DARUNAVIR/COBICISTAT

MODULE 2.6.4

PHARMACOKINETICS WRITTEN SUMMARY

TABLE OF CONTENTS

IN-TEXT FIGURES **5**

IN-TEXT TABLES **6**

ABBREVIATIONS **7**

1. BRIEF SUMMARY **9**

2. METHODS OF ANALYSIS **14**
2.1. Darunavir 14
2.2. Cobicistat 15

3. ABSORPTION **15**
3.1. Absorption 15
3.1.1. Caco2- Cells 15
3.1.1.1. Darunavir 15
3.1.1.2. Cobicistat 16
3.1.2. Studies in Preclinical Species 16
3.1.2.1. Darunavir 16
3.1.2.1.1. Mice *17*
3.1.2.1.2. Rats *18*
3.1.2.1.3. Rabbits *21*
3.1.2.1.4. Dogs *21*
3.1.2.1.5. Minipigs *22*
3.1.2.2. Cobicistat 23
3.1.2.2.1. Single-Dose *23*
3.1.2.2.2. Repeated-Dose *25*
3.2. Kinetic Parameters, Bioequivalence and/or Bioavailability 27
3.2.1. Darunavir 27
3.2.2. Cobicistat 28

4. DISTRIBUTION **28**
4.1. Tissue Distribution 28
4.1.1. Darunavir 28
4.1.1.1. Single Dose 28
4.1.1.2. Repeated Dose 30
4.1.2. Cobicistat 31
4.2. Protein Binding and Distribution in Blood Cells 32
4.2.1. Darunavir 32
4.2.2. Cobicistat 33
4.3. Placental Transfer 34

5. METABOLISM **35**
5.1. Chemical Structures and Quantities of Metabolites in Biological Samples – In Vivo Metabolism 35
5.1.1. Darunavir 35
5.1.1.1. Studies in Mice 35
5.1.1.2. Studies in Rats 35
5.1.1.3. Studies in Dogs 36
5.1.1.4. Studies in Humans 37
5.1.2. Cobicistat 38
5.1.2.1. Studies in Mice 38
5.1.2.2. Studies in Rats 38
5.1.2.3. Studies in Dogs 39

5.1.2.4. Studies in Humans ... 39
5.2. Possible Metabolic Pathways ... 39
5.2.1. Darunavir ... 39
5.2.2. Cobicistat ... 42
5.3. Presystemic Metabolism (GI/Hepatic First-Pass Effects) ... 44
5.4. In Vitro Metabolism, Including P450 Studies ... 44
5.4.1. Darunavir ... 44
5.4.1.1. In Vitro Metabolic Pathway ... 44
5.4.1.2. CYP450 Isozymes Involved in the Oxidative Metabolism of Darunavir ... 47
5.4.2. Cobicistat ... 47
5.4.2.1. In Vitro Metabolic Pathway ... 47
5.4.2.2. CYP450 Isozymes Involved in the Oxidative Metabolism of Cobicistat ... 49
5.5. Enzyme Induction and Inhibition ... 49
5.5.1. Darunavir ... 49
5.5.1.1. Ex-vivo Studies Measuring Enzyme Activities in animals ... 49
5.5.1.2. In Vitro Induction of Human CYP450 Enzymes ... 50
5.5.1.2.1. In Vitro Study Measuring CYP mRNA Induction in Human Hepatocytes ... 50
5.5.1.2.2. In Vitro Study Measuring CYP Activity Induction in Human Hepatocytes ... 50
5.5.1.3. In Vitro Inhibition ... 51
5.5.1.3.1. Inhibition of P450 Isozymes by Darunavir ... 51
5.5.1.3.2. In Vitro Inhibition of Human UGT1A1 ... 51
5.5.2. Cobicistat ... 52
5.5.2.1. Ex-vivo Studies Measuring Enzyme Activities in animals ... 52
5.5.2.2. In Vitro Induction ... 53
5.5.2.2.1. Induction of Metabolizing Enzymes of Rat by COBI In Vitro ... 53
5.5.2.2.2. Xenobiotic Receptor Activation by COBI ... 54
5.5.2.2.3. In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes ... 54
5.5.2.3. Inhibition of P450 Isozymes by Cobicistat ... 56
5.5.2.3.1. Inhibition of CYP3A Activity by COBI ... 56
5.5.2.3.2. Inhibition of Other Human Cytochromes P450 ... 57
5.5.2.3.3. Inhibition of Human Cytochromes P450 by Human Metabolites of COBI ... 57
5.5.2.3.4. In Vitro Inhibition of Human UGT1A1 ... 58

6. EXCRETION ... 58
6.1. Routes and Extent of Excretion ... 58
6.1.1. Darunavir ... 58
6.1.2. Cobicistat ... 59
6.2. Excretion in Milk ... 61
6.2.1. Darunavir ... 61
6.2.2. Cobicistat ... 61

7. PHARMACOKINETIC DRUG INTERACTIONS ... 61
7.1. Darunavir ... 61
7.2. Cobicistat ... 61

8. OTHER PHARMACOKINETIC STUDIES: Membrane transporters ... 61
8.1.1. Darunavir ... 61
8.1.2. Cobicistat ... 62
8.1.2.1. Inhibition of Individual Recombinant Expressed Human Drug Transporters ... 62

8.1.2.2. Inhibition of Bidirectional Transport of MDR1 and BCRP Substrates Through Caco-2 Cell Monolayers 64
8.1.2.3. Potential for COBI to be a Substrate for Human OCT2 64
8.1.2.4. Potential for COBI to be a Substrate for Human MDR1 or BCRP 65

9. DISCUSSION AND CONCLUSIONS 65
9.1.1. Darunavir 65
9.1.2. Cobicistat 67

10. TABLES AND FIGURES 70

11. REFERENCES 70

IN-TEXT FIGURES

Figure 1: Tissue to Blood AUC_{0-24h} Ratios of Total Radioactivity after Oral Administration of ^{14}C-DRV Alone or in Combination with RTV in Male Pigmented Long-Evans Rats 29
Figure 2: Tissue to Blood AUC_{0-8h} Ratios of Total Radioactivity after Oral Administration of ^{14}C-DRV Alone or in Combination with RTV in Pregnant Rats 30
Figure 3: *In Vivo* Metabolic Pathways of DRV in Animals and Humans[63,64,65,66] 41
Figure 4: Common Primary and Secondary Pathways for Metabolism of COBI by Mouse, Rat, Dog, and Human In Vivo 42
Figure 5: In Vitro Metabolic Pathways of DRV in Adult Animals and Humans[67] 46
Figure 6: Common Primary Pathways for Metabolism of COBI by Mouse, Rat, Dog, and Human In Vitro 48

IN-TEXT TABLES

Table 1: Pharmacokinetic Parameters of DRV in Mice with and without RTV Co-administration[11] 18

Table 2: Pharmacokinetic Parameters of DRV in Rats With[34] and Without[20] RTV Co-administration for 6 Months. 20

Table 3: Pharmacokinetic Parameters of DRV in Dogs with[47,49] or without RTV[41,43,44,45] Co-administration for 2 Weeks 22

Table 4: Plasma Toxicokinetic Parameters for DRV in Female Minipigs 23

Table 5: Mean Plasma Pharmacokinetic Parameters for COBI Following 30-Minute Intravenous Infusion at 1 mg/kg to Sprague Dawley Rats, Beagle Dogs, and Cynomolgus Monkeys (mean ! SD, N = 3) 24

Table 6: Mean Plasma Pharmacokinetic Parameters Following Oral Administration of COBI in Solution to Male Sprague-Dawley Rats, Beagle Dogs, and Cynomolgus Monkeys (mean ! SD, N = 3) 24

Table 7: Plasma Protein Binding and Blood Distribution of DRV in Various Species [61] ... 33

Table 8: Protein Binding for COBI in Mouse, Rat, Dog, Monkey, and Human Plasma Determined by Equilibrium Dialysis (mean ! SD, n = 3) 33

Table 9: Whole Blood to Plasma Concentration Ratios of Radioactivity after Oral Administration of ^{14}C-COBI 34

Table 10: Percentage of Administered ^{14}C-DRV Dose Metabolized per Major Pathway in the Rat Dosed With and Without RTV[63], Dog[65] and in Human With and Without RTV[66] 40

Table 11: Cross-Species Comparison of COBI Metabolites 43

Table 12: Urinary and Fecal Excretion of the Radioactivity Following Single Oral Administration of ^{14}C-DRV in Rat[63], and Dog[65] 59

Table 13: Effects of COBI and RTV on the Activities of Human Transporters 63

ABBREVIATIONS

A-B	apical to basal
AhP	aryl hydrocarbon receptor (AHR gene product)
AP	apical side of monolayer
ATP	adenosine triphosphate
ATV	atazanavir (Reyataz®, Bristol-Myers Squibb)
AUC	area under the concentration-time curve
B-A	basal to apical
BCRP	breast cancer resistance protein (ABCG2)
BSEP	bile salt export pump (ABCB11)
BL	basolateral side of monolayer
Caco-2	human colonic adenocarcinoma cell line
cDNA	complementary deoxyribose nucleic acid
CHO	Chinese hamster ovary cell line
Cl	clearance
C_{max}	maximum plasma concentration
COBI	cobicistat (GS-9350)
CYP	cytochrome(s) P450
DMSO	dimethyl sulfoxide
DRV	darunavir
EDTA	ethylenediamine tetraacetic acid
ER	efflux ratio = secretory/absorptive permeation
f_u	fraction unbound
F	oral bioavailability of the drug (%)
GI	gastrointestinal
GLP	good laboratory practice
HEK293	human embryonic kidney 293 cells
HIV	human immunodeficiency virus
HLMs	human liver microsomes
$[I]_1$	inhibitor concentration corresponding to steady state C_{max}
$[I]_2$	inhibitor concentration corresponding to theoretical maximum concentration in the intestinal lumen
IC_{50}	concentration required to produce 50% inhibition
Ki	inhibition constant
K_I	affinity constant for enzyme inactivation
k_{inact}	theoretical maximum enzyme inactivation rate
LC	liquid chromatography
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry

LLC-PK1	porcine kidney cell line
MATE1	multidrug and toxin extrusion protein 1 (SLC47A1)
MATE2-K	multidrug and toxin extrusion protein 2-K (SLC47A2)
MDCK II	Madin-Darby canine kidney cell line
MDR1	P-glycoprotein (Pgp, ABCB1 gene product)
mRNA	messenger ribonucleic acid
MRP1	multi-drug resistance-associated protein-1 (ABCC1)
MRP2	multi-drug resistance-associated protein-2 (ABCC2, cMOAT)
MRP4	multi-drug resistance-associated protein-4 (ABCC4)
MS	mass spectrometry
NADPH	β-nicotinamide adenine dinucleotide phosphate (reduced form)
NC	not calculated
ND	not detectable / not determined
OAT1	organic anion transporter 1 (SLC22A6)
OAT3	organic anion transporter 3 (SLC22A8)
OATP	organic anion transporting polypeptide (SLCO or SLC22A gene products)
OATP1B1	organic anion transporting polypeptide 1B1 (SLCO1B1)
OATP1B3	organic anion transporting polypeptide 1B3 (SLCO1B3)
OCT1	organic cation transporter 1 (SLC22A1)
OCT2	organic cation transporter 2 (SLC22A2)
OCTN1	organic cation transporter novel, type 1 (SLC22A4)
P_{app}	apparent permeability
PEG400	polyethylene glycol 400
Pgp	P-glycoprotein
PI	protease inhibitor
PXR	pregnane X receptor (SXR, NR1I2 gene product)
QWBA	quantitative whole-body autoradiography
RTV	ritonavir
S_2	Schneider 2 cell line
SD	standard deviation
t1/2	elimination half-life
tmax	time after administration at which Cmax is reached
TR	total radioactivity
UGT	uridine diphosphate glucuronosyl transferase
Vdss	volume of distribution at steady state

1. BRIEF SUMMARY

The darunavir/cobicistat (**DRV/COBI**) fixed dose combination tablet contains 800 mg of the medicinal product DRV and 150 mg of the medicinal product COBI and is developed by the Applicant in collaboration with Gilead Sciences Inc. (Gilead). Darunavir is a registered human immunodeficiency virus type 1 (HIV-1) protease inhibitor (PI) and COBI is a pharmacokinetic enhancer that is recently approved. 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 beyond the pharmacoenhancing effect of COBI on DRV. In this summary the nonclinical drug absorption and disposition profiles of DRV (also named TMC114 or Prezista®) and COBI (a structural analog of ritonavir [RTV] also named GS-9350 or GS-340649) are summarized. Both compounds have been investigated separately and therefore the individual DRV and COBI data are described in this document.

The original nonclinical programs reported here are consistent with the best scientific principles and international guidelines. The pivotal studies have been conducted according to Good Laboratory Practice standards.

The pharmacokinetic studies for DRV and COBI are listed in the overview table (Tabulated Summary 2.6.5.1).

Darunavir

The drug substance is a solvate (ethanolate). The dose levels used in all studies in this document are expressed as the nominal dose.

Darunavir has been assessed in comprehensive nonclinical pharmacokinetic studies. Results from pharmacokinetic studies of DRV are summarized below:

Darunavir absorption was rapid following oral administration in all species, based on observed t_{max} values (0.5 to 6 hours). The absolute oral bioavailability was 37 to 58% in rats and very likely was influenced by the extent of the first pass effect as demonstrated by the

presence of a large amount of metabolites in the bile. Bioavailability was higher in dogs and ranged between 60 to 122%. In rats and dogs, the plasma clearance and the volume of distribution were moderate to high. The elimination was rapid across all species.

Across the dose range studied, the kinetics of DRV was less than dose-proportional in mice, rats and dogs after single oral administration, especially at the high dose levels in line with the low solubility of the compound. In adult rodents, repeated oral dosing resulted in a decrease in systemic exposure to DRV, possibly due to induction of the enzymes involved in the metabolism of DRV. Ex vivo induction studies showed DRV was an inducer of cytochrome P450 (CYP) 3A isoenzyme in rodents. Uridine diphosphate glucuronosyl transferase (UGT) activity was additionally induced in rats. These effects were in line with the toxicology findings observed in the liver and thyroid in rodents. In dogs, no decrease in exposure and enzymatic induction was observed after repeated administration. Ritonavir, a strong inhibitor of CYP3A, had a modest effect on DRV exposure in mice (2-fold increase) and rats (4-fold increase) but had no clear effect in dogs and in minipigs. The highest impact, however, was in rabbits where a 15-fold increase in exposure was seen. In humans, however, the impact was noticeable. RTV markedly reduced the metabolism of DRV and consequently increased its oral bioavailability from 37 to 82%.

In adult 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. In pregnant rats, ^{14}C-DRV was slightly distributed in placenta and fetus. Total radioactivity (TR) exposure in fetus was about 13 to 27 % of that in maternal blood, while in placenta it was the same as in blood. Exposure (plasma, brain and liver) to DRV was age-dependent in juvenile rats when measured between Days 5-11 and Days 12-26. No consistent difference in DRV plasma exposure was seen in rats aged between Day 5 and 11 though. Plasma, liver and

brain exposure decreased between Day 12 and Day 26 of age due to the maturation of the liver enzymes involved in the elimination of DRV and also of the blood-brain barrier. At an age range of 23 to 50 days, exposures were broadly similar to those observed in adult rats. The plasma protein binding was moderate to high in all tested species (i.e., mouse, rat, rabbit, dog and human). The free fraction ranged from 5% (rat) to 38% (rabbit), and was 5 % in humans. Plasma protein binding in most species was concentration dependent.

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. 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 observed. Metabolic pathways proposed on the basis of in vitro studies were consistent with those observed following in vivo studies in rats, dogs and humans. Therefore, the in vitro metabolism data provide a valuable comparison between the various species, including the mouse and rabbit in which the metabolism of DRV was not studied in vivo.

In all examined species (rat, dog and human), the predominant route of excretion for ^{14}C-DRV was via the feces and amounted to 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 in humans 12.2%. 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 rats, DRV was probably also excreted via milk.

In human liver, CYP3A was almost exclusively involved in the metabolism of DRV. Darunavir inhibited CYP3A in human liver microsomes (HLM) with an inhibition constant (K_i) value of 0.40 μM. Given this low value, this inhibition is considered to be clinically relevant. The K_i values for the other P450 enzymes (CYP1A2,

CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1) were at least 60-fold higher, indicating a much lower affinity and less potential for clinically relevant interactions. Darunavir has only a mild inhibitory potency towards UGT1A1. Darunavir was also an inducer of CYP3A4 mRNA expression level as well as activity in human hepatocytes.

Cobicistat

Cobicistat has been assessed in comprehensive nonclinical pharmacokinetic studies. Results from pharmacokinetic studies of COBI are summarized below:

Estimates of fraction absorbed in vivo, derived from bioavailability corrected for predicted first-pass metabolism, or from recovery of radiolabel in bile or urine, were all > 50%. After moderate doses, oral bioavailability in nonclinical species was low due to metabolic instability and resulting high first-pass elimination. Cobicistat can act as a substrate for MDR1 and BCRP intestinal efflux transporters, but this likely did not play a significant role during its absorption as COBI had high passive permeability, as demonstrated in Caco-2 cells.

After oral dosing with ^{14}C-COBI, radioactivity was widely distributed, and volumes of distribution of COBI were close to those for body water. Cobicistat was relatively excluded from the cellular fraction of blood (whole blood to plasma ratios are ~0.6) and from brain, testes, and the eye. Exclusion from the brain may be due to the action of MDR1 and/or BCRP at the blood:brain barrier. Cobicistat showed preferential binding in melanin-containing tissues, but this was reversible. Cobicistat showed moderately high plasma binding.

Interpreting the metabolism of COBI was complicated by concurrent mechanism-based inhibition of human CYP3A enzymes. This attribute was species-specific, as COBI showed high clearance in nonclinical species due to a lack of self-inhibition of metabolism. The primary routes of metabolism of COBI were oxidation by CYP3A (major) and CYP2D6 (minor) enzymes. Metabolites M21, M26, and M31 were identified in mouse, rat, dog, and human samples in vitro, and were later identified in excreta from these species. One other primary

metabolite, M39, was also identified in all species in vivo. Other metabolites arise from secondary metabolism, due to combinations of these primary pathways, and from other minor primary metabolites. Parent COBI was the major component circulating in plasma in all species.

After oral dosing of mice, rats, dogs, and humans with ^{14}C-COBI, the majority of radiolabel was recovered in the feces or bile with little in the urine. Total recovery of radiolabel was high for all species. Excretion of COBI into milk was detected in rats.

The intended pharmacologic action of COBI was inhibition of human CYP3A enzymes. In that regard, COBI was a potent mechanism-based inhibitor of human CYP3A and showed activity against a wide range of CYP3A activities. All 3 of the metabolites initially identified (M21, M26, and M31) were weaker inhibitors than COBI and were very unlikely to contribute to the pharmacologic effect, especially considering their low plasma concentrations. Inhibition of human cytochrome P450 enzymes showed high selectivity, with insignificant or very weak inhibition of CYP1A2, CYP2C8, CYP2C9, and CYP2C19, weak inhibition of CYP2D6, and modest inhibition of CYP2B6. Cobicistat was also a weak inhibitor of human UGT1A1. In this regard, COBI showed greater selectivity than RTV, which inhibited CYP2C8 and CYP2C9 and was a more potent inhibitor of CYP2D6 and UGT1A1.

At concentrations achieved in plasma at the proposed therapeutic dose, COBI did not inhibit the drug transporters MDR1, MRP1, MRP2, BCRP, OAT1, or OAT3. With respect to renal transporters, COBI was a weak inhibitor of MRP4, MATE2 K and OCT2, and a more potent inhibitor of MATE1 and OCTN1, with similar potencies to RTV.

With respect to hepatic uptake transporters, COBI was a moderate inhibitor of OATP1B1 and OATP1B3. At high concentrations, achievable briefly in the intestinal lumen during drug absorption, COBI can inhibit intestinal efflux transporters, such as MDR1 and BCRP. Cobicistat did not activate human aryl hydrocarbon receptor (AhR) and did not induce human CYP1A2 activity or mRNA. Cobicistat was a very weak activator of human pregnane X receptor

(PXR), and affects CYP3A4 mRNA and CYP3A immunodetectable protein only at high concentrations. Cobicistat thus had lower liability for drug interactions than RTV, which was a more potent PXR activator. Interestingly, COBI and RTV showed similar, moderately potent ability to activate rat PXR, and this was manifest as increased CYP3A activity in hepatic microsomal fraction from rats and mice after repeat dose treatment. Such species differences in PXR activation, caused by differences in the ligand binding domain of the receptor, are well understood[1].

In conclusion, COBI was a potent, selective mechanism-based inhibitor of human CYP3A enzymes with low potential for other drug-drug interactions (inhibition of other cytochromes P450, UGT1A1, or drug transporters, and induction of enzymes and transporters).

2. METHODS OF ANALYSIS

2.1. Darunavir

Several bioanalytical assays were developed to support DRV toxicokinetic and pharmacokinetic programs. The performance of most of these assays was characterized by validation processes, in line with the internal procedures, and bioanalytical guidelines.[1] The liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method was used to determine DRV and/or RTV in mouse, rat, rabbit; minipigs and dog heparin plasma and/or ethylenediamine tetraacetic aced (EDTA) plasma. Tissue samples were analyzed with a non-validated qualified research method based on the validated plasma method.

The stability of DRV and RTV was assessed in different solvents (methanol and acetonitrile), biological matrices (heparin and EDTA blood and plasma at several temperatures) and also during sample handling and processing. Both test articles were found to be sufficiently stable under the conditions tested and this allowed the use of each assay under normal laboratory testing conditions.

Concerning radio-carbon labeled DRV, the DRV molecule was uniformly labeled with ^{14}C in the aniline moiety, which was metabolically stable.[2]

2.2. Cobicistat

Appropriate bioanalytical assays were developed to support COBI toxicokinetic and pharmacokinetic programs. Fully validated LC-MS/MS methods were used to quantify COBI in GLP toxicokinetic studies in plasma and milk. The lower limit of quantification was 5 ng/mL for all matrices. The COBI metabolite, GS-9612 (M31), was also quantified in plasma using fully validated methods and the lower limit of quantification was 1 ng/mL. In non-GLP pharmacokinetic and toxicokinetic studies the LC-MS/MS analytical methods for COBI were evaluated for selectivity, linearity, intra-assay accuracy and precision.

For studies employing radiodetection COBI was labeled with ^{14}C in the methyl carbon of the N methylurea moiety. This site was metabolically stable.

