_{inact}$ | inactivation constant |
| KLH | keyhole limpet hemocyanin |
| LD | lactation day |
| LV | left ventricular |
| MAPD | monophasic action potential duration |
| MATE | multidrug and toxin extrusion protein |
| MBI | mechanism based inhibitor |
| MDR1 | multidrug resistance protein 1 (P-glycoprotein, Pgp) |
| MFD | maximum feasible dose |
| MRP1, 2, or 4 | multidrug resistance associated protein 1, 2, or 4 (ABCC1, 2, or 4) |

| | |
|---|---|
| MTD | maximum tolerated dose |
| NOAEL | no observed adverse effect level |
| NOEL | no observed effect level |
| OAT1 | organic anion transporter |
| OATP | organic anion transporter protein |
| OCT | organic cation transporter |
| OCTN | organic cation transporter novel |
| PEG | polyethylene glycol |
| P-gp | p-glycoprotein |
| PR | interval representing the time between the onset of atrial depolarization and the onset of ventricular depolarization |
| PXR | pregnane X receptor |
| q.d. | quaque die, once daily |
| QT | interval representing the time for both ventricular depolarization and repolarization to occur |
| RBC | red blood cells |
| RTV | ritonavir |
| T4 | thyroxine |
| TDAR | T-cell dependent antibody response |
| TSH | thyroid-stimulating hormone |
| UGT | uridine diphosphate glucuronosyltransferase |

# MODULE 2.4.1

# OVERVIEW OF THE NONCLINICAL TESTING STRATEGY AND DOSSIER APPROACH

# 1. OVERVIEW OF THE NONCLINICAL TESTING STRATEGY AND DOSSIER APPROACH

This nonclinical overview supports the use of the fixed dose combination (FDC) tablet including 800 mg darunavir (DRV) and 150 mg cobicistat (COBI) for the treatment of HIV-1 infection.

DRV (PREZISTA®, formerly known as TMC114) is an HIV protease inhibitor. DRV 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 (q.d.) is recommended in ARV treatment-naive patients and ARV treatment-experienced patients without DRV resistance associated mutations.

COBI (also known as GS-9350) is a new chemical entity developed by Gilead Sciences Inc. (Gilead) for use as a pharmacoenhancer (booster) to increase systemic exposure levels of co administered ARVs metabolized by CYP3A enzymes, including elvitegravir (EVG), atazanavir (ATV) and DRV. It is a structural analogue of RTV and a potent inhibitor of CYP3A. Cobicistat has recently been registered to be concurrently administered as a pharmacokinetic enhancer of DRV and ATV.

A FDC oral tablet, combining DRV 800 mg and COBI 150 mg q.d., has been developed to be used in the same adult population for which DRV/RTV 800/100 mg q.d. is recommended and approved. The intended indication for the FDC of DRV/COBI 800/150 mg does not represent a new combination regimen but 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.

The legal basis for this DRV/COBI FDC MAA in the European Union is Article 10b of Directive 2001/83/EC. To support this Article 10b application, a full dossier is submitted. Hence, all data on the FDC have to be submitted, as per Annex I, Part I of the Directive. In line with Notice To Applicants, Volume 2A, Procedures for marketing authorization, Chapter 1 Marketing Authorisation, studies on the single individual substances are included in this application for DRV/COBI fixed-combination to justify the absence of certain specific data on the combination and to ensure a full “stand-alone” dossier.

DRV/COBI: 2.4 Nonclinical Overview

The nonclinical development program of the DRV/COBI FDC is entirely based on the development programs of the individual compounds DRV (in combination with RTV) and COBI and support the use of the FDC tablet DRV/COBI as proposed in the Prescribing Information in Module 1. In accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/[redacted], [redacted], Section 4.2.1 "Fixed combination of compounds already approved as free combination therapy), no safety studies in animals were performed with DRV in combination with COBI.

A comprehensive package of nonclinical safety data is available for DRV, including studies of DRV in combination with RTV. Similarly, a comprehensive nonclinical program has been carried out by Gilead, documenting the safety pharmacology, nonclinical pharmacokinetics and toxicology of COBI, both as a single agent and in co-administration with ATV.

The program and design of the studies of each compound were consistent with the best scientific principles and international guidelines. Pivotal safety studies have been conducted in compliance with Good Laboratory Practices (GLP) standards issued by the Organization for Economic Cooperation and Development (OECD Principles of GLP). The animal species used in the various studies were from recognized sources and are standard models for nonclinical safety testing. An overview of studies conducted with DRV or COBI, with information on the GLP status, is given in Tabulated Summaries 2.6.3.1 Pharmacology: Overview, 2.6.5.1 Pharmacokinetics: Overview and 2.6.7.1 Toxicology: Overview.

In this document, a summary of these nonclinical data is described for each single agent and a nonclinical assessment on the combined use of DRV and COBI is given in the integrated overview and conclusion. 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. Moreover, the mechanism of action and pharmacokinetic profile of COBI are different in humans and animals, and therefore the use of nonclinical studies to assess effects of the interaction of COBI with other compounds may not adequately predict effects in humans.

In Appendix 1 an overview is given of nonclinical studies not included in the current submission dossier with justification for their absence for DRV and COBI.

Appendix 2 provides a list of all nonclinical studies conducted with the single individual substances that are considered relevant to support the marketing authorization application for the DRV/COBI FDC. The study identifier and study title are provided, as well as the initial MAA in which the reports reside. This overview further indicates which studies are included in the FDC MAA as they are considered "pivotal" (key) for the benefit-risk assessment of the FDC, and which studies are considered "supportive". For studies that are not re-submitted in the FDC MAA (available upon request), the location in the single agent MAA has been specified.

Throughout this overview, safety margins are presented based on comparison of plasma exposure values in animals with the human exposure values derived from GS-US-216-0130 substudy after treatment with DRV/COBI at 800/150 mg q.d. for 24 weeks in HIV-1 infected patients. For DRV at this dose level, the mean maximum plasma concentration ($C_{max}$) value was 7.66 !g/mL and the area under the concentration-time curve ($AUC_{0-24h}$) was 81.6 !g.h/mL. For COBI, the corresponding $C_{max}$ and $AUC_{0-24h}$ values were 0.99 µg/mL and 7.6 !g.h/mL, respectively.

# MODULE 2.4.2

# PHARMACOLOGY

# 2. PHARMACOLOGY

## 2.1. Primary and Secondary Pharmacodynamics

Darunavir is an inhibitor of the dimerization and of the catalytic activity of the 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 virus particles. Darunavir has potent in vitro activity against both wild type and multi-drug resistant HIV-1 strains.

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).

For more details about primary and secondary pharmacodynamics of DRV and COBI, please refer to Module 2.6.2 Pharmacology Written Summary and Module 2.7.2 Summary of Virology.

## 2.2. Safety Pharmacology

Darunavir and COBI were studied in a series of in vitro and in vivo safety pharmacology studies (Module 2.6.2 Pharmacology Written Summary). The studies conducted are listed in Overview Table 2.6.3.1.

### 2.2.1. Darunavir

Darunavir has been tested in 2 in vitro safety pharmacology studies at concentrations significantly exceeding the DRV 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 using 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) showed no significant effect on membrane potassium current in human-ether-à-go-go-gene (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 corresponding to 13-fold the clinical concentration (Module 2.6.2 Pharmacology Written Summary/Section 4.1).

In vivo, DRV had no effect on cardio-hemodynamic and electrocardiogram (ECG) parameters in telemetered dogs 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. Although DRV peak plasma levels in dogs are higher (2.0- to 2.2-fold) than that attained in humans (mean $C_{max}$ = 7.66 µg/mL) at the therapeutic dose (DRV/COBI 800/150 mg q.d), 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 (Module 2.6.2 Pharmacology Written Summary/Section 4.1.2.1).

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 (Module 2.6.2 Pharmacology Written Summary/Sections 4.1.2.2, 4.1.2.3 and 4.1.2.4).

Overall, DRV safety pharmacology studies did not detect any significant nonclinical safety signals.

### 2.2.2. Cobicistat

Safety pharmacology studies were conducted to determine the potential effects of COBI on the central nervous, cardiovascular and respiratory systems. In the rat central nervous system (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 no observed adverse effect level (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[1,2,3]. 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). (Module 2.6.2 Pharmacology Written Summary/Sections 4.2.2.1 and 4.2.2.3).

Patch clamp studies indicated that COBI inhibited the hERG potassium current ($IC_{50}$ 1.8 µM) and the hCav1.2 L type calcium channel ($IC_{50}$ 6 µM), but was a weak inhibitor of the hNav1.5 sodium channel ($IC_{50}$ 86.5 µM). In rabbit Purkinje fibers

(protein-free environment), which are considered more sensitive to drug-induced action potential duration (APD) prolongation and early after depolarizations (EADs) than fibers isolated from dog and several other species[4], COBI caused a shortening of the APD at ≥ 1 µM (0.78 µg/mL); there was no evidence of triangulation, instability, or alternans predictive of prolongation of the QT interval (Module 2.6.2 Pharmacology Written Summary/Section 4.2.1).

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 left ventricular [LV] function) at concentrations ≥ 1.5 µM. When hearts were exposed to COBI in combination with ATV, effects on PR interval and LV function were similar to the decreases noted with COBI alone. Cobicistat had no notable effects alone, or in combination with ATV, on QRS and QT intervals, monophasic action potential duration (MAPD), or triangulation; and there were no EADs (Module 2.6.2 Pharmacology Written Summary/Section 4.2.1.3).

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[5,6]. 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[7,8]. (Module 2.6.2 Pharmacology Written Summary/Section 4.2.2.2).

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 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[9,10].

COBI has shown the potential to decrease LV function and prolong the PR interval in the isolated rabbit heart at ≥ 1 µM (0.78 µg/mL), which is approximately 10-fold above the anticipated clinical unbound exposure at the 150 mg COBI dose (Module 2.6.2 Pharmacology Written Summary/Section 4.2.1.2). 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 (Module 2.6.4 Pharmacokinetics Written Summary/Section 4.2.2), the potential of COBI to decrease LV function and prolong PR is expected to be low in patients. In a thorough QT clinical study. 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 at baseline and after receiving 150 mg COBI for at least 15 days indicated no clinically significant change in LV function.

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.

# MODULE 2.4.3

# PHARMACOKINETICS

# 3. PHARMACOKINETICS

The nonclinical pharmacokinetics of DRV and COBI were investigated in a series of in vitro and in vivo studies and are described in detail in the Pharmacokinetics Written Summary (Module 2.6.4). The studies conducted are listed in Tabulated Summary 2.6.5.1.

## 3.1. Absorption

### 3.1.1. Darunavir

The transepithelial permeability of DRV was intermediate to high in Caco-2 cells and passive transcellular diffusion is proposed as the predominant mechanism for DRV intestinal absorption. One or more efflux transporters modulated DRV permeation, but the impact of these systems was limited at higher DRV concentrations (> 100 µM or 55 µg/mL). (Module 2.6.4 Pharmacokinetics Written Summary/Section 3.1.1.1).

In animals, DRV absorption was rapid following oral administration in all species with observed values for time to reach maximum plasma concentration in the range of 0.5 to 6 hours. The absolute oral bioavailability was 37% to 58% in adult rats and was very likely influenced by the high first pass effect as demonstrated by the presence of a large amount of metabolites in the bile. Bioavailability was higher in dogs (60 to 122%). In rats and dogs, the plasma clearance and the volume of distribution were moderate to high. The elimination was rapid in all species (Module 2.6.4 Pharmacokinetics Written Summary/Section 3.1.2.1).

After single oral administration and across the dose range studied, the plasma kinetics of DRV in mice, rats, rabbits, minipigs and dogs was less than dose proportional, especially at high dose levels, in line with the low solubility of the compound.

In adult rodents including pregnant rats, repeated oral dosing resulted in a decrease in systemic exposure, mainly due to induction of CYP3A iso-enzymes, which are extensively involved in the metabolism of DRV. In dogs, no decrease in exposure or enzymatic induction was observed after repeated administration. No conclusion could be drawn from the minipig study (Module 2.6.4 Pharmacokinetics Written Summary/Section 5.5.1).

### 3.1.2. Cobicistat

In Caco-2 cells, COBI showed high forward permeability and no evidence for efflux (Module 2.6.4 Pharmacokinetics Written Summary/Section 3.1.1.2).

Single-dose pharmacokinetics were studied in rats, dogs, and monkeys. Clearance values were high relative to hepatic blood flow (likely due to the lack of self-limiting enzyme inactivation in nonclinical species) and volumes of distribution were similar to those for total body water. After oral dosing, bioavailability was low or low/moderate, likely due to high first-pass elimination (Module 2.6.4 Pharmacokinetics Written Summary/Section 3.1.2.2).

After repeated oral dosing, there were species differences in autoinduction during these studies, with hepatic microsomal fractions from treated mice and rats showing higher levels of CYP3A, but with no increases in treated dogs. This is consistent with the species differences observed in induction studies in vitro where COBI was found to activate rat pregnane X receptor (PXR) but did not activate human PXR or induce human drug metabolizing enzymes or multidrug resistance protein 1 (MDR1) in hepatocytes (Module 2.6.4 Pharmacokinetics Written Summary/Section 5.5.2.).

## 3.2. Plasma Protein Binding and Tissue Distribution

### 3.2.1. Darunavir

The plasma protein binding of DRV was moderate to high in all tested species (i.e., mouse, rat, rabbit, dog and human). In human plasma, DRV was mostly bound to $\alpha_1$-acid glycoprotein and to a lesser extent to albumin. The free fraction ranged from 5% (rat) to 38% (rabbit), and was 5% in humans. Plasma protein binding in most species was concentration-dependent (Module 2.6.4 Pharmacokinetics Written Summary/Section 4.2.1). The blood to plasma concentration ratios ranged from 0.64 to 1.11 across all species at 0.5 µg/mL, indicating some distribution of DRV to blood cells, especially in the rabbit and dog in which the plasma protein binding was lower than in the other species

After oral administration of $^{14}$C-DRV in rats, the tissue distribution of $^{14}$C-DRV was extensive and rapid. The highest concentrations of radioactivity were measured in the liver and adrenal gland. No undue retention or accumulation was observed, except in melanin-rich tissues such as the pigmented parts of the eye. However, from these tissues a gradual decrease of the radioactivity levels could be demonstrated, showing

the reversibility of this binding (Module 2.6.4 Pharmacokinetics Written Summary/ Section 4.1.1).

### 3.2.2. Cobicistat

Binding of COBI in plasma was moderately high, yielding a fraction unbound of 6.3% in human plasma at 1 µM COBI (0.78 µg/mL). Binding to mouse, rat, and monkey plasma was similar and showed modest concentration-dependence, but binding in dog and human plasma was largely unchanged over the range 1–30 µM. Fraction unbound values were similar in nonclinical species (mean values 4.75%–6.54%) to humans. In addition, mean fraction unbound values were between 2.49% and 2.71% in normal subjects, 3.23% in subjects with moderate hepatic impairment, and 2.47% in subjects with severe renal impairment. COBI does not distribute well into the cellular fraction of blood from mouse, rat, dog, or human (Module 2.6.4 Pharmacokinetics Written Summary/Section 4.2.2).

After oral administration of $^{14}$C-COBI to albino and pigmented rats, radioactivity was rapidly and widely distributed to most tissues. Generally, the radioactivity was preferentially distributed into glandular tissues and organs of elimination. The tissues showing the highest concentrations of radioactivity, excluding the gastro intestinal tract, included liver, adrenal, kidney, and pituitary. The tissues with the lowest $C_{max}$ values were eye, spinal cord, and brain, bone, and secondary sex organs. Low levels of radioactivity in the brain, spinal cord, and testes suggest minimal transport across the blood:brain and blood:testes barriers. Compared to albino rats, the pigmented rats showed very similar patterns of distribution of radioactivity, but with higher concentrations in the uveal tract of the eye. There were also higher concentrations of radioactivity in pigmented skin compared to nonpigmented skin, suggesting that COBI was associated with melanin. Tissue concentrations of radioactivity declined largely in parallel with those in plasma. In pigmented animals there was more prolonged retention of radioactivity in melanin-containing tissues, but dosimetry analysis confirmed that concentrations were declining and association with the tissues was reversible (Module 2.6.4 Pharmacokinetics Written Summary/Section 4.1.2).