3. ABSORPTION

3.1. Absorption

3.1.1. Caco2- Cells

3.1.1.1. *Darunavir*

Caco-2 human colon tumor cells were used as an in vitro model for evaluating the intestinal absorption of DRV and to assess the possible role of P-glycoprotein (PgP) in DRV transport alone and in combination with RTV[3,4]. ^{14}C- DRV was applied to either the apical (AP) or basolateral (BL) side of the monolayers to measure AP to BL (i.e. absorptive) transport, or BL to AP (i.e. secretory) transport, respectively (Tabulated Summaries 2.6.5.3.A and 2.6.5.3.B).

Darunavir showed an intermediate to high transepithelial permeability, with an absorptive average apparent permeability coefficient (P_{app}) ranging from 5.6 x 10^{-6} to 17.4 x 10^{-6} cm/s. Darunavir permeation was highly polarized initially with efflux ratio (ER= secretory/absorptive permeation) values up to 17. At later time points, DRV transport was moderately polarized with ER values up to 6. Both transport polarity and bi-directional transport rates were concentration-dependent (3-300 µM or 1.64-164 µg/mL), supporting the involvement of an efflux transporter. When PgP inhibitors (100 µM verapamil; 100 µM RTV) were added to the incubation medium, a reduction of DRV ER at

30 µM was observed from 5.4 to 3.1 and 2.1 with verapamil and RTV, respectively. Passive transcellular diffusion is proposed as the predominant mechanism for transepithelial DRV permeation. 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). Bi-directional transport experiments with the PgP substrate taxol demonstrated that DRV has PgP inhibitory properties with an apparent IC_{50} of 32.9 µM (18 µg/mL). A substantial inhibition of the efflux transport of taxol was also observed with RTV having an IC_{50} of 15.8 µM.

Given the fact that DRV solubility and dissolution rate in the gastro-intestinal (GI) environment are not limiting factors at the therapeutic dose levels, it can be concluded that DRV will exhibit sufficient membrane permeability to obtain adequate intestinal absorption. Inhibition of transepithelial permeation of P-gP substrates by DRV cannot be excluded, but for most drugs a possible effect is unlikely to be clinically significant with regard to intestinal absorption.

3.1.1.2. *Cobicistat*

The cellular permeability of COBI was assessed in vitro using Caco-2 monolayers, at an initial COBI concentration of 1 µM (0.78 µg/ml) [2] (Tabulated Summary 2.6.5.3.I). Transfer rates in both the A-B (forward) and B-A (reverse) directions were assessed.

At a target concentration of 1 µM (0.78 µg/ml), COBI had high forward permeability (7.61×10^{-6} cm/s), with little evidence for significant efflux (ratio = 1.1). The potential inhibition of intestinal MDR1 or BCRP by COBI is described Section 8.1.2.1.

3.1.2. Studies in Preclinical Species

3.1.2.1. *Darunavir*

Darunavir has been administered once or on several consecutive days to mice, rats, rabbits, dogs and minipigs in order to examine pharmacokinetics or toxicokinetics (as part of toxicology studies). Also several combination studies with DRV and RTV have been performed. The compound was dissolved in polyethylene glycol 400 (PEG400).

3.1.2.1.1. Mice

Darunavir alone

A **single oral dose** of DRV at 100, 300 and 1000 mg/kg in CD-1 mice resulted in a rapid to moderate absorption, with C_{max} and AUC values of 55 and 86 µg/mL and 199 and 360 µg.h/mL in males and females, respectively, at 1000 mg/kg[5] (Tabulated Summary 2.6.5.3.C).

Multiple dosing[6,7,8,9] (Tabulated Summaries 2.6.5.4.B, 2.6.5.4.C, 2.6.5.7.A and 2.6.5.4.AC) of DRV up to 24 months or administering DRV orally to pregnant mice for 10 days resulted in rapid absorption of the compound in males and females at all examined dose levels. AUC values increased more than dose-proportionally up to 450 mg/kg/day and less than dose-proportionally at higher dose levels. Repeated oral dosing resulted in a decrease in AUC (by up to 3-fold at high dose levels), likely due to induction of liver enzymes involved in the metabolism of DRV. Generally, C_{max} and AUC values were higher in females than in males by up to 2.8-fold at highest dose levels. Elimination was rapid with estimated $t_{1/2}$ values ranging between 0.6 and 2.8 hours. The systemic exposure to DRV after single or repeated administration during gestation was comparable to the systemic exposure in non-pregnant females over the dose range 150 to 1000 mg/kg/day.

Additionally, a 1-week study was performed in male CD-1 mice using dietary admixture (150, 1000 and 5000 mg/kg/day)[10] (Tabulated Summary 2.6.5.4.A). No gain in DRV systemic exposure was observed with the dietary administration relative to gavage with PEG400.

Darunavir in combination with RTV

Some studies were conducted to investigate whether RTV could be used as a pharmacokinetic enhancer for DRV in mice. The pharmacokinetics of DRV (1000 mg/kg/day) in the presence of RTV (50 mg/kg/day) was investigated in a 2-week oral study in male and female Swiss Albino mice[11] and in a 10-day reproduction toxicity dose range finding study in female mice[8] (Tabulated Summaries 2.6.5.4.D and 2.6.5.7.A). In mice, daily co-administration of RTV did not result in major increase in DRV systemic exposure (by 2-fold in both sexes (Table 1). Darunavir systemic exposure decreased after repeated

administration with and without RTV co-administration, although the decrease seemed slightly less extensive in the presence of RTV. The systemic exposure to DRV in the presence of RTV was comparable in pregnant and non-pregnant females after single and repeated oral administration.

Table 1: Pharmacokinetic Parameters of DRV in Mice with and without RTV Co-administration[11]

Duration of dosing	Dose (mg/kg/day)		C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
	DRV	RTV	Male	Female	Male	Female
Day 1	1000	0	18.9	22.0	65.8	109
	1000	50	13.7	19.3	83.3	137
Day 14	1000	0	9.41	13.6	34.9[b]	55.9
	1000	50	16.0	15.2	65.3	82.1

[a] : $AUC_{0-\infty}$ after single dose or AUC_{0-24h} after repeated dose ; [b] : AUC_{0-8h}

3.1.2.1.2. Rats

Darunavir alone

Single dose pharmacokinetics of DRV was investigated in oral studies in male and female Wistar rats. Darunavir was rapidly absorbed and showed non-linear kinetics. At a dose level exceeding 500 mg/kg, no increase in systemic exposure was observed. Elimination was rapid ($t_{1/2}$ = 0.2-2.9 hours). No consistent differences in plasma kinetics were observed between males and females[12,13] (Tabulated Summaries 2.6.5.3.D and 2.6.5.3.E). In addition, single dose pharmacokinetics after intravenous administration of DRV was investigated in 2 studies[14,15] (Tabulated Summary 2.6.5.3.F and 2.6.5.4.O). From the study[15] with the suitable formulation, plasma clearance (Cl) ranged between 1.6 and 3.0 L/h/kg and the volume of distribution at steady-state (Vd_{ss}) ranged between 1.0 and 2.1 L/kg. The calculated Cl approximated liver blood flow, indicative of high DRV hepatic extraction. No consistent differences in plasma kinetics were observed between males and females. The absolute oral bioavailability was 37 and 58% in male and female rats, respectively (Tabulated Summary 2.6.5.4.G).

Multiple dose of DRV were orally administered in male and female rats up to 24 months[16,17,18,19,20,21] (Tabulated Summaries 2.6.5.4.F, 2.6.5.4.M, 2.6.5.4.AF, 2.6.5.4.G, 2.6.5.4.I and 2.6.5.4.AG) and in

pregnant rats in developmental toxicity studies[22,23,24] (Tabulated Summary 2.6.5.7.B, Tabulated Summaries 2.6.5.7.E and 2.6.5.7.F). Pharmacokinetics was also assessed after single or repeated oral administration in a combined pre- and post-natal development and juvenile toxicity study[25] (Tabulated Summary 2.6.5.7.C), as well as in 3 follow-up juvenile studies[26,27,28] (Tabulated Summary 2.6.5.3.H, Tabulated Summaries 2.6.5.4.AD and 2.6.5.4.AE). All the studies were performed in Sprague Dawley rats except the 2-week oral study where Wistar rats were used.

Darunavir was rapidly absorbed after repeated administration. C_{max} and AUC values increased more than dose-proportionally up to 100 mg/kg/day and less than dose-proportionally at higher dose levels. A decrease in systemic exposure to DRV was observed (by up to 2.5-fold at high dose levels), likely due to induction of liver enzymes involved in the metabolism of DRV. No consistent differences in plasma kinetics were observed between males and females. Elimination was rapid ($t_{1/2}$ = 1.1-6.8 h). Furthermore the systemic exposure to DRV was comparable in pregnant and non-pregnant females.

After repeated direct DRV administration in juvenile rats (from day 12 to day 25 of age), although available data were limited, C_{max} and AUC_{0-5h} values were up to 4-fold-higher in day 12 of age relative to day 26. Pups aged 5 days or 11 days were also administered DRV once (40, 80 and 120 or 160 mg/kg/day) and showed no consistent difference in DRV AUC. Dosing from Day 23 to Day 50 of age (DRV or DRV/RTV) resulted in exposures broadly similar to those observed in adult rats for DRV alone or in combination with RTV. Overall, the above data indicate that exposure to DRV in juvenile rats is age-dependent and influenced by the postnatal maturation of the liver enzymes involved in elimination of DRV.

Additionally, a 1-week study[29] in males (100, 500 and 2500 mg/kg/day), and a 3-month study[30] in males and females (260, 1040, and 2600 mg/kg/day) were performed using dietary admixture (Tabulated Summaries 2.6.5.4.E and 2.6.5.4.H). No gain in DRV

systemic exposure was observed with the dietary administration relative to gavage with PEG400

Darunavir in combination with RTV

Combination studies of DRV with RTV up to 6 months [31,32,33,34] (Tabulated Summaries 2.6.5.4.J, 2.6.5.4.K, 2.6.5.4.L and 2.6.5.4.N) were conducted in Wistar rats. When comparing data from the 6-month study[20] with DRV alone and the 6-month combination study[34] (Table 2), RTV appeared to have no consistent effect on C_{max} of DRV. However, RTV increased DRV AUC, by up to 4-fold after 6 months of administration. Without RTV co-administration, DRV systemic exposure decreased after repeated administration, especially at the high dose levels, whereas, in the presence of RTV systemic exposure did not decrease. No consistent differences in plasma kinetics of DRV were observed between male and female rats.

Table 2: Pharmacokinetic Parameters of DRV in Rats With[34] and Without[20] RTV Co-administration for 6 Months.

Duration of dosing	DRV dose (mg/kg/day)	Sex	C_{max} (µg/mL)		AUC[b] (µg.h/mL)	
			-RTV	+RTV	-RTV	+RTV
Day 1	20	M	1.48	2.00	3.34	10.9
	20	F	2.03	1.54	6.46	7.79
	100	M	6.67	4.87	28.1	66.3
	100	F	7.62	4.29	44.0	60.5
	500[a]	M	12.1	11.4	164	194
	500[a]	F	11.6	7.81	140	163
	1000[a]	M	-	9.74	-	179
	1000[a]	F	-	10.8	-	192
Week 26	20	M	1.03	1.66	2.92	11.2
	20	F	1.77	1.95	4.38	13.4
	100	M	7.11	5.30	31.6	69.0
	100	F	12.8	6.42	35.8	70.4
	500[a]	M	10.3	11.4	63.8	183
	500[a]	F	24.5	26.6	121	318
	1000[a]	M	-	14.4	-	198
	1000[a]	F	-	24.8	-	297

[a] : Dose of RTV: 75 mg/kg/day, in all other cases 50 mg/kg/day RTV was used. [b] : on day 1 $AUC_{0-\infty}$, if not accurate AUC_{last}; at week 26 AUC_{last}. - : not tested

3.1.2.1.3. Rabbits

Darunavir alone

Non-pregnant female New Zealand White rabbits were administered **single** oral doses[35] (Tabulated Summary 2.6.5.4.P) of DRV (250, 500 and 1000 mg/kg) formulated in carboxymethylcellulose/Tween 80/water. The absorption rate was rapid. At 1000 mg/kg, C_{max} was 0.28 µg/mL and AUC ranged between 0.6 and 1.1 µg.h/mL. Elimination was rapid ($t_{1/2}$ = 0.69-5.0 h).

Repeated dosing of DRV were administered in non-pregnant (5 and 7 days)[35,36] (Tabulated Summaries 2.6.5.4.P and Tabulated Summary 2.6.5.4.Q) and pregnant rabbits (from gestation Day 8-20)[37] (Tabulated Summary 2.6.5.7.D). Absorption of DRV was rapid (t_{max} values =0.5-2 h). AUC values increased less than dose proportionally in non-pregnant and pregnant animals. Elimination was rapid in all females ($t_{1/2}$ values = 0.6-4.3 h).

Darunavir in combination with RTV

The combination of DRV and RTV[36] resulted in 15-fold increases in plasma DRV C_{max} and AUC values compared to DRV alone, in rabbits.

3.1.2.1.4. Dogs

Darunavir alone

Male and female Beagle dogs were administered **single** oral doses of DRV (20, 80 and 320 mg/kg), which was rapidly absorbed (t_{max} =1-1.3 h)[38] (Tabulated Summary 2.6.5.3.G). The highest C_{max} and AUC in males and females together were 19.1 µg/mL and 74.6 µg.h/mL at 80 mg/kg, respectively. Systemic exposure increased dose-proportionally up to 80 mg/kg but above this dose level, no further increase was observed. Elimination was rapid ($t_{1/2}$ = 0.6-0.9 h). In addition, male and female Beagle dogs were administered single intravenous doses of DRV in 2 studies[39,40] (Tabulated Summaries 2.6.5.4.AA and 2.6.5.4.AB). From the study[40] with the suitable formulation, plasma Cl ranged from 1.3 to 2.2 L/h/kg and Vd_{ss} ranged from 0.7 to 1.9 L/kg. No consistent differences in plasma kinetics were

observed between males and females. The absolute oral bioavailability of DRV was 60 and 122% in males and females, respectively. (Tabulated Summary 2.6.5.4.R).

Darunavir was given **repeatedly** for 2 weeks (30, 60, 120, 360 mg/kg/day)[41,42] 3[43], 6[44] and 12[45] months (30, 60, 120 mg/kg/day) to dogs. T_{max} values of 0.5 to 4 hours indicated fast absorption. AUC values increased dose proportionally at 12 months and generally remained constant upon repeated administration. Exposure values, however, were variable within a given dose and within the studies. Vomiting incidents may have contributed to the data variability generated in dogs. No consistent differences in plasma kinetics were observed between males and females.

Darunavir in combination with RTV

Multiple dose pharmacokinetics of DRV in combination with RTV[46,47,48,49] was investigated whether RTV could be used as a pharmacokinetic enhancer for DRV (Tabulated Summaries 2.6.5.4.W to 2.6.5.4.Z).

Co-administration of RTV in the dog, at various doses using once or twice daily regimen, did not increase the systemic exposure to DRV. The influence of RTV co-administration is shown in Table 3.

Table 3: Pharmacokinetic Parameters of DRV in Dogs with[47,49] or without RTV[41,43,44,45] Co-administration for 2 Weeks

Doses of DRV or DRV/RTV (mg/kg/day)	C_{max} (µg/mL)		AUC_{last} (µg.h/mL)	
	M	F	M	F
30	4.23-11.5	3.59-11.4	9.23-31.6	8.43-21.8
60	8.83-15.4	8.09-17.4	20.1-71.4	24.3-53.1
120	12.2-27.8	10.5-25.9	52.7-130	25.5-100
120 /10	16.9-23.9	12.7-22.3	54.0-103	54.2-105

The mean range of C_{max} and AUC obtained at the end of the study.

3.1.2.1.5. Minipigs

Non-pregnant female Göttingen SPF minipigs were dosed with DRV alone or in combination with RTV during 1 day (bid dosing) or during 6 days (bid dosing). Due to poor tolerability the latter dosing regimen

was changed and minipigs were dosed q.d. for 7 days[50] (Tabulated Summary 2.6.5.4.AH).

After twice daily dosing of DRV alone on day 1, mean exposure to DRV increased less than dose proportionally between 500 and 1000 mg/kg twice daily of DRV. No clear effect of RTV on DRV exposure was observed in both phases (Table 4). No conclusion could be drawn on the time-effect.

Table 4: Plasma Toxicokinetic Parameters for DRV in Female Minipigs

	First day of dosing		Last day of dosing	
Doses (mg/kg)	C_{max}	AUC_{0-24h}	C_{max}	AUC_{0-24h}
Single dose				
2 x 250 DRV	12.0 [a]	190		
2 x 500 DRV	15.0 [a]	238		
2 x 250 DRV + 2 x 50	11.4 [a]	181		
From days 29 to 34				
2 x 250 DRV	8.0[a]	109		
2 x 500 DRV	14.0[a]	159		
2 x 250 DRV + 2 x 50	8.7[a]	115		
From days 35 to 41				
250 DRV			6.0	59
500 DRV			8.1	82
250 DRV + 50 RTV			5.6	71

[a] : after the second dosing; Minipigs (6 out 9) vomited after the second dose occasion on Day 1 and Day 29

3.1.2.2. *Cobicistat*

3.1.2.2.1. Single-Dose

The mean plasma pharmacokinetic parameters for COBI following **intravenous administration** to Sprague Dawley rats[3] (Tabulated Summary 2.6.5.3.K), beagle dogs[4] (Tabulated Summary 2.6.5.3.L), and cynomolgus monkeys[5] (Tabulated Summary 2.6.5.3.M) are summarized in Table 5.

Table 5: Mean Plasma Pharmacokinetic Parameters for COBI Following 30-Minute Intravenous Infusion at 1 mg/kg to Sprague Dawley Rats, Beagle Dogs, and Cynomolgus Monkeys (mean ± SD, N = 3)

Species	Sex	C_{max} (nM)	$AUC_{0-\infty}$ (nM.h)	CL (L/h/kg)	V_{ss} (L/kg)	$t_{½}$ (h)
Rat	Male	664 ± 31.4	351 ± 12.6	3.59 ± 0.14	0.76 ± 0.14	0.40 ± 0.02
	Female	890 ± 74.3	566 ± 50.1	2.37 ± 0.18	0.70 ± 0.09	0.35 ± 0.01
Dog	Male	924 ± 267	565 ± 155	2.18 ± 0.69	1.33 ± 0.69	1.02 ± 0.04
Monkey	Male	1222 ± 41.1	977 ± 83.7	1.36 ± 0.14	1.31 ± 0.12	1.42 ± 0.07

SD = standard deviation

The CL of COBI was high in males of all species and was close to hepatic blood flow in each case. Female rats showed lower CL than males. The Vd_{ss} was equal to (rat), or somewhat larger (other species) than the volume of total body water

The mean plasma pharmacokinetic parameters of COBI following **oral administration** in solution to fasted male Sprague Dawley rats[3], beagle dogs[4], and cynomolgus monkeys[5] summarized in Table 6 (Tabulated Summaries 2.6.5.3.K, 2.6.5.3.L, and 2.6.5.3.M, respectively).

Table 6: Mean Plasma Pharmacokinetic Parameters Following Oral Administration of COBI in Solution to Male Sprague-Dawley Rats, Beagle Dogs, and Cynomolgus Monkeys (mean ± SD, N = 3)

Species	Dose (mg/kg)	t_{max} (h)	C_{max} (nM)	$t_{½}$ (h)	$AUC_{0-\infty}$ (nM.h)	%F
Rat	5	0.50 ± 0.00	764 ± 506	0.92 ± 0.22	594 ± 42.6	33 ± 3
Dog	5	1.00 ± 0.43	313 ± 186	1.12 ± 0.14	331 ± 130	11 ± 4
Monkey	6	2.17 ± 1.76	161 ± 102	1.36 ± 0.21	445 ± 280	7.3 ± 4.6

SD = standard deviation

At doses of 5 or 6 mg/kg, oral bioavailability of COBI was moderate in the rat (33%) and low in the dog and monkey (11% and 7%,

respectively). The high CL values in these species indicate the potential for high hepatic metabolic first-pass extraction following oral absorption in these species. Comparing bioavailability and predicted hepatic extraction values, it is likely that a substantial proportion (50%) of the dose was absorbed from the GI tract.

Exploratory single-dose escalation studies were also performed in male and female Balb/cBy x C57BL/6 F1-Tg(HRAS)2Jic hybrid mice[6] (Model 001178-W; wild type for rasH2 transgenic mice), male and female rats[3], and male dogs[4] (Tabulated Summaries 2.6.5.3.J, 2.6.5.3.K, and 2.6.5.3.L, respectively). In the mice, there were no remarkable sex differences in exposure, and exposure increased in a roughly dose proportional manner from 30 to 100 mg/kg. In the male rat and the male dog, there was a greater than proportional increase in AUC_{0-t} as the dose was increased from 5 to 25 mg/kg and 10 to 30 mg/kg, respectively, likely reflecting saturation of first-pass metabolism. Following saturation, the change in AUC_{0-t} was near proportional as the dose was increased further, from 25 to 100 mg/kg in the male rat and 30 to 100 mg/kg in the male dog. In the female rat, as seen after intravenous administration, exposures were higher than in the males at both doses consistent with the known gender difference in CYP3A expression in this species. The increase in AUC_{0-t} in female rats, when the dose was increased from 25 to 110 mg/kg, was greater than dose proportional.

3.1.2.2.2. Repeated-Dose

Multiple dose in vivo toxicokinetic studies were performed in mouse, rat, and dog in support of safety evaluation. The results are presented in detail in Module 2.6.6/Section3.2 and general conclusions from representative studies are noted below.

Similar to the single-dose study in CByB6F1-Tg(HRAS)2Jic mice, in CD 1 mice treated daily with COBI for 13 weeks[7] (Tabulated Summary 2.6.7.10) and 104 weeks[8] (Tabulated Summary 2.6.7.10.C) there were no notable sex differences in exposure. Exposure was greater than dose-proportional when the dose was increased from 5 to 15 mg/kg, likely due to saturation of first-pass metabolism, and then roughly dose-proportional. After treatment for 13 weeks, there were

modest increases in hepatic microsomal CYP2B and CYP3A activities, but these were only manifest at the highest dose. This is consistent with COBI activating rodent PXR[9] (Tabulated Summary 2.6.5.12.P), which regulates these enzymes. Despite the increase in enzyme activity, exposure did not change appreciably in these animals after 13 weeks of dosing, likely due to continued saturation of metabolism. Similar toxicokinetic results were found in a 4-week dose range-finding study in CByB6F1-Tg(HRAS)2Jic mice[10] (Tabulated Summary 2.6.7.10). After treatment for 104 weeks, potential accumulation of COBI was observed after multiple dosing.

Similar to the results seen in the single-dose study described above, in rats treated with COBI for 26 weeks[11] (Tabulated Summary 2.6.7.7.H) and 104 weeks[12] (Tabulated Summary 2.6.7.10.D) there was an approximate 2-fold sex difference in exposure (females > males). After daily oral dosing with COBI for 26 weeks, there were modest increases in hepatic microsomal CYP3A activity in both males (at 100 mg/kg/day) and females (at 30 and 100 mg/kg/day). This is consistent with the ability of COBI to activate rat PXR[9] (Tabulated Summary 2.6.5.12.P) which regulates CYP3A enzymes. Increases in hepatic microsomal CYP1A activity (up to 3.5-fold compared to vehicle treated animals) were also seen, but the magnitude of the changes is very small compared to the typical response to a potent CYP1A inducer. Increasing rat CYP1A activity through AhR activation by COBI would be a species-specific phenomenon as there were no notable increases in CYP1A activity after multiple dosing studies in mice or dogs, and COBI does not activate human AhR[13] (Tabulated Summary 2.6.5.12.N) or increase CYP1A2 activity in human hepatocytes[14] (Tabulated Summary 2.6.5.12.O). After treatment for 104 weeks, accumulation of COBI was observed after multiple dosing, notably in males at 10 and 25 mg/kg/day, and in females at 5 mg/kg/day.

In dogs treated daily with COBI at doses of 5, 15, or 45 mg/kg/day for 4 weeks[15] (Tabulated Summary 2.6.7.7.J) or 5, 10, or 20 mg/kg/day for 39 weeks[16] (Tabulated Summary 2.6.7.7.K), there were no notable sex differences in exposure. As seen in the single-dose studies described above, exposure increased in a greater than dose-proportional manner

from 5 to 15 mg/kg/day (4-week study) or to 20 mg/kg/day (39 week study), likely due to saturation of metabolism, and then was roughly proportional from 15 to 45 mg/kg (4-week study). Dosing for 4 weeks led to an apparent decrease in hepatic microsomal CYP3A activity. As discussed in detail in Section 5.5.2.3.1, unlike its effects in humans, COBI is not an effective mechanism-based CYP3A enzyme inhibitor in nonclinical species, but it is a potent reversible CYP3A inhibitor. In vitro studies[17] (Tabulated Summary 2.6.5.12.J) showed that dog hepatic microsomal CYP3A activity is very sensitive to inhibition by COBI (IC_{50}: 0.12 µM). The apparent reduction in CYP3A activity may be due to the presence of low levels of residual COBI in the microsomal fraction.

Multiple-dose in vivo toxicokinetic studies were also performed with COBI in combination with other agents. These studies were performed in support of safety evaluation and were not intended as pharmacokinetic drug interaction studies as, due to the species differences in CYP3A inhibition noted above, the results would not be representative of those expected in humans. In rats treated for 90 days[18] (Tabulated Summary 2.6.7.7.I), atazanavir (ATV) exposures were higher when co-dosed with COBI than when dosed alone, consistent with inhibition of CYP3A-dependent metabolism of ATV.

3.2. Kinetic Parameters, Bioequivalence and/or Bioavailability

3.2.1. Darunavir

In the rat[15,18] , the absolute oral bioavailability of DRV was 37 to 58%, whereas in the dog[40,41,] it was 60 and 122%, in males and females, respectively (Tabulated Summary 2.6.5.4.R).

For DRV, oral single-dose data are also available from studies comparing different capsule and tablet formulations under fasted or fed conditions in dogs[51,52,53,54,55,56]. No consistent effect of food was observed on the tested formulations. Overall, exposure values observed with these concepts were low relative to gavage administration with PEG400. Therefore, these formulations were not considered further for preclinical testing.

3.2.2. Cobicistat

At doses of 5 or 6 mg/kg, oral bioavailability of COBI was moderate in the rat (33%) and low in the dog and monkey (11% and 7%, respectively).

4. DISTRIBUTION

4.1. Tissue Distribution

4.1.1. Darunavir

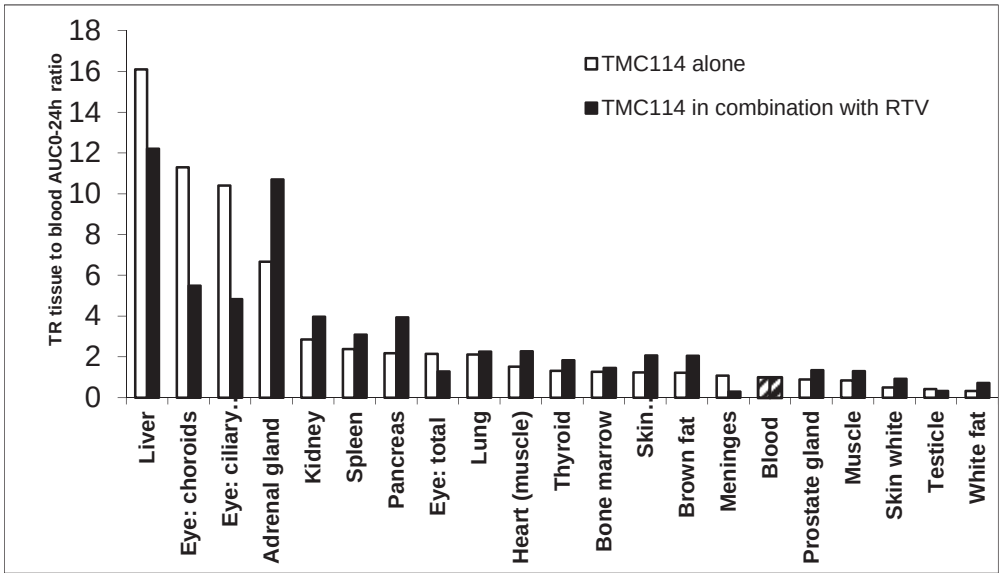
4.1.1.1. *Single Dose*

After a single dose of DRV at 1000 mg/kg, brain and liver tissues were taken in **juvenile rats**[22] aged 12 or 26 days (Tabulated Summary 2.6.5.7.B). On day 12 of age, $AUC_{0\text{-}5h}$ was 215 to 249 µg.h/mL in plasma, 1200 to 1790 µg.h/g in liver and 111 to 133 µg.h/g in brain. On day 26, these values were lower: $AUC_{0\text{-}5h}$ was 59.6 to 102 µg.h/mL in plasma , 371 to 604 µg.h/g in liver and 4.7 to 7.1 µg.h/g in brain. The tissue to plasma AUC ratios were lower in brain on day 26 of age relative to day 12 and were similar in liver between the two age groups. These data indicate that brain and liver DRV tissue concentrations in rats are age dependent and likely influenced by maturation of blood-brain barrier and liver enzymes involved in the metabolism of DRV.

Tissue distribution was also studied in **adult rats**, receiving a single dose of ^{14}C-labelled DRV at 40 mg/kg, with or without RTV (25 or 50 mg/kg/day for 3 days), using quantitative whole-body autoradiography (QWBA). Studies were performed in pigmented male Long Evans rats[57] and in pregnant Sprague-Dawley rats[58] (Tabulated summaries 2.6.5.5.A and 2.6.5.5.B). Distribution to placenta and fetuses was examined in the latter study. Tissue distribution was investigated at several time points, up to 24 and 336 hours, in pregnant Sprague Dawley rats and male Long Evans rats, respectively. In pigmented male Long Evans rats, peak total radioactivity (TR) levels in blood and most tissues were observed 4 hours after ^{14}C-DRV administration (with and without RTV), indicating a rapid absorption and tissue distribution. Without RTV treatment, the highest concentrations of radioactivity were measured in the liver and adrenal gland and were, respectively, 16 and 7-fold higher than levels observed in blood. TR levels in the spleen, pancreas, lung and kidney were

about 2 to 3-fold higher than those in blood. Lowest quantifiable concentrations were found in non-pigmented skin, testicle and white fat. TR levels in all other tissues were either slightly higher or lower than TR levels in blood. Levels in non-pigmented tissues generally declined at a similar rate as those in blood. Treatment with RTV did not have a major effect on tissue to blood ratios (Figure 1) but resulted in increased TR levels in blood and most non-pigmented tissues at the early sampling times. However, TR levels seemed to decrease faster in this dosage group resulting in lower TR levels at later sampling times. In pigmented tissues, in animals dosed with and without RTV, highest TR levels were found in pigmented parts of the eye, i.e. the choroid and ciliary body. In all animals, no undue retention or accumulation of radioactivity was observed in any of investigated tissues 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. Co-treatment with RTV resulted in clearly lower levels in pigmented tissues at later time points.

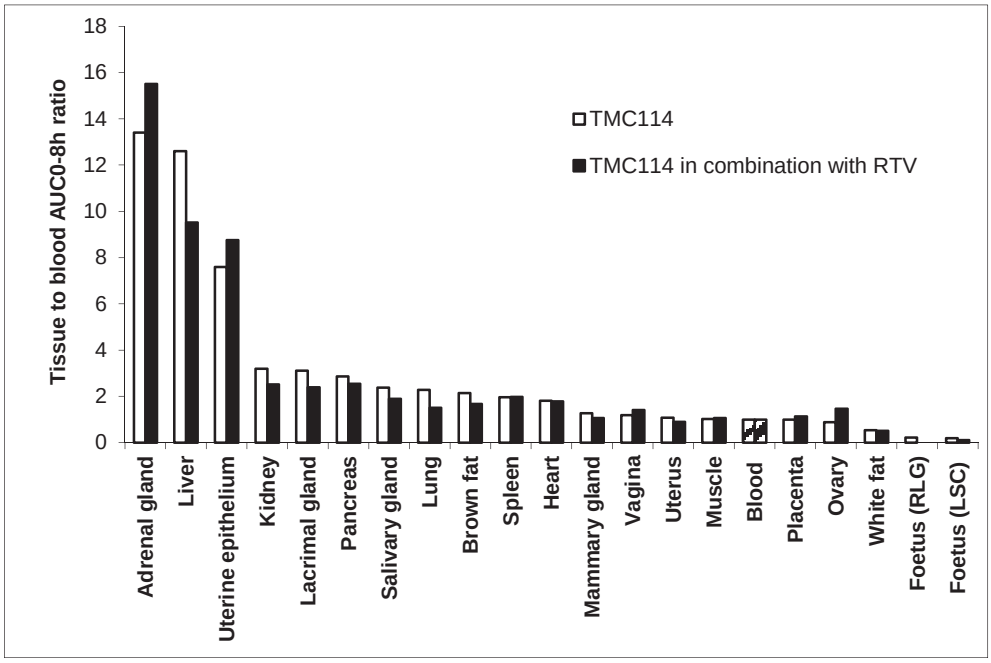
Figure 1: **Tissue to Blood $AUC_{0\text{-}24h}$ Ratios of Total Radioactivity after Oral Administration of ^{14}C-DRV Alone or in Combination with RTV in Male Pigmented Long-Evans Rats**



In pregnant Sprague-Dawley rats, peak TR levels in blood and most of tissues were observed within 1-8 hours after ^{14}C-DRV administration with and without RTV. The highest amounts of TR were again

measured in the adrenal gland and liver and were, respectively, 15- and 11- fold higher than levels observed in blood (Figure 2). Concentrations of TR in the pancreas, kidney, lacrimal gland, spleen, salivary gland, heart, brown fat and lung were about 2 to 3-fold higher than those in blood. TR levels in all other tissues were lower than those in blood. In procreative tissues, highest TR amounts were found in uterine epithelium, about 8-fold higher than in blood. TR exposure in fetus was about 13 to 27% of that in maternal blood. TR exposure in mammary glands was 30% higher and in placenta was equal to TR exposure in blood. Tissue to blood ratios were similar with or without RTV. The TR concentrations however were lower in animals treated with RTV, by about 2.5 fold on average.

Figure 2: **Tissue to Blood AUC_{0-8h} Ratios of Total Radioactivity after Oral Administration of ^{14}C-DRV Alone or in Combination with RTV in Pregnant Rats**



4.1.1.2. *Repeated Dose*

The tissue distribution in Wistar rats with unlabelled DRV and without RTV, was investigated after repeated oral administration at 250 mg/kg/day for 7 consecutive days.[59] Two days after the last dose, plasma concentrations were below the lower limit of quantification.

The highest quantities of DRV were found in the GI contents, adrenals and liver (3900 and 123 and 92 ng/g, respectively).

4.1.2. Cobicistat

^{14}C-COBI was administered orally as a solution to male albino Sprague Dawley rats[19] (Tabulated Summary 2.6.5.5.C) and pigmented Long Evans rats[20] (Tabulated Summary 2.6.5.5.D) at a target dose of 10 mg/kg and 200-250 μCi/kg. The distribution of radioactivity was then determined by QWBA.

After oral administration of ^{14}C-COBI to rats, radioactivity was widely distributed to most tissues by 0.25 hour postdose. Almost all of the tissues reached maximum radioactive concentration by 1 hour postdose. Generally, the radioactivity was preferentially distributed into glandular tissues and organs of elimination. The tissues showing the highest concentrations of radioactivity, excluding the GI tract, included liver, adrenal, kidney, and pituitary. The tissues with the lowest C_{max} values were eye, spinal cord, and brain, bone, seminal vesicles, epididymis, and testes (with concentrations all < 400 ng COBI equivalent/g tissue). 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.

In the Sprague-Dawley rats, clearance from most tissues was not complete by 24 hours postdose; however, radioactivity showed a time-dependent decrease in all tissues examined over the sampling period. Also, in an excretion study an average total of 6.9% of dosed radiolabel was recovered in excreta between 24 and 168 hours postdose (see Section 6.1.2). In the Long Evans rats, there was detectable radioactivity in pigmented tissues and some other tissues at 72 hours postdose, but dosimetry analysis showed that concentrations were declining, indicating association with the tissues was reversible.

4.2. Protein Binding and Distribution in Blood Cells

4.2.1. Darunavir

The plasma protein binding of DRV was studied, in vitro, by equilibrium dialysis for 4 hours at 37° C. One study was performed with unlabelled DRV, using plasma samples obtained from male Wistar rats, male Beagle dogs and male healthy subjects.[60] In the second study, plasma samples from male and female CD-1 mice, male and female Sprague-Dawley rats, female New Zealand white rabbits, male Beagle dogs and healthy male subjects, were fortified with ^{14}C-labelled DRV.[61] The distribution of ^{14}C-labelled DRV in blood was also examined (Tabulated Summaries 2.6.5.6.A and 2.6.5.6.B).

Results are summarized in Table 7. The results of plasma protein binding were comparable per species in both studies. Plasma protein binding was highest in rats and humans (~95%), followed by mice (~90%), dogs (~80%), and rabbits (~63%), at 0.5 µg/mL DRV plasma concentration. For most species, the plasma protein binding appeared to be dependent on DRV concentration, especially at levels higher than 5 µg/mL, and in the rat also at lower levels. In rat plasma, the binding was 95% at 0.052 µg/mL and 55 to 60% at 37.5 µg/mL. In human plasma, the binding was 94% at 0.052 µg/mL and 75% at 18.8 µg/mL. In rabbit plasma, the protein binding was concentration independent over a range of 0.052 to 4.69 µg/mL. In animal species where plasma protein binding was investigated in males and females (rats and mice), no gender-related differences were detected. In human plasma, DRV was mostly bound to (1-acid glycoprotein and to a lesser extent to albumin.

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.

Table 7: Plasma Protein Binding and Blood Distribution of DRV in Various Species [61]

	Human (M)	Dog (M)	Rabbit (F)	Rat		Mouse	
				(M)	(F)	(M)	(F)
Plasma protein binding (%)	95.3	80.7	62.5	94.6	95.0	89.6	90.4
Free fraction (%)	4.7	19.3	37.5	5.4	5.0	10.4	9.6
Blood-to-plasma ratio	0.64	0.85	1.11	0.70	0.70	0.79	0.77
Distribution to plasma water (%)	3.8	10.7	21.2	4.7	4.6	8.0	7.4
Plasma proteins (%)	77.4	45.1	35.4	82.9	85.9	68.4	69.9
Blood cells (%)	18.8	44.2	43.4	12.4	9.5	23.6	22.7

M: male; F: Female.
The DRV concentrations tested were 0.500 µg base-eq./mL in mice, rats and humans and 0.346 µg base-eq./mL in dogs.

4.2.2. Cobicistat

The binding of COBI in plasma from CD-1 mouse[21], Sprague-Dawley rat, beagle dog, cynomolgus monkey, and human[22] (Tabulated Summary 2.6.5.6.C) was assessed by equilibrium dialysis against isotonic phosphate buffer at 37°C for 3 hours (the time determined to achieve equilibration). The data are summarized in Table 8.

The plasma protein binding of COBI was determined to be moderately high in all species, ranging from 90.9% to 97.7% over the concentration range 1 to 30 µM (0.78 to 23 µg/mL). The fraction unbound in human plasma at a COBI concentration of 1 µM (representative of that seen in vivo) was 6.3%. Binding to mouse, rat, and monkey plasma showed modest concentration dependence.

Table 8: Protein Binding for COBI in Mouse, Rat, Dog, Monkey, and Human Plasma Determined by Equilibrium Dialysis (mean ! SD, n = 3)

	Fraction Unbound (%)			
Plasma source	**1 #M COBI**	**10 #M COBI**	**30 #M COBI**	**Mean**
Mouse	3.31 ! 0.14	4.78 ! 0.27	6.15 ! 0.48	4.75
Rat	2.33 ! 0.06	5.34 ! 0.24	8.51 ! 0.48	5.40
Dog	5.68 ! 0.60	6.46 ! 0.60	6.33 ! 0.40	6.16
Monkey	4.31 ! 0.50	6.17 ! 0.50	9.13 ! 0.30	6.54
Human	6.33 ! 0.80	8.92 ! 0.90	7.54 ! 0.60	7.60

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 (Studies GS-US-183-0133[23] and GS US 216-0124[24], respectively).

Whole blood to plasma concentration ratios for COBI can be estimated from the distribution of radioactivity within blood obtained from in vivo studies with ^{14}C-COBI. Representative results are summarized in Table 9.

Blood to plasma ratios are all low, indicating that COBI does not distribute well into the cellular fraction of blood (Tabulated summaries 2.6.5.9.F, 2.6.5.5.C, 2.6.5.5.D and 2.6.5.13.D).

Table 9: Whole Blood to Plasma Concentration Ratios of Radioactivity after Oral Administration of ^{14}C-COBI

Species	Value
CD-1 mouse	0.562 at 1 h (n = 3) [25]
Sprague-Dawley rat	0.605 at 1 h (n = 3) [19]
Long Evans rat	0.600 at 1 h (n = 1) [20]
Beagle dog	0.508 at 1 h (n = 3) [26]
Human	0.589 at 1.5 h (n = 8) [27]

4.3. Placental Transfer

The placental transfer of **DRV** was investigated during the QWBA study in pregnant **rats**[58] (Section 4.1.1.1) (Tabulated summary 2.6.5.5.B). Low concentrations of ^{14}C-DRV were found in placenta and fetus: TR exposures in placenta and fetus were, respectively, equal to and 13 to 27% of those in maternal blood. The administration of RTV did not alter placental and fetal tissue to blood ratios.

Only limited data on distribution to the fetus are available in **rabbits**. Fetal transfer was investigated in pregnant New Zealand White rabbits 2 hours after repeated oral administration of unlabelled DRV at 1000 mg/kg/day from day 8 of gestation to day 20[62] (Tabulated summary 2.6.5.8). Plasma concentrations in the rabbit fetuses were significantly lower than the maternal plasma concentration at 2 hours after dosing. The highest concentration in the fetus (0.0442 µg/mL)

was about 35-fold lower than the maternal plasma concentration and in one out of three fetuses no DRV could be detected. Amnion fluid concentrations were below or close to the lower limit of quantification (0.010 µg/ml).

No placental transfer study was performed with COBI.

5. METABOLISM

5.1. Chemical Structures and Quantities of Metabolites in Biological Samples – In Vivo Metabolism

5.1.1. Darunavir

The metabolic stability of the ^{14}C label of DRV was investigated following a single oral dose of ^{14}C-DRV at 40 mg/kg in Sprague-Dawley rats[2]. The recovery of $^{14}CO_2$ from the expired air collected for 26 hours after administration was negligible. This indicated that the ^{14}C-label was metabolically stable

5.1.1.1. *Studies in Mice*

No studies in mice were performed with DRV.

5.1.1.2. *Studies in Rats*

Two metabolism and excretion studies were conducted with ^{14}C-DRV in Sprague-Dawley rats[63,64] after single **oral** administration at 40 mg/kg. In the first study DRV was dosed alone or with RTV (25 mg/kg/day dosed for 3 days) (Tabulated Summaries 2.6.5.9.B, 2.6.5.9.C, 2.6.5.11.A and 2.6.5.11.B).

Orally administered ^{14}C-DRV was extensively metabolized. Unchanged DRV was mainly excreted in feces: 6.3% and 12.3% of the dose in male and female non-boosted rats (DRV alone) and 20% and 16% in male and female boosted rats (DRV with RTV), respectively. In urine, unchanged compound amounted to less than 1.2%. Unchanged drug accounted for the largest fraction of the radioactivity in plasma of boosted as well as of non-boosted rats. Some circulating metabolites were detected but each of them accounted for less than 9 % of the plasma radioactivity. The metabolite profile of DRV, dosed without RTV, was qualitatively comparable in male and female rats, although some quantitative differences were observed. Metabolites M6 and M23 were the major metabolites in females (14.5% of the dose)

and males (4.4% of the dose) (See Figure 3). Other metabolites included M2 and M21 (2.8% and 6.5% of the dose in males and females, respectively), M33 (3.8% and 4.9% of the dose in males and females, respectively) or M29 (3.6% and 4.2% of the dose in males and females, respectively), M25 together with M27 and M28 (4.7% and 3.2% of the dose in males and females, respectively), M12 (3.1% and 1.5% of the dose in males and females, respectively) and M6 together with M15 and M19 (6.4% and 6.2% of the dose in males and females, respectively). M30 was a minor pathway in males and females (1.2% and 2.1% of the dose, respectively). When co-administered with RTV, the metabolic profile of DRV was both qualitatively and quantitatively comparable in both sexes. Treatment with RTV had inhibitory effects on the aromatic hydroxylation, oxidative ring opening and carbamate hydrolysis pathways in male and female rats. Some metabolites were more obviously present in boosted rats than in non-boosted rats, especially metabolites formed by aliphatic and alicyclic hydroxylation and by N-acetylation. The occurrence of other Phase II metabolites in excreta was very limited. In the second study, the biliary metabolite profile was investigated in the rat[64]. Only about 1% of the dose was recovered unchanged in the bile. The biliary metabolic profile was qualitatively similar to the fecal metabolic profile reported in the previous study[63] except for the presence of glucuronide conjugated metabolites in the bile. There were at least 5 different glucuronide metabolites in the bile, which were not observed previously in the feces, most probably due to hydrolysis by the)-glucuronidase enzymes present in the intestinal lumen.