## 3.3. Metabolism and Interaction Potential

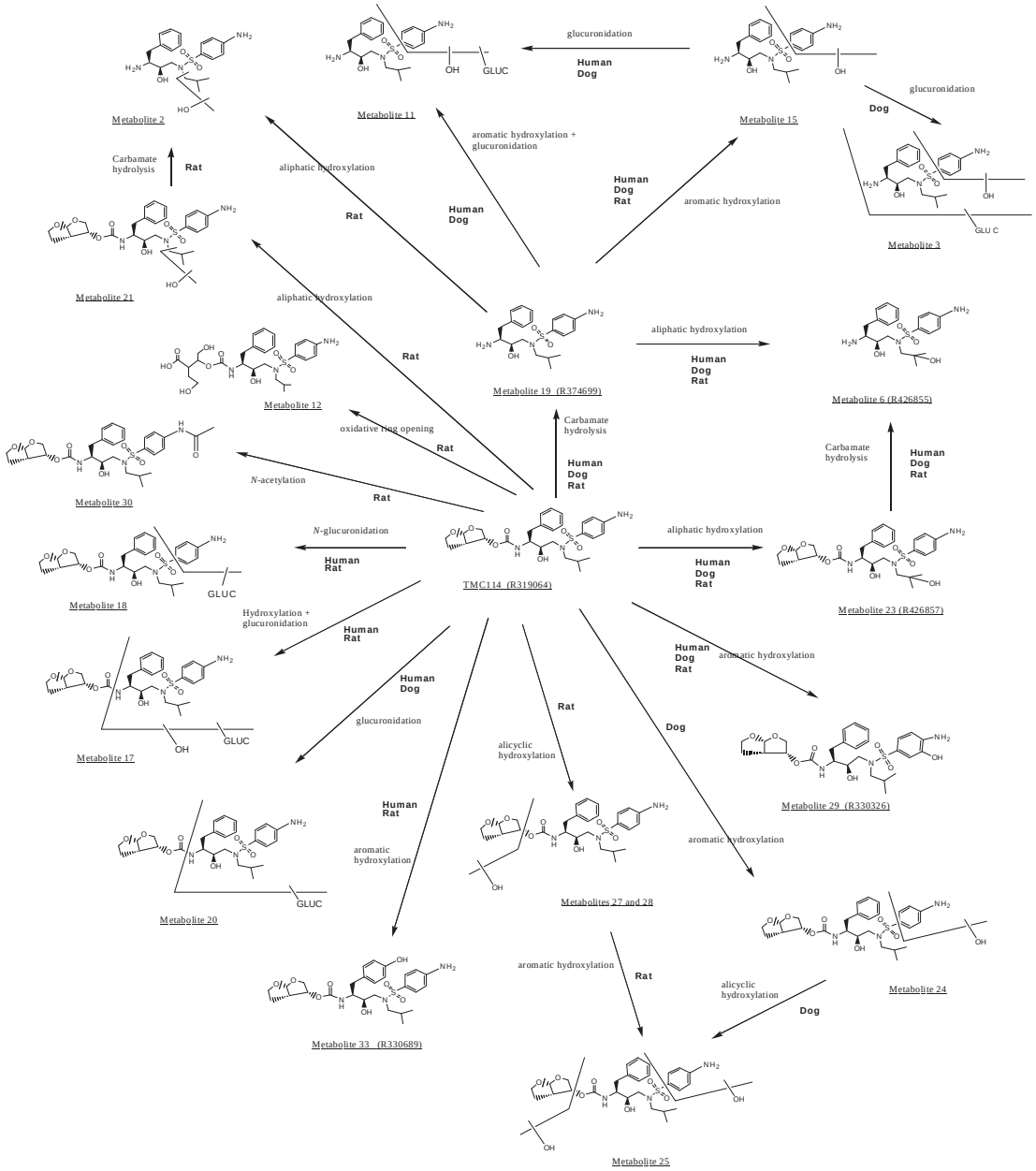
### 3.3.1. Darunavir

The metabolism of DRV following single oral administration was extensive and qualitatively similar in all species, including humans. In vitro and in vivo studies in

rats, dogs and humans identified three major Phase I metabolic reactions: carbamate hydrolysis, aliphatic hydroxylation at the isobutyl moiety and aromatic hydroxylation at the aniline moiety (see Figure 1). In dogs and humans, the major Phase I metabolic pathway was the carbamate hydrolysis whereas in rats, hydroxylation in a different part of the molecule was more important. Phase II glucuronidation was a minor pathway in rats, dogs and humans. No unique human metabolites were identified. Metabolic pathways proposed on the basis of in vitro studies were consistent with those observed following in vivo studies in rats, dogs and humans. (Module 2.6.4 Pharmacokinetics Written Summary/Section 5).

In human liver, CYP3A was almost exclusively involved in the metabolism of DRV. DRV inhibited CYP3A in human liver microsomes with an inhibitory constant ($K_i$) value of 0.40 µM (0.22 µg/mL). Given this low value, this inhibition is considered to be clinically relevant. The $K_i$ values for the other CYP450 enzymes (CYP2B6, CYP2C9, CYP2C1, CYP2D6, CYP2C8 and uridine disphosphate glucuronosyltransferase (UGT) 1A1) were at least 60-fold higher, indicating a much lower affinity and less potential for clinically relevant interactions. DRV also showed a concentration dependent effect on CYP3A4 induction in vitro. This effect may have limited clinical relevance, as induction was significant in vitro at concentrations several fold higher than those attained in the clinic (Module 2.6.4 Pharmacokinetics Written Summary/ Section 5.5.1.2). In Caco-2 cells, DRV has P-glycoprotein (P-gp) inhibitory properties with an apparent $IC_{50}$ of 32.9 µM (18.0 µg/mL) (Module 2.6.4 Pharmacokinetics Written Summary/Section 3.1.1.1). Darunavir has low potential to inhibit organic cation transporter (OCT) 2 and multidrug and toxin extrusion protein (MATE) 1 (Module 2.6.4/ Pharmacokinetics Written Summary/Section 8.1.1). A comprehensive drug-drug interaction clinical program with DRV/RTV was conducted, including a cocktail study to assess the effects of DRV/RTV on the metabolism of probe substrates. Interactions are most likely a result of the RTV component because of its inhibitory effects on CYP2D6, CYP3A and certain transporters, and/or its induction effects on CYP2C9, CYP2C19 and UGT[5]. Further details are provided in Module 2.7.2 Summary of Clinical Pharmacology studies/ Section 3.3.2.

**Figure 1: In Vivo Metabolic Pathways of DRV in Animals and Humans**

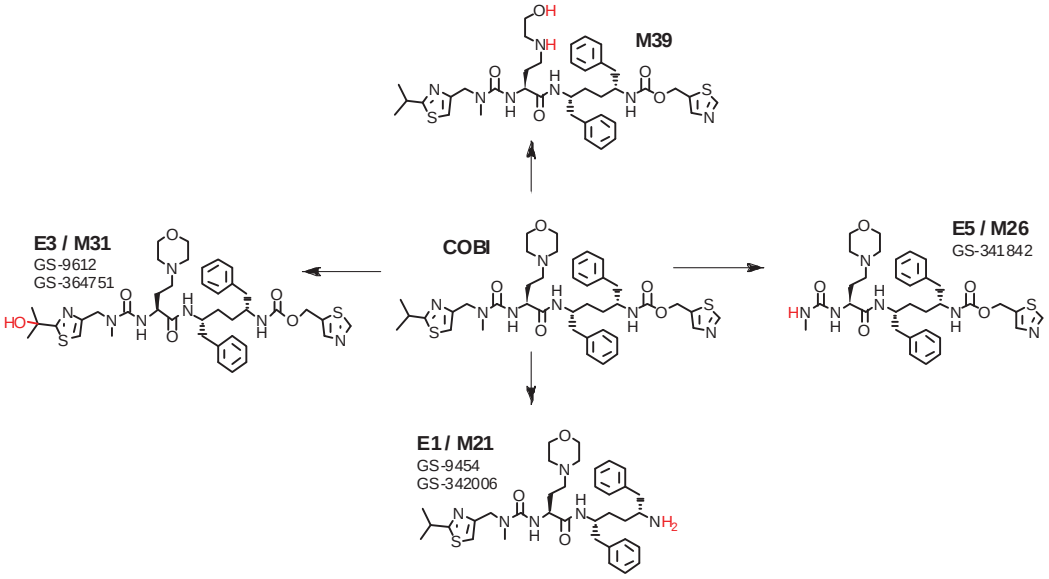



### 3.3.2. Cobicistat

The primary metabolic pathways for COBI are illustrated in Figure 2 and are methine oxidation of the isopropyl moiety (M31, GS-9612), cleavage adjacent to the methylurea (M26, GS-341842), cleavage of the carbamate (M21, GS-9454), and cleavage and deethylation of the morpholine (M39). Combinations of these routes and other routes of oxidative metabolism were also detected. Oxidation is primarily catalyzed by CYP3A, which can generate all metabolites, with a minor role for

CYP2D6 (which contributes to the generation of M31). In vitro metabolism in nonclinical species was relatively rapid but rates of metabolism by human hepatic microsomal fractions, human hepatocytes, and recombinant CYP3A4 were relatively slow due to concurrent CYP3A inactivation. After oral administration of $^{14}$C-COBI to mice, COBI was the most abundant analyte in plasma. Parent COBI, M21, and M31 were the most abundant analytes in feces, with M39 also being significant in dog feces. Profiles in bile from rats and dogs were complex, with many small peaks being detected (each accounting for ≤ 5.3% of the dose) (Module 2.6.4 Pharmacokinetics Written Summary/Section 5).

**Figure 2: Proposed Primary Metabolic Pathway of COBI**




Cobicistat is a potent inhibitor of human CYP3A with inactivation kinetics ($k_{inact}$ 0.47 min-1, $K_I$ 1.1 µM), similar to those of RTV. Inhibition of CYP3A is relatively specific as COBI did not inhibit human CYP1A2, CYP2C9, or CYP2C19, is a very weak inhibitor of CYP2C8 ($IC_{50}$ 30.1 µM), a weak inhibitor of CYP2D6 ($IC_{50}$ 9.2 µM), and a modest inhibitor of CYP2B6 ($IC_{50}$ 2.8 µM). This is in contrast to RTV, which is a more potent inhibitor of CYP2D6 ($IC_{50}$ 3.4 µM), CYP2C9 ($IC_{50}$ 3.9 µM), and CYP2C8 ($IC_{50}$ 5.5 µM)[5,6]. This higher specificity for COBI has been confirmed in clinical drug interaction studies in which COBI had no effect on the pharmacokinetics of efavirenz (CYP2B6) and little effect on the pharmacokinetics of desipramine (CYP2D6). Cobicistat is a weak inhibitor of human hepatic microsomal UGT1A1 activity ($IC_{50}$ 16.3 µM), being less potent than RTV ($IC_{50}$ 4.7 µM). Cobicistat treatment should thus not raise serum bilirubin concentrations due to

inhibition of UGT1A1 (Module 2.6.4 Pharmacokinetics Written Summary/Section 5.5.2.3).

To aid in the interpretation of the data and allow a quantitative estimate of the potential drug interaction liability from the $IC_{50}$ values, the key human pharmacokinetic data are summarized in Table 1.

**Table 1: Clinical Concentrations for Drug Interaction Liability Assessment**

| Parameter | Value | Rationale |
|---|---|---|
| Total $C_{max}$ ($[I]_1$) | 1.27 µM | $C_{max}$ 0.99 µg/mL |
| $C_{max,u}$ | 0.080 µM | $f_u$ 6.33% at 1 µM |
| $[I]_2$ | 770 µM | 150 mg/250 mL |

$f_u$ = fraction unbound; the $f_u$ value is from AD-216-2026 study $[I]_1$ = inhibitor concentration corresponding to steady state $C_{max}$; $[I]_2$ = inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen

In xenobiotic receptor transactivation studies, COBI showed no ability to activate human aryl hydrocarbon receptor and was a very weak activator of human PXR (2.2-fold activation at 10 µM, compared to 10.1-fold activation by 10 µM RTV). This was confirmed in human hepatocyte studies where COBI, at concentrations up to 30 µM, increased CYP1A2 activity and mRNA and protein by < 2% of the positive control and increased CYP3A4 mRNA expression by an average of 27.4%. CYP3A activity was below that of the vehicle control, due to inhibition by COBI, but a slight increase in immunodetectable CYP3A was detected. Other targets for induction (UGT1A1 mRNA, MDR1 mRNA, and CYP2B6 mRNA and protein) were all unaffected or weakly affected by COBI treatment (Module 2.6.4 Pharmacokinetics Written Summary/Section 5.5.2).

In contrast to its lack of effect on human PXR, COBI activates rodent PXR and increases the expression of proteins regulated by this receptor, such as rat CYP3A, UGT1A1, and presumably organic anion transporter protein (OATP) 2 (Module 2.6.4 Pharmacokinetics Written Summary/ Section 5.5.2.2).

At systemic concentrations achieved in plasma at the 150 mg COBI dose, COBI would not inhibit the drug transporters MDR1, multidrug resistance associated protein (MRP) 1, MRP2, breast cancer resistance protein (BCRP), organic anion transporter (OAT) 1, or OAT3 ($[I]_1/IC_{50} < 0.1$). However, at concentrations achievable briefly in the intestinal lumen during absorption ($[I]_2$ = 770 µM) COBI can

inhibit intestinal efflux transporters such as MDR1 and BCRP ($[I]_2/IC_{50} > 10$). This was consistent with a clinical drug interaction study in which COBI was found to increase the $C_{max}$ of the MDR1 substrate, digoxin, but to have less effect on the area under the curve. With respect to hepatic uptake transporters, COBI is a moderate inhibitor of OATP1B1 and OATP1B3 ($[I]_1/IC_{50}$ 0.4 and 0.7, respectively) (Module 2.6.4 Pharmacokinetics Written Summary/Section 8.1.2). Inhibition of OATP transporters was consistent with a clinical drug interaction study in which, after dosing with 150 mg EVG and 150 mg COBI, there was a modest increase (38%) in exposure of co dosed rosuvastatin that was not considered clinically relevant.

With respect to renal transporters, COBI is a weak inhibitor of MRP4, MATE 2K and OCT2, and a more potent inhibitor of MATE1 and organic cation transporter novel, type 1 (OCTN1), with similar potencies to RTV (Module 2.6.4 Pharmacokinetics Written Summary/Section 8.1.2). Since OCT2 and MATE1 transporters appear to play a role in the active tubular secretion of creatinine by the kidney[11,12,13], inhibition of these transporters by COBI provides a plausible explanation for the clinical finding of a reduction in renal creatinine clearance without a change in glomerular filtration rate, i.e., COBI effects the active secretion of creatinine, but not passive filtration. This phenomenon has been reported for a variety of other compounds including cimetidine[14], trimethoprim[15], pyrimethamine[16], amiodarone[17], ranolazine[18], dronedarone[19], rilpivirine[20], dolutegravir[21], the antitubercular agent PA-824[22], the fluoroquinolone DX-619[23], and the thrombin inhibitor AZD0837[24].

## 3.4. Excretion

### 3.4.1. Darunavir

In all examined species, the predominant route of excretion for $^{14}$C-DRV was via the feces (94% in rats, 86% in dogs and 82% in humans). Urinary excretion was about 4% of the administered dose in rats and dogs but was higher (12.2%) in humans. Unchanged DRV was mainly excreted in feces and amounted to up to 12.3% in rats, 26% in dogs and 6.8% in humans. In plasma, unchanged compound accounted for the largest fraction of the radioactivity in the 3 species. (Module 2.6.4 Pharmacokinetics Written Summary/Section 6.1.

In rats, DRV was excreted in milk with milk to plasma AUC ratios up to 2.3 in dams (Module 2.6.4 Pharmacokinetics Written Summary/Section 6.2.1). The systemic exposure in pups exposed to DRV via milk was very limited when compared to levels observed in dams or in pups after direct dosing.

### 3.4.2. Cobicistat

After oral administration of $^{14}$C-COBI to mice, rats, and dogs, recovery of radioactivity was high (≥ 86.1% in all groups) with the majority being found in feces (≤ 2.06% in urine). Recovery was largely complete by 48 hours postdose. After oral administration of $^{14}$C-COBI to bile duct cannulated animals, an average of 69.3% and 63.9% of dosed radioactivity was recovered in bile in rats and dogs, respectively (Module 2.6.4 Pharmacokinetics Written Summary/Section 6.1.2).

Cobicistat was present in milk samples 2 hours post dose on lactation day (LD) 10 with milk to plasma ratios ranging from 1.3 to 1.9. (Module 2.6.4 Pharmacokinetics Written Summary/Section 6.2.2).

## 3.5. Other pharmacokinetics

### 3.5.1. Effect of RTV on DRV Pharmacokinetics

The impact of RTV, a structural analogue of COBI and a potent MBI of CYP3A, on the pharmacokinetics of DRV was evaluated in a series of experiments in mice, rats, rabbits, minipigs and dogs. RTV had a modest effect on DRV exposure in mice (2-fold increase) and (pregnant) rats (4-fold increase) but had no clear effect in minipigs and dogs. In juvenile rats (23 to 50 days of age) DRV exposure (AUC) increased by approximately 2-fold when given in combination with RTV. The highest impact, however, was in rabbits where a 15-fold increase in exposure was seen. (Module 2.6.4 Pharmacokinetics Written Summary/Section 3.1.2.1). In pigmented and pregnant rats, the blood/tissue ratios were similar after oral administration of DRV alone or in combination with RTV. (Module 2.6.4 Pharmacokinetics Written Summary/Section 4.1.1). In line with its CYP3A effect, RTV had inhibitory effects on different metabolic pathways in rats such as aromatic hydroxylation, oxidative ring opening and carbamate hydrolysis. No difference in excretion of radioactivity was seen after single oral administration of $^{14}$C-DRV alone or in combination with RTV. (Module 2.6.4 Pharmacokinetics Written Summary/Section 6.1.1). Overall, RTV had limited or no impact on exposure, distribution, metabolism and elimination of DRV in mice, rats, dogs and minipigs. In humans, however, the impact was noticeable. RTV markedly reduced the metabolism of DRV and consequently increased its oral bioavailability from 37% to 82%.

### 3.5.2. Effect of COBI on ATV Pharmacokinetics

Multiple-dose GLP toxicology studies in rats were also performed with COBI in combination with ATV. In general, COBI only modestly increased ATV steady state exposures compared to ATV when dosed alone, consistent with its dual action as a reversible CYP3A inhibitor and a P450 inducer in rodents.

# MODULE 2.4.4

# TOXICOLOGY

# 4. TOXICOLOGY

## 4.1. Single Dose Toxicity Studies

### 4.1.1. Darunavir

DRV was generally well tolerated after single oral doses up to 100 mg/kg in mice, 2000 mg/kg in rats and 80 mg/kg in dogs. (Module 2.6.6 Toxicology Written Summary/Section 2.1).

### 4.1.2. Cobicistat

The single-dose toxicity of COBI was low; the maximum tolerated dose (MTD) was 100 mg/kg in mice (moribund euthanasia occurred at 300 mg/kg), and the NOAEL was 500 mg/kg in rats (Module 2.6.6 Toxicology Written Summary/Section 2.2).

## 4.2. Repeated Dose Toxicity Studies

### 4.2.1. Mouse Studies

#### *4.2.1.1. Darunavir*

In a 2-week study, the NOAEL was not established in this preliminary study but at 50 or 150 mg/kg/day, the changes were minimal. In a 3-month repeated dose study, no NOAEL was established (Module 2.6.6 Toxicology Written Summary/Section 5.1).

#### *4.2.1.2. Cobicistat*

In a 4-week non-pivotal toxicity study using wild type mice, the NOAEL was considered to be 100 mg/kg/day. In the 13-week mouse study, the NOAELs were considered to be 5 mg/kg/day in males and 50 mg/kg/day in females (Module 2.6.6 Toxicology Written Summary/Section 5.2).

### 4.2.2. Rat Studies

#### *4.2.2.1. Darunavir*

In rats, the oral (gavage) toxicity of DRV alone was evaluated in studies up to 6-months at doses up to 500 mg/kg/day (Module 2.6.6 Toxicology Written Summary/Section 3.1.1.1). The oral toxicity of various combinations of DRV and RTV were also evaluated in 3 studies up to 6-months at doses up to 1000/75 mg/kg/day DRV/RTV (Module 2.6.6 Toxicology Written Summary/Section 3.1.1.2).