5.1.1.3. *Studies in Dogs*

The excretion and metabolism in male dogs were studied after a single oral administration of ^{14}C-DRV at 30 mg/kg[65]. (Tabulated Summaries 2.6.5.9.D and 2.6.5.11.D).

^{14}C-DRV was moderately metabolized in dogs. About 26% of the radioactive dose was excreted as unchanged drug and almost exclusively in feces. Darunavir was the major radioactive compound in plasma up to 4 hours post dosing but from 8 hours circulating radioactivity was mainly accounted for by metabolites of DRV. The major plasma metabolites were M15 and M19 (See also Figure 3).

M15 was the major metabolite in urine, whereas DRV was the major radioactive compound excreted in feces. Carbamate hydrolysis appears to be the most important metabolic pathway in dogs (10% of the administered dose). Hydroxylations of metabolites at the isobutyl function and at the aniline group and glucuronidation of metabolites at the aniline moiety were also observed. Combinations of hydroxylation and glucuronidation were reported for specific metabolites.

5.1.1.4. *Studies in Humans*

The metabolism of DRV was investigated in male subjects following a single oral dose of 400 mg alone and in combination with RTV (100 mg b.i.d administered 2 days before and until 6 days after DRV administration)[66]. This study was performed under fasted conditions using ^{14}C-DRV oral solution in 8 male subjects (n=4 per treatment group). (Tabulated Summaries 2.6.5.9.E and 2.6.5.11.E).

^{14}C-DRV was extensively metabolized in non-boosted subjects: only about 8% of the dose was excreted unchanged. Co-administration with RTV markedly reduced metabolism: 48.8% of the dose was excreted unchanged. Unchanged DRV was mainly excreted via feces in non-boosted and boosted subjects (6.8% and 41%, respectively). In urine, unchanged compound amounted to 1.2% and 7.7% in non-boosted and boosted subjects, respectively. In all subjects, unchanged drug accounted for the largest fraction of the radioactivity in pooled plasma samples. The most predominant metabolites were M19, M6, M11 and M15 accounting for 13.2% of the dose (see Figure 3). Other major metabolites included M23 together with M6 (9.8% of the dose) and M29 (4.5% of the dose). M33 was a minor metabolic pathway in the non-boosted subjects (1.6% of the dose) and the excretion of glucuronide metabolites could hardly be observed (0.57% of the dose). In boosted subjects, co-administration of RTV resulted in significant inhibition of the major metabolism pathways. M33 was not inhibited in boosted subjects (1.7% of the dose) and the excretion of glucuronide metabolites (2.2% of the dose) was markedly higher than in non-boosted subjects. No chiral inversion of DRV occurred at the alcohol function in vivo.

The antiviral activities of 3 metabolites (19, 29 and 33) were tested and none of them showed antiviral activity similar to DRV.

5.1.2. Cobicistat

5.1.2.1. *Studies in Mice*

The metabolic profiles of COBI were determined after administration of a single oral dose (target 30 mg/kg) of ^{14}C-COBI to Hsd:ICR(CD-1) mice[25] (Tabulated Summary 2.6.5.9.F).

In plasma, parent drug contributed a large majority (approximately 86% to 91%) of the circulating radioactivity, and M21 and M31 were the most abundant circulating metabolites. In urine collected 0–24 hours postdose, the predominant radioactive peak was M21 and accounted for 0.66% of dosed radioactivity (see also Figure 4). Unchanged parent and all other observed metabolites were each present at ≤ 0.13% of the dose. In feces collected 0–48 hours postdose, unchanged parent drug accounted for 14.5% of the dose. M21 and M31 were the most abundant metabolites and accounted for 13.4% and 5.21% of the dose, respectively. All other metabolites were each < 5% of the dose.

5.1.2.2. *Studies in Rats*

The metabolic profiles of radioactivity derived from ^{14}C-COBI in plasma, bile, urine, and feces following administration of an oral dose (target 10 mg/kg) to rats[28] were evaluated (Tabulated Summary 2.6.5.9.G).

After oral administration, most of the circulating radioactivity was associated with COBI. In addition to unchanged parent drug, M21 was the major circulating metabolite in plasma. In urine, low levels of unchanged parent drug were detected (0.05% of the dose in urine collected 0–24 hours postdose;), while M21 and M31 were the most abundant metabolites, and accounted for 0.43% and 0.21%, respectively, of the dose administered to intact rats (see Figure 4). M21 and M31 were the major metabolites in feces from intact rats, accounting for 11.4% and 7.22% of the dose, respectively. Unchanged parent drug accounted for 5.67% of the dose. No unchanged COBI was detected in bile. M21, M26, M14, M28, and M39 were the major metabolites in this matrix.

5.1.2.3. *Studies in Dogs*

The metabolic profiles of plasma, urine, feces, and bile were determined after administration of a single oral dose (target 5 mg/kg) of ^{14}C-COBI to beagle dogs[29] (Tabulated Summary 2.6.5.9.H).

Cobicistat was the major component circulating in plasma, with M21, M31, and M37 also being detected. Cobicistat was also detected in urine, but the most abundant analytes in that matrix were M56 (a structurally unassigned highly polar peak), M21, and M31 (all < 0.2% of the dose) (see Figure 4). In feces, the most abundant analytes were M21, M31, M39, and COBI, accounting for 12.4%, 8.76%, 8.63%, and 7.15% of the dose recovered within 48 hours, respectively. In bile, the profile was more complex, with only M21 exceeding 5% of the dose. Unchanged COBI accounted for 1.65% of the dose in this matrix.

5.1.2.4. *Studies in Humans*

Following administration of ^{14}C-COBI to humans[27], radioprofiling of pooled (n = 8 subjects) plasma collected revealed COBI as the predominant analyte.

Comparing $AUC_{0\text{-}24h}$ of COBI with that for TR suggests that COBI accounted for 98.6% of the radioactivity over 24 hours. No other peak exceeded 10% of sample radioactivity at any time point (1–24 hours). The majority of the radiolabel was recovered in feces; an average of 85.34% of the dose by 240 hours. Radioanalysis of fecal samples (0-240 hours) revealed that COBI was the most abundant component (26.9% of the dose) with M31, M21, M39, M14, and M26 also being detected (14.0%, 5.47%, 2.41%, 2.40%, and 2.37% of the dose, respectively). All other analytes accounted for < 2% of dosed radioactivity. An average of 7.37% of dosed radioactivity was recovered in urine by 24 hours postdose. The majority (5.45% of the dose) was COBI, with M31 and M21 being the most abundant metabolites (0.7% and 0.09% of the dose, respectively).

5.2. Possible Metabolic Pathways

5.2.1. Darunavir

A number of DRV metabolites were identified in the in-vivo studies in rats[63,64], dogs[65] and humans[66]; the structure of each metabolite and the in vivo metabolic pathways are presented in Figure 3. Darunavir is

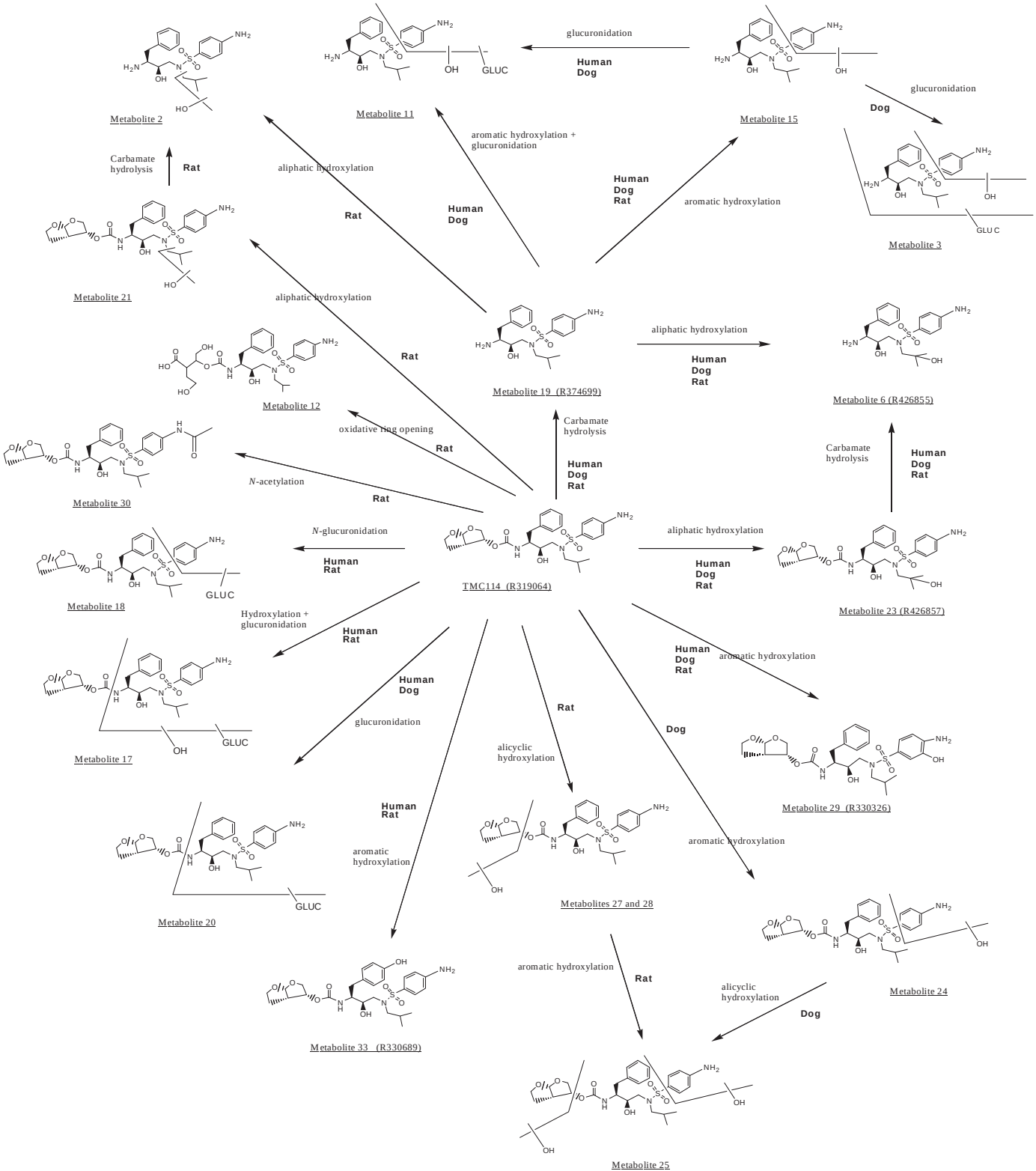
metabolized by Phase I and Phase II biotransformation mechanisms. The most prevalent metabolic pathways were Phase I biotransformations including carbamate hydrolysis, aliphatic hydroxylation at the isobutyl moiety and aromatic hydroxylation at the aniline moiety. Phase II glucuronidation was a minor pathway in rats, dogs and humans. In the rats and humans, co-administration of RTV resulted in the inhibition of certain metabolic pathways mainly carbamate hydrolysis. Metabolic pathways proposed on the basis of in vitro studies were consistent with those observed following in vivo studies. Therefore, the in vitro metabolism data provide a valuable comparison between the various species, including the mouse and rabbit in which the metabolism of DRV was not studied in vivo.

Table 10: Percentage of Administered ^{14}C-DRV Dose Metabolized per Major Pathway in the Rat Dosed With and Without RTV[63], Dog[65] and in Human With and Without RTV[66]

	Rats				Dogs	Humans	
Pathway	DRV		DRV + RTV		DRV	DRV	DRV + RTV
	M	F	M	F	M	M	M
Aliphatic hydroxylation to tertiary alcohol	4.4	14.5	19.6	18.6	4.7	9.8	2.3
Aliphatic hydroxylation to primary or sec. alcohol	2.8	6.5	6.4	5.5		-	-
Aromatic hydroxylation at the benzylic moiety	3.8	4.9	2.9	2.2		1.6	1.7
Aromatic hydroxylation at the aniline moiety	3.6	4.2	1.9	2.1	<5.5	4.5	1.4
Alicyclic hydroxylation at the furan moiety	4.7	3.2	4.8	4.1	<2.2	-	-
Oxidative ring opening	3.1	1.5	0.63	1.0		-	-
Carbamate hydrolysis	6.4	6.2	2.7	2.3	10	13.2	0.67
N-acetylation	1.2	2.1	3.8	4.5		-	-
Glucuronidation	-	-	-	0.14	3.8	0.57	2.2

- : Not detected

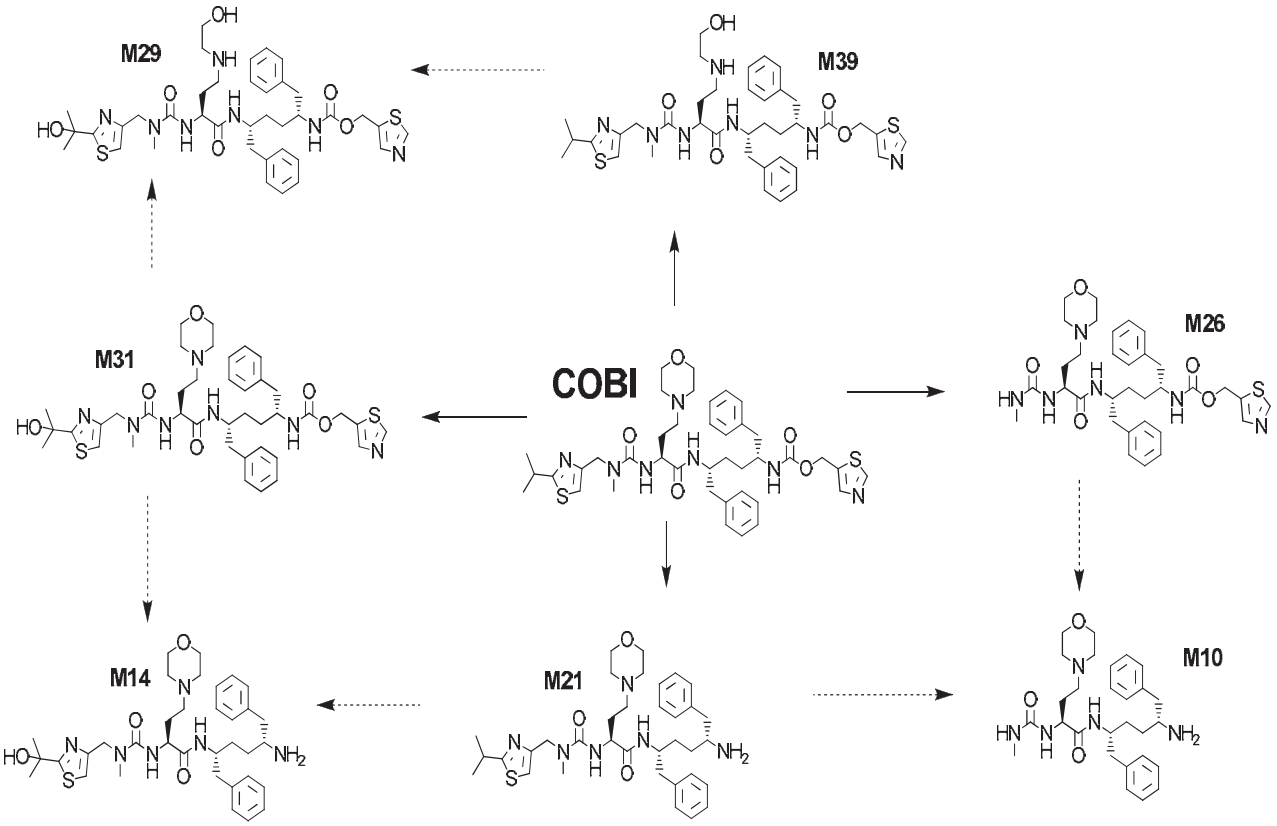
Figure 3: ***In Vivo* Metabolic Pathways of DRV in Animals and Humans**[63,64,65,66]



5.2.2. Cobicistat

Three primary metabolites (M21, M26, and M31) were common to all species in vivo and in vitro and are illustrated in Figure 4. M39, found in all species, is formed by de-ethylation of the morpholine moiety and may involve a 2-step reaction, but no intermediate metabolite was detected and thus this is included here as a primary metabolite. The most common secondary metabolites were formed by combinations of these primary reactions and all possible pairwise combinations of the common primary reactions, M21, M26, M31, and M39 were detected. No direct conjugates of COBI were detected. Common metabolites are illustrated in Figure 4.

Figure 4: Common Primary and Secondary Pathways for Metabolism of COBI by Mouse, Rat, Dog, and Human In Vivo



COBI and all metabolites were detected in samples from mouse, rat, dog and human, except M29 (not in human).

Dashed arrows indicate combinations of primary metabolic pathways, not proven routes of metabolism.

For metabolites for which some structural information was proposed, Table 11 provides a comparison of their presence in plasma and/or excreta of mouse, rat, dog, and human. Cobicistat was detected in all samples apart from rat bile. Urine and plasma generally had similar profiles, with COBI, M21, and M31 being the most significant analytes. The common primary metabolites, M21 and M31, were found in all urine, feces, and bile samples, while M26 and M39 were limited to feces and bile. M14 was the most common secondary metabolite.

Table 11: Cross-Species Comparison of COBI Metabolites

Analyte	Class	Route(s)	Mouse	Rat	Dog	Human
COBI	Parent	Unmodified	PUF	PUF	PUFB	PUF
M10	Secondary	Carbamate cleavage + Dealkylation at N-methylurea	UF	UFB	UFB	UF
M14	Secondary	Carbamate cleavage + Isopropyl oxidation	UF	UFB	UFB	UF
M21	Primary	Carbamate cleavage	PUF	PUFB	PUFB	UF
M26	Primary	Dealkylation at N-methylurea	UF	UFB	UFB	F
M28	Secondary	Dioxidation of isopropyl	-	UFB	-	-
M29	Secondary	Isopropyl oxidation + Deethylation of morpholine	F	FB	FB	-
M31	Primary	Isopropyl methine oxidation	PUF	PUFB	PUFB	PUF
M39	Primary	Deethylation of morpholine	F	UFB	UFB	F
M48	Secondary	Isopropyl oxidation + Core oxidation	F	-	FB	F
M50	Secondary	Isopropyl oxidation + Core oxidation	F	-	FB	-
M65	Primary	Core oxidation (aromatic)	F	-	FB	-

B: detected in bile; F: detected in feces; P: detected in plasma; U: detected in urine

5.3. Presystemic Metabolism (GI/Hepatic First-Pass Effects)

No studies were performed with **DRV** to investigate possible presystemic metabolism. However, the high extent of metabolism contributed to the low bioavailability (37 to 58 %) of DRV in rats. Large numbers of metabolites were detected in the bile including glucuronide metabolites which had not been observed in the feces due to hydrolysis by)-glucuronidases. The bioavailability (60 to 122%) of DRV was higher in dogs and the contribution of the first pass effect seemed lower.

After moderate doses of **COBI**, oral bioavailability in nonclinical species was low due to metabolic instability and resulting high first-pass elimination. No studies were performed with COBI to investigate possible presystemic metabolism because of the species difference in the mechanism of enzyme inhibition there was no valid animal model to study presystemic metabolism in vivo.

5.4. In Vitro Metabolism, Including P450 Studies

5.4.1. Darunavir

5.4.1.1. *In Vitro Metabolic Pathway*

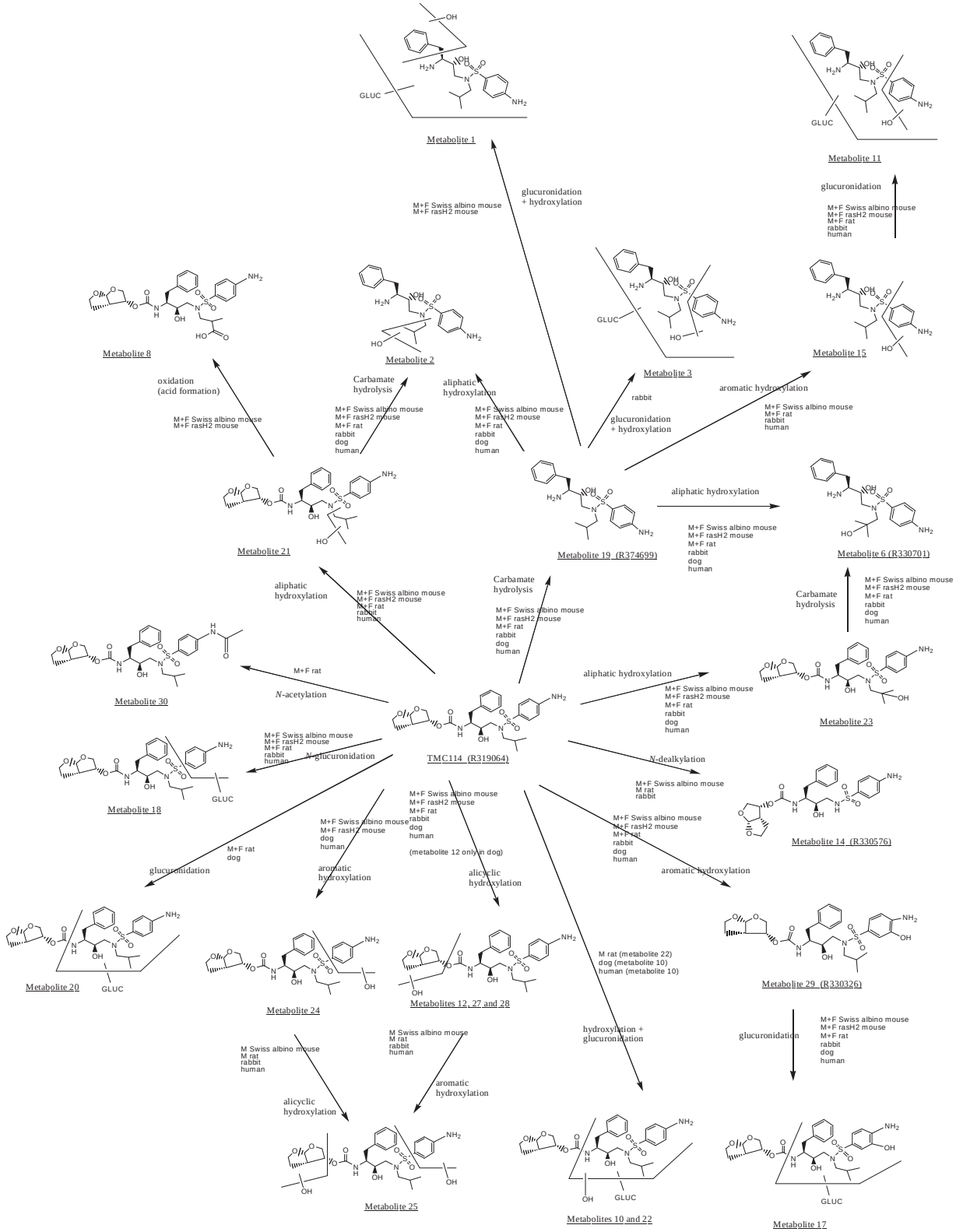
The in vitro metabolism of ^{14}C-DRV was studied in hepatocytes and liver subcellular fractions of male and female CD-1 and rasH2 mice, male and female Sprague Dawley rats, female rabbits, male dogs, and man[67] (Tabulated Summary 2.6.5.10.A).

In each species, a large number of metabolites were detected (Figure 5). Aliphatic hydroxylation occurred at the isobutyl function (M21 and M23), aromatic hydroxylation at the aniline moiety (M24 and M29), and alicyclic hydroxylation at the hexahydro-furo-[2,3-b]furan moiety (M12, M27 and M28). Several of the hydroxylated metabolites were further metabolised by either hydroxylation (M25), oxidation to an acid metabolite (M8), or carbamate hydrolysis (M2). Several metabolites also originated from aliphatic or aromatic hydroxylation, alone or in combination with glucuronidation, of the carbamate hydrolysed metabolite of DRV. Darunavir itself was also glucuronidated, either at the aniline moiety (M18) or at the [(4-amino-benzenesulfonyl)-isobutyl-amino]-1-benzyl-2-hydroxy-propyl moiety

(M20). Hydroxylation in combination with glucuronidation of the latter moiety was also observed (M17). The different hydroxylated metabolites of DRV were all detected in man and also in all animal species. Secondary metabolites, originating from further metabolism of the hydroxylated metabolites of DRV, were observed in man and also in one or more of the animal species. Carbamate hydrolysis occurred in man and in all animal species. Subsequent biotransformation via hydroxylation and glucuronidation was also observed in man and in several of the animal species. Some metabolites, namely an acid metabolite and one of the glucuronidated hydroxylated carbamate-hydrolysed metabolites, were specific for Swiss albino mice and rasH2 transgenic mice. *N*-acetylation was only observed in the rat. Some gender related differences in DRV metabolism were seen in rats and mice; M25 was detected in male mice and rats only, and M22 and 14 only in male rats. In general, all identified metabolites that were detected in human matrices were also detected in at least one animal species.

The in vitro metabolism of ^{14}C-DRV was also studied in liver sub-cellular fractions of male and female juvenile Sprague Dawley rats (aged 12 and 26 days) in a toxicology study[22] and compared with the metabolism of DRV in normal adult male and female Sprague Dawley rats[68] (Tabulated Summaries 2.6.5.10.B and 2.6.5.11.C). Several metabolites were not formed in the liver of juvenile rats at day 12 of age. As a result, this age group had limited metabolic capacity relative to older animals (26 day old and adults). Moreover, no gender related differences were observed in the metabolism of DRV in juvenile rats (12 and 26 days old), unlike in adult rats where such differences were seen.

Figure 5: In Vitro Metabolic Pathways of DRV in Adult Animals and Humans[67]



5.4.1.2. *CYP450 Isozymes Involved in the Oxidative Metabolism of Darunavir*

Two in vitro metabolism studies were performed to characterize the cytochrome P450 isozymes involved in the oxidative metabolism of DRV[69,70]. In these studies, the inhibition of DRV metabolism by known chemical inhibitors of cytochrome P450 isozymes was assessed in HLM (Tabulated Summaries 2.6.5.10.C and 2.6.5.10.D).

From both studies it can be concluded that out of the various human cytochrome P450 isozymes, CYP3A showed dominant metabolic activity towards DRV. Darunavir metabolism in HLM to M6, M19, M23, M27, M28 and M29 was markedly inhibited by CYP3A diagnostic inhibitors (ketoconazole, troleandomycin, RTV). The results of expressed CYP isoforms indicate that the metabolic reactions for the formation of the six metabolites (carbamate hydrolysis as well as aliphatic, alicyclic and aromatic hydroxylation) were catalyzed by CYP3A. The formation of M29 was also catalyzed to some extent by CYP2C19 and CYP2D6 isoforms along with CYP3A isoforms. Other CYP isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8/9 and CYP2E1) were not involved in the metabolism of DRV. In the correlation experiment, the metabolism rates of DRV and its metabolites were also correlated with CYP isoform specific activities of a panel of 10 batches of characterized HLMs. For DRV and its metabolites, positive correlation coefficients (> 0.50) were found with CYP3A isoform activities only.

Based on these results, it can be concluded that the oxidative metabolism of DRV in man is almost exclusively catalyzed by CYP3A.

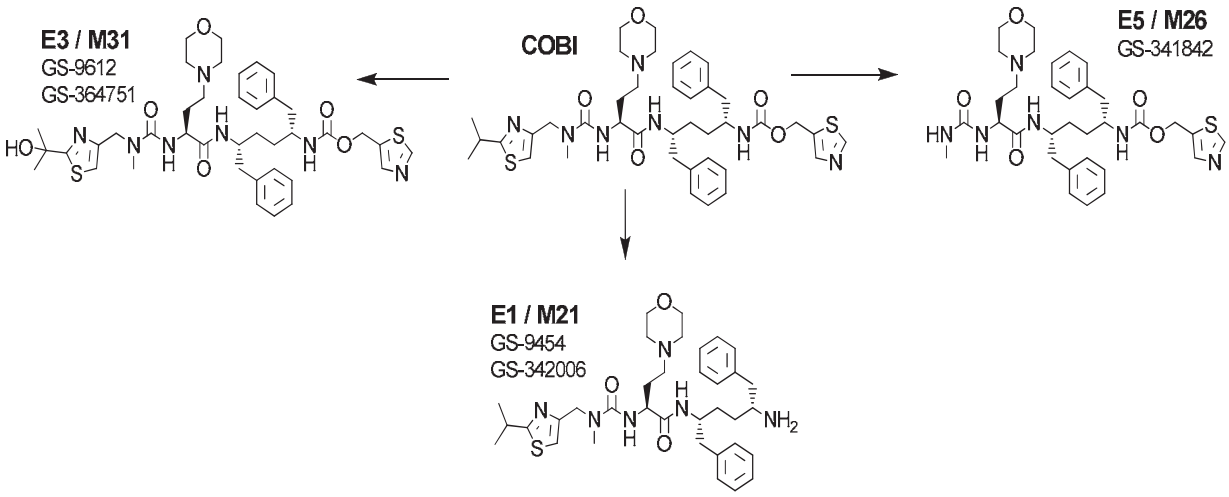
5.4.2. Cobicistat

5.4.2.1. *In Vitro Metabolic Pathway*

Preliminary identification of COBI metabolites was performed using samples generated in vitro by human hepatocytes, and mouse[30], rat, dog, and human[31,32] hepatic microsomal fractions. (Tabulated Summary 2.6.5.10.E). In all species, apart from dog, metabolism of COBI could be accounted for by the generation of 3 metabolites. These metabolites were initially denoted E1, E3, and E5, but during comprehensive cross-species radioprofiling of samples generated in

vivo, the metabolites were named M21, M31, and M26, respectively. In dog microsomal samples, the pattern was more complex, but M21, M31, and M26 were still the most abundant metabolites. The assigned structures of M21, M31, and M26 are illustrated in Figure 6. Further metabolite identification studies using samples generated in vivo (see below) also found these 3 metabolites to be the most abundant.

Figure 6: Common Primary Pathways for Metabolism of COBI by Mouse, Rat, Dog, and Human In Vitro



The rates of hepatic metabolism of COBI were assessed in vitro in cryopreserved human hepatocytes and hepatic microsomes from mouse[30], rat, dog, monkey, and human[31] (Tabulated Summary 2.6.5.10.E).

The in vitro half-life for COBI when incubated with human hepatocytes was 12.7 hours, yielding a predicted human hepatic clearance of 0.19 L/h/kg. With microsomal fractions, the rank order for species (by increasing predicted hepatic extraction) was CD-1 mouse < human < Sprague Dawley rat < beagle dog < cynomolgus monkey. Prediction of human clearance of COBI is rendered complex by enzyme inactivation occurring during the incubations (see section 5.5.2.3.1).

5.4.2.2. *CYP450 Isozymes Involved in the Oxidative Metabolism of Cobicistat*

The rates of metabolism of COBI and RTV were determined by incubating the compounds with cDNA expressed human CYP450 enzyme preparations coexpressed with human NADPH cytochrome P450 reductase[33] (Tabulated Summary 2.6.5.10.F). Cobicistat was a substrate for CYP2D6 and CYP3A4, but there was no significant metabolism by the other 3 enzymes tested (CYP1A2, CYP2C9 and CYP2C19). It was consistent with another study[34] confirming that CYP3A4 is the major catalyst of COBI metabolism by human hepatic microsomal fraction. In addition, neither recombinant human CYP2B6 nor CYP2C8 metabolised COBI to a significant extent, despite efficient turnover of positive control substrates[35]. Ritonavir was also metabolized by CYP2D6 and CYP3A4 and there was also detectable metabolism by CYP2C19. The apparent slow rates of metabolism of COBI and RTV by CYP3A4 are likely due to self-limiting inhibition during the incubation

5.5. Enzyme Induction and Inhibition

5.5.1. Darunavir

5.5.1.1. *Ex-vivo Studies Measuring Enzyme Activities in animals*

Ex-vivo studies were conducted in mice, rats and dogs to determine if daily oral administration of DRV during 1 month (50 and 500 mg/kg/day), 3 months (up to 1000 mg/kg/day), 4 weeks (up to 500 mg/kg/day; with and without RTV), and 12 months (up to 120 mg/kg/day), respectively, would affect hepatic enzyme activity[71,72,73,74] (Tabulated Summary 2.6.5.12.A, 2.6.5.12.B, 2.6.5.12.C and 2.6.5.12.G).

Similar CYP450 isozyme induction patterns were found in mice and rats: DRV induced CYP3A and is probably a weak inducer of CYP2B and possibly other CYP subfamily forms. UGT activity was additionally induced in rats. In dogs, no induction effects were observed.

5.5.1.2. *In Vitro Induction of Human CYP450 Enzymes*

5.5.1.2.1. In Vitro Study Measuring CYP mRNA Induction in Human Hepatocytes

The potential of DRV to induce CYP enzymes was determined in primary hepatocyte cultures from cryopreserved human hepatocytes originating from 3 different donors and compared to data obtained with the positive controls omeprazole, rifampicin, phenobarbital and RTV[75]. The concentrations of DRV in the culture medium were 2.5, 10, 25, or 50 µM (28 µg/mL). Induction of CYP enzymes was assessed at the end of the 48 hour treatment period, by measurement of mRNA expression with TaqMan quantitative RT-polymerase chain reaction. Cytotoxicity was also determined by measurement of intracellular Adenosine triphosphate (ATP) content (Tabulated Summary 2.6.5.12.D).

Treatment of the hepatocytes did not result in a significant cytotoxic and different concentrations of DRV resulted in a consistent increase in the intracellular ATP content.

Treatment of the cells with different concentrations of DRV resulted in a minor statistically non-significant increase of CYP1A2 mRNA expression at the highest concentration only. At that concentration, treatment with DRV resulted in a significant increase of CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, and CYP3A4 mRNA expression (2.4-up to 5.9-fold; 5.5-fold for CYP3A4). The rank order of induction potential of DRV was: rifampicin > RTV ≅ DRV > phenobarbital. It is unclear, however, if the observed induction in mRNA levels will translate to induction in the activity of individual CYP450 isoenzymes.

5.5.1.2.2. In Vitro Study Measuring CYP Activity Induction in Human Hepatocytes

The potential of DRV to induce CYP enzymes was determined in primary hepatocyte cultures from cryopreserved human hepatocytes originating from 3 different donors and compared to data obtained with the positive controls omeprazole and rifampicin[76]. Human hepatocytes were treated for 3 consecutive days with vehicle (DMSO), DRV (2.5 and 50 µM (28 µg/mL)), omeprazole (25 µM) or rifampicin (50 µM).

Induction of CYP activities (CYP1A2, CYP2B6, CYP2C19 and CYP3A4) was assessed at the end of the 72 hour treatment period using the corresponding CYP probe substrates (7-ethoxy resorufin (CYP1A2), s-mephenytoin (CYP2B6 and CYP2C19) and testosterone (CYP3A4). Spectrofluorometry and LC-MS/MS assays were used to measure the products of probe substrates to determine the CYP activity of the hepatocytes. (Tabulated Summary 2.6.5.12.E)

Darunavir showed a concentration dependent effect on induction of CYP3A4 in human hepatocytes. The mean fold induction of CYP3A4 activity was 2.2 and 6.8 at 2.5 and 50 µM (28 µg/mL) of DRV, respectively. The compound was not an inducer of CYP1A2 and CYP2C19 in human hepatocytes, while CYP2B6 was only marginally increased.

5.5.1.3. *In Vitro Inhibition*

5.5.1.3.1. Inhibition of P450 Isozymes by Darunavir

Darunavir was tested for its effect on the metabolism of various human CYP450 probe substrates to gain information about the possibility of clinical relevant interactions with other drugs[77,78] (Tabulated Summaries 2.6.5.12.F and 2.6.5.12.H). For that purpose, incubations with P450 probe substrates selective towards CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A were performed in HLMs in the absence and presence of DRV. The extent of inhibition was evaluated with clinically relevant concentrations of DRV.

The results showed that CYP3A is most potently inhibited by DRV and this inhibition might be clinically relevant since the K_i value is relatively low (0.4 #M; 0.22 µg/mL). The K_i values for the other CYP450 isoenzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1) were at least 60-fold higher, indicating a much lower affinity and less potential for interaction.

5.5.1.3.2. In Vitro Inhibition of Human UGT1A1

The potential inhibition of UGT1A1-mediated bilirubin glucuronidation by DRV in a pooled batch of HLM was investigated[78]. (Tabulated Summary 2.6.5.12.H).

The Ki values of DRV and RTV for UGT1A1 amounted to 201 ! 18 µM and 7.2 ! 0.8 µM respectively, indicating that DRV has only a mild inhibitory potency towards bilirubin glucuronidation and that RTV is a much stronger inhibitor.

5.5.2. Cobicistat

5.5.2.1. *Ex-vivo Studies Measuring Enzyme Activities in animals*

Administration of COBI at dose levels of 5, 15, and 50 mg/kg/day for 13 weeks[7] (Tabulated Summary 2.6.7.10) to male and female **mice** for resulted in no notable (≥ 2 fold) change compared to control animals in protein yield, total CYP450 content, or CYP1A, CYP2D, CYP2E, CYP4A, and UGT activities. No notable (≥ 2 fold) changes compared to control animals in CYP2B and CYP3A activities were observed in male and female animals at 5 and 15 mg/kg/day. However, notable increases (3.4- and 3.7-fold, and 3.0- and 2.5 fold in males and females, respectively) in CYP2B and CYP3A activities were observed at 50 mg/kg/day.

In a non-GLP study in **rats**[36] (Tabulated Summary 2.6.7.6) after oral administration of COBI or RTV for 14 days, analysis of liver microsomal enzymes showed no notable changes in total cytochrome P450 content, and CYP1A, CYP2B, CYP2E, and UGT activities in COBI and RTV treated animals. However, at 175 or 300 mg/kg/day COBI, slight decreases in total protein, and slight to marked decreases in CYP4A and CYP3A activities were observed. At 100 mg/kg/day RTV, there were marked decreases in CYP3A activity. Slight increases in CYP3A activity at 30 and 100 mg/kg/day COBI likely reflect induction due to the activation of rat pregnane X receptor (PXR), which has been demonstrated in vitro (see Section 5.5.2.2.1). The reduction in CYP3A activity may be due to a combination of the high exposure to the compounds, the weak, but detectable, mechanism-based inhibition of rat CYP3A activity noted in vitro, and the reversible inhibition by COBI or RTV caused by carryover of drug remaining in the liver when the microsomal fractions were prepared.

Administration of COBI to male and female **rats** at dose levels of 10, 30, and 100 mg/kg/day for 26 weeks[11] (Tabulated Summary 2.6.7.7.H)

resulted in no notable changes in protein yield, total CYP450 content, and CYP2B, CYP2E, CYP4A, and UGT activities. However, notable to marked increases in CYP1A and CYP3A activities were observed in females at 30 mg/kg/day, and in males and females at 100 mg/kg/day. Decreases in CYP2C activity were noted in males, with notable increases in females at 100 mg/kg/day. The maximum increase in CYP1A activity (3.5-fold increase in males and 2.4-fold in females) represents only a small fraction of that achievable by a positive control suggesting that COBI is a weak inducer of CYP1A in this species.

Administration of COBI and ATV either alone or in combination to male and female **rats** for 13 weeks[18] (Tabulated Summary 2.6.7.7.I) resulted in no notable (+ 2-fold increase) in protein yield, total cytochrome P450 content, CYP1A, CYP2B, CYP2B/2C, CYP2E, CYP4A, and UGT activities. Administration of 30 mg/kg/day COBI to male rats resulted in a notable increase (2.1-fold) in CYP3A activity. In females, there was a marked increase (5.6-fold) in CYP3A activity in the 30 mg/kg/day COBI group, and notable increases (3.5- and 2.6-fold, respectively) for the 30/20 and 30/50 mg/kg/day COBI/ATV combination groups.

In the 4 week study in **dogs**[15] (Tabulated Summary 2.6.7.7.J), analysis of liver microsomal enzymes resulted in no notable change in protein yield, total cytochrome P450 content, or CYP1A, CYP2B, CYP2E, CYP4A, and UGT activities. However, CYP3A activity was decreased in all COBI dose groups. Following a 4 week recovery phase, the observed decrease in CYP3A was found to be reversible. The apparent reduced microsomal CYP3A activity may reflect inhibition by COBI remaining in the liver when the microsomal fractions were prepared.

5.5.2.2. *In Vitro Induction*

5.5.2.2.1. Induction of Metabolizing Enzymes of Rat by COBI In Vitro

To better understand the changes in microsomal cytochrome P450 levels seen in multiple-dose rodent toxicology studies, the potential for induction of rat drug metabolizing enzymes and transporters through the activation of PXR by COBI was assessed in vitro using a reporter cell line[9] (Tabulated Summary 2.6.5.12.P). Both COBI and RTV

activated rat PXR in a concentration-dependent manner, with potencies similar to that of the moderately potent inducer, miconazole. Concentrations of COBI and RTV up to 30 µM had no significant effect on cell viability. At a COBI concentration of 100 µM (78 µg/mL), the relative viability was reduced to 77% of the 1% (v/v) DMSO vehicle control.

The results suggest that, in contrast to its lack of effect on human PXR (Section 5.5.2.2.2), COBI has the potential to activate rat PXR and increase the expression of proteins regulated by this receptor, such as rat CYP3A, UGT1A1, and OATP2, in repeat-dose toxicology studies (see Section 5.5.2.2.2).

5.5.2.2.2. Xenobiotic Receptor Activation by COBI

The potential for COBI to induce human drug metabolizing enzymes and transporters through activation of the human AhR or the human PXR was initially evaluated by receptor transactivation analysis (Tabulated Summary 2.6.5.12.N)[13]. The studies were performed using human hepatoma cell lines transfected with expression vectors for the receptors and containing the promoter of an appropriate responsive gene linked to firefly luciferase as a reporter gene. The cell lines were DRE, expressing AhR and with the human CYP1A2 promoter and DPX2 (PXR with CYP3A4 promoter).

At concentrations up to 10 µM, neither COBI nor RTV showed significant activation of AhR. In contrast, RTV showed significant activation of PXR (10-fold at 10 µM), while COBI was much weaker (2.2 fold at 10 µM (5.5 µg/ml)). Cobicistat is therefore expected to have little liability to cause clinically relevant drug drug interactions through PXR activation and is very unlikely to activate AhR at clinically used doses. The lack of efficacy at human PXR was species specific as COBI, like RTV, was found to activate rat PXR in a cell-based system (Section 5.5.2.2.1).

5.5.2.2.3. In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes

The potential of COBI to induce drug metabolizing enzymes and transporters was further evaluated in primary cultures of human

hepatocytes[14] (Tabulated Summary 2.6.5.12.O). Cobicistat (1, 3, 10, and 30 μM) and known inducers (3-methylcholanthrene, phenobarbital, and rifampicin) were incubated in cultures of human hepatocytes from 3 separate donors for 3 consecutive days. The activities of CYP1A2, CYP2B6, and CYP3A were determined using selective metabolite markers (CYP1A2-catalyzed acetaminophen formation from phenacetin; CYP2B6-catalyzed hydroxybupropion formation from bupropion; and CYP3A-catalyzed 6β-hydroxytestosterone from testosterone). Other endpoints assessed were mRNA expression levels for CYP1A2, CYP2B6, CYP3A4, UGT1A1, and MDR1 and immunodetectable CYP3A protein.

Cobicistat was not cytotoxic to hepatocytes at concentrations up to 30 μM (16.5 μg/mL). No clear concentration related increases of CYP1A2, CYP2B6, or CYP3A enzyme activity were observed with any of the concentrations of COBI examined in any of the human donor preparations. Maximal increases in enzyme activity in individual donors were 4.2%, 20.5%, and 8.2% of the positive controls for CYP1A2, CYP2B6, and CYP3A, respectively. For CYP3A activity, most activities were below those of the vehicle control, likely due to enzyme inactivation. The lack of response of CYP1A2, a sensitive marker for AhR activation, and CYP2B6, a sensitive marker for CAR activation, was confirmed at the mRNA level. The mRNA expression for CYP3A4, a sensitive marker for PXR activation, was increased weakly in a concentration-dependent manner, reaching an average of 27.4% of the positive control at 10 μM COBI. Immunodetectable CYP3A protein was also increased in a concentration-dependent manner. Secondary markers for PXR activation, UGT1A1 and MDR1 mRNA expression, were increased only at high concentrations of COBI (MDR1) in a hepatocyte donor-dependent manner (UGT1A1).