In the rat studies with DRV alone, no deaths associated with DRV occurred, but there were a number of accidental gavage deaths that were considered related to the viscous and irritant nature of the vehicle (PEG400). There were no relevant clinical signs or effects on body weight or food consumption and no abnormalities were detected during ophthalmoscopy. The hematopoietic system was affected, as demonstrated by a limited decrease (about 10%) in red blood cell (RBC) counts and related parameters, and an increase in reticulocytes (up to 79%) and bilirubin levels (up to 7-fold) at 500 mg/kg/day. These changes are indicative of an increased RBC turnover, which was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen. Activated partial thromboplastin time (APTT) was prolonged at 100 and 500 mg/kg/day (by about 50%), but was not associated with any gross or microscopic evidence of bleeding. There was also an increase in platelet counts at 500 mg/kg/day. Liver weight increases correlated with hepatocellular hypertrophy and sometimes with vacuolation. The hepatocellular hypertrophy is considered to reflect an adaptive response to enzyme induction after administration of a xenobiotic, rather than a direct adverse effect of DRV. Besides bilirubin, several clinical biochemistry parameters (lipids and proteins at 500 mg/kg/day) were altered, which was considered secondary to the liver effects. Diffuse follicular hypertrophy and hyperplasia in the thyroid at 1000 mg/kg/day were observed in females in different studies, but not in the 6-month study. It is possible that this is a rodent specific adaptive response to liver enzyme induction and an enhanced metabolism of thyroid hormones. An ex vivo-liver enzyme induction and inhibition study showed that DRV was an inducer of CYP3A4 isoenzyme and UGT, in line with the liver and thyroid findings (Module 2.6.4 Pharmacokinetics Written Summary/Section 5.5.1). No relevant changes were seen in the 3- and 6-month studies at the NOAEL of 20 mg/kg/day. In rats, the exposures based on plasma DRV AUC values at the NOAEL dose from the 6-month study were between 0.04- to 0.05-fold, relative to that observed in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d; Section 4.8.1.5 and Table 2).

In rat studies of DRV in combination with RTV, similar changes to those with DRV alone were observed, but there were differences in the detail of the changes. In the 6-month combination study, 33 animals were found dead or killed before scheduled necropsy. The majority of deaths were considered accidental (due to twice daily gavage dosing of irritant formulations), but the cause of 12 deaths was not evident. As most of these 12 animals were dosed at 75 mg/kg/day RTV, a relationship with RTV cannot be excluded. A reduction of body weight gain was evident in male animals

given 500/75 and 1000/75 mg/kg/day, but not in animals given RTV alone, indicating an effect of DRV or the combination and the MTD had been achieved. In addition to the hematopoietic changes observed in all treated groups (20/50, 100/50, 500/75 and 1000/75 mg/kg/day), APTT and prothrombin time initially increased and decreased thereafter. Increases in liver transaminase activities, centrilobular hypertrophy accompanied by multinucleated hepatocytes and some single cell necrosis were observed as a result of an increase in DRV exposure and presence of RTV. The transaminase increases seen with RTV showed an inverse relationship with the DRV dose which might be related to decreases in RTV systemic exposure at higher DRV dose levels. In addition, histopathological changes (increased incidence/severity of islet fibrosis/siderocytes in males) were observed in the pancreas after treatment with DRV/RTV. In these studies, it was not possible to establish a NOAEL. The combination of DRV with RTV resulted in sustained systemic exposure to DRV. In addition, the exacerbation of changes related to DRV was dose related and no new target organs were identified. It is considered that there is evidence of a small increase in effect of the combination of DRV and RTV in the 6-month study on RBC parameters, liver and thyroid. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV and were similar to the effects seen with each compound alone. In the 6-month study, exposure to DRV expressed as $AUC_{0\text{-}24h}$ was in the range of 11.2 to 318 µg.h/mL at the end of the study. These exposures were between 0.14- and 3.9-fold, relative to that observed in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d.; see also Section 4.8.1.5 and Table 2).

#### *4.2.2.2. Cobicistat*

In the 4-week and 26-week oral dose rat studies (Module 2.6.6 Toxicology Written Summary/Section 3.2), increases in liver and thyroid weights were associated with CYP3A enzyme induction, hepatocellular hypertrophy, thyroid hormone changes (decreased thyroxine [T4]; increased thyroid stimulating hormone [TSH]) and thyroid follicular cell hyperplasia/hypertrophy. These findings were reversible, and were not considered adverse. However, 1 high dose male animal had a follicular cell carcinoma in the thyroid in the 26-week study. The liver and thyroid effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction and thyroid hormone imbalance (decreases in T4 and increases in TSH), respectively[25,26,27,28,29,30,31,32]. Hematological and clinical chemistry changes were not considered adverse. Hematological changes (not exceeding 10% versus controls) included slightly lower mean values for erythrocyte count, hemoglobin, hematocrit, mean corpuscular

volume, and mean corpuscular hemoglobin, and slightly higher mean platelet counts. Serum chemistry changes observed after 13 and/or 26 weeks of dosing included slightly higher mean gamma glutamyltransferase, cholesterol, total protein, albumin, globulin, and calcium. After a 13-week recovery period, cholesterol and total protein values remained slightly higher in high-dose females, whereas other values were generally comparable to control values, indicating reversibility. Urinalysis and urine chemistry changes, noted primarily in high-dose rats at 100 mg/kg/day, included slightly higher electrolyte excretion and slightly lower electrolyte concentrations, consistent with findings of lower urine osmolality, higher urine volume and/or pH. These changes showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and blood urea nitrogen (BUN), and were without histopathological correlates in the kidney. The NOAEL for COBI in the 26-week rat study is considered to be 30 mg/kg/day, based on significant decreases in body weight and food consumption, slight changes in hematological parameters, and increases in urine volume at 100 mg/kg/day. In rats, the exposures based on plasma AUC values at the NOAEL doses in the longest duration studies were approximately 1.3- to 1.8-fold higher than the AUC in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d.) (see also Section 4.8.2.2 and Table 3).

### 4.2.3. Dog Studies

#### *4.2.3.1. Darunavir*

In dogs, the oral (gavage) toxicity of DRV alone was evaluated in studies up to 12-month duration at doses up to 360 mg/kg/day (Module 2.6.6 Toxicology Written Summary/Section 3.1.2.1). Various combinations of DRV with RTV were investigated in studies up to 2-week duration (Module 2.6.6 Toxicology Written Summary/Section 3.1.2.2).

In the dog studies with DRV alone, there were only limited effects up to the highest dose of 120 mg/kg/day. In dogs, after 120 mg/kg/day for 6 months there was no significant toxicology effect. After 12 months of treatment a limited response in the liver was observed. In the 12 month study, the major change was limited liver enlargement (13% to 22%) in some animals, which occurred generally in the absence of histopathological observations. At the end of the treatment, there was only increased hepatocellular pigment and vacuolation observed with a limited increase in alkaline phosphatase (up to 45%) levels at 60 and 120 mg/kg/day. In the 12-month dog study, the NOAEL was 30 mg/kg/day, based on the absence of any relevant liver

enzyme and histopathological changes. At NOAEL dose, the exposures based on plasma DRV AUC values were between 0.26- to 0.39-fold, relative to that observed in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d; Section 4.8.1.5 and Table 2). In this study at 120 mg/kg/day at the end of the dosing period, DRV exposures, expressed as mean $AUC_{0\text{-}24h}$, were 130 and 100 µg.h/mL in males and females respectively. These exposures were higher (up to 1.6-fold) relative to that observed in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d.).

In order to increase exposure to DRV, two 2-week studies were conducted with various combinations of DRV with RTV. Dogs did not tolerate DRV co administered with RTV well. Vomiting and a body weight decrease were observed. As these clinical effects would be unacceptable in a chronic study, no further studies with DRV/RTV in dogs were conducted.

#### *4.2.3.2. Cobicistat*

In dogs, apart from salivation and vomiting associated with dosing, treatment with COBI was well tolerated at doses up to 15 mg/kg/day in the 4-week study, and up to 10 mg/kg/day in the 39-week study (Module 2.6.6 Toxicology Written Summary/Section 3.2). Changes in the thymus and adrenal gland in dogs observed in high dose animals after 13-weeks of dosing were absent after 39-weeks of dosing, and were considered stress related, and not a direct effect of COBI. In dogs administered 20 mg/kg/day for 39-weeks, clinical signs (salivation, emesis, fecal changes), decreases in body weight and food consumption, nonadverse changes in clinical pathology parameters, and minimal, adaptive changes in the liver (increased weights, hypertrophy) were noted. After 39-weeks of dosing at 10 mg/kg/day, effects were limited to minimal hepatocellular hypertrophy in males, and slightly increased liver weights in females. Clinical pathology changes in the 28-day study included minimal-to-mild increases in bilirubin, alanine aminotransferase (ALT), and alkaline phosphatase activities. In the 39-week study, slightly higher platelets counts, slightly higher alkaline phosphatase, and slightly lower total protein and albumin were observed; these changes were reversible following cessation of dosing. Urinalysis and urine chemistry changes, noted in female dogs at 20 mg/kg/day, included slightly higher electrolyte excretion and slightly lower electrolyte concentrations, consistent with findings of lower urine osmolality, higher urine volume and/or pH. These changes showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and BUN, and were without histopathological correlates in the kidney. In dogs, a

greater incidence of bilirubinuria was noted in males at 20 mg/kg/day during the 39-week study; no changes were observed at recovery. Based on these findings, the NOAEL for COBI when administered daily by oral gavage to dogs for up to 39-weeks is 10 mg/kg/day. In dogs, the exposures based on plasma COBI AUC values at the NOAEL doses in the longest duration studies were approximately 2.2- to 2.6-fold higher than the AUC in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d.) (see Section 4.8.2.2 and Table 3).

## 4.3. Genotoxicity Studies

### 4.3.1. Darunavir

In vitro and in vivo genotoxicity tests have shown that DRV has no genotoxic potential. (Module 2.6.6 Toxicology Written Summary/Section 4.1)

### 4.3.2. Cobicistat

No significant effects were observed in genotoxicity (Module 2.6.6 Toxicology Written Summary/Section 4.2).

## 4.4. Carcinogenicity

### 4.4.1. Darunavir

DRV was evaluated for carcinogenic potential by oral gavage administration to mice and rats up to 24-months. Daily doses up to 1000 mg/kg were administered to mice and doses up to 500 mg/kg were administered to rats (Module 2.6.6 Toxicology Written Summary/Section 5.1). A dose-related increase in the incidences of hepatocellular adenomas and carcinomas was observed in males and females of both species. Thyroid follicular cell adenomas were noted in male rats. Administration of DRV did not cause a statistically significant increase in the incidence of any other benign or malignant neoplasm in mice or rats. The observed hepatocellular findings in rodents were associated with liver enzyme induction and are considered to be of limited relevance to humans. (Module 2.6.6 Toxicology Written Summary/Section 9.1). At the highest tested doses, the systemic exposures (based on AUC) were between 0.6- and 0.8-fold (mice) and 1.1-fold (rats), of that observed in humans at the therapeutic dose (DRV/COBI 800/150 mg q.d.) (see Section 4.8.1.5 and Table 2)

#### 4.4.2. Cobicistat

In the 104-week carcinogenicity study in mice with COBI, no drug-related increase in tumor incidence was observed at exposures 10 to 23 times (males and females, respectively) the human systemic exposure at the therapeutic daily dose (see Section 4.8.2.2 and Table 3). In the 104-week carcinogenicity study in rats, increases in follicular cell adenomas and/or carcinomas in the thyroid gland were observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans[27,29,30]. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 3 times the human systemic exposure at the therapeutic daily dose (see Section 4.8.2.2 and Table 3). In rats, COBI induces hepatic CYP3A activity due to a species-specific activation of PXR, which does not occur in humans. The observed toxicity profile on the thyroid is rodent-specific and it is unlikely that COBI presents a risk to the human thyroid. These effects, associated with liver enzyme induction, bear no relevance for man as a similar association between liver enzyme induction and carcinogenesis does not exist in man. Module 2.6.6 Toxicology Written Summary/Section 9.2)

## 4.5. Reproductive Toxicity Studies

#### 4.5.1. Darunavir

The reproduction and developmental toxicity studies demonstrated that there was no effect of DRV or DRV/RTV on fertility or early embryonic development in rats and DRV showed no teratogenic potential in mice, rats, and rabbits (Module 2.6.6 Toxicology Written Summary/Section 6.1). In embryo-fetal development studies, the NOAELs were considered at 1000 mg/kg in mouse and rabbits where exposures were approximately 0.78-fold and 0.074-fold higher, respectively than the human therapeutic exposures (see Section 4.8.1.5 and Table 2). In rats, the NOAEL for maternal toxicity was considered to be less than 600/100 mg/kg/day DRV/RTV and the NOAEL for embryo-fetal development was considered to be 600/100 mg/kg/day (DRV/RTV) where maternal exposures were approximately 2.5-fold (Day of gestation (GD) 11) and 3.2-fold (GD17) higher, than the human therapeutic exposure (see Section 4.8.1.5 and Table 2).

In the pre- and postnatal development study in rats, DRV caused a transient reduction in pup body weight gain, which was attributed to exposure to DRV during lactation

(Module 2.6.6 Toxicology Written Summary/Section 6.1).The toxicity of DRV was also investigated in a juvenile study where male and female rats received dose levels up to 500 mg/kg/day from 23 days of age until sexual maturity (50-day old). In addition, several single and repeated oral dose studies were conducted in juvenile rats as young as 5 days of age. In studies where dosing commenced at less than 23 days of age (equivalent to less than 2 years of age in humans), a NOAEL was not established. Mortality was observed at most dose levels (from 20 mg/kg/day up to 1000 mg/kg/day) and in some cases convulsions and other apparent central nervous system effects preceded these deaths. High plasma, liver and brain exposure values were observed, which were both dose- and age-dependent and considerably greater than those observed in adult rats. These findings were attributed to the ontogeny of CYP liver enzymes involved in the metabolism of DRV and the immaturity of the blood brain barrier.

### 4.5.2. Cobicistat

No adverse effects were observed in a rat fertility study with COBI; the no observed effect level (NOEL) for reproductive parameters was 100 mg/kg/day at exposures approximately 2.3-fold higher than human therapeutic exposures. No teratogenic effects were observed in rat and rabbit developmental toxicity studies (Module 2.6.6 Toxicology Written Summary/Section 6.2). In rats at 125 mg/kg/day, increases in postimplantation loss and decreased fetal weights were associated with significant maternal toxicity (adverse clinical signs, decreased body weight and food consumption). The NOEL/NOAELs in the rat and rabbit studies were 50 and 100 mg/kg/day, respectively, where exposures were approximately 1.9 and 4.7 fold higher, respectively, than human therapeutic exposures (see Section 4.8.2.2 and Table 3). In the pre/postnatal study, the maternal NOAEL for general toxicity was 30 mg/kg/day, and the NOAEL for reproduction in the dams and viability and growth of the offspring was 75 mg/kg/day, the highest dose tested (exposures on LD 10 were 1.3-fold human therapeutic exposures). In the juvenile toxicity phase of the pre/postnatal study in rats with COBI, the NOAEL for toxicity of COBI is 75 mg/kg/day for juvenile rats where exposures were 2.7-fold higher (for males and females) than therapeutic adult human exposures at the therapeutic dose (DRV/COBI 800/150 mg q.d.).

## 4.6. Local Tolerance

### 4.6.1. Darunavir

Local tolerance studies showed , no skin irritation and sensitization and eye irritation. (Module 2.6.6 Toxicology Written Summary/Section 7.1).

### 4.6.2. Cobicistat

Cobicistat was mildly irritating to skin, not a severe irritant to eyes, and showed no potential for phototoxicity (Module 2.6.6 Toxicology Written Summary/Section 7.2)

## 4.7. Other Toxicity Studies

### 4.7.1. Immunotoxicity

#### *4.7.1.1. Darunavir*

DRV formulated in PEG400 was administered daily, alone or in combination with RTV, in a 4-week rat study at doses of DRV alone up to 500 mg/kg/day, or DRV/RTV at 100/50 mg/kg/day. Changes in the hematopoietic system and liver (as seen in the repeated dose toxicity studies) were present but the immune response of treated animals, as measured by immunoglobulin (Ig) M production, was not affected. The NOAEL was 20 mg/kg/day (Module 2.6.6 Toxicology Written Summary/Section 8.1.1).

#### *4.7.1.2. Cobicistat*

The immunotoxicity of COBI was evaluated in a 28 day T-cell dependent antibody response (TDAR) study in rats at doses up to 150 mg/kg/day (Module 2.6.6 Toxicology Written Summary/Section 8.2.1). Immunosuppressive effects were noted in females at 50 and 150 mg/kg/day, based on a decreased response to keyhole limpet hemocyanin (KLH) immunization (lower anti-KLH immunoglobulin G [IgG] antibody titers). Decreased anti-KLH IgG responses in males did not reach statistical significance at 150 mg/kg/day. No COBI-related changes in the anti KLH IgM response in males and females were noted. In addition, clinical signs, decreases in body weight gain and/or food consumption, increases in liver and thyroid weights, and lymphoid depletion of germinal centers in the spleen were observed at 50 and/or 150 mg/kg/day. The NOEL for the TDAR is considered to be 20 mg/kg/day in females, and 50 mg/kg/day in males, and the NOAEL was 20 mg/kg/day in both sexes. Additional immunohistochemical analysis of spleens from all animals was conducted, as described in the ICH S8 Guideline, Immunotoxicity Studies for Human

Pharmaceuticals[33]. Formalin-fixed, paraffin-embedded tissues (spleen) were evaluated for detection of B cells (KiB1R-positive), T cells (CD3-positive), and germinal centers (PNA-positive). Immunohistochemical findings observed in COBI-treated animals did not correlate with the decreases noted in anti-KLH IgG levels, as greater decreases in anti KLH IgG levels were observed in females versus males, but immunohistochemical trends were noted only in males. In females, there was a notable lack of any dose-response with respect to the immunohistochemical changes.