Collectively these data corroborate the analyses performed by xenobiotic receptor transactivation analysis (Section 5.5.2.2.2): COBI does not activate human AhR and is a very weak activator of human PXR. At plasma concentrations found in humans, COBI would be expected to have no effect on the expression of secondary targets of PXR, such as CYP2C9, CYP2C19, UGT1A1, and MDR1, and would have very little effect on the expression of CYP3A4 mRNA. Any

effect on CYP3A enzyme activity would be masked by mechanism based inhibition

5.5.2.3. *Inhibition of P450 Isozymes by Cobicistat*

5.5.2.3.1. Inhibition of CYP3A Activity by COBI

The intended pharmacological effect of COBI is inhibition of human CYP3A enzyme activity. CYP3A inhibition studies in human hepatic microsomal fractions, using an established clinical CYP3A inhibitor, RTV, as a comparator, were performed to test the generality of CYP3A inhibition, mechanism of inhibition, and enzyme inactivation parameters[37] (Tabulated Summary 2.6.5.12.I).

Cobicistat was a potent inhibitor of all human hepatic microsomal CYP3A activities tested, including established CYP3A probe activities (midazolam 1' hydroxylase, testosterone 6) hydroxylase, and terfenadine t-butyl hydroxylase), and clinically relevant interactions (elvitegravir hydroxylase, atazanavir oxidase, telepravir oxidase). Studies with midazolam 1' hydroxylase and testosterone 6) hydroxylase showed that the apparent inhibitory potency could be increased in a preincubation time-dependent and NADPH cofactor-dependent manner, suggesting that COBI is a mechanism-based inhibitor of human CYP3A enzymes. Detailed enzyme inactivation kinetic studies were performed with COBI and RTV. Cobicistat was found to be an efficient inactivator of human hepatic microsomal CYP3A activity, with kinetic parameters (k_{inact} = 0.47 min^{-1}, K_I = 1.1 μM) similar to those of RTV (k_{inact} = 0.23 min^{-1}, K_I = 0.26 μM), but with a higher theoretical maximal inactivation rate and a lower affinity.

To allow an understanding of the non-dose-linear pharmacokinetics seen in nonclinical species, and the pharmacokinetic drug interactions in the ATV/COBI combination toxicology studies, the effect of COBI on rat, dog, and monkey hepatic microsomal CYP3A activity was assessed[17] (Tabulated Summary 2.6.5.12.J). Midazolam 1' hydroxylase was used as the probe activity and RTV was tested in parallel. Both RTV and COBI were potent inhibitors of midazolam 1'-hydroxylase activity in all 3 species. In dog and monkey, there was no evidence for preincubation time-dependence, indicating that these compounds were

potent reversible inhibitors, but not mechanism-based inhibitors in these species. Inactivation of hepatic microsomal activity of the rat was much less efficient than human (k_{inact} values 10 fold lower), suggesting that reversible inhibition would predominate in the rat.

5.5.2.3.2. Inhibition of Other Human Cytochromes P450

Having demonstrated that COBI is a potent mechanism-based inhibitor of human CYP3A activities, the specificity of inhibition was assessed using pooled human hepatic microsomal fractions and enzyme-specific activities[38,39] (Tabulated Summary 2.6.5.12.K). Ritonavir was tested in parallel as a relevant comparator.

As reported above (Section 5.5.2.3.1), both COBI and RTV were potent inhibitors of CYP3A activities in vitro, with IC_{50} values less than 0.2 µM. At concentrations up to 25 µM (13.8 µg/mL), neither COBI nor RTV inhibited CYP1A2 or CYP2C19 activity. In contrast to RTV, COBI did not inhibit CYP2C9, and COBI was a weaker inhibitor of CYP2D6. Cobicistat is a potent inhibitor of human CYP3A activity and a weak inhibitor of CYP2D6 activity. Cobicistat was a weak inhibitor of CYP2C8 activity in vitro, with potency lower than RTV. Although COBI was a stronger inhibitor of CYP2B6 activity, with a potency very similar to RTV, the IC_{50} is above the human plasma C_{max} value. At clinically relevant plasma concentrations, COBI is unlikely to cause drug interactions by inhibition of CYP2C8 and interactions with CYP2D6 and CYP2B6.

5.5.2.3.3. Inhibition of Human Cytochromes P450 by Human Metabolites of COBI

Three metabolites of COBI were initially identified during incubations with both human hepatocytes and human hepatic microsomal fractions. These metabolites are also the most abundant in human feces and urine (see Section 5.1.2.4). The effects of these metabolites on the activities of 5 major human drug metabolizing cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP3A4 and CYP2D6) were assessed[40] (Tabulated Summary 2.6.5.12.L). Multiple CYP3A activities were tested because of the known substrate-dependent inhibition potency of this enzyme.

Metabolites M21 (E1, GS-342006; cleavage at carbamate) and M26 (E5, GS-341842; dealkylation at urea) showed substantially less inhibition of human CYP3A activity compared to COBI and are thus unlikely to contribute to the pharmacologic effect. These metabolites also show no ability to inhibit other drug metabolizing cytochromes P450. Metabolite M31 (E3, GS-364751; oxidation of isopropylthiazole) is an inhibitor of human CYP3A activity, but somewhat weaker than COBI. M31 is also a more potent inhibitor of CYP2C19 and CYP2D6 activities. The contribution of M31 to the pharmacologic effect of COBI and the potential to cause drug interactions through inhibition of CYP2D6 activity is unlikely to be significant as circulating concentrations of this metabolite are very low. In addition, none of these 3 metabolites inhibited CYP2B6 (IC_{50} > 25 μM) and all three showed weak or no inhibition of CYP2C8 (IC_{50} values > 25, 13.7 and 10.9 μM for M21, M26 and M31, respectively)[41].

5.5.2.3.4. In Vitro Inhibition of Human UGT1A1

The potential for COBI to inhibit the catalytic activity of human UGT1A1 was evaluated[42] (Tabulated Summary 2.6.5.12.M). The rates of formation of β-estradiol-3-glucuronide from β estradiol substrate by hepatic microsomal fractions were determined in the presence and absence of COBI and IC_{50} values were determined where possible. Ritonavir and ATV were used as comparators. Cobicistat was a weak inhibitor of human UGT1A1 activity, being 19.6-fold less potent than the positive control, ATV, and 3.4-fold less potent than RTV.

6. EXCRETION

6.1. Routes and Extent of Excretion

6.1.1. Darunavir

Excretion was evaluated after single oral administration of ^{14}C-DRV in male and female Sprague-Dawley rats at 40 mg/kg (with or without RTV at 25 mg/kg/day)[63] and in male dogs at 30 mg/kg[65]. Excretion of the radioactivity was monitored over a 96-hour and 168-hour period in rat and dog, respectively. Biliary excretion was examined in male rats dosed at 40 mg/kg[64] (Tabulated Summaries 2.6.5.9.B, 2.6.5.9.C, 2.6.5.11.A and 2.6.5.11.B).

The predominant route of excretion of ^{14}C-DRV was via feces. It averaged 94% in male and female rats. The excretion of radioactivity in the feces was rapid and accounted for more 80% in the first 24-hour collection period. In the rat, co-administration with RTV had no substantial effect on the excretion mass balance (Table 12) and the elimination rate of ^{14}C-DRV. There were no significant differences in excretion of the radioactivity between male and female rats. Excretion in the dog was monitored over a 168-hour period, and 86% of the administered radioactivity was recovered from the feces (Table 12). Fecal excretion was rapid and accounted for 70% in the first 24-hour period. Urinary excretion was limited and represented 4.2% and 3.9% of the administered dose in rats and dogs, respectively. In a biliary excretion study in Sprague-Dawley rats, an average of 54% of the radioactivity was excreted in the bile during the 24h period after DRV administration.

Table 12: Urinary and Fecal Excretion of the Radioactivity Following Single Oral Administration of ^{14}C-DRV in Rat[63], and Dog[65]

% of administered dose	Rat				Dog
	M		F		M
	DRV	DRV + RTV	DRV	DRV + RTV	DRV
urine	3.87	5.76	4.48	6.47	3.93
feces	92.61	94.75	93.72	88.54	85.86
cage washings	0.24	0.15	0.21	0.25	1.56
total	**96.72**	**100.66**	**98.41**	**95.27**	**91.38**

M: male; F: female

In man[66], urinary excretion was quantitatively higher than in rats and dogs, accounting for 12.2% of the radioactive dose. Nevertheless, fecal elimination was still the predominant route of excretion, accounting for 81.7% of the dose. Excretion of the radioactivity did not change when DRV was co-administered with RTV.

6.1.2. Cobicistat

The excretion of radioactivity was determined after administration of a single oral dose of ^{14}C-COBI to Hsd:ICR(CD-1) mice[25] (target 30 mg/kg), male Sprague-Dawley rats[19] (10 mg/kg) and to male beagle dogs[26] at 5 mg/kg (Tabulated Summaries 2.6.5.13.B, 2.6.5.13.C; and 2.6.5.13.D). In addition, ^{14}C-COBI was dosed orally to male bile duct-

cannulated Sprague-Dawley rats[19] at 10 mg/kg (Tabulated Summary 2.6.5.14.A) and to male bile duct-cannulated beagle dogs at 10 mg/kg[43] (Tabulated Summary 2.6.5.14.B).

In **mice,** radioactivity derived from ^{14}C-COBI was rapidly excreted, primarily within the first 24 hours after dosing. An average of 85.9% of the administered radioactivity was excreted in feces and 2.00% was excreted in urine by 168 hours postdose. Average overall recovery of radioactivity was 88.7%.

In unmodified **rats**, recovery in excreta collected up to 168 hours postdose was high (mean ! SD = 93.5% ! 1.6% of dose, n = 3). The majority of the radioactivity (>90%) was recovered in the first 48 hours, with 89.6% in the feces and 1.9% in the urine. In bile duct-cannulated rats, recovery of radioactivity in the excreta (93.2% ! 2.84%) was almost identical to that of unmodified rats, with an average of 69.3% in the bile and 4.2% in the urine; indicating that at least 73.5% of dosed radioactivity was absorbed and that biliary excretion was the major route of elimination of radioactivity. Excretion of radioactivity in bile was relatively rapid with an average of 57.6% by 8 hours postdose. Only 2.5% of dosed radioactivity was recovered in excreta 48 to 168 hours after dosing.

In unmodified **dogs**, recovery in excreta collected up to 168 hours postdose was high (mean ! SD & 86.12% ! 0.96% of dose, n = 3). The majority of the radioactivity (, 80%) was recovered in the first 48 hours, with 76.8% in the feces and 1.8% in the urine. In bile duct-cannulated beagle dogs, of the 3 dogs dosed, 1 was an outlier in which no radioactivity was detected in plasma or blood at any time point, and 90.19% of dosed radioactivity was found in the first urine sample, collected 0–12 hours postdose. The data for the other 2 dogs were very comparable. In those 2 dogs, mean total recovery was high (90.3%), with 63.9% in bile and 1.88% in urine, suggesting at least 65.8% of dosed radioactivity was absorbed. More than half of dosed radioactivity was recovered by 8 hours postdose and the majority was recovered by 72 hours postdose (only 3.2% recovered in excreta after 72 hours).

6.2. Excretion in Milk

6.2.1. Darunavir

Darunavir was administered orally by gavage in rat dams at 1000 mg/kg for 4 days (Days 2-5, 5-8 and 8-11).[79]

Milk to plasma $AUC_{0\text{-}8h}$ ratios, which are used as an index for DRV distribution to milk, were 2.3, 1.9 and 1.7, indicating DRV excretion in milk. In pups, DRV concentrations were much lower relative to levels observed in dams. Therefore it was concluded that systemic exposure in pups exposed to DRV via milk was limited (Tabulated Summary 2.6.5.13.A).

6.2.2. Cobicistat

The excretion of COBI in rat milk was examined as part of a postnatal development study[44]. Two hours after treatment of lactating females (postnatal Day 10) with COBI, mean milk/plasma concentration ratios were 1.3, 1.9, and 1.7 after doses of 10, 30, and 75 mg/kg/day, respectively (Tabulated Summary 2.6.7.14.C), indicating that COBI is distributed into milk in this species.

7. PHARMACOKINETIC DRUG INTERACTIONS

7.1. Darunavir

The potency of known anti-HIV compounds (delavirdine, saquinavir, RTV, indinavir, nelfinavir, amprenavir, and atazanavir) to inhibit hepatic metabolism of DRV was investigated, in vitro, using HLM[70,80].

All tested anti-HIV compounds inhibited the metabolism of DRV. Based on K_i values, RTV, indinavir and nelfinavir are likely to inhibit DRV metabolism, in vivo. In line with expectations, RTV was the most potent inhibitor of DRV metabolism.

7.2. Cobicistat

No studies were performed.

8. OTHER PHARMACOKINETIC STUDIES: Membrane transporters

8.1.1. Darunavir

The potential for DRV to inhibit human drug transporters was assessed in OCT2- and MATE1-transfected CHO cells[81]. The positive control

inhibitors were verapamil (100 µM) for OCT2 and quinidine (100 µM) for MATE1. The highest concentration of DRV tested was 100 µM.

OCT2-transfected CHO cells showed a flux 15.9-fold higher than wild-type cells. The DRV IC_{50} was higher than 100 µM (maximum effect was 30.66% ± 5.46 inhibition at 100 µM). MATE1-transfected CHO cells showed a flux 10.9-fold higher than wild-type cells. The DRV IC_{50} was higher than 100 µM (maximum effect was 44.37% ± 5.19 inhibition at 100 µM). These results showed that DRV has a low potential to inhibit OCT2 and MATE1 transporters (Tabulated Summary 2.6.5.15.A).

8.1.2. Cobicistat

8.1.2.1. *Inhibition of Individual Recombinant Expressed Human Drug Transporters*

The potential for COBI to inhibit human drug transporters was assessed in cell lines or vesicles expressing individual recombinant proteins. Transporters tested, and their respective positive control inhibitors, were MDR1 (20 µM verapamil), MRP1 (100 µM caffeic acid phenethyl ester), MRP2 (100 µM MK571), MRP4 (150 µM MK571), BCRP (2 µM fumitremorgin C), OAT1 (200 µM benzbromarone), OAT3 (200 µM probenecid), OCT2 (100 µM verapamil), OCTN1 (100 µM verapamil), MATE1 (10 µM cimetidine), MATE2 K (100 µM cimetidine), OATP1B1 (50 µM rifampicin), and OATP1B3 (50 µM rifampicin) [45,46,47,48,49,50,51]. The data are summarized in Table 13 (Tabulated Summaries 2.6.5.15.E, 2.6.5.15.L, 2.6.5.15.F, 2.6.5.15.K, 2.6.5.15.H, 2.6.5.15.J, 2.6.5.15.I and 2.6.5.15.G).

All positive control inhibitors reduced transport of their respective substrates, confirming the sensitivities of the cell lines to inhibition. Cobicistat showed negligible or weak inhibition of the efflux transporters MDR1, MRP1, MRP2, MRP4, BCRP, and MATE2 K, and the renal uptake transporters OAT1 and OAT3. Cobicistat is a weak inhibitor of the renal uptake transporter, OCT2, and a more potent inhibitor of the hepatic uptake transporters OATP1B1 and OATP1B3, and the renal efflux transporters OCTN1 and MATE1. Inhibition of MDR1 and BCRP activity is discussed in more detail in Section 8.1.2.4.

Table 13: Effects of COBI and RTV on the Activities of Human Transporters

Transporter	Cell line	Substrate (concentration)	IC_{50} (µM)	
			COBI	RTV
MDR1[45]	MDCK II	calcein AM (10 µM)	22.5 – 45.0[a]	10.0 – 20.0[a]
MRP1[45]	MDCK II	calcein AM (10 µM)	45.0 – 90.0[a]	10.0 – 20.0[a]
MRP2[45]	MDCK II	calcein[b]	45.0 – 90.0[a]	> 20[d]
MRP4[46]	LLC-PK1[c]	DHEAS (0.02 µM)	20.7	> 20[d]
BCRP[47]	MDCK II	Hoechst 33342 (10 µM)	59.0	> 20[d]
OAT1[46]	CHO	p-aminohippurate (5 µM)	> 100[d]	> 20[d]
OAT3[46]	HEK293	estrone 3-sulfate (0.2 µM)	> 100[d]	8.46
OCT2[48]	CHO	metformin (2 µM)	8.24	22.6
OCTN1[49]	S_2	tetraethylammonium (5 µM)	2.49	2.08
MATE1[50]	HEK293	tetraethylammonium (5 µM)	1.87	1.34
MATE2-K[50]	HEK293	tetraethylammonium (5 µM)	33.5	100
OATP1B1[51]	CHO	Fluo 3 (2 µM)	3.50	2.05
OATP1B3[51]	CHO	Fluo 3 (2 µM)	1.88	1.83

AM = acetomethoxy ester; BCRP = breast cancer resistance protein; COBI = cobicistat; DHEAS = 5-dehydroepiandrosterone sulfate; MATE1 = multidrug and toxin extrusion protein 1; MATE2-K = multidrug and toxin extrusion protein 2-K; MDR1 = P-glycoprotein (multidrug resistance protein 1); MRP = multi-drug resistance-associated protein; OAT = organic anion transporter; OATP = organic anion transporting polypeptide; OCT2 = organic cation transporter 2; OCTN1 = novel organic cation transporter N1; RTV = ritonavir; [a] : Range of tested concentrations bracketing 50% inhibition (IC_{50} not calculated); [b] : Generated from 10 µM calcein AM; [c] : Study performed with vesicles derived from the cell line; [d]: Maximum concentration tested

The potential of COBI to inhibit hepatic organic cation uptake (OCT1) and bile salt export pump (BSEP) was investigated into OCT1 over-expressing cells or BSEP-containing membrane vesicles[52]. The inhibition of OCT1 by COBI was similar to that observed for RTV (IC_{50} of 14.7 µM and ~ 20 µM, respectively). Cobicistat was found to be a weaker inhibitor of BSEP than RTV (IC_{50} of 6.5 µM and 1.8 µM, respectively)

8.1.2.2. *Inhibition of Bidirectional Transport of MDR1 and BCRP Substrates Through Caco-2 Cell Monolayers*

The potential for inhibition of intestinal MDR1 or BCRP by COBI was assessed by determining its effects on the bidirectional transport of the selective substrates, digoxin and prazosin, respectively[53,54] (Tabulated Summaries 2.6.5.15.B and 2.6.5.15.C, respectively). A high concentration (90 µM (50 µg/mL)) of COBI reduced the efflux of digoxin to the same extent as the selective MDR1 inhibitor, cyclosporine A, and another known inhibitor, RTV. Similarly, the high concentration (90 µM) of COBI reduced the polarized transport of the BCRP substrate, prazosin, through Caco-2 cells. This suggests that high concentrations of COBI, such as those present in the intestinal lumen during drug absorption, could inhibit intestinal MDR1 and BCRP. However, as demonstrated above (Section 8.1.2.1), COBI is a weak inhibitor of both of these transporters and so would have not be expected to have any effect systemically at concentrations achievable in plasma.

8.1.2.3. *Potential for COBI to be a Substrate for Human OCT2*

An attempt was made to determine if COBI and RTV are substrates for the human renal uptake transporter, OCT2[55] (Tabulated Summary 2.6.5.15.M). ^{14}C-COBI or ^{3}H-RTV were incubated with either wild type CHO K1 cells or CHO-OCT2 cells expressing human OCT2 and the concentration-dependence and time-dependence of accumulation determined. The effects of an OCT2 inhibitor (100 µM verapamil) were also assessed.

The positive control OCT2 substrate, metformin (2 µM), showed 36-fold higher relative accumulation in OCT2-expressing cells compared to wild type cells, and this was almost completely inhibited by verapamil. In contrast to metformin, both COBI and RTV showed high accumulation in wild type cells. Maximum relative accumulation of COBI and RTV was 1.4-fold and 2.0-fold, respectively, and this was relatively insensitive to inhibition by verapamil. Thus, since both COBI and RTV showed rapid OCT2-independent uptake, it was not

possible to determine a role for OCT2 in their cellular uptake using this system.

8.1.2.4. *Potential for COBI to be a Substrate for Human MDR1 or BCRP*

The potential for COBI to be a substrate for human MDR1 or human BCRP was assessed in MDCK II cells expressing those transporters[56] (Tabulated Summary 2.6.5.15.D). The efflux ratio for COBI in MDR1-transfected cells and BCRP transfected cells was higher (16-fold and 1.9-fold, respectively) than in the corresponding wild type cells, suggesting that COBI can act as a substrate for these transporters. As further confirmation, the efflux in transfected cells could be reduced by selective inhibitors of each transporter. The actual role of these transporters in the disposition of COBI is difficult to determine because, as shown above, COBI shows high cellular permeability in Caco-2 cells, resulting in relatively little polarized transport.

9. DISCUSSION AND CONCLUSIONS

9.1.1. Darunavir

Darunavir has been assessed in comprehensive nonclinical pharmacokinetic studies. Results from pharmacokinetic studies of DRV are summarized below

Darunavir absorption was rapid following oral administration in all species, based on observed t_{max} values (0.5 to 6 hours). The absolute oral bioavailability was 37 to 58% in rats and very likely was influenced by the extent of the first pass effect as demonstrated by the presence of a large amount of metabolites in the bile. Bioavailability was higher in dogs and ranged between 60 to 122%. In rats and dogs, the plasma clearance and the volume of distribution were moderate to high. The elimination was rapid across all species.

Across the dose range studied, the kinetics of DRV was less than dose-proportional in mice, rats and dogs after single oral administration, especially at the high dose levels in line with the low solubility of the compound. In adult rodents, repeated oral dosing resulted in a decrease in systemic exposure to DRV, possibly due to induction of the enzymes involved in the metabolism of DRV. Ex vivo induction

studies showed DRV was an inducer of CYP3A isoenzyme in rodents. UGT activity was additionally induced in rats. These effects were in line with the toxicology findings observed in the liver and thyroid in rodents. In dogs, no decrease in exposure and enzymatic induction was observed after repeated administration. Ritonavir, a strong inhibitor of CYP3A, had a modest effect on DRV exposure in mice (2-fold increase) and rats (4-fold increase) but had no clear effect in dogs and in minipigs. The highest impact, however, was in rabbits where a 15-fold increase in exposure was seen. In humans, however, the impact was noticeable. RTV markedly reduced the metabolism of DRV and consequently increased its oral bioavailability from 37 to 82%.