To further assess the potential for immunotoxicity associated with COBI, immune tissues (spleen, thymus, lymph nodes, and Peyers patches) from rats administered COBI in the 4 week and 26-week toxicity studies were subjected to a pathology peer-review. Evaluation was conducted according to recommendations for rat lymphoid tissue evaluation and included an estimate of the numbers of germinal centers in the spleen for each animal[34,35,36]. The Peer Reviewing Pathologist was in agreement with the overall interpretations and conclusion of the histopathology diagnoses and conclusions of the studies regarding the lack of treatment related effects on rat lymphoid tissues. The NOAELs for COBI in the 4 week and 26-week studies were 50 and 30 mg/kg/day, respectively.

In the 26-week rat study with COBI, peripheral blood immunophenotyping was conducted during Week 26 and prior to recovery sacrifice (Recovery Week 13). Total T cells, helper T cells, cytotoxic T cells, B cells and NK cells were quantified using flow cytometry. No adverse effects on mean immunophenotyping values were noted.

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

In summary, for COBI, decreased IgG levels were noted in female rats at dose levels (≥ 50 mg/kg/day) above the NOAEL for systemic toxicity in long term studies (30 mg/kg/day). However, in standard toxicity studies with COBI in mice (up to 13

weeks dosing), rats (up to 26 weeks dosing) and dogs (up to 39 weeks dosing), and at higher dose levels and exposures, no signs of immune function changes have been observed.

### 4.7.2. Drug Substance Impurities

All specified impurities for the 2 compounds are qualified according to the ICHQ3A guideline (Module 2.6.6 Toxicology Written Summary/Section 8.1.3 and 8.2.3).

## 4.8. Toxicology Evaluation

### 4.8.1. Darunavir

#### *4.8.1.1. Mortality*

Lethality, related to DRV, could not be demonstrated following single or repeated oral and intravenous treatment in adult mouse, rat and dog. Throughout all of the rodent toxicology studies there were no mortalities associated with DRV, but animals were found dead or were killed prematurely during the various studies. The majority of these deaths were associated with the dosing procedures with single daily doses or twice-daily gavage dosing where RTV was used or, in some cases, blood collection procedures. With the former, the irritant and viscous nature of the vehicle, PEG400 was considered contributory. In the 6-month rat study with DRV administered in combination with RTV, the cause of death was not evident in some animals and a relationship between mortality and RTV treatment at 75 mg/kg/day, could not be excluded based on the distribution of the deaths. In dogs, there were also isolated incidence of accidental deaths associated with gavage dosing but none were related to DRV treatment.

Mortality was reported in juvenile rats between day 12 and day 25 of age following single and repeated dosing at 40 mg/kg/day and above. The exact cause of death was not established and clinical signs observed prior to mortality were among others distended abdomens, unsteady gait, convulsions, reduced activity and labored breathing. In contrast, a DRV dose of 1000 mg/kg given at day 26 of age to rats was without clinical signs or mortality. Exposure in juvenile animals directly dosed was dose and age dependent and mortality seen at this age range might be attributed to high DRV exposure caused by the immaturity of liver enzymes. Exposure at 1000 mg/kg on day 12 was at least 3-fold higher than that observed in the clinic at the recommended dose.

#### *4.8.1.2. Assessment of Target Organ of Toxicity*

No target organs of toxicity were identified in dogs. The key target organ/systems identified in rodents following oral administration of DRV and/or RTV were the hematopoietic system, the blood coagulation system, liver and thyroid.

The hematopoietic system was affected in rats and mice. These changes included a variable, but slight decrease (about 10%) in some or all of the RBC counts, hemoglobin and hematocrit. There was also an increase in reticulocytes and bilirubin levels. These changes are indicative of an increased RBC turnover, which was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen, sometimes accompanied by an increase in spleen weight. In rats, the effects occurred at doses up to 500 mg/kg/day ($AUC_{0\text{-}24h}$ 63.8 µg.h/mL in males and 121 µg.h/mL in females at the end of the 6-month study). The NOAEL of this finding, after DRV alone was 20 mg/kg/day and corresponded to exposures ($AUC_{0\text{-}24h}$) ranged between 3 and 4 µg.h/mL. The RBC system has been identified as a target organ of toxicity in rodents with other protease inhibitors[1]. No changes in RBC-related parameters have been observed with DRV in dogs where exposure was generally higher (up to 1.6-fold) than exposures in patients. No clinically relevant adverse effects on the hematopoietic system have been observed in clinical studies conducted to date with DRV (Module 2.7.4 Summary of Clinical Safety/Sections 3.1 and 5).

The blood coagulation system was affected[2]. In rats platelet counts were increased and there was a prolongation in the APTT observed at 100 and 500 mg/kg/day. The NOAEL of this finding, after DRV alone was 20 mg/kg/day and corresponded to exposures ($AUC_{0\text{-}24h}$) ranged between 3 and 4 µg.h/mL. The increase in the APTT observed was not associated with any gross or microscopic evidence of bleeding. APTT and PT were decreased in the 6-month combination study with RTV in rats, whereas in dogs no such changes were seen. No clinically relevant adverse effects on the blood coagulation system have been observed in clinical studies conducted to date with DRV (Module 2.7.4 Summary of Clinical Safety/Sections 3.1 and 5).

No changes in the immune response were observed for DRV.

Liver changes were observed in rats and mice and these included increases in weight and hepatocellular hypertrophy sometimes with vacuolation of centrilobular hepatocytes at doses of 100 mg/kg/day and above. The hepatocellular hypertrophy is considered mild in nature and reflects an adaptive response to enzyme induction

(CYP3A and UGT) seen after DRV administration rather than a direct adverse effect[3]. In rats, several clinical biochemistry parameters were altered by treatment with DRV, mainly increases in cholesterol and proteins (e.g. total protein) and decreases in triglycerides. These lipid and protein changes were probably secondary to the effects on the liver. In dogs, changes in the liver were observed only after 12 months of treatment and were limited to a slight increase in liver weight associated with hepatocellular vacuolation and pigmentation at an $AUC_{last}$ of 100 to 130 µg.h/mL. Increases in liver transaminases in animal studies were generally mild or absent. In the clinic, liver abnormalities, mainly increases of AST and ALT were generally mild to moderate, and drug-induced hepatitis (e.g. acute hepatitis, cytolytic hepatitis) have been reported with DRV/RTV. (Module 2.7.4 Summary of Clinical Safety/Sections 3.1 and 5).

Thyroid homeostasis was altered to various extends in rats (and mice) as seen by diffuse follicular hypertrophy and hyperplasia. It is likely that this is a rodent-specific adaptive response to liver enzyme induction and an enhanced metabolism of thyroid hormones. Both CYP P450 and UGT are responsible for eliminating numerous substrates, both endogenous and exogenous, rendering them water-soluble and excretable in urine and bile. Increased elimination of thyroid hormones, especially T4, via UGT induction, may stimulate the thyroid and result in the changes seen. Both of these enzymes were induced in rats following repeated oral administration with DRV alone and in combination with RTV. Thyroid changes were not observed in the dog. No clinically relevant adverse effects on thyroid homeastasis have been observed in clinical studies conducted to date with DRV (Module 2.7.4 Summary of Clinical Safety/Sections 3.1 and 5).

The combination of DRV with RTV in rats resulted in slight increased exposure to DRV. Given the complex nature of the combination studies and the potential effects of both compounds these results should be considered in comparison with other studies. If this is done then it is considered that there is evidence of a small increase in effect in the 6-month study with the combination of DRV and RTV on RBC parameters, liver and thyroid. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV but were not of a different order of magnitude observed in animals with each compound alone. At doses of 500/75 and 1000/75 mg/kg/day exposure to DRV expressed as $AUC_{last}$ was similar and in the range of 183 to 318 µg.h/mL, at the end of the study. These values were about 4 times higher than those observed with DRV alone. As the full clinical development of DRV was done with RTV, the comparative information on safety of DRV versus the

DRV/RTV combination is only available from Phase I trials. These trials showed no synergistic effect.

#### *4.8.1.3. Assessment of Reproduction and Development Toxicology*

There was no evidence of an effect on fertility or early embryonic development and DRV showed no teratogenic potential.

In the rat pre- and postnatal development study, reduction in pup body weight gain associated with a slight delay in developmental milestones was observed with DRV alone (at 200 mg/kg/day and above) or in combination with RTV (at 1000/75(50) mg/kg/day) during lactation. This was due to exposure of pups to drug substances via the milk as shown in the dose range finding study. Sexual development, fertility or mating performance of offspring was not affected by maternal treatment with DRV alone or in combination with RTV or RTV alone. No post-weaning functions were affected at any dose level.

The juvenile data available to date show that in juvenile rats from 23 days of age DRV has a toxicity profile and exposure that is comparable to that observed in adult rats. In studies where dosing commenced at less than 23 days of age (equivalent to less than 2 years of age in humans) mortality was observed and in some animals, convulsions and other apparent CNS effects preceded these deaths. Within this age range, high plasma, liver and brain AUC values were observed, which were both dose- and age-dependent and considerably greater than those observed in adults. These findings were attributed to the immaturity of the liver enzymes and of the blood brain barrier. The above observations in juvenile rats provide evidence of a potential safety risk as a result of drug-brain accumulation in young children (below 3 years of age).[9,10,11]

#### *4.8.1.4. Assessment of Genotoxicity and Carcinogenicity*

Genotoxicity tests, in vitro and in vivo, have shown DRV to be free of genotoxic potential.

Darunavir was evaluated for carcinogenic potential by oral gavage administration to mice and rats up to 24-months. Daily doses of 150, 450 and 1000 mg/kg were administered to mice and doses of 50, 150 and 500 mg/kg were administered to rats. A dose-related increase in the incidences of hepatocellular adenomas and carcinomas were observed in males and females of both species. Thyroid follicular cell adenomas

were noted in male rats. Administration of DRV did not cause a statistically significant increase in the incidence of any other benign or malignant neoplasm in mice or rats. The observed hepatocellular findings in rodents were associated with liver enzyme induction and are considered to be of limited relevance to humans[7,8,9]. Repeated administration of DRV to rats caused hepatic microsomal UGT induction and increased thyroid hormone elimination, which predispose rats, but not humans, to thyroid neoplasms. At the highest tested doses, the systemic exposures (based on AUC) were between 0.6- and 0.8-fold (mice) and 1.1-fold (rats), of that observed in humans at the recommended therapeutic dose (see Section 4.8.1.5 and Table 2).

#### *4.8.1.5. Safety Margin*

In the chronic toxicity studies, the highest tested DRV doses were limited by the maximum feasible dose (MFD) associated with PEG400 administration, the vehicle that was used in nonclinical oral studies. At the MFD in rodents, high DRV exposures were achieved at the start of the studies, but these levels were not maintained due to auto-induction (i.e. DRV's activity to increase its own metabolism as a result of CYP3A induction). RTV, as a potent CYP3A4 inhibitor, maintained DRV exposure in rats following repeated administration. A NOAEL was not established in the rat combination studies due to limited toxicity effects seen on liver and hematopoietic systems. However, at the MFD, DRV exposure was about 2- to 4-fold higher than the human exposure (DRV/COBI 800/150 mg q.d.).

In an attempt to increase exposure in nonclinical studies, other routes of administration such as dietary and intravenous were tested. Both routes yielded no further gain in DRV AUC.

The animal/human exposure ratios can be found in Table 2.

**Table 2: Estimated Safety Margins for DRV 800 mg Based on Exposure (AUC) at Animal NOAEL**

| Species Gender | Study Type | NOAEL Dose (mg/kg/day) | $AUC_{0-t}$ (µg.h/mL) | Safety Margin[a] |
|---|---|---|---|---|
| **Mice** | | | | |
| Male - Female | 24-Month Carcinogenicity (Day 188) | 1000 [b] | 48.1- 63.8- | 0.59 – 0.78 X |
| Female (Pf) | Embryo- Fetal Development (GD15) | 1000 | 63.9 | 0.78 X |
| **Rat** | | | | |
| Male - Female | 26-week Toxicity | 20 | 2.9 – 4.4 | 0.04 – 0.05 X |
| Male - Female | 24-Month Carcinogenicity (Day 184) | 500 [b] | 90.3 -89.5 | 1.1 X |
| Female (Pf) | Embryo- Fetal Development | 2x300 (DRV) + 2 x 50 (RTV) | 202 (GD11) 261 (GD17) | 2.5 X 3.2 X |
| Male - Female | 13-week Combination Toxicity | 20 [c] (DRV) + 50 (RTV) | 11.2 – 13.4 | 0.14 – 0.16 X |
| **Rabbit** | | | | |
| Female (Pf) | DRF of Prenatal Development Toxicity (GD20) | 1000 | 6.00 | 0.074 X |
| **Dog** | | | | |
| Male - Female | 12-month Toxicity | 30 | 21.2 – 31.6 | 0.26 – 0.39 X |

RTV, ritonavir; NOAEL, no observed adverse effect level, Pf : pregnant female
[a]: Human $AUC_{0-24h}$ 81.6 µg.h/mL (GS-US-216-0130 substudy); [b] : the highest dose tested; [c] : in this study no NOAEL was determined , this dose corresponded to the low dose.

### 4.8.2. Cobicistat

#### *4.8.2.1. Target Organ Effects*

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

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

In rats, increases (up to 38%) in mean platelet counts were noted in the 4-week study at doses ≥ 50 mg/kg/day, and in the 26-week study in males at ≥ 30 mg/kg/day and in females at 100 mg/kg/day. In dogs, increases in platelet counts (up to 43%) were

noted after 13, 26 and 39 weeks dosing in high dose (20 mg/kg/day) females, and in 10 mg/kg/day females at Week 26 only. Further, in dogs, minimal decreases (up to 15%) in APTT were noted in 10 mg/kg/day females and in both sexes at 20 mg/kg/day. Similar changes were not observed in the 4 week dog study at doses up to 45/30 mg/kg/day. In all cases, there were no associated effects on bleeding, the changes were reversible, and they were not considered adverse. Since these changes only occurred at high doses, the relevance of these limited changes is questionable.

Slight but statistically significant increases in cholesterol were observed in the 13-week mouse study (up to 46% increase at dose levels ≥ 15 mg/kg/day) and in female rats in the 26 week study (up to 35% increase at dose levels ≥ 30 mg/kg/day;. Cobicistat is a rat PXR inducer, with activation similar to known PXR inducers (RTV and miconazole). Induction of PXR can reduce Cyp7a1 transcription and cholesterol 7a-hydroxylase activity, which are involved in the conversion of cholesterol to bile acids[37]. Down-regulation of Cyp7a1 results in less cholesterol being converted to bile acids and consequently more free cholesterol. An approximate 6 fold induction of rPXR was observed with COBI at 10 µM. In the 13-week mouse and 26-week rat toxicity studies, COBI $C_{max}$ values associated with cholesterol changes were 2.7 to 11.4 µg/mL (3.5 to 14.7 µM), suggesting adequate PXR activation to explain the slight increase in cholesterol levels observed with COBI. At clinically-relevant concentrations, COBI would not activate human PXR. In Phase 3 studies with COBI-boosted ATV, mean values for fasting glucose and lipid parameters remained in the normal range.

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

Results from a rat immunotoxicity study showed lower anti KLH IgG antibody titers in females at ≥ 50 mg/kg/day. There were no COBI-related changes in the anti KLH IgM antibody response at any dose in either sex. The NOEL for the T cell dependent antibody response is considered to be 20 mg/kg/day in females and 50 mg/kg/day in

males. However, in standard 13 week mouse, 26 week rat and 39 week dog toxicity studies at doses up to 50, 100 and 20 mg/kg/day in mouse, rat and dog, respectively, microscopic changes suggestive of immunotoxicity were not been observed in lymphoid organs. Further, immunophenotyping of peripheral blood cells evaluated in the chronic rat and dog toxicity studies did not reveal any adverse effects, and there were no signs of potential immunosuppression as assessed by animal health status (ie, no signs of opportunistic infections) and clinical chemistry and hematological analyses. The clinical significance of the decrease in anti KLH IgG levels in a single study in female rats is unclear considering that no adverse effects on hematological parameters, IgG levels, or rate of infections considered related to study drug that could be suggestive of immunosuppression have been observed in clinical studies conducted with COBI, mean values for fasting glucose and lipid parameters remained in the normal range.

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

In the 13-week mouse study, mild to marked elevations in ALT and AST were noted in males at 15 and 50 mg/kg/day, respectively. These changes were associated with increases in liver weight, microsomal enzyme induction, and minimal hepatocellular hypertrophy at 50 mg/kg/day. Female mice were notably less sensitive; a marked elevation in ALT and AST was noted in only one high dose female (50 mg/kg/day). Similar liver findings were noted in the 4-week toxicity in HRAS wild type mice. In rats, increases in liver weights were associated with hepatocellular hypertrophy. These findings were reversible and were not considered adverse. The liver effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction[31,32]. Cobicistat induces hepatic CYP3A activity in mice and in rats likely due to a species specific activation of rodent PXR (Section 3.3.2).

In dogs, minimal to mild increases in bilirubin, ALT, and alkaline phosphatase (ALP) activities, increased liver weights, and hepatocyte vacuolation were noted after 4 weeks dosing at 45 or 45/30 mg/kg/day. In the 39 week study, there were no notable serum chemistry changes; however, minimal liver changes (hepatocellular

hypertrophy) were observed in males at 10 mg/kg/day, and in both sexes at 20 mg/kg/day. These hepatic changes observed in the 39 week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing[31,32].

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

Effects on the thyroid glands in rats in the 26 week study were characterized by decreases in T4 in males at 100 mg/kg/day, increases in TSH in 10 mg/kg/day females and in both sexes at 30 and 100 mg/kg/day, increased thyroid weights at 30 and 100 mg/kg/day, and thyroid follicular cell hypertrophy (in one female at 10 and 30 mg/kg/day, and in most male and female animals at 100 mg/kg/day). These findings were reversible and were not considered adverse. However, one high dose 100 mg/kg/day male in the 26-week study also had a follicular cell carcinoma in the thyroid.