In adult 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. In pregnant rats, ^{14}C-DRV was slightly distributed in placenta and fetus. Total radioactivity exposure in fetus was about 13 to 27 % of that in maternal blood, while in placenta it was the same as in blood.

Exposure (plasma, brain and liver) to DRV was age-dependent in juvenile rats when measured between Days 5-11 and Days 12-26. No consistent difference in DRV plasma exposure was seen in rats aged between Day 5 and 11 though. Plasma, liver and brain exposure decreased between Day 12 and Day 26 of age due to the maturation of the liver enzymes involved in the elimination of DRV and also of the blood-brain barrier. At an age range of 23 to 50 days, exposures were broadly similar to those observed in adult rats. The plasma protein binding was moderate to high in all tested species (i.e., mouse, rat, rabbit, dog and human). The free fraction ranged from 5% (rat) to 38% (rabbit), and was 5 % in humans. Plasma protein binding in most species was concentration dependent.

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. 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 observed. Metabolic pathways proposed on the basis of in vitro studies were consistent with those observed following in vivo studies in rats, dogs and humans. Therefore, the in vitro metabolism data provide a valuable comparison between the various species, including the mouse and rabbit in which the metabolism of DRV was not studied in vivo.

In all examined species (rat, dog and human), the predominant route of excretion for ^{14}C-DRV was via the feces and amounted to 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 in humans 12.2%. 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 rats, DRV was probably also excreted via milk.

In human liver, CYP3A was almost exclusively involved in the metabolism of DRV. Darunavir inhibited CYP3A in HLM with a K_i value of 0.40 μM. Given this low value, this inhibition is considered to be clinically relevant. The K_i values for the other P450 enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1) were at least 60-fold higher, indicating a much lower affinity and less potential for clinically relevant interactions. Darunavir has only a mild inhibitory potency towards bilirubin glucuronidation Darunavir was also an inducer of CYP3A4 mRNA expression level as well as activity in human hepatocytes.

9.1.2. Cobicistat

Cobicistat has been assessed in comprehensive nonclinical pharmacokinetic studies. Results from pharmacokinetic studies of COBI are summarized below.

Estimates of fraction absorbed in vivo, derived from bioavailability corrected for predicted first-pass metabolism, or from recovery of radiolabel in bile or urine, were all > 50%. After moderate doses, oral bioavailability in nonclinical species was low due to metabolic instability and resulting high first-pass elimination. Cobicistat can act as a substrate for MDR1 and BCRP intestinal efflux transporters, but this likely did not play a significant role during its absorption as COBI had high passive permeability, as demonstrated in Caco-2 cells.

After oral dosing with ^{14}C-COBI, radioactivity was widely distributed, and volumes of distribution of COBI were close to those for body water. Cobicistat was relatively excluded from the cellular fraction of blood (whole blood to plasma ratios are ~ 0.6) and from brain, testes, and the eye. Exclusion from the brain may be due to the action of MDR1 and/or BCRP at the blood:brain barrier. Cobicistat showed preferential binding in melanin-containing tissues, but this was reversible. Cobicistat showed moderately high plasma binding.

Interpreting the metabolism of COBI was complicated by concurrent mechanism-based inhibition of human CYP3A enzymes. This attribute was species-specific, as COBI showed high clearance in nonclinical species due to a lack of self-inhibition of metabolism. The primary routes of metabolism of COBI were oxidation by CYP3A (major) and CYP2D6 (minor) enzymes. Metabolites M21, M26, and M31 were identified in mouse, rat, dog, and human samples in vitro, and were later identified in excreta from these species. One other primary metabolite, M39, was also identified in all species in vivo. Other metabolites arise from secondary metabolism, due to combinations of these primary pathways, and from other minor primary metabolites. Parent COBI was the major component circulating in plasma in all species.

After oral dosing of mice, rats, dogs, and humans with ^{14}C-COBI, the majority of radiolabel was recovered in the feces or bile with little in the urine. Total recovery of radiolabel was high for all species. Excretion of COBI into milk was detected in rats.

The intended pharmacologic action of COBI was inhibition of human CYP3A enzymes. In that regard, COBI was a potent mechanism-based

inhibitor of human CYP3A and showed activity against a wide range of CYP3A activities. All 3 of the metabolites initially identified (M21, M26, and M31) were weaker inhibitors than COBI and were very unlikely to contribute to the pharmacologic effect, especially considering their low plasma concentrations. Inhibition of human cytochrome P450 enzymes showed high selectivity, with insignificant or very weak inhibition of CYP1A2, CYP2C8, CYP2C9, and CYP2C19, weak inhibition of CYP2D6,and modest inhibition of CYP2B6. Cobicistat was also a weak inhibitor of human UGT1A1. In this regard, COBI showed greater selectivity than RTV, which inhibited CYP2C8 and CYP2C9 and was a more potent inhibitor of CYP2D6 and UGT1A1.

At concentrations achieved in plasma at the proposed therapeutic dose, COBI did not inhibit the drug transporters MDR1, MRP1, MRP2, BCRP, OAT1, or OAT3. With respect to renal transporters, COBI was a weak inhibitor of MRP4, MATE2 K and OCT2, and a more potent inhibitor of MATE1 and OCTN1, with similar potencies to RTV.

With respect to hepatic uptake transporters, COBI was a moderate inhibitor of OATP1B1 and OATP1B3. At high concentrations, achievable briefly in the intestinal lumen during drug absorption, COBI can inhibit intestinal efflux transporters, such as MDR1 and BCRP. Cobicistat did not activate human AhR and did not induce human CYP1A2 activity or mRNA. Cobicistat was a very weak activator of human PXR, and affects CYP3A4 mRNA and CYP3A immunodetectable protein only at high concentrations. Cobicistat thus had lower liability for drug interactions than RTV, which was a more potent PXR activator. Interestingly, COBI and RTV showed similar, moderately potent ability to activate rat PXR, and this was manifest as increased CYP3A activity in hepatic microsomal fraction from rats and mice after repeat dose treatment. Such species differences in PXR activation, caused by differences in the ligand binding domain of the receptor, are well understood[1].

In conclusion, COBI was a potent, selective mechanism-based inhibitor of human CYP3A enzymes with low potential for other drug-

drug interactions (inhibition of other cytochromes P450, UGT1A1, or drug transporters, and induction of enzymes and transporters).

10. TABLES AND FIGURES

In-text tables and figures are included at appropriate points throughout the summary within the text. Furthermore data and figures can be found under 2.6.5 Pharmacokinetics Tabulated Summaries.

11. REFERENCES

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

Darunavir Studies

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72. Nonclinical Pharmacokinetics Report TMC114-NC208 (FK4928; [redacted] 5304/1). 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. [redacted] Ltd (Nov [redacted]).

73. Nonclinical Pharmacokinetics Report TMC114-NC209 (FK4941; [redacted] 5317/1). 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. [redacted] Ltd (Mar [redacted]).

74. Nonclinical Pharmacokinetics Report TMC114-NC388 (FK6155; [redacted] 5358/1). 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. [redacted]. Nonclinical (Apr [redacted]).

75. Nonclinical Pharmacokinetics Report TMC114-NC171 (FK4909). In vitro study on the potential of TMC114 to induce CYP mRNA in cryopreserved human hepatocytes. J&JPRD (Aug [redacted]).

76. Nonclinical Pharmacokinetics Report TMC114-NC247 (FK5470). An in vitro study to assess the potential of TMC114 to induce CYP enzyme activities in cryopreserved human hepatocytes. J&JPRD (Sep [redacted]).

77. Nonclinical Pharmacokinetics Report TMC114-NC123 (TNO45033; [redacted] V3635/01). Inhibition of human cytochrome P450 enzymes by TMC114 *in vitro*. [redacted]. (Aug [redacted]).

Report Title

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80. Nonclinical Pharmacokinetics Report TMC114-NC134 (TNO3917; [redacted] V3917). Interaction of 6 anti-HIV compounds with the cytochrome P450-mediated metabolism of TMC114 *in vitro*. [redacted]. (Jan [redacted]).

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1. LeCluyse EL. Pregnane X receptor: molecular basis for species differences in CYP3A induction by xenobiotics. Chem Biol Interact 2001;134 (3):283-9.

2. Nonclinical Pharmacokinetics Report AD-216-2023. Permeability of GS-9350 across Caco-2 cell monolayers.

3. Nonclinical Pharmacokinetics Report AD-216-2020. Pharmacokinetics of GS-9350 in Rats.

4. Nonclinical Pharmacokinetics Report AD-216-2021. Pharmacokinetics of GS-9350 in Beagle Dogs.

5. Nonclinical Pharmacokinetics Report AD-216-2022. AD-216-2022 Pharmacokinetics of GS-9350 in cynomolgus monkeys.

6. Nonclinical Pharmacokinetics Report 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.

7. Nonclinical Toxicology Study TX-216-2026. 3-Month Oral Gavage Toxicity and Toxicokinetic Study with COBI in CD-1 Mice

8. **Nonclinical Toxicology Report TX-216-2030. 104 week Oral Gavage Study Carcinogenicity Study with GS-9350 in Mice.**

9. **Nonclinical Pharmacokinetics Report AD-216-2039. Induction of metabolizing enzymes of rat by GS-9350 in vitro.**

10. **Nonclinical Pharmacokinetics Report AD-216-2041 Drug interaction properties of putative human metabolites of GS-9350.**

11. **Nonclinical Toxicology Report TX-216-2017. 26-Week Oral Gavage Toxicity and Toxicokinetic Study with COBI in Rats with a 13-Week Recovery Period.**

12. **Nonclinical Toxicology Report TX-216-2031. 104 week Oral Gavage Study Carcinogenicity Study with GS-9350 in Rats.**

13. **Nonclinical Pharmacokinetics Report AD-216-2027 Induction of metabolizing enzymes by GS-9350 in vitro.**

14. **Nonclinical Pharmacokinetics Report AD-216-2071. In Vitro Assessment of the Induction Potential of GS-9350 in Primary Cultures of Human Hepatocytes.**

15. Nonclinical Toxicology Report TX-216-2005. 4-Week Oral Gavage Toxicity and Toxicokinetic Study with COBI in Dogs with a 4-Week Recovery Phase.

16. **Nonclinical Toxicology Report 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.**

17. **Nonclinical Pharmacokinetics Report. AD-216-2040 Inhibition of CYP3A activity in rat, dog and monkey by GS-9350 in vitro.**

18. **Nonclinical Toxicology Report TX-216-2024. 90-Day Oral Gavage Bridging Study with GS-9350 and Atazanavir in Rats with a 1-Month Recovery Period.**

19. **Nonclinical Pharmacokinetics Report AD-216-2034. PK, distribution, metabolism, excretion [^{14}C]-GS-9350 in rats.**

20. **Nonclinical Pharmacokinetics Report AD-216-2060. A Whole-Body Autoradiography (WBA) of Rats Following Oral Administration of [^{14}C]-GS-9350.**

21. **Nonclinical Pharmacokinetics Report AD-216-2076. Plasma Protein Binding of GS-9350 in CD-1 Mice.**

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23. **Nonclinical Pharmacokinetics Report GS-US-183-0133. 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.**

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25. Nonclinical Pharmacokinetics Report AD-216-2073. Pharmacokinetics, Metabolism, and Excretion of [^{14}C]GS-9350 Following Oral Administration to Mice.

26. **Nonclinical Pharmacokinetics Report AD-216-2067. Mass Balance of Radioactivity after Oral Administration of [^{14}C] GS-9350 to Naïve Male Beagle Dogs.**

27. **Clinical Report GS-US-216-0111. A Phase 1 Study to Evaluate the Pharmacokinetics, Metabolism and Excretion of GS-9350 (Mass Balance Study)**

28. **Nonclinical Pharmacokinetics Report AD-216-2082.Radioprofiling and Metabolite Identification Following Oral Administration of [^{14}C]GS-9350 to rats.**

29. **Nonclinical Pharmacokinetics Report. AD-216-2101 Radioprofiling and Metabolite Identification Following Oral Administration of [^{14}C]GS-9350 to intact and bile duct cannulated dogs.**

30. Nonclinical Pharmacokinetics Report AD-216-2074. Identification of Major Metabolites of GS-9350 in CD-1 Mouse Microsomes In Vitro.

*新薬承認情報提供時に置き換え

31. **Nonclinical Pharmacokinetics Report AD-216-2024. In vitro metabolism of GS-9350 in hepatocytes and hepatic subcellular fractions from rat, dog, monkey and human.**

32. **Nonclinical Pharmacokinetics Report. AD-216-2038 Identification of Major Metabolites of GS-9350 In Vitro.**

33. **Nonclinical Pharmacokinetics Report. AD-216-2025 Cytochrome P450 phenotyping for GS-9350**

34. **Nonclinical Pharmacokinetics Report AD-216-2106. Metabolite Profiles of Cobicistat Generated by Human CYP2D6 and CYP3A4.**

35. **Nonclinical Pharmacokinetics Report AD-216-2108. The Potential for Cytochromes P450 CYP2B6 and CYP2C8 to Metabolize Cobicistat.**

36. Nonclinical Toxicology Report TX-216-2001. 14 Day Oral Gavage Toxicity and Toxicokinetics Study with GS-9350 and GS-017415 in Rats.

37. **Nonclinical Pharmacokinetics Report AD-216-2028. Inhibition of human CYP3A activity by GS-9350 in vitro**

38. **Nonclinical Pharmacokinetics Report AD-216-2029. In vitro assessment of human liver cytochrome P450 inhibition potential of GS-9350**

39. **Nonclinical Pharmacokinetics Report AD-216-2070. In Vitro Assessment of Human Liver CYP2B6 and CYP2C8 Inhibition Potential of GS-9350**

40. **Nonclinical Pharmacokinetics Report AD-216-2041. Drug interaction properties of putative human metabolites of GS-9350**

41. **Nonclinical Pharmacokinetics Report AD-216-2107. In Vitro Assessment of the Potential for Cobicistat Metabolites to Inhibit CYP2B6, CYP2C8 or UGT1A1**

42. **Nonclinical Pharmacokinetics Report AD-216-2075. In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-9350**

43. **Nonclinical Pharmacokinetics Report AD-216-2068. Mass Balance of Radioactivity after Oral Administration of [14C] GS-9350 to Naïve Male Bile Duct-Cannulated Beagle Dogs**

44. **Nonclinical Toxicology Report TX-216-2033. Perinatal/Postnatal study with GS-9350 and Juvenile Toxicity**

45. **Nonclinical Pharmacokinetics Report AD-216-2030. Interaction of GS-9350 with MRP1, MRP2 and Pgp.**

46. **Nonclinical Pharmacokinetics Report AD-216-2105. Inhibition of OAT1, OAT3 and MRP4 by GS-9350 and ritonavir.**

47. **Nonclinical Pharmacokinetics Report AD-216-2099. Inhibition of BCRP by GS-9350 and ritonavir.**

48. **Nonclinical Pharmacokinetics Report AD-216-2093. In vitro interaction study of GS-9350 with human OCT2 uptake transporter.**

49. **Nonclinical Pharmacokinetics Report AD-216-2098. Effects of GS-017415 and GS-340649 on uptake into OCTN1 expressing cells.**

50. **Nonclinical Pharmacokinetics Report AD-216-2094. In vitro interaction study of GS-9350 with human MATE1 and MATE2-K transporters.**

51. **Nonclinical Pharmacokinetics Report AD-216-2100. Inhibition of OATPB1 and OATPB3 by GS-9350, ritonavir and lopinavir.**

52. **Nonclinical Pharmacokinetics Report AD-236-2008. In vitro Inhibition Studies of Stribild Components with Human OCT1 and BSEP Transporters.**

53. **Nonclinical Pharmacokinetics Report AD-216-2072. Inhibition of P-glycoprotein-Dependent Bi-Directional Transport of Digoxin Through Monolayers of Caco-2 Cells by GS-9350**

54. **Nonclinical Pharmacokinetics Report AD-216-2104. Inhibition of BCRP-Dependent Bi-Directional Transport of Prazosin through Monolayers of Caco-2 Cells by Cobicistat**

55. **Nonclinical Pharmacokinetics Report AD-216-2095. Potential for GS-9350 and ritonavir to be substrates for human OCT2**

56. **Nonclinical Pharmacokinetics Report AD-216-2103. Bidirectional Permeability of Cobicistat Through Monolayers of P-glycoprotein- and BCRP- Overexpressing Cells.**

MODULE 2.6.5

PHARMACOKINETICS TABULATED SUMMARY

2.6.5.1 Pharmacokinetics: Overview-Darunavir

Test article: darunavir

Type of Study	Species/ Strain	Route/method of administration (vehicle/formulation)	GLP compliance	Testing Facility	Study/ Report Number	Location in CTD
Absorption						
Drug permeability and transport	CaCo-2 cells	In vitro	N	J&J PRD	TMC114-NC137	4.2.2.2
Drug permeability and transport	CaCo-2 cells	In vitro	N	J&J PRD	PEPI-NC119	4.2.2.2
Absorption, Single Dose	Mouse (CD1)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC111	4.2.3.1
Absorption, Single Dose	Rat (Wistar)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC101	4.2.3.1
Absorption, Single Dose	Rat (Wistar)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC104	4.2.3.1
Absorption, Single Dose	Rat (Wistar)	Intravenous (50% PEG400, 10% ethanol, 40% sodium chloride)	N	[redacted] BV	TMC114-NC110	4.2.3.1
Absorption, Single Dose	Rat (pup)/ Sprague-Dawley	Oral/Gavage	N	J&J PRD	TMC114-NC240	4.2.3.5.4
Absorption, Dose Escalation	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC102	4.2.3.1
Absorption, Repeat Dose (7 days)	Mouse (CD1)	Diet admixture	N	J&J PRD	TMC114-NC200	4.2.2.2
Absorption, Repeat Dose (1 month)	Mouse (CB6F1-non Tgras H2)	Oral/gavage (PEG400)	Y	J&J PRD	TMC114-NC194	4.2.3.4.3
Absorption, Repeat Dose (3 month)	Mouse (CD1)	Oral/gavage (PEG400)	Y	J&J PRD	TMC114-NC157	4.2.3.4.1
Absorption, Repeat Dose (14 days) with ritonavir	Mouse (Swiss)	Oral/gavage (PEG400)	N	J&J PRD	TMC114-NC223	4.2.2.2
Absorption, Repeat Dose (2 years)	Mouse/ CD1	Oral/Gavage (PEG400)	Y	J&J PRD	TMC114-NC159	4.2.3.4.1
Absorption, Repeat Dose (7 days)	Rat (Sprague Dawley)	Diet admixture	N	J&J PRD	TMC114-NC201	4.2.2.2
Absorption, Repeat Dose (14 days)	Rat (Wistar)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC107	4.2.3.2

2.6.5.1 Pharmacokinetics: Overview-Darunavir (Continued)

Test article: darunavir

Type of Study	Species/ Strain	Route/method of administration (vehicle/formulation)	GLP compliance	Testing Facility	Study/ Report Number	Location in CTD
Absorption (Continued)						
Absorption, Repeat Dose (2 weeks)	Rat (pup)/ Sprague-Dawley	Oral/Gavage (PEG400)	N	J&J PRD	TMC114-NC248	4.2.3.5.4
Absorption, Repeat Dose (2 weeks)	Pregnant Rat/ Sprague Dawley	Oral/Gavage (PEG400)	N	J&J PRD	TMC114-NC397	4.2.3.5.2
Absorption, Repeat Dose (2weeks))	Pregnant Rat/ Sprague Dawley	Oral/Gavage (PEG400)	Y	J&J PRD	TMC114-NC398	4.2.3.5.2
Absorption, Repeat Dose (1 month)	Rat (pup)/ Sprague-Dawley	Oral/Gavage (PEG400)	Y	J&J PRD	TMC114-NC241	4.2.3.5.4
Absorption, Repeat Dose (1 month)	Rat/ Sprague-Dawley	Oral (Mechanistic)	Y	J&J PRD	TMC114-NC162	4.2.3.7.3
Absorption, Repeat Dose (3 months)	Rat (Sprague-Dawley)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC130	4.2.3.2
Absorption, Repeat Dose (3 months)	Rat (Sprague-Dawley)	Diet admixture	Y	J&J PRD	TMC114-NC196	4.2.3.4.3
Absorption, Repeat Dose (6 months)	Rat (Sprague-Dawley)	Oral/gavage (PEG400)	Y	J&J PRD	TMC114-NC132	4.2.3.2
Absorption, Repeat Dose (2 years)	Rat/ Sprague-Dawley	Oral/Gavage (PEG400)	Y	J&J PRD	TMC114-NC158	4.2.3.4.1
Absorption, Repeat Dose (7 days) with ritonavir	Rat (Wistar)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC139	4.2.2.2
Absorption, Repeat Dose (14 days) with ritonavir	Rat (Wistar)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC141	4.2.3.2
Absorption, Repeat Dose (14 days) with ritonavir	Rat (Wistar)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC143	4.2.3.2
Absorption, Repeat Dose (1 month) with ritonavir	Rat (Sprague-Dawley)	Oral/gavage (PEG400)	Y	J&J PRD	TMC114-NC187	4.2.3.7.2
Absorption, Repeat Dose (6 months) with ritonavir	Rat (Wistar)	Oral/gavage (PEG400)	Y	[redacted] BV	TMC114-NC146	4.2.3.2
Absorption, Repeat Dose (14 days)	Rat (Sprague Dawley)	Intravenous (15% ! cyclodextrin)	Y	J&J PRD	TMC114-NC160	4.2.3.2
Absorption, Repeat Dose (7 days)	Rabbit (New Zealand White)	Oral/gavage (CMC/ Tween in water)	N	[redacted] BV	TMC114-NC124	4.2.3.5.2