In the 104-week rat carcinogenicity study, neoplastic findings in the thyroid consisted of significant positive trends in thyroid follicular adenoma, carcinoma, and combined adenoma and carcinoma in males and/or females, with significant increases in adenoma for males at 50 mg/kg/day, and in combined adenoma/carcinoma for males at ≥ 25 mg/kg/day and for females at 30 mg/kg/day. The increase in thyroid follicular tumors was associated with follicular cell hypertrophy at ≥ 25 and ≥ 15 mg/kg/day in males and females, respectively.

Thyroid effects were slightly more pronounced in females possibly due to the higher exposures achieved in this sex. There are no indications that COBI has a direct effect on the thyroid gland, or a particular affinity for thyroid tissue. These clinical and anatomic pathology changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction (Section 3.3) and thyroid hormone imbalance. The thyroid effects are considered rodent specific, and predispose rats, but not humans, to thyroid neoplasms. It is unlikely that COBI presents a risk to the human

thyroid[25,26,27,28,29,30]. No clinically relevant adverse effects on thyroid function have been observed in clinical studies conducted to date with COBI.

Urinalysis and urine chemistry changes, noted primarily in high-dose rats (at 100 mg/kg/day) and in dogs (at ≥ 30 mg/kg/day), included higher urine volume, lower urine specific gravity, and increases in electrolyte excretion. These changes showed no progression after long term dosing, were not associated with remarkable serum clinical chemistry changes, including serum electrolytes, serum creatinine and BUN, were without histopathological correlates, and were reversible. Although the mechanism associated with these urinalysis changes is not understood (there were no treatment-related changes in serum vasopressin or aldosterone levels in the 26-week rat study), similar findings have been reported with other structurally-related agents, including ATV[38,39] and RTV[40]. In the 13-week combination toxicity study in rats with COBI and ATV, increases in urine volume were noted in groups given COBI and ATV alone, and in combination. However, these effects were slight, not additive when COBI and ATV were administered in combination, nor associated with microscopic correlates and were reversible; therefore these changes were not considered adverse.

#### *4.8.2.2. Safety Margins*

A comprehensive program of nonclinical studies with COBI has been conducted. In repeat-dose studies (up to 13 weeks in mice, up to 26 weeks in rats; up to 39 weeks in dogs), target organs identified were liver (mouse, rat, and dog) and thyroid (rat). The liver effects in mice and rats are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction[31,32]. In dogs, the hepatic changes observed in the 39-week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing[31,32]. The thyroid changes in rats are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance[25,26,27,28,29,30]. The thyroid effects are considered rodent specific and predispose rats, but not humans, to thyroid neoplasms.

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

These animal/human exposure ratios can be found in Table 3.

**Table 3: Estimated Safety Margins for COBI 150 mg Based on Exposure (AUC) at Animal NOAEL**

| Species Gender | Study Type | NOAEL Dose (mg/kg/day) | $AUC_{0-t}$ (µg.h/mL) | Safety Margin[a] |
|---|---|---|---|---|
| **Mice** | | | | |
| Male - Female | 104-week Carcinogenicity (week 29) | 50 -100[b] | 75 -174 | 10 -23 X |
| | | | | |
| **Rat** | | | | |
| Male - Female | 26-week Toxicity | 30 | 9.9 – 13.3 | 1.3 – 1.8 X |
| Male - Female | 104-week Carcinogenicity (week 26) | 50 – 30[b] | 22.6 – 19.9 | 3 -2.6 X |
| Female (Pf) | Embryo-Fetal Development (GD17) | 50 | 14.8 | 1.9 X |
| **Rat + 50 mg/kg ATV** | | | | |
| Male - Female | 13-week Combination Toxicity | 30 | 6.1 – 6.3 | 0.8 – 0.8 X |
| **Rabbit** | | | | |
| Female (Pf) | Embryo-Fetal Development (GD20) | 100 | 35.7 | 4.7 X |
| **Dog** | | | | |
| Male - Female | 39-week Toxicity | 10 | 19.6 – 16.8 | 2.6 – 2.2 X |

ATV, atazanavir; COBI, cobicistat; NOAEL, no observed adverse effect level; Pf : pregnant female
[a]: Human $AUC_{0-24h}$ =7.6 µg.h/mL (GS-US-216-0130 substudy); [b] : the highest dose tested

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

# MODULE 2.4.5

# INTEGRATED OVERVIEW AND CONCLUSIONS

# 5. INTEGRATED OVERVIEW AND CONCLUSIONS

This nonclinical overview supports the use of a fixed dose combination tablet including 800 mg DRV and 150 mg COBI for the treatment of HIV-1 infection.
A comprehensive package of nonclinical safety data is available for DRV and COBI, both as a single agent and the use of each compound separately has already been approved.

Overall, DRV safety pharmacology studies did not detect any significant nonclinical safety signals[4].
Although COBI showed the potential to decrease LV function and prolong the PR interval in isolated rabbit hearts, the TQT study, and ECGs and echocardiograms conducted during clinical development did not reveal clinically-significant changes in these parameters. When COBI was tested in combination with ATV in isolated rabbit hearts, there were no clearly additive effects on LV function or prolongation of the PR interval.
Given the lack of effects for DRV on the cardiovascular system, the potential for cardiovascular effects when administered with COBI is considered low.

After oral administration, DRV was rapidly absorbed and eliminated. DRV is primarily eliminated by metabolism via CYP3A iso-enzyme. Several metabolic pathways were identified in vivo and they were qualitatively similar in tested species including human. DRV was an inhibitor of CYP3A4 at clinically relevant concentrations and also showed a concentration-dependent effect on CYP3A4 induction in vitro. This effect may have limited clinical relevance, as induction was significant in vitro at concentrations several fold higher than those attained in the clinic. Exposure in rodents including pregnant rats decreased following repeated oral administration due to the induction of CYP3A. RTV, a structural analogue of COBI and a potent MBI of CYP3A, had a modest effect on DRV exposure in mice (2-fold increase), rats (4-fold increase) including pregnant rats but had no clear effect in minipigs and dogs. In humans, however, the impact was noticeable. RTV markedly reduced the metabolism of DRV and consequently increased its oral bioavailability from 37% to 82%.
COBI is a potent inhibitor of human CYP3A enzymes. In rodents and dogs, COBI is a weak competitive inhibitor of CYP3A enzymes, and primarily acts as a species-specific CYP inducer in rodents via activation of PXR. As such, the mechanism of action and pharmacokinetic profile of COBI are different in humans and animals, and therefore the use of nonclinical studies to assess effects of the interaction of COBI

with DRV may not adequately predict effects in humans. In addition, DRV/COBI combination is not anticipated to produce any new human metabolites.
The clinical drug-drug interaction potential of DRV/COBI is described in Module 2.5 Clinical overview/Section 3.1.7.

For DRV, repeated dose toxicity studies up to 6 months in rats and 12 months in dogs identified only limited effects of treatment with DRV. The key target organs identified in rats after administration of DRV were the hematopoietic system, the blood coagulation system, liver and thyroid. The effects seen in the liver and thyroid (cellular hypertrophy with increase in organ weight) were consistent with the liver enzyme inducing property of DRV. Limited changes in liver were observed in dogs after 12 months of treatment. In a 6-month study in rats, the combination of DRV with RTV showed a small increase in effect on RBC count parameters, liver and thyroid in rats. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV, and were similar to the effects noted with each compound alone. There were no unexpected toxicities with the combination treatment. The hematological, liver and thyroid changes appeared to be not clinically relevant, as in clinical practice, DRV in combination with RTV is generally safe and well tolerated in HIV-infected patients. Due to the poor tolerability and no clear boosting effect of RTV on DRV in dogs and minipigs, these species were not considered suitable for further assessment of DRV.
For COBI, in repeated-dose toxicity studies up to 6 months in rats and up to 9 months in dogs, the target organs identified for COBI were the liver (rat and dog) and thyroid (rat). Slight, non-adverse hematological changes were noted in rats, slight clinical chemistry changes were observed in rats and dogs, and increases in urine volume and urine chemistry changes were noted primarily at high doses in rats and dogs. In rats, the thyroid changes are considered rodent-specific, secondary to microsomal enzyme induction and thyroid imbalance. Liver changes in rats, and dogs included microsomal enzyme induction, increased weights, and hepatocellular hypertrophy and/or vacuolation. Combination toxicity studies of COBI with ATV in rats did not result in unexpected or additive toxicity. Although COBI was associated with urinalysis and urine chemistry changes at high doses in rats and dogs, these changes were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and BUN, and were without morphological evidence of kidney damage. Cobicistat is a weak inhibitor of human renal transporters OCT2, MRP4, and MATE2-K, and is a more potent inhibitor of OCTN1 and MATE1, with similar potencies being found for RTV. These data, along with the clinical findings,

suggest that COBI reversibly blocks secretion of creatinine in humans. In light of the potential for overlapping liver toxicity and the similarity of the urinalysis findings in rats, a 90-day combination toxicity study in rats of COBI coadministered with ATV was conducted. This combination toxicity study with COBI and ATV did not reveal any new or additive toxicities. In addition, COBI decreased IgG levels in female rats, only. No clinical relevant changes suggestive of immunosuppression have been observed in clinical studies conducted with to date with COBI.

Although the nonclinical toxicity profiles of DRV and COBI are similar, there are no clinically significant overlapping toxicities. For DRV, target organs were the hematopoietic system, the blood coagulation system, liver, and thyroid in rodents. The hematological changes, and the adaptive liver and thyroid changes appear to be not clinically relevant, as in clinical practice, DRV in combination with RTV is generally safe and well tolerated in HIV-infected patients. Potential toxicities related to COBI observed in nonclinical toxicology studies (hematology, clinical chemistry, and urinalysis changes; lower IgG antibody titers; and adaptive liver and thyroid changes) have not been observed in clinical studies with COBI.

Combined dosing of DRV with COBI is not anticipated to alter the genotoxicity profiles of the individual agents. Darunavir and COBI demonstrated low carcinogenic potential in conventional 2-year studies. It is unlikely that combination dosing would change these profiles, and no exacerbation of toxicity is expected.

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

In view of the similarity of the boosting effect between RTV and COBI across species and the preclinical toxicity profiles of DRV and COBI, there are no anticipated clinically relevant pharmacological or toxicological interactions expected with the combined use of DRV and COBI beyond the pharmacoenhancing effect of COBI on DRV. Moreover, in clinic, after administration of DRV in combination with COBI, the clinical findings were consistent with the known DRV and COBI safety profiles and no new safety findings were observed.

In addition, the absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/[redacted], [redacted]).

Therefore, the comprehensive preclinical programs of the individual agents DRV and COBI support the use of the fixed dose combination tablet DRV/COBI as proposed in the Prescribing Information in Module 1.

# MODULE 2.4.6

# LIST OF LITERATURE CITATIONS

# 6. LIST OF LITERATURE CITATIONS

All literature references are considered supportive but have been included for the convenience of the reviewer

**Darunavir references**


1. Vogel M, Rockstroh JK. Safety of lopinavir/ritonavir for the treatment of HIV-infection. Expert Opin. Drug Saf. 2005; 4(3): 403-420.

2. J.A.Racoosin, C.M. Kessler. Bleeding episodes in HIV-positive patients taking HIV-protease inhibitors: a case series. Haemophilia. 5(4):266-9, 1999 Jul.

3. Greaves P. Hepatocellular hypertrophy and hyperplasia. In: Histopathology of Preclinical Toxicity Studies. Interpretation and Relevance in Drug Safety Evaluation, 2nd ed. Amsterdam: Elsevier; 2000: 445-515.

4. ICH guideline: Safety Pharmacology Studies for Human Pharmaceuticals. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Guideline S7A, Jul 2001.

5. Fichtenbaum CJ, Gerber JG. Interactions between antiretroviral drugs and drugs used for the therapy of the metabolic complications encountered during HIV infection. Clin Pharmacokinet 2002; 41(14): 1195-2111.

6. Dixit V, Hariparsad N, Li F, et al. Cytochrome P450 enzymes and transporters induced by anti-human immunodeficiency virus protease inhibitors in human hepatocytes: implications for predicting clinical drug interactions. Drug Metabolism and Disposition 2007; 35(10): 1853-1859.

7. Greaves P. Liver and Pancreas. In: Histopathology of Preclinical Toxicity Studies, Interpretation and Relevance in Drug Safety Evaluation, 3rd ed. Academic Press Elsevier; 2007: 457-569.

8. Grasso P, Hinton RH. Evidence for and possible mechanisms of non-genotoxic carcinogenesis in rodent liver. Mutation Research 1991; 248: 271-290.

9. Holsapple MP, Pitot HC, Cohen SH, et al. Mode of action in relevance of rodent liver tumors to human cancer risk. Toxicological Sciences 2006; 89(1): 51-56.

10. Davies TS, Monro A. Marketed human pharmaceuticals reported to be tumorigenic in rodents. Journal of the American College of Toxicology 1995; 14(2): 90-107.

11. Lamminpaa A, Pukkala E, Teppo L, Neuvonen PJ. Cancer incidence among patients using antiepileptic drugs: a long-term follow-up of 28,000 patients. Eur J Clin Pharmacol 2002; 58: 137-141.

**Cobicistat References**

1. Watkinson WP, Gordon CJ. Caveats regarding the use of the laboratory rat as a model for acute toxicological studies: modulation of the toxic response via physiological and behavioral mechanisms. Toxicology 1993;81 (1):15-31.

2. Watkinson WP, Campen MJ, Wichers LB, Nolan JP, Costa DL. Cardiac and thermoregulatory responses to inhaled pollutants in healthy and compromised rodents: modulation via interaction with environmental factors. Environ Res 2003;92 (1):35-47.

3. Redfern WS, Strang I, Storey S, Heys C, Barnard C, Lawton K, et al. Spectrum of effects detected in the rat functional observational battery following oral administration of non-CNS targeted compounds. J Pharmacol Toxicol Methods 2005;52 (1):77-82.

4. Lu HR, Marien R, Saels A, De Clerck F. Species plays an important role in drug-induced prolongation of action potential duration and early afterdepolarizations in isolated Purkinje fibers. J Cardiovasc Electrophysiol 2001;12 (1):93-102.

5. Sasaki H, Shimizu N, Suganami H, Yamamoto K. QT PRODACT: inter-facility variability in electrocardiographic and hemodynamic parameters in conscious dogs and monkeys. J Pharmacol Sci 2005;99 (5):513-22.

6. Soloviev MV, Hamlin RL, Shellhammer LJ, Barrett RM, Wally RA, Birchmeier PA, et al. Variations in hemodynamic parameters and ECG in healthy, conscious, freely moving telemetrized beagle dogs. Cardiovasc Toxicol 2006;6 (1):51-62.

7. Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovasc Res 2003;58 (1):32-45.

8. Toyoshima S, Kanno A, Kitayama T, Sekiya K, Nakai K, Haruna M, et al. QT PRODACT: in vivo QT assay in the conscious dog for assessing the potential for QT interval prolongation by human pharmaceuticals. J Pharmacol Sci 2005;99 (5):459-71.

9. Dai DZ. Two patterns of ion channelopathy in the myocardium: perspectives for development of anti-arrhythmic agents. Curr Opin Investig Drugs 2005;6 (3):289-97.

10. Guth BD. Preclinical cardiovascular risk assessment in modern drug development. Toxicol Sci 2007;97 (1):4-20.

11. Urakami Y, Kimura N, Okuda M, Inui K. Creatinine transport by basolateral organic cation transporter hOCT2 in the human kidney. Pharm Res 2004;21 (6):976-81.

12. Sato T, Masuda S, Yonezawa A, Tanihara Y, Katsura T, Inui K. Transcellular transport of organic cations in double-transfected MDCK cells expressing human organic cation transporters hOCT1/hMATE1 and hOCT2/hMATE1. Biochem Pharmacol 2008;76 (7):894-903.

13. Tanihara Y, Masuda S, Sato T, Katsura T, Ogawa O, Inui K. Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters. Biochem Pharmacol 2007;74 (2):359-71.

14. van Acker BA, Koomen GC, Koopman MG, de Waart DR, Arisz L. Creatinine clearance during cimetidine administration for measurement of glomerular filtration rate. Lancet 1992;340 (8831):1326-9.

15. Naderer O, Nafziger AN, Bertino JS, Jr. Effects of moderate-dose versus high-dose trimethoprim on serum creatinine and creatinine clearance and adverse reactions. Antimicrob Agents Chemother 1997;41 (11):2466-70.

16. Opravil M, Keusch G, Luthy R. Pyrimethamine inhibits renal secretion of creatinine. Antimicrob Agents Chemother 1993;37 (5):1056-60.

17. Pollak PT, Sharma AD, Carruthers SG. Creatinine elevation in patients receiving amiodarone correlates with serum amiodarone concentration. Br J Clin Pharmacol 1993;36 (2):125-7.

18. RANEXA® (ranolazine) extended-release tablets. US Prescribing Information. Gilead Sciences, Inc. , Foster City, CA. Revised July 2011.

19. Tschuppert Y, Buclin T, Rothuizen LE, Decosterd LA, Galleyrand J, Gaud C, et al. Effect of dronedarone on renal function in healthy subjects. Br J Clin Pharmacol 2007;64 (6):785-91.

20. Edurant (rilpivirine) Tablets. US Prescribing Information. Tibotec Pharmaceuticals, Raritan, NJ, USA. May 2011.

21. Min S, Carrod A, Curtis L, Stainsby C, Brothers C, Yeo J, et al. Safety profile of dolutegravir (DTG, S/GSK1349572), in combination with other antiretrovirals in antiretroviral (ART)-naïve and ART-experienced adults from two phase IIb studies [Poster Exhibition TUPE238]. 6th IAS Conference on HIV Pathogenesis, Treatment and Prevention; 2011 July 17-20; Rome, Italy.

22. Ginsberg AM, Laurenzi MW, Rouse DJ, Whitney KD, Spigelman MK. Assessment of the effects of the nitroimidazo-oxazine PA-824 on renal function in healthy subjects. Antimicrob Agents Chemother 2009;53 (9):3726-33.

23. Imamura Y, Murayama N, Okudaira N, Kurihara A, Okazaki O, Izumi T, et al. Prediction of fluoroquinolone-induced elevation in serum creatinine levels: a case of drug-endogenous substance interaction involving the inhibition of renal secretion. Clin Pharmacol Ther 2011;89 (1):81-8.

24. Schutzer KM, Svensson MK, Zetterstrand S, Eriksson UG, Wahlander K. Reversible elevations of serum creatinine levels but no effect on glomerular filtration during treatment with the direct thrombin inhibitor AZD0837. Eur J Clin Pharmacol 2010;66 (9):903-10.

25. Burns-Naas LA, Zorbas M, Jessen B, Evering W, Stevens G, Ivett JL, et al. Increase in thyroid follicular cell tumors in nelfinavir-treated rats observed in a 2-year carcinogenicity study is consistent with a rat-specific mechanism of thyroid neoplasia. Hum Exp Toxicol 2005;24 (12):643-54.

26. Capen CC. Overview of structural and functional lesions in endocrine organs of animals. Toxicol Pathol 2001;29 (1):8-33.

27. Wu KM, Farrelly JG. Preclinical development of new drugs that enhance thyroid hormone metabolism and clearance: inadequacy of using rats as an animal model for predicting human risks in an IND and NDA. Am J Ther 2006;13 (2):141-4.

28. McClain RM. Mechanistic considerations for the relevance of animal data on thyroid neoplasia to human risk assessment. Mutat Res 1995;333 (1-2):131-42.

29. Cohen SM, Klaunig J, Meek ME, Hill RN, Pastoor T, Lehman-McKeeman L, et al. Evaluating the human relevance of chemically induced animal tumors. Toxicol Sci 2004;78 (2):181-6.

30. Meek ME, Bucher JR, Cohen SM, Dellarco V, Hill RN, Lehman-McKeeman LD, et al. A framework for human relevance analysis of information on carcinogenic modes of action. Crit Rev Toxicol 2003;33 (6):591-653.

31. Ennulat D, Walker D, Clemo F, Magid-Slav M, Ledieu D, Graham M, et al. Effects of hepatic drug-metabolizing enzyme induction on clinical pathology parameters in animals and man. Toxicol Pathol 2010;38 (5):810-28.

32. Boone L, Meyer D, Cusick P, Ennulat D, Bolliger AP, Everds N, et al. Selection and interpretation of clinical pathology indicators of hepatic injury in preclinical studies. Veterinary clinical pathology / American Society for Veterinary Clinical Pathology 2005;34 (3):182-8.

33. U.S. Department of Health and Human Services Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Center for Biologics Evaluation and Research (CBER). Guidance for Industry. S8 Immunotoxicity Studies for Human Pharmaceuticals. April, 2006.

34. Haley P, Perry R, Ennulat D, Frame S, Johnson C, Lapointe JM, et al. STP position paper: best practice guideline for the routine pathology evaluation of the immune system. Toxicol Pathol 2005;33 (3):404-7; discussion 8.

35. Germolec DR, Nyska A, Kashon M, Kuper CF, Portier C, Kommineni C, et al. Extended histopathology in immunotoxicity testing: interlaboratory validation studies. Toxicol Sci 2004;78 (1):107-15.

36. Kuper CF, de Heer E, Van Loveren H, Vos JG. Immune System. In: Haschek, WM, Rousseaux, CG, Wallig, MA, editors. Handbook of Toxicologic Pathology, Second Edition. Academic Press 2002. p. 585-646.

37. Staudinger J, Liu Y, Madan A, Habeebu S, Klaassen CD. Coordinate regulation of xenobiotic and bile acid homeostasis by pregnane X receptor. Drug Metab Dispos 2001;29 (11):1467-72.

38. Atazanavir (Reyataz®) European Public Assessment Report (EPAR): Scientific Discussion. European Medicines Agency (EMEA) Web site. http://www.emea.europa.eu/humandocs/PDFs/EPAR/reyataz/586503en6.pdf. Accessed March 26, 2009.

39. REYATAZ 100 mg hard capsules: Summary of Product Characteristics. Bristol-Myers Squibb Pharma EEIG. Uxbridge, UK. Revised November 2011.

40. Center for Drug Evaluation and Research. Application Number: 21-976 - Darunavir: clinical pharmacology/toxicology review. December 23, 2005.

# 7. APPENDIX

**Appendix 1: Justification for Absence of Documents in Module 4 for Darunavir, Cobicistat and the Fixed Dose Combination Darunavir/Cobicistat.**

| **Darunavir** | | |
|---|---|---|
| **Module** | | **Reason** |
| 4.2.1.4 | Pharmacodynamic Drug Interactions | Studies on pharmacodynamic drug interactions are included in Module 2.7.2. Summary of Virology. |
| 4.2.2.1 | Analytical Methods and Validation Reports | No new studies on the FDC have been conducted and during the Presubmission meetings with the (Co-)Rapporteurs it was agreed that a short summary in Module 2.6.4 Pharmacokinetics Written Summary was considered sufficient |
| 4.2.2.5 | Excretion | No formal excretion studies were conducted. Excretion analyses were part of metabolism (Module 4.2.2.4) studies |
| 4.2.2.6 | Pharmacokinetic Drug Interaction (nonclinical) | Studies were performed and were reported in Module 2.6.4/Section 7. |
| 4.2.2.7 | Other Pharmacokinetic Studies | Studies were performed and were reported in Module 2.6.4/Section 8. |
| 4.2.3.4.2 | Short- or Medium Term Studies | No short- or medium term carcinogenicity studies have been performed since 6 month rat and 3 month mouse studies were performed and reported in Module 2.6.6, sections 3.1.1 and 5.1.1.1, respectively. |
| 4.2.3.7.1 | Antigenicity | In accordance with the ICH guideline, data from the general toxicology studies are considered sufficient to evaluate antigenic potential of DRV. Based on these data, it was concluded that there are no indications that DRV has an antigenic potential and therefore no additional studies were performed. |
| 4.2.3.7.4 | Dependence | No studies on dependence were conducted as DRV is intended to be used as an anti-HIV. |
| 4.2.3.7.5 | Metabolites | Metabolite studies were not conducted as the unchanged DRV was more abundant than any metabolite. |

Appendix 1: Justification for Absence of Documents in Module 4 for Darunavir, Cobicistat and the Fixed Dose Combination Darunavir/Cobicistat.

| **Cobicistat** | | |
|---|---|---|
| **Module** | | **Reason** |
| 4.2.1.1 | Primary Pharmacodynamics | No specific primary pharmacodynamics study has been performed. A study describing inhibition of human CYP3A activity by COBI is submitted under 4.2.2.4 (AD-216-2028). |
| 4.2.1.4 | Pharmacodynamic Drug Interactions | Studies on pharmacodynamic drug interactions are included in Module 2.7.2. Summary of Virology. |
| 4.2.2.1 | Analytical Methods and Validation Reports | No new studies on the FDC have been conducted and during the Presubmission meetings with the (Co-)Rapporteurs it was agreed that a short summary in Module 2.6.4 Pharmacokinetics Written Summary was considered sufficient |
| 4.2.2.6 | Pharmacokinetic Drug Interaction (nonclinical) | Studies were performed and were reported in Module 2.6.4/Section 8. |
| 4.2.2.7 | Other Pharmacokinetic Studies | Studies were performed and were reported in Module 2.6.4/Section 8. |
| 4.2.3.4.2 | Short- or Medium Term Studies | No short- or medium term carcinogenicity studies, or other carcinogenicity studies have been performed. A 13-week study in the mouse and a 26-week study (with 13 weeks recovery) in the rat were performed and reported in Module 2.6.6, Section 5.2.1.1 and 3.2.1.1, respectively. |
| 4.2.3.5.4 | Studies in which the Offspring are Dosed | The potential effects of COBI in juvenile animals were evaluated in the pre- and postnatal development toxicity study in rats, described in Module 2.6.6/Section 6.2.3. |
| 4.2.3.7.1 | Antigenicity | In accordance with the ICH guideline, data from the general toxicology studies are considered sufficient to evaluate antigenic potential of COBI. Based on these data, it was concluded that there are no indications that COBI has an antigenic potential and therefore no additional studies were performed. |
| 4.2.3.7.3 | Mechanistic Studies | No specific mechanistic studies were conducted for COBI. |
| 4.2.3.7.4 | Dependence | Drugs that inhibit cytochrome P450, or structurally-related HIV protease inhibitors, have no known properties that would suggest development of dependence. There was no evidence of development of dependence in nonclinical studies with COBI and tissue distribution studies in rats indicated minimal transport across the blood:brain barrier (Module2.6.4/Section 4.1.2). Consequently, dependency studies are not considered warranted. |
| 4.2.3.7.5 | Metabolites | As there are no major human metabolites of COBI, and the most abundant metabolites were similar across species, no separate safety studies on COBI metabolites have been conducted. |

| **Fixed Dose Combination Darunavir /Cobicistat** |
|---|
| 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. Moreover, the mechanism of action and pharmacokinetic profile of COBI are different in humans and animals, and therefore the use of nonclinical studies to assess effects of the interaction of COBI with other compounds may not adequately predict effects in humans. In addition, the absence of nonclinical safety studies with the combination is in accordance with the CHMP Guideline on the Non-Clinical Development of Fixed Combinations of Medicinal Products (EMEA/CHMP/SWP/258498/[redacted], [redacted] [redacted]). |

**Appendix 2: List of Nonclinical Studies Conducted With Darunavir and Cobicistat**

| Module 4: Nonclinical Study Reports | | | | |
|---|---|---|---|---|
| **Submission Component and location within FDC MAA** | **Compound** | **Key / Supportive** | **Single agent MAA (if not re-submitted in FDC MAA)** | **Location in single agent MAA (if not re-submitted in FDC MAA)** |
| **4.2 Study Reports** | | | | |
| **4.2.1 Pharmacology** | | | | |
| **4.2.1.1 Primary Pharmacodynamics** | | | | |
| **TMC114-20050003-VRR -** Inhibition of the activity of human proteases by TMC114: Part 1 | darunavir | Key | | |
| **TMC114-20050004-VRR -** Inhibition of the activity of human proteases by TMC114: Part 2 | darunavir | Key | | |
| **TMC114-20050005-VRR -** Drug susceptibility profile of TMC114 against a large panel of HIV-1 quasispecies observed in isolates from clinical samples | darunavir | Key | | |
| **TMC114-20050006-VRR -** Antiviral activity of TMC114 in combination with currently approved HIV-1 inhibitors | darunavir | Key | | |
| **TMC114-20050007-VRR -** Characterization of the interaction between TMC114 and wildtype HIV-1 protease. Determination of Association and Dissociation Constants**.** | darunavir | Key | | |
| **TMC114-20050009-VRR -** Influence of the time of addition of TMC114 and determination of TMC114 HIV-1 protease inhibitory constant | darunavir | Key | | |
| **TMC114-20050010-VRR -** Antiviral activity of TMC114 in the presence of human serum proteins | darunavir | Key | | |
| **TMC114-20050011-VRR -** Antiviral activity of TMC114 against HIV-1 primary isolates from group M and O in human PBMCs | darunavir | Key | | |
| **TMC114-20050012-Virology Report -** In vitro selection of resistant HIV-1 starting from wildtype HIV-1 isolates, in the presence of TMC114 or other protease inhibitors | darunavir | Key | | |
| **TMC114-20050013-VRR -** In vitro selection of resistant HIV-1 starting from HIV-1 isolates containing mutations associated with resistance to protease inhibitors in the presence of TMC114 | darunavir | Key | | |
| **TMC114-20050014-VRR -** Antiviral activity of TMC114 against wild type HIV-1, and HIV-2, and SIV in T cell lines, peripheral blood mononuclear cells and monocyte/macrophages | darunavir | Key | | |
| **TMC114-20050016-VRR -** In vitro cytotoxicity of TMC114 in different cell lines | darunavir | Key | | |
| **TMC114-20050017-VRR -** Antiviral Activity of TMC114 Against a Screening Panel of Recombinant Clinical Isolates With Various Degrees of Resistance to Protease Inhibitors | darunavir | Key | | |
| **TMC125-20060004-VRR-Virology Report -** Antiviral Activity of TMC125 in Combination With Currently Approved HIV-1 Inhibitors | darunavir | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-20050002-VRR -** Antiviral activity of TMC114 against a panel of protease inhibitors and multidrug resistant HIV-1 primary isolates in human peripheral blood mononuclear cells | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M5.3.5.4 |
| **TMC114-20050008-VRR -** Antiviral activity of TMC114 and other registered HIV-1 protease inhibitors at different multiplicities of infection | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M5.3.5.4 |
| **TMC114-20050019-VRR -** Antiviral Activity of TMC114 on HIV-1/HXB2 harboring site directed mutations in the protease gene | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M5.3.5.4 |
| **4.2.1.2 Secondary Pharmacodynamics** | | | | |
| **TMC114-NC118-NCP -** Pharmascreen®: Evaluation of the activity of TMC114 in tissue, animal and anti-infective in vitro assays. | darunavir | Key | | |
| **PC-216-2001 -** Activity of cobi against HIV-1 and host proteases | cobicistat | Key | | |
| **PC-216-2002 -** Anti-HIV Activity of GS-9350 in MT-2 cells | cobicistat | Key | | |
| **PC-216-2003 -** Cytotoxicity profile of GS-9350 | cobicistat | Key | | |
| **PC-216-2005 -** Antiviral Activity of HIV Inhibitors in Combination with GS-9350 | cobicistat | Key | | |
| **PC-216-2011 -** Testing of Gilead Compound GS-9350 Against Seventeen HIV-1 Isolates and Two HIV-2 Isolates in Fresh Human PBMCs | cobicistat | Key | | |
| **PC-216-2004 -** In vitro effects of GS-9350 on adipocytes | cobicistat | Supportive | TYBOST MAA | M4.2.1.2 |
| **TX-168-2007 -** LeadProfilingScreen Data Report for Gilead Sciences, Inc.; GS-340649 | cobicistat | Supportive | TYBOST MAA | M4.2.1.2 |
| **TX-168-2011 -** Individual Tests Data Report for Gilead Sciences, Inc.; GS-340649 | cobicistat | Supportive | TYBOST MAA | M4.2.1.2 |
| **PC-137-2004 -** LeadProfilingScreen Data Report for Gilead Sciences, Inc.; GS-017415 | cobicistat | Supportive | TYBOST MAA | M4.2.1.2 |
| **PC-216-2006 -** Activity of GS-9350 against other Human Viruses | cobicistat | Supportive | TYBOST MAA | M4.2.1.3 and M5.3.5.4 |
| **PC-168-2005-** Individual Tests Data Report for Gilead Sciences, Inc.; GS-017415 | cobicistat | Supportive | TYBOST MAA | M4.2.1.2 |
| **4.2.1.3 Safety Pharmacology** | | | | |
| **TMC114-NC103-NCP -** Effect of TMC114 on hERG currents recorded from stably transfected HEK293 cells. | darunavir | Key | | |
| **TMC114-NC105-NCP -** Effect of TMC114 on action potential parameters in sheep isolated cardiac purkinje fibers. | darunavir | Key | | |
| **TMC114-NC108-NCP -** Cardiovascular effects of TMC114 in conscious, telemetered Beagle dogs**.** | darunavir | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC116-NCP -** Safety pharmacology study: neurobehavioral observations and automated motor activity assessment after single dose oral administration of TMC114 in the rat**.** | darunavir | Key | | |
| **TMC114-NC117-NCP -** Safety pharmacology studies: respiratory assessment after single dose oral administration of TMC114 in the rat. | darunavir | Key | | |
| **TMC114-NC120-NCP -** General pharmacology studies: gastro-intestinal transit time study after oral administration of TMC114 in albino rats**.** | darunavir | Key | | |
| **PC-216-2007 -** Effects of COBI on Isolated Hearts (non-GLP) | cobicistat | Key | | |
| **PC-216-2009 -** An Examination of the Cardiovascular Effects of COBI, ATV and COBI + ATV on the Isolated Heart of the Female Rabbit (Langendorff Method) (non-GLP) | cobicistat | Key | | |
| **TX-216-2006 -** A Pharmacological Assessment of the Effect of COBI on the Central Nervous System of the Albino Rat (GLP) | cobicistat | Key | | |
| **TX-216-2007 -** A Pharmacological Assessment of the Effect of COBI on the Respiratory System of the Albino Rat (GLP) | cobicistat | Key | | |
| **TX-216-2008 -** A Pharmacological Assessment of the Effect of COBI on the Cardiovascular System of the Beagle Dog Using Telemetry (GLP) | cobicistat | Key | | |
| **TX-216-2009 -** Evaluation of Effects of COBI on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells (GLP) | cobicistat | Key | | |
| **TX-216-2015 -** Effects of GS-9350 and GS-017415 on Cardiac Ion Channels Expressed in Human Embryonic Kidney Cells | cobicistat | Key | | |
| **TX-168-2012 -** Effect of GS-340649 and GS-017415 on Action Potentials in Isolated Rabbit Cardiac Purkinje Fibers (non-GLP) | cobicistat | Key | | |
| **4.2.1.4 Pharmacodynamic Drug Interaction** | | | | |
| Studies on pharmacodynamic drug interactions are included in Module 2.7.2. Summary of Virology. | | | | |
| **4.2.2 Pharmacokinetics** | | | | |
| **4.2.2.1 Analytical Methods and Validation Reports** | No new studies on the FDC have been conducted and during the Presubmission meetings with the (Co-)Rapporteurs it was agreed that a short summary in Module 2.6.4 Pharmacokinetics Written Summary was considered sufficient | | | |
| **4.2.2.2 Absorption** | | | | |
| **TMC114-NC106-TSR -** Subacute 14-Day oral toxicity study with TMC114 by daily gavage in the dog | darunavir | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC106-NCTK -** Subacute 14-Day oral toxicity study with TMC114 by daily gavage in the dog | darunavir | Key | | |
| **TMC114-NC137-NCPK** - Determination of the in vitro transport characteristics of TMC114, evaluation of the possible interaction of TMC114 as substrate and/or inhibitor with human Pglycoprotein, and assessment of the effect of the anti-HIV drug ritonavir on TMC114 transport: an in vitro study in Caco-2 monolayers | darunavir | Key | | |
| **TMC114-NC144-TSR -** Subacute 14-Day Oral Toxicity Study with TMC114 and Ritonavir by Daily Gavage or Capsules in the Dog | darunavir | Key | | |
| **TMC114-NC144-NCTK -** Subacute 14-Day Oral Toxicity Study with TMC114 and Ritonavir by Daily Gavage or Capsules in the Dog | darunavir | Key | | |
| **TMC114-NC144-NCPCD -** Subacute 14-Day Oral Toxicity Study with TMC114 and Ritonavir by Daily Gavage or Capsules in the Dog | darunavir | Key | | |
| **TMC114-NC147-NCBA -** Bioavailability of TMC114 after coadministration of ritonavir in the dog | darunavir | Key | | |
| **TMC114-NC147-NCPK -** Bioavailability of TMC114 after coadministration of ritonavir in the dog | darunavir | Key | | |
| **TMC114-NC147-NCPCD -** Bioavailability of TMC114 after coadministration of ritonavir in the dog | darunavir | Key | | |
| **TMC114-NC223-NCPK -** Drug-drug interaction effect (boosting effect) of ritonavir at 50 mg/kg on the pharmacokinetics and relative bioavailability of TMC114 in male and female SPF Albino Swiss mice after single and 14-day repeated oral administration of TMC114 at 1000 mg eq./kg | darunavir | Key | | |
| **AD-216-2020 -** Pharmacokinetics of GS-9350 in Rats | cobicistat | Key | | |
| **AD-216-2021 -** Pharmacokinetics of GS-9350 in Beagle Dogs | cobicistat | Key | | |
| **AD-216-2023 -** Permeability of GS-9350 across Caco-2 cell monolayers | cobicistat | Key | | |
| **PC-216-2013 -** Single dose PK study in wild-type transgenic mice with GS-9350 | cobicistat | Key | | |
| **PC-216-2013-PK -** Determination of the Pharmacokinetics of GS-9350 Following a Single Oral Gavage Dose to Male and Female 001178-W (wild-type) Mice | cobicistat | Key | | |
| **PEPI-NC119-NCPK -** Evaluation of the possible effects of ritonavir on bi-directional transport of TMC114, taxol and saquinavir across Caco-2 monolayers. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC121-NCBA -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC121-NCPK -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC121-NCPCD -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC122-NCBA -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC122-NCPK -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC122-NCPCD -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC135-NCBA -** Comparative bioavailability study with TMC114 in the dog, comparison between different formulations and nutritional status | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC135-NCPK -** Comparative bioavailability study with TMC114 in the dog, comparison between different formulations and nutritional status | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC135-NCPCD -** Comparative bioavailability study with TMC114 in the dog, comparison between different formulations and nutritional status | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC138-NCBA -** Bioavailability of TMC114 after coadministration of ritonavir in the dog**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC138-NCPK -** Bioavailability of TMC114 after coadministration of ritonavir in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC138-NCPCD -** Bioavailability of TMC114 after coadministration of ritonavir in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC139-NCBA -** Bioavailability of TMC114 after coadministration of ritonavir in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC139-NCPK -** Bioavailability of TMC114 after coadministration of ritonavir in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC139-NCPCD -** Bioavailability of TMC114 after coadministration of ritonavir in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC142-NCBA -** Comparative bioavailability study with TMC114 in the dog, comparison between different formulations and nutritional status | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC142-NCPK -** Comparative bioavailability study with TMC114 in the dog, comparison between different formulations and nutritional status | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC142-NCPCD** - Comparative bioavailability study with TMC114 in the dog, comparison between different formulations and nutritional status | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC149-NCBA -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC149-NCPK -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC149-NCPCD -** Comparative bioavailability study with TMC114 in the dog | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC200-NCPK -** Pilot study on the bioavailability and plasma pharmacokinetics following oral administration by dietary admixture for 7 days to male mice. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC201-NCPK -** Pilot study on the bioavailability and plasma pharmacokinetics following oral administration by dietary admixture for 7 days to male rats**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **TMC114-NC211-NCPK -** A pilot study on the pharmacokinetics and relative bioavailability of TMC114 in male Beagle dogs after single oral administration of different concepts of oral formulations of TMC114 at 200 mg eq. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.2 |
| **AD-216-2022 -** Pharmacokinetics of GS-9350 in cynomolgus monkeys | cobicistat | Supportive | TYBOST MAA | M4.2.2.2 |
| **4.2.2.3 Distribution** | | | | |
| **TMC114-NC192-NCPK** - Tissue distribution of 14C-TMC114, as studied by whole-body autoradiography, in the pigmented male rat after single oral administration of 14C-TMC114 at 40 mg/kg, either alone or in combination with ritonavir at 25 mg/kg/day for 3 days. | darunavir | Key | | |
| **TMC114-NC205-NCPK -** Tissue distribution and placental transfer of 14C-TMC114, as studied by whole-body autoradiography, in the Sprague-Dawley rat after single oral administration of 14C-TMC114 at 40 mg/kg, either alone or in combination with ritonavir at 50 mg/kg/day for 3 days. | darunavir | Key | | |
| **TMC114-NC215-NCPK** - The plasma protein binding and blood distribution of TMC114 in animals and man. | darunavir | Key | | |
| **AD-216-2026 - Plasma protein binding of GS-9350** | cobicistat | Key | | |
| **AD-216-2034 -** PK, distribution, metabolism, excretion 14C-GS-9350 in rats | cobicistat | Key | | |
| **AD-216-2060 -** Whole-Body Autoradiography (WBA) of Rats Following Oral Administration of 14C-GS-9350 | cobicistat | Key | | |
| **AD-216-2076 -** Plasma Protein Binding of GS-9350 in CD-1 Mice | cobicistat | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **60N-1103A** - Determination of EX vivo Protein Binding of 不純物CE* and GS-9350 in Human Plasma Samples from Subjects with Normal and Impaired Hepatic Function in Support of GS-US-183-0133 | cobicistat | Key | | |
| **60N-1103B** - Determination of Ex vivo Protein Binding of 不純物CE* and GS-9350 in Human Plasma Samples from Subjects with Normal and Impaired Renal Function in Support of GS-US-216-0124 | cobicistat | Key | | |
| **TMC114-NC113-NCDME**- The in vitro binding of TMC114 to plasma proteins of rat, dog and human. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.3 |
| **TMC114-NC119-NCADME** - Absorption, distribution, metabolism and excretion of repeated oral doses of TMC114 in the Wistar rat**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.3 |
| **TMC114-NC119-NCPK** - Absorption, distribution, metabolism and excretion of repeated oral doses of TMC114 in the Wistar rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.3 |
| **TMC114-NC119-NCPCD** - Absorption, distribution, metabolism and excretion of repeated oral doses of TMC114 in the Wistar rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.3 |
| **4.2.2.4 Metabolism** | | | | |
| **TMC114-NC123-NCDME** - Inhibition of human cytochrome P450 enzymes by TMC114 in vitro**.** | darunavir | Key | | |
| **TMC114-NC152-NCPK** - The metabolism and excretion of 14C-TMC114 in the male and female Sprague-Dawley rat after single oral administration of 14C-TMC114 at 40 mg (active moiety-eq.)/kg, either alone or in combination with ritonavir at 25 mg/kg/day for 3 days**.** | darunavir | Key | | |
| **TMC114-NC153-NCPK** - The absorption, metabolism and excretion of TMC114 in the male Beagle dog after a single oral dose of 14C-TMC114 at 30 mg (active moiety-eq.)/kg. | darunavir | Key | | |
| **TMC114-NC154-NCPK** - The in-vitro metabolism of 14C-TMC114 in hepatocytes and liver subcellular fractions of male and female Swiss albino mice, male and female black Agouti rasH2 microinjected mice, male and female rats, female rabbit, male dog and man. | darunavir | Key | | |
| **TMC114-NC164-NCPK** - Biliary excretion and identification of biliary metabolites of 14C-TMC114 in the male Sprague-Dawley rat after a single oral dose of 14C-TMC114 at 40 mg (active moiety-eq.)/kg. | darunavir | Key | | |
| **TMC114-NC171-NCPK** - In vitro study on the potential of TMC114 to induce CYP mRNA in cryopreserved human hepatocytes | darunavir | Key | | |

*新薬承認情報提供時に置き換え

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC202-NCPK -** An in vitro study to (a) determine the kinetics of TMC114 metabolism in human liver microsomes; (b) identify the microsomal cytochrome P-450 iso-enzymes mediating TMC114 metabolism (reaction phenotyping) and (c) determine the inhibitory potency of ATV on the metabolism of TMC114. | darunavir | Key | | |
| **TMC114-NC213-NCPK -** The absorption, metabolism and excretion of TMC114 after a single oral dose of 400 mg base-eq. in healthy male subjects with and without ritonavir pre-treatment (clinical trial TMC114-C109). | darunavir | Key | | |
| **TMC114-NC247-NCPK -** An in vitro study to assess the potential of TMC114 to induce CYP enzyme activities in cryopreserved human hepatocytes | darunavir | Key | | |
| **TMC114-NC392-NCPK -** An in-vitro study on the inhibition of CYP2C8 mediated paclitaxel 6-a-hydroxylation by TMC114, and on the inhibition of UGT1A1 mediated bilirubin glucuronidation by TMC114 and ritonavir. | darunavir | Key | | |
| **AD-216-2024 -** In vitro metabolism of GS-9350 in hepatocytes and hepatic subcellular fractions from rat, dog, monkey and human**.** | cobicistat | Key | | |
| **AD-216-2025 -** Cytochrome P450 phenotyping for GS-9350 | cobicistat | Key | | |
| **AD-216-2027 -** Induction of metabolizing enzymes by GS-9350 in vitro | cobicistat | Key | | |
| **AD-216-2028 -** Inhibition of human CYP3A activity by GS-9350 in vitro | cobicistat | Key | | |
| **AD-216-2029 -** In vitro assessment of human liver cytochrome P450 inhibition potential of GS-9350 | cobicistat | Key | | |
| **AD-216-2038 -** Identification of Major Metabolites of GS-9350 In Vitro | cobicistat | Key | | |
| **AD-216-2039 -** Induction of metabolizing enzymes of rat by GS-9350 in vitro | cobicistat | Key | | |
| **AD-216-2040 -** Inhibition of CYP3A activity in rat, dog and monkey by GS-9350 in vitro | cobicistat | Key | | |
| **AD-216-2041 -** Drug interaction properties of putative human metabolites of GS-9350 | cobicistat | Key | | |
| **AD-216-2070 -** In Vitro Assessment of Human Liver CYP2B6 and CYP2C8 Inhibition Potential of GS-9350 | cobicistat | Key | | |
| **AD-216-2071** - In Vitro Assessment of the Induction Potential of GS-9350 in Primary Cultures of Human Hepatocytes | cobicistat | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **AD-216-2075** - In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-9350 | cobicistat | Key | | |
| **AD-216-2082** - Radioprofiling and Metabolite Identification Following Oral Administration of [14C]GS-9350 to rats | cobicistat | Key | | |
| **AD-216-2101** - Radioprofiling and Metabolite Identification Following Oral Administration of [14C]GS-9350 to intact and bile duct cannulated dogs | cobicistat | Key | | |
| **AD-216-2106** - Metabolite Profiles of Cobicistat Generated by Human CYP2D6 and CYP3A4 | cobicistat | Key | | |
| **AD-216-2107** - In Vitro Assessment of the Potential for Cobicistat Metabolites to Inhibit CYP2B6, CYP2C8 or UGT1A1 | cobicistat | Key | | |
| **AD-216-2108** - The Potential for Cytochromes P450 CYP2B6 and CYP2C8 to Metabolize Cobicistat | cobicistat | Key | | |
| **TMC114-NC191-NCPK** - The metabolic stability of 14C-labelled TMC114 in male Sprague-Dawley rats after a single oral administration at 40 mg (active moiety-eq.)/kg | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **TMC114-NC112-NCDME**- Characterization of cytochrome P450 enzymes involved in the in vitro metabolism of TMC114, and metabolite profiling in rat, dog and human liver microsomes. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **TMC114-NC226-NCPK** - A study of the effects of TMC114 on some hepatic enzyme activities after oral administration for three months at doses of 0, 150, 450 and 1000 mg/kg/day to male and female Swiss Albino CD-1 mice. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **TMC114-NC208-NCPK** - A study of the effects of TMC114 and ritonavir on some hepatic enzyme activities after oral administration for four weeks at doses of 0, 20, 100 and 500 mg/kg/day TMC114, 50 mg/kg/day ritonavir and 100 mg/kg/day TMC114 and 50 mg/kg/day ritonavir to male and female Sprague-Dawley rats. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **TMC114-NC388-NCPK**- A study of the effects of TMC114 on some hepatic enzyme activities after oral administration for one month at doses of 0,50, and 500 mg equivalents/kg/day to male and female Sprague-Dawley rats. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **TMC114-NC209-NCPK** - A study of the effects of TMC114 on some hepatic enzyme activities after Oral administration for twelve months at doses of 0, 30, 60 and 120 mg/kg/day to male and female Beagle dogs. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **TMC114-NC246-NCPK** - The in-vitro metabolism of 14C-TMC114 in liver subcellular fractions of juvenile and adult Sprague-Dawley rats. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **AD-216-2073** -Pharmacokinetics, Metabolism, and Excretion of [14C]GS-9350 Following Oral Administration to Mice | cobicistat | Supportive | TYBOST MAA | M4.2.2.4 and M4.4.2.5 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **AD-216-2074 -** Identification of Major Metabolites of GS-9350 in CD-1 Mouse Microsomes In Vitro | cobicistat | Supportive | TYBOST MAA | M4.2.2.4 |
| **4.2.2.5 Excretion** | | | | |
| **AD-216-2067 -** Mass Balance of Radioactivity after Oral Administration of [14C] GS-9350 to Naïve Male Beagle Dogs | cobicistat | Key | | |
| **AD-216-2068 -** Mass Balance of Radioactivity after Oral Administration of [14C] GS-9350 to Naïve Male Bile Duct-Cannulated Beagle Dogs | cobicistat | Key | | |
| **AD-216-2034 PK -** Distribution, Metabolism, Excretion of 14C-GS-9350 following oral administration in rats | cobicistat | Key | | |
| **TMC114-NC249-NCPK -** Preliminary milk transfer/profile study in the rat**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.5 |
| **4.2.2.6 Pharmacokinetic Drug Interactions** | | | | |
| **AD-216-2030 -** Interaction of GS-9350 with MRP1, MRP2 and Pgp | cobicistat | Key | | |
| **AD-216-2072 -** Inhibition of P-glycoprotein-Dependent Bi-Directional Transport of Digoxin Through Monolayers of Caco-2 Cells by GS-9350 | cobicistat | Key | | |
| **AD-216-2093 -** In vitro interaction study of GS-9350 with human OCT2 uptake transporter | cobicistat | Key | | |
| **AD-216-2094 -** In vitro interaction study of GS-9350 with human MATE1 and MATE2-K transporters | cobicistat | Key | | |
| **AD-216-2095 -** Potential for GS-9350 and ritonavir to be substrates for human OCT2 | cobicistat | Key | | |
| **AD-216-2098 -** Effects of GS-017415 and GS-340649 on uptake into OCTN1 expressing cells | cobicistat | Key | | |
| **AD-216-2099 -** Inhibition of BCRP by GS-9350 and ritonavir | cobicistat | Key | | |
| **AD-216-2100 -** Inhibition of OATP1B1 and OATP1B3 by GS-9350, ritonavir and lopinavir | cobicistat | Key | | |
| **AD-216-2103 -** Bidirectional Permeability of Cobicistat Through Monolayers of P-glycoprotein- and BCRP- Overexpressing Cells | cobicistat | Key | | |
| **AD-216-2104 -** Inhibition of BCRP-Dependent Bi-Directional Transport of Prazosin through Monolayers of Caco-2 Cells by Cobicistat | cobicistat | Key | | |
| **AD-216-2105 -** Inhibition of OAT1, OAT3 and MRP4 by GS-9350 and ritonavir | cobicistat | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **AD-216-2109 -** In Vitro Inhibition Studies of Darunavir with Human OCT2 and MATE1 Transporters | darunavir | Key | | |
| **AD-236-2008 -** In Vitro Inhibition Studies of Quad Components with Human OCT1 and BSEP Transporters | cobicistat | Key | | |
| **TMC114-NC134-NCDI -** Interaction of 6 anti-HIV compounds with the cytochrome P450-mediated metabolism of TMC114 in vitro**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.2.4 |
| **4.2.2.7 Other Pharmacokinetic Studies** | | | | |
| Studies were performed and were reported in Module 2.6.4/Section 8. | | | | |
| **4.2.3 Toxicology** | | | | |
| **4.2.3.1 Single-Dose Toxicity** | | | | |
| **TMC114-NC111-TSR -** Acute oral toxicity study with TMC114 in the mouse**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC111-NCTK -** Acute oral toxicity study with TMC114 in the mouse**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC101-TSR -**Acute oral toxicity study with TMC114 in the rat**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC101-NCTK -**Acute oral toxicity study with TMC114 in the rat**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC104-TSR -** Acute oral toxicity study with TMC114 in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC104-NCTK -** Acute oral toxicity study with TMC114 in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC110-TSR -** Acute intravenous toxicity study with TMC114 in the rat | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC110-NCTK -** Acute intravenous toxicity study with TMC114 in the rat | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC102-TSR -** TMC114: Range finding oral toxicity study in male and female Beagle dogs. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC102-NCTK -** TMC114: Range finding oral toxicity study in male and female Beagle dogs. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC109-TSR -** Single and repeated dose intravenous toxicity of TMC114 in male and female Beagle dogs. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TMC114-NC109-NCTK -** Single and repeated dose intravenous toxicity of TMC114 in male and female Beagle dogs. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.1 |
| **TX-216-2003 -** TX-216-2003 Single Dose Oral Gavage Toxicity Study with COBI in Rats (GLP**)** | cobicistat | Supportive | TYBOST MAA | M4.2.3.1 |
| **4.2.3.2 Repeat-Dose Toxicity** | | | | |
| **TMC114-NC132-TSR -** Six month oral (gavage) repeat dose toxicity study in the rat. | darunavir | Key | | |
| **TMC114-NC132-NCPK -** Six month oral (gavage) repeat dose toxicity study in the rat. | darunavir | Key | | |
| **TMC114-NC132-NCTK -** Six month oral (gavage) repeat dose toxicity study in the rat**.** | darunavir | Key | | |
| **TMC114-NC132-NCPCD -** Six month oral (gavage) repeat dose toxicity study in the rat**.** | darunavir | Key | | |
| **TMC114-NC146-TSR -** 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Key | | |
| **TMC114-NC146-NCTK -** 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Key | | |
| **TMC114-NC146-NCPCD -** 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Key | | |
| **TMC114-NC145-TSR -** Twelve-month oral (gavage) toxicology study in the dog including toxicokinetic sampling. | darunavir | Key | | |
| **TMC114-NC145-NCTK -** Twelve-month oral (gavage) toxicology study in the dog including toxicokinetic sampling. | darunavir | Key | | |
| **TMC114-NC145-NCPCD -** Twelve-month oral (gavage) toxicology study in the dog including toxicokinetic sampling. | darunavir | Key | | |
| **TX-216-2017 -** 26-Week Oral Gavage Toxicity and Toxicokinetic Study with COBI in Rats with a 13-Week Recovery Period | cobicistat | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TX-216-2024 -** 90-Day Oral Gavage Bridging Study with GS-9350 and Atazanavir in Rats with a 1-Month Recovery Period | cobicistat | Key | | |
| **TX-216-2016 -** 39-Week Oral Gavage Toxicity and Toxicokinetic Study with COBI in Dogs with 13-Week Interim Necropsy and a 13-Week Recovery Period | cobicistat | Key | | |
| **TMC114-NC107-TSR -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC107-NCTK -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC130-TSR -** Three month oral (gavage) repeat dose toxicity study in the rat | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC130-NCTK -** Three month oral (gavage) repeat dose toxicity study in the rat | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC160-TSR -** 2-Week repeated dose intravenous toxicity study in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC141-TSR -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC141-NCTK -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC141-NCPCD -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC143-TSR -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC143-NCTK -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC143-NCPCD -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC106-TSR -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC106-NCTK -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC173-TSR** - 14 Day oral (gavage) bioavailability and tolerance study in the Beagle dog**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC173-NCTK -** 14 Day oral (gavage) bioavailability and tolerance study in the Beagle dog**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC173-NCPCD -** 14 Day oral (gavage) bioavailability and tolerance study in the Beagle dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC131-TSR -** Three month oral (gavage) repeat dose toxicity study in the Beagle dog**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC131-NCTK -** Three month oral (gavage) repeat dose toxicity study in the Beagle dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC133-TSR -** Six month oral (gavage) repeat dose toxicity study in the Beagle dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC133-NCTK -** Six month oral (gavage) repeat dose toxicity study in the Beagle dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC133-NCPCD -** Six month oral (gavage) repeat dose toxicity study in the Beagle dog**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC199-TSR -** 2-Week repeated dose intravenous toxicity study in the Beagle dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC140-TSR -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC140-NCPK -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC140-NCTK -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TMC114-NC140-NCPCD -** Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in the dog. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.2 |
| **TX-216-2001 -** 14 Day Oral Gavage Toxicity and Toxicokinetics Study with GS-9350 and GS-017415 in Rats | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2001-TK -** 14-day Oral Gavage Toxicity and Toxicokinetics Range-Finding Study with GS-9350 in Male Rats: Toxicokinetic Analysis of GS-9350 in Plasma | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TX-216-2004 -** 4-Week Oral Gavage Toxicity and Toxicokinetic Study with COBI in Rats with a 4-Week Recovery | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2002 -** 7-Day Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 and GS-017415 in Dogs | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2002-TK -** A 7-day Toxicity and Toxicokinetic Study of GS-9350 in Dogs: Toxicokinetic Analysis of GS-9350 in Plasma | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2005 -** 4-Week Oral Gavage Toxicity and Toxicokinetic Study with COBI in Dogs with a 4-Week Recovery Phase | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2027 -** 5-day oral toxicity and toxicokinetic study of COBI and ATV in female Sprague-Dawley rats | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **4.2.3.3. Genotoxcity** | | | | |
| **4.2.3.3.1 In vitro** | | | | |
| **TMC114-[redacted]293063-TSR -** Evaluation of the mutagenic activity of TMC114 in the Salmonella typhymurium reverse mutation assay and the Escherichia coli reverse mutation assay (with independent repeat). | darunavir | Key | | |
| **TMC114-[redacted]294288-TSR -** Evaluation of the ability of TMC114 to induce chromosome aberrations in cultured peripheral human lymphocytes**.** | darunavir | Key | | |
| **TX-216-2010 -** GS-9350 Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay (GLP) | cobicistat | Key | | |
| **TX-216-2011 -** GS-9350 L5178Y Mouse Lymphoma Forward Mutation Assay (GLP) | cobicistat | Key | | |
| **4.2.3.3.2 In vivo** | | | | |
| **TMC114-NC114-TSR -** Micronucleus test in bone marrow cells of the mouse with TMC114**.** | darunavir | Key | | |
| **TX-216-2012 -** GS-9350 Rat Micronucleus Test (GLP) | cobicistat | Key | | |
| **4.2.3.4 Carcinogenicity** | | | | |
| **4.2.3.4.1 Long-term studies** | | | | |
| **TMC114-TiDP3-NC158-TSR -** 24-month repeated dose oral carcinogenicity study of TMC114 in the rat. | darunavir | Key | | |
| **TMC114-TiDP3-NC159-TSR -** 24-month repeated dose oral carcinogenicity study of TMC114 in the mouse. | darunavir | Key | | |
| **TX-216-2031 -** 104 week Oral Gavage Study Carcinogenicity Study with GS-9350 in Rats | cobicistat | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TX-216-2030 -** 104 week Oral Gavage Study Carcinogenicity Study with GS-9350 in Mice | cobicistat | Key | | |
| **TMC114-NC157-TSR-** 3-Month repeated dose oral toxicity study in the Swiss mouse. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.4.1 |
| **TMC114-NC190-TSR -** 2-Week repeated dose oral toxicity study in the Swiss mouse. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.4.1 |
| **TX-216-2025 -** 2-Week Non-GLP Oral Gavage Dose Range-Finding Toxicity and Toxicokinetic Study of GS-9350 in CD-1 Mice | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2026 -** 3-Month Oral Gavage Toxicity and Toxicokinetic Study with COBI in CD-1 Mice | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2032 -** 14-Day Oral Gavage Toxicity and Toxicokinetic Study of GS-9350 in Mice | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **TX-216-2041,** 4-week dose range finding oral gavage toxicity and toxicokinetic study with COBI in Tg(HRAS) (wild type) mice | cobicistat | Supportive | TYBOST MAA | M4.2.3.2 |
| **4.2.3.4.2 Short -or medium-term studies** | | | | |
| For DRV, no short- or medium term carcinogenicity studies have been performed since 6 month rat and 3 month mouse studies were performed and reported in Module 2.6.6, sections 3.1.1 and 5.1.1.1, respectively. For COBI, no short- or medium term carcinogenicity studies, or other carcinogenicity studies have been performed. A 13-week study in the mouse and a 26-week study (with 13 weeks recovery) in the rat were performed and reported in Module 2.6.6, Section 5.2.1.1 and 3.2.1.1, respectively. | | | | |
| **4.2.3.4.3 Other Studies** | | | | |
| **TMC114-NC194-TSR -** 4-week toxicity study by oral route (gavage) in CB6F1-nonTgrasH2 mice**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.4.3 |
| **TMC114-NC196-TSR -** 13-Week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.4.3 |
| **4.2.3.5 Reproductive and Developmental Toxicity** | | | | |
| **4.2.3.5.1 Fertility and early embryonic development** | | | | |
| **TMC114-NC129-TSR -** The effects of TMC114 on fertility and early embryonic development in Sprague-Dawley rats. | darunavir | Key | | |
| **TX-216-2023 -** Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats | cobicistat | Key | | |
| **4.2.3.5.2 Embryofetal development** | | | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC128-TSR -** Study of the effect of TMC114 on embryo-fetal development in Sprague-Dawley rats. | darunavir | Key | | |
| **TMC114-TiDP3-NC398-TSR -** Oral Developmental Toxicity Study of TMC114 in the Rat. | darunavir | Key | | |
| **TMC114-NC126-TSR -** Study of the effect of TMC114 on embryo-fetal development in New Zealand White rabbits. | darunavir | Key | | |
| **TMC114-NC126-NCTK -** Study of the effect of TMC114 on embryo-fetal development in New Zealand White rabbits. | darunavir | Key | | |
| **TMC114-NC126-NCPCD -** Study of the effect of TMC114 on embryo-fetal development in New Zealand White rabbits. | darunavir | Key | | |
| **TMC114-NC172-TSR -** Oral (gavage) developmental toxicity study in the mouse. | darunavir | Key | | |
| **TMC114-NC399-NCPK -** Combined pharmacokinetic / DRF study in female minipigs**.** | darunavir | Key | | |
| **TX-216-2020 -** Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rats | cobicistat | Key | | |
| **TX-216-2021 -** Oral Gavage Study for Effects on Embryo-fetal Development and Toxicokinetics with GS-9350 in Rabbits | cobicistat | Key | | |
| **TMC114-NC124-TSR -** Multiple dose toxicity study of TMC114 in New Zealand White rabbits**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC124-NCPK -** Multiple dose toxicity study of TMC114 in New Zealand White rabbits**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC124-NCPCD -** Multiple dose toxicity study of TMC114 in New Zealand White rabbits**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC125-TSR -** Dose range finding study of prenatal developmental toxicity of TMC114 in New Zealand White rabbits**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC125-NCTK -** Dose range finding study of prenatal developmental toxicity of TMC114 in New Zealand White rabbits**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC127-TSR -** Dose range finding study of prenatal developmental toxicity of TMC114 in Sprague-Dawley rats. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC127-NCTK -** Dose range finding study of prenatal developmental toxicity of TMC114 in Sprague-Dawley rats. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-NC175-TSR -** Oral (gavage) developmental toxicity dose range finding study in the mouse**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC189-TSR-** 5-Day Repeated Dose Oral Toxicity Study in the Female Rabbit**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TMC114-TiDP3-NC397-TSR-** Pilot oral developmental toxicity study of TMC114 in the rat**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.2 |
| **TX-216-2018-** Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetic Study with GS-9350 in Rats | cobicistat | Supportive | TYBOST MAA | M4.2.3.5.2 |
| **TX-216-2019-** Oral Gavage Dose Range-finding Developmental Toxicity and Toxicokinetic Study with GS-9350 in Rabbits | cobicistat | Supportive | TYBOST MAA | M4.2.3.5.2 |
| **4.2.3.5.3 Prenatal and postnatal development, including maternal function** | | | | |
| **TMC114-NC156-TSR -** Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat**.** | darunavir | Key | | |
| **TMC114-NC178-NCPCD -** Oral (gavage) pre- and post-natal developmental toxicity and juvenile toxicity dose range finding study in the rat. | darunavir | Key | | |
| **TMC114-NC178-NCTIS -** Oral (gavage) pre- and post-natal developmental toxicity and juvenile toxicity dose range finding study in the rat. | darunavir | Key | | |
| **TMC114-NC178-TSR -** Oral (gavage) pre- and post-natal developmental toxicity and juvenile toxicity dose range finding study in the rat. | darunavir | Key | | |
| **TX-216-2033 -** Perinatal/Postnatal study with GS-9350 and Juvenile Toxicity | cobicistat | Key | | |
| **4.2.3.5.4 Studies in which the offspring (juvenile animals) are dose and/or further evaluated** | | | | |
| **TMC114-NC240-TSR -** Juvenile tolerance study in rats. [redacted] | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.4 |
| **TMC114-NC241-TSR -** TMC114 Toxicity study in the juvenile rat by oral (gavage) administration**.** | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.5.4 |
| **TMC114-NC248 -** TMC114 Range finding study in the juvenile rat by oral (gavage) administration**.** | darunavir | Supportive | Not applicable | Available upon request |
| **4.2.3.6 Local Tolerance** | | | | |
| **TMC114-NC245-TSR -** Local Lymph Node Assay (LLNA) | darunavir | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC166-TSR** - In vitro Bovine Corneal Opacity Permeability Eye Irritation test | darunavir | Key | | |
| **TMC114-NC330-TSR** - Evaluation of skin sensitization potential in mice using the local lymph node assay (LLNA). | darunavir | Key | | |
| **TMC114-TiDP3-NC316** - Primary skin irritation study in rabbits (4-hours Semi Occlusive Application) | darunavir | Key | | |
| **TX-216-2042** - Local Lymph Node Assay in mice with GS-9350 | cobicistat | Key | | |
| **TX-216-2043** - BCOP with GS-9350 | cobicistat | Key | | |
| **TX-216-2044** - Dermal Irritation with GS-9350 | cobicistat | Key | | |
| **4.2.3.7 Other Toxicity Studies** | | | | |
| **4.2.3.7.1 Antigenicity** | | | | |
| In accordance with the ICH guideline, data from the general toxicology studies are considered sufficient to evaluate antigenic potential of DRV and COBI. Based on these data, it was concluded that there are no indications that DRV or COBI has an antigenic potential and therefore no additional studies were performed. | | | | |
| **4.2.3.7.2 Immunotoxicity** | | | | |
| **TMC114-NC187-TSR** - 4-Week immunotoxicity study by oral route (gavage) in rats. | darunavir | Key | | |
| **TX-216-2022** - 4-Week Oral Gavage T-Cell Dependent Antibody Assay with GS-9350 in Rats | cobicistat | Key | | |
| **4.2.3.7.3 Mechanistic Studies** | | | | |
| **TMC114-NC162-TSR** - 1-month repeated dosed mechanistic oral toxicity study of TMC114 in the rat. | darunavir | Supportive | PREZISTA EMEA/H/C/000707 | M4.2.3.7.3 |
| **4.2.3.7.4 Dependence** | | | | |
| Drugs that inhibit cytochrome P450, or structurally-related HIV protease inhibitors, have no known properties that would suggest development of dependence. No studies on dependence were conducted as DRV is intended to be used as an anti-HIV. There was no evidence of development of dependence in nonclinical studies with COBI and tissue distribution studies in rats indicated minimal transport across the blood:brain barrier (Module2.6.4/Section 4.1.2). Consequently, dependency studies are not considered warranted. | | | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **4.2.3.7.5 Metabolites** | | | | |
| For DRV, metabolite studies were not conducted as the unchanged DRV was more abundant than any metabolite. As there are no major human metabolites of COBI, and the most abundant metabolites were similar across species, no separate safety studies on COBI metabolites have been conducted either. | | | | |
| **4.2.3.7.6 Impurities** | | | | |
| **TMC114-TiDP3-NC354 -** In vitro Mammalian Chromosome Aberration Test with TMC114 spiked with 2 % impurities in Human Lymphocytes | darunavir | Key | | |
| **TMC114-TiDP3-NC355 -** In vitro Mammalian Chromosome Aberration Test with TMC114 spiked with 2 % impurities in Human Lymphocytes | darunavir | Key | | |
| **TMC114-TiDP3-NC336 -** In Vitro Bacterial Reverse Mutation Test with TMC114 spiked with 2% impurities in Salmonella typhimurium | darunavir | Key | | |
| **TMC114-TiDP3-NC337 -** In vitro mammalian chromosome aberration test in cultured human lymphocytes | darunavir | Key | | |
| **TMC114-NC338-TSR -** 2-Week Repeated Dose Oral Toxicity Study of TMC114 in the Beagle Dog | darunavir | Key | | |
| **TMC114-TiDP3-NC358 -** In vitro bacterial reverse mutation test with TMC114 spiked with 3% impurities in Salmonella typhimurium | darunavir | Key | | |
| **TMC114-TiDP3-NC357 -** In Vitro Mammalian Chromosome Aberration Test with TMC114 spiked with 3% impurities in Human Lymphocytes | darunavir | Key | | |
| **TMC114-NC227-TSR -** 2-Week repeated dose oral toxicity study in the rat. | darunavir | Key | | |
| **TMC114-NC228-TSR -** Bacterial reverse mutation test. | darunavir | Key | | |
| **TMC114-NC242-TSR -** 2-Week repeated dose oral toxicity study in the rat**.** | darunavir | Key | | |
| **TMC114-NC244-TSR -** In vitro bacterial reverse mutation test with Salmonella typhimurium. | darunavir | Key | | |
| **TMC114-TiDP3-NC359-TSR-Compl -** In Vitro Bacterial Reverse Mutation Test with TMC114 spiked with 3% impurities in Salmonella typhimurium | darunavir | Key | | |
| **TMC114-NC150-TSR -** Evaluation of the mutagenic activity of TMC114 in the Salmonella Thyphimurium reverse mutation assay in tester strain TA98 and TA100**.** | darunavir | Key | | |
| **TMC114-NC170-TSR -** Evaluation of the mutagenic activity of three batches of TMC114 ethanolate in the Salmonella Typhimurium reverse mutation assay tester strain TA98 and TA100**.** | darunavir | Key | | |

| Submission Component and location within FDC MAA | Compound | Key / Supportive | Single agent MAA (if not re-submitted in FDC MAA) | Location in single agent MAA (if not re-submitted in FDC MAA) |
|---|---|---|---|---|
| **TMC114-NC176-TSR -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog. | darunavir | Key | | |
| **TMC114-NC176-NCTK-Compl -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog. | darunavir | Key | | |
| **TMC114-NC176-NCPCD -** Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog. | darunavir | Key | | |
| **TX-216-2045 -** Impurity of GS-9350 drug product | cobicistat | Key | | |
| **TX-216-2046 -** In silico profiling of impurities of COBI | cobicistat | Key | | |
| **TX-216-2054 -** In silico Evaluation of Potential Genotixicity and Carcinogencity of 不純物CF* | cobicistat | Key | | |
| **TX-216-2052 -** 不純物CA* , 不純物CB* 不純物CC* and 不純物CD* Bacterial Reverse Mutation Test | cobicistat | Key | | |
| **TX-216-2053 -** 不純物CA* , 不純物CB* 不純物CC* and 不純物CD* Mammalian Cell Mutation Test | cobicistat | Key | | |
| **4.2.3.7.7 Other** | | | | |
| **TMC114-TiDP3-NC394 -** In vitro effects of lopinavir (JNJ-1382028-AAA-, tipranavir and darunavir (JNJ-25875382-AAA-29852452, TMC114) on human platelet function using collagen, adenoside diphosphate and platelet activing factor as agonists for platelet aggregation**.** | darunavir | Supportive | Not applicable | Available upon request |
| **4.3 Literature References** | | | All literature references are considered supportive but have been included for the convenience of the reviewer | |

*新薬承認情報提供時に置き換え