2.6.5.1 Pharmacokinetics: Overview-Darunavir (Continued)

Test article: darunavir

Type of Study	Species/ Strain	Route/method of administration (vehicle/formulation)	GLP compliance	Testing Facility	Study/ Report Number	Location in CTD
Absorption (Continued)						
Absorption, Repeat Dose (5 days) with ritonavir	Rabbit (New Zealand White)	Oral/gavage (HPMC/ Tween in water)	N	J&J PRD	TMC114-NC189	4.2.3.5.2
Absorption, Repeat Dose (14 days)	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC106	4.2.2.2
Absorption, Single or Repeat Dose (14 days)	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC173	4.2.3.2
Absorption, Repeat Dose (3 months)	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC131	4.2.3.2
Absorption, Repeat Dose (6 months)	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC133	4.2.3.2
Absorption, Repeat Dose (12 months)	Dog (Beagle)	Oral/gavage (PEG400)	Y	[redacted] BV	TMC114-NC145	4.2.3.2
Absorption, Repeat Dose (4 days) with ritonavir	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC147	4.2.2.2
Absorption, Repeat Dose (7 days) with ritonavir	Dog (Beagle)	Oral/gavage (PEG400)	N	[redacted] BV	TMC114-NC138	4.2.2.2
Absorption, Repeat Dose (14 days) with ritonavir	Dog (Beagle)	Oral/gavage (PEG400)	Y	J&J PRD	TMC114-NC140	4.2.3.2
Absorption, Repeat Dose (14 days) with ritonavir	Dog (Beagle)	Oral/bid capsules and gavage (PEG400)	N	[redacted] BV	TMC114-NC144	4.2.2.2
Absorption, Single or Repeat Dose (3 days)	Dog (Beagle)	Intravenous (50% PEG400, 10% ethanol, 40% sodium chloride)	N	[redacted] BV	TMC114-NC109	4.2.3.1
Absorption, Repeated Dose (14 days)	Dog (Beagle)	Intravenous (15% ! cyclodextrin)	Y	J&J PRD	TMC114-NC199	4.2.3.2
Absorption with ritonavir	Pregnant Mouse (CD1)	Oral/gavage (PEG400)	Y	[redacted]	TMC114-NC172	4.2.3.5.2
Absorption	Pregnant Rat (Sprague-Dawley)	Oral/gavage (PEG400)	N	[redacted]	TMC114-NC127	4.2.3.5.2
Absorption (pre and postnatal)	Pregnant Rat and Pups (Sprague-Dawley)	Oral/gavage (PEG400)	N	[redacted]	TMC114-NC178	4.2.3.5.3

2.6.5.1 Pharmacokinetics: Overview-Darunavir (Continued)

Test article: darunavir

Type of Study	Species/ Strain	Route/method of administration (vehicle/formulation)	GLP compliance	Testing Facility	Study/ Report Number	Location in CTD
Absorption (Continued)						
Absorption	Pregnant Rabbit (New Zealand White)	Oral/gavage (1% CMC/0.2% Tween 80)	N	[redacted]	TMC114-NC125	4.2.3.5.2
Absorption, Repeat Dose (2 weeks)	Female Minipig/ Göttingen SPF	Oral/gavage (PEG400)	N	[redacted].	TMC114-NC399	4.2.3.5.2
Comparative bioavailability	Dog (Beagle)	Oral/gavage (PEG400, capsule, solution)	N	[redacted]	TMC114-NC121	4.2.2.2
Comparative bioavailability	Dog (Beagle)	Oral/gavage (PEG400,capsule, tablet, solution)	N	[redacted]	TMC114-NC122	4.2.2.2
Comparative bioavailability	Dog (Beagle)	Oral/gavage (capsule, tablet, solution)	N	[redacted]	TMC114-NC135	4.2.2.2
Comparative bioavailability	Dog (Beagle)	Oral/gavage (capsule, suspension, solution)	N	[redacted]	TMC114-NC142	4.2.2.2
Comparative bioavailability	Dog (Beagle)	Oral/gavage (capsule, solution)	N	[redacted]	TMC114-NC149	4.2.2.2
Comparative bioavailability	Dog (Beagle)	Oral/gavage (capsule, tablet)	N	J&JPRD	TMC114-NC211	4.2.2.2
Distribution						
Tissue Distribution (single dose, with ritonavir)	Rat (Long Evans)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC192	4.2.2.3
Tissue Distribution (single dose, with ritonavir)	Pregnant Rat (Sprague-Dawley)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC205	4.2.2.3
Tissue Distribution (repeated dose, with or without ritonavir)	Rat (Wistar)	Oral/gavage (PEG400)	Y	[redacted]	TMC114-NC119	4.2.2.3
Protein Binding	Rat, Dog, Human	In vitro	Y	[redacted]	TMC114-NC113	4.2.2.3
Protein Binding Blood Distribution	Mouse, Rat, Rabbit, Dog, Human	In vitro (^{14}C-TMC114)	N	J&J PRD	TMC114-NC215	4.2.2.3
Tissue Distribution (repeated dose, 14 days)	Pregnant Rabbit (New Zealand White)	Oral/gavage (1% CMC/0.2% Tween 80)	N	[redacted]	TMC114-NC126	4.2.3.5.2

2.6.5.1 Pharmacokinetics: Overview-Darunavir (Continued)

Test article: darunavir

Type of Study	Species/ Strain	Route/method of administration (vehicle/formulation)	GLP compliance	Testing Facility	Study/ Report Number	Location in CTD
Metabolism						
Metabolism Excretion (single dose)	Rat (Sprague Dawley)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC191	4.2.2.4
Metabolism Excretion (single dose with ritonavir)	Rat (Sprague Dawley)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC152	4.2.2.4
Metabolism Excretion in bile (single dose)	Rat (Sprague Dawley)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC164	4.2.2.4
Metabolism Excretion (single dose)	Dog (Beagle)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC153	4.2.2.4
Metabolism Excretion (single dose with ritonavir)	Human	Oral (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC213	4.2.2.4
Metabolism	Mouse, rat, rabbit, dog, human	In vitro: hepatocytes, subcellular liver fractions	N	J&J PRD	TMC114-NC154	4.2.2.4
Metabolism	young and adult rat	In vitro: subcellular liver fractions	N	J&J PRD	TMC114-NC246	4.2.2.4
Metabolism	Rat, dog, human	In vitro: hepatic microsomes	Y	[redacted]	TMC114-NC112	4.2.2.4
Metabolism	Cytochrome P450	In vitro: E. coli expressed CYP isoforms	N	J&J PRD	TMC114-NC202	4.2.2.4
Metabolism (induction/inhibition, 3 months)	Mouse	In vitro: hepatic microsomes	Y	[redacted]	TMC114-NC226	4.2.2.4
Metabolism (induction/inhibition, 1 month, with ritonavir)	Rat	In vitro: hepatic microsomes	Y	[redacted]	TMC114-NC208	4.2.2.4
Metabolism (induction/inhibition, 12 months)	Dog	In vitro: hepatic microsomes	Y	[redacted]	TMC114-NC209	4.2.2.4
Metabolism: Ex vivo inhibition/induction	Rat/ Sprague-Dawley	Oral/Gavage (PEG400)	Y	[redacted]	TMC114-NC388	4.2.2.4
Metabolism Inhibition	Human	Pooled liver microsomes – in vitro (CYP2C8 and UDP-GT1A1)	N	J&J PRD	TMC114-NC392	4.2.2.4

2.6.5.1 Pharmacokinetics: Overview-Darunavir (Continued)

Test article: darunavir

Type of Study	Species/ Strain	Route/method of administration (vehicle/formulation)	GLP compliance	Testing Facility	Study/ Report Number	Location in CTD
Metabolism (Continued)						
Metabolism (induction/inhibition)	Human CYP450	In vitro	N	J&J PRD	TMC114-NC171	4.2.2.4
Metabolism (induction)	Human CYP450	In vitro	N	J&J PRD	TMC114-NC247	4.2.2.4
Metabolism (inhibition)	Human CYP450	In vitro	Y	[redacted]	TMC114-NC123	4.2.2.4
Excretion						
Excretion Metabolism (single dose with ritonavir)	Rat (Sprague Dawley)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC152	4.2.2.4
Excretion in bile Metabolism (single dose)	Rat (Sprague Dawley)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC164	4.2.2.4
Excretion in Milk	Rat/ Sprague-Dawley	Oral/Gavage (PEG400)	No	J&J PRD	TMC114-NC249	4.2.2.5
Excretion Metabolism (single dose)	Dog (Beagle)	Oral/gavage (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC153	4.2.2.4
Excretion Metabolism (single dose with ritonavir)	Human	Oral (^{14}C-TMC114, PEG400)	N	J&J PRD	TMC114-NC213	4.2.2.4
Pharmacokinetic Drug Interactions						
Metabolism (drug-drug interaction)	Human CYP450	In vitro	Y	[redacted]	TMC114-NC134	4.2.2.6
Inhibition of OCT2 and MATE1 transporters by DRV	CHO cells	In vitro	N	[redacted]	AD-216-2109	4.2.2.6

2.6.5.1 Pharmacokinetics: Overview-Cobicistat

Test Article: Cobicistat

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. Location in CTD
Analytical Methods and Validation					
Absorption					
Permeability Across Caco-2 Cell Monolayer	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2023 4.2.2.2
Single Dose Pharmacokinetics	No	Mouse	Oral	[redacted], USA	PC-216-2013-PK 4.2.2.2
Single Dose Pharmacokinetics	No	Rat	IV, Oral	[redacted], USA	AD-216-2020 4.2.2.2
Single Dose Pharmacokinetics	No	Dog	IV, Oral	[redacted], USA	AD-216-2021 4.2.2.2
Single Dose Pharmacokinetics	No	Cynomolgus Monkey	IV, Oral	[redacted], USA	AD-216-2022 4.2.2.2
Distribution					
Plasma Protein Binding (Mouse)	No	In Vitro	In Vitro	[redacted], USA	AD-216-2076 4.2.2.3
Plasma Protein Binding (Other Species)	No	In Vitro	In Vitro	[redacted], USA	AD-216-2026 4.2.2.3
Absorption and Disposition of Radioactivity (Albino Rat)	No	Rat	Oral	[redacted] Inc., [redacted], USA	AD-216-2034 4.2.2.3
Tissue Distribution of	No	Rat	Oral	[redacted] Inc., [redacted],	AD-216-2060

Test Article: Cobicistat

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. Location in CTD
Radioactivity (Pigmented Rat)				[redacted], USA	4.2.2.3
Ex Vivo Protein Binding (Human, normal and impaired hepatic function)	No	Human	Ex Vivo	[redacted]	60N-1103A 4.2.2.3
Ex Vivo Protein Binding (Human, normal and impaired renal function)	No	Human	Ex Vivo	[redacted]	60N-1103B 4.2.2.3
Metabolism					
Pharmacokinetics, metabolism, and excretion of radioactivity	No	Mouse	Oral	[redacted] Inc., [redacted], [redacted], USA	AD-216-2073 4.2.2.4
Radioprofiling and Metabolite Identification	No	Rat	Oral	[redacted] Inc., [redacted], [redacted], USA	AD-216-2082 4.2.2.4
Radioprofiling and Metabolite Identification	No	Dog	Oral	[redacted] Inc., [redacted], [redacted], USA	AD-216-2101 4.2.2.4
Metabolite identification in vitro (Mouse)	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2074 4.2.2.4
Cytochrome P450 phenotyping	No	In Vitro	In Vitro	[redacted], [redacted], UK	AD-216-2025 4.2.2.4
Metabolite identification in vitro (rat, dog, human)	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2038 4.2.2.4

Test Article: Cobicistat

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. Location in CTD
In Vitro Metabolism in Hepatocytes and Hepatic Subcellular Fractions from Rat, Dog, Monkey, and Human	No	Hepatocytes and hepatic subcellular fractions	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2024 4.2.2.4
Metabolite Profiles generated by Human CYP2D6 and CYP3A4	No	Human hepatic microsomal fraction	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2106 4.2.2.4
In Vitro Inhibition of CYP2B6, CYP2C8 and UGT1A1	No	Pooled human liver microsomes	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA/[redacted] UK	AD-216-2107 4.2.2.4
Metabolism of COBI by CYP2B6 and CYP2C8	No	Human CYP enzyme preparations	In Vitro	[redacted] UK	AD-216-2108 4.2.2.4
Human CYP3A inhibition potential	No	In Vitro	In Vitro	[redacted], [redacted], UK/ Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2028 4.2.2.4
Nonhuman CYP3A Inhibition Potential	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2040 4.2.2.4
Cytochrome P450 Inhibition Potential	No	In Vitro	In Vitro	[redacted], [redacted], UK	AD-216-2029 4.2.2.4
Human CYP2B6 and CYP2C8 Inhibition Potential	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2070 4.2.2.4
Drug Interaction Properties of COBI Metabolites	No	In Vitro	In Vitro	[redacted], [redacted], UK/[redacted], [redacted], [redacted], USA/ Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2041 4.2.2.4

Test Article: Cobicistat

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. Location in CTD
Human UGT1A1 inhibition potential	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2075 4.2.2.4
Induction of metabolizing enzymes (Xenobiotic Receptors)	No	In Vitro	In Vitro	[redacted], USA	AD-216-2027 4.2.2.4
Induction potential in primary cultures of human hepatocytes	No	In Vitro	In Vitro	[redacted], USA	AD-216-2071 4.2.2.4
Induction of Rat Metabolizing Enzymes in vitro (Rat PXR)	No	In Vitro	In Vitro	[redacted], USA	AD-216-2039 4.2.2.4
Excretion					
Pharmacokinetics, metabolism, and excretion of radioactivity	No	Mouse	Oral	[redacted] Inc., [redacted], USA	AD-216-2073 4.2.2.4

Test Article: Cobicistat

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. Location in CTD
Pharmacokinetics, distribution, metabolism, and excretion of radioactivity	No	Rat	Oral	[redacted] Inc., [redacted], [redacted], USA	AD-216-2034 4.2.2.5
Mass balance of radioactivity (intact dogs)	No	Dog	Oral	[redacted], [redacted], [redacted], USA	AD-216-2067 4.2.2.5
Mass balance of radioactivity (bile duct-cannulated dogs)	No	Dog	Oral	[redacted], [redacted], [redacted], USA	AD-216-2068 4.2.2.5
Pharmacokinetic Drug Interactions					
Potential to be a substrate for human OCT2	No	In Vitro	In Vitro	[redacted], [redacted], [redacted], USA/ [redacted], [redacted], Hungary	AD-216-2095 4.2.2.6
Inhibition of Pgp-dependent bidirectional transport of digoxin through Caco-2 monolayers	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2072 4.2.2.6
Inhibition of BCRP-Dependent Bidirectional Transport	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2104 4.2.2.6
Interaction with MRP1, MRP2, and Pgp	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2030 4.2.2.6
Inhibition of BCRP	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2099 4.2.2.6
Inhibition of OATP1B1 and OATP1B3	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2100 4.2.2.6

Test Article: Cobicistat

Type of Study/Description	GLP[a]	Test System	Method of Administration	Testing Facility	Gilead Study No. Location in CTD
Interaction with human OCT2 uptake transporter	No	In Vitro	In Vitro	[redacted], USA/ [redacted], Hungary	AD-216-2093 4.2.2.6
Interaction with human MATE1 and MATE2-K transporters	No	In Vitro	In Vitro	[redacted], USA/ [redacted] Co., [redacted], Japan	AD-216-2094 4.2.2.6
Effects on uptake into OCTN1 expressing cells	No	In Vitro	In Vitro	[redacted], USA/ [redacted] Co., [redacted], Japan	AD-216-2098 4.2.2.6
Human OAT1, OAT3 and MRP4 transporter inhibition potential	No	In Vitro	In Vitro	[redacted], USA/ [redacted], Hungary	AD-216-2105 4.2.2.6
Permeability in MDR1 and BCRP Overexpressing Cells	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-216-2103 4.2.2.6
Inhibition of OCT1 and BSEP	No	In Vitro	In Vitro	Gilead Sciences, Inc., Foster City, CA, USA	AD-236-2008 4.2.2.6
Other Pharmacokinetic Studies					
None					

COBI = cobicistat; EVG = elvitegravir; FTC = emtricitabine; TDF = tenofovir disoproxil fumarate; TFV = tenofovir

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

2.6.5.2 Pharmacokinetics: Analytical Methods and Validation Reports

Darunavir; Cobicistat: Not applicable

2.6.5.3.A Pharmacokinetics: Caco-2 Permeability

Test article: darunavir

Location in CTD	**4.2.2.2**	
Study No.	**TMC114-NC137**	
Type of Study: Transepithelial permeation of TMC114 across Caco-2 cell monolayers		
Method : Caco-2 cells were maintained for 21 days on cell culture inserts. ^{14}C-TMC114 (3-300 µM) was added to the apical or basolateral compartment and transport was measured for 15, 45, 90 and 120 min. ^{14}C-alniditan, ^{3}H-levocabastine and ^{3}H-theophylline at a final concentration of 20 µM were included as control compounds for low, medium and high permeability. The involvement of P-glycoprotein and other efflux transporters (Multidrug Resistance-associated Protein-2 and Breast Cancer Resistance Protein) in efflux of TMC114 was assessed by measuring the effects of 100 µM verapamil or 100 µM ritonavir on TMC114 transport.		
Condition, Compound	**P_{app} (10^{-6} cm/s) ± SD**	**Efflux Ratio (B to A/A to B)**
	Apical to Basolateral / **Basolateral to Apical**	
3 µM TMC114	7.3 ± 0.5 / 70.7 ± 3.8	9.7
10 µM TMC114	5.6 ± 1.2 / 74.3 ± 3.8	13.4
30 µM TMC114	10.7 ± 1.5 / 57.1 ± 6.7	5.4
100 µM TMC114	12.1 ± 3.7 / 45.1 ± 1.7	3.7
300 µM TMC114	17.4 ± 4.6 / 34.9 ± 0.5	2.0
30 µM TMC114 + 100 µM verapamil	14.9 ± 1.7 / 45.9 ± 3.1	3.1
30 µM TMC114 + 100 µM ritonavir	13.0 ± 3.1 / 27.3 ± 3.4	2.1
20 µM alniditan	1.4 ± 1.0 / 1.04 ± 0.1	0.7
20 µM levocabastine	13.5 ± 3.2 / 34.9 ± 1.5	2.6
20 µM theophylline	36.7 ± 8.3 / 56.4 ± 7.2	1.5
Additional Information Apical to Basolateral (absorptive) P_{app} values were calculated from the slopes of 15-45-90 min transport-time profiles (except for levocabastine and theophylline, where 0-15 min transport-time profiles were used). Basolateral to Apical (secretory) P_{app} values were calculated from the slopes of 0-15 min transport time profiles. All incubations in this experiment were conducted in the presence of Hank's Balanced Salt Solution +10 % Fetal Calf Serum, at pH 6.5 (+ 25 mM MES) in the apical compartment and at pH 7.4 in the basolateral compartment. The average Mannitol permeability value across all incubation conditions (including all conditions with either TMC114 or any of the reference compounds in both transport directions) was 0.8×10^{-6} cm/s and the highest average apparent Mannitol permeability value measured in the present study was 2.1×10^{-6} cm/s (for incubations with Alniditan). This indicates that tight junctional and cell monolayer integrity were maintained for all incubations conducted in the present study.		

P_{app} : Apparent permeability coefficient; MES : 2-(N-morpholino)ethanesulfonic acid

2.6.5.3.A Pharmacokinetics: Caco-2 Permeability

Test article: darunavir

Location in CTD	**4.2.2.2**		
Study No.	**TMC114-NC137**		
Type of Study: P-glycoprotein inhibitory effect of TMC114 in Caco-2 monolayers **Method :** Caco-2 cells were maintained for 21 days on cell culture inserts. ^{3}H-taxol (75.8 nM) was added to the apical or basolateral compartment and transport was determined in the absence and presence of TMC114 (1, 3, 10, 30, 100 µM) over 120 min incubation period. Measurement of bi-directional Taxol transport in the presence of 100 µM verapamil was used as a positive control for inhibition of P-glycoprotein mediated transport across Caco-2 monolayers.			
Condition, Compound	**Taxol P_{app} (10^{-6} cm/s) ! SD**		**Taxol Efflux Ratio (B to A/A to B)**
	Apical to Basolateral	**Basolateral to Apical**	
Control (^{3}H-taxol only)	1.04 ! 0.10	19.8 ! 0.7	19
1 µM TMC114	0.96 ! 0.06	14.8 ! 0.7	15
3 µM TMC114	1.13 ! 0.03	17.0 ! 0.5	15
10 µM TMC114	1.28 ! 0.02	14.8 ! 1.9	12
30 µM TMC114	1.35 ! 0.04	10.6 ! 0.9	8
100 µM TMC114	1.55 ! 0.16	5.48 ! 0.7	4
100 µM verapamil	1.65 ! 0.07	7.43 ! 0.2	4
Additional Information ^{3}H-Taxol Papp values were calculated from the slopes of 0-120 min transport-time profiles. All incubations in this experiment were conducted in the presence of Hank's Balanced Salt Solution +10 % Fetal Calf Serum, at pH 6.5 (+ 25 mM MES) in the apical compartment and at pH 7.4 in the basolateral compartment.			

P_{app} : Apparent permeability coefficient; MES : 2-(N-morpholino)ethanesulfonic acid

2.6.5.3.B Pharmacokinetics: Caco-2 Permeability

Test article : darunavir

Location in CTD	**4.2.2.2**
Study No.	**PEPI-NC119**

Type of Study: Transepithelial permeation of TMC114, taxol and saquinavir in presence of ritonavir across Caco-2 cell monolayers

Method : Caco-2 cells were maintained for 19-21 days on cell culture inserts. ^{14}C-TMC114 (30 µM), ^{3}H-taxol (59 nM and 30 µM) and saquinavir (30 µM) were added to the apical or basolateral compartment in presence of ritonavir (1-100 µM). Transport was measured for 15, 45, 90 minutes. ^{14}C-mannitol and ^{3}H-theophylline were included as control to check the integrity for each individual insert.

Results in presence of ritonavir concentrations (1-100 µM):

Compounds	**IC_{50} (µM)**	**E_o (%)**	**E_{max} (%)**
taxol (59 nM)	12.7	83	83
taxol (30 µM)	15.8	98	98
saquinavir (30 µM)	24.1	88	86
TMC114 (30 µM)	12.3	93	93

Additional Information :

The average absorptive permeability values across all incubation conditions were 0.13 ! 0.06 10^{-6} cm/s and 1.4 ! 0.2 10^{-6} cm/s for ^{14}C-mannitol and ^{3}H-theophylline, respectively. This indicates that tight junctional and cell monolayer integrity were maintained for all incubations conducted in the present study.

2.6.5.3.C Pharmacokinetics: Absorption after a Single Dose in Mice

Test article: darunavir

Location in CTD	**4.2.3.1**					
Study No.	**TMC114-NC111**					
Species	**Mouse (CD1)**					
Feeding Condition	not fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	M/5	F/5	M/5	F/5	M/5	F/5
Dose[1] (mg/kg)	100	100	300	300	1000	1000
Concentration (mg/mL)	5	5	15	15	50	50
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	7.63	9.67	36.5	30.1	55.0	85.9
t_{max} (h)	1	1	6	2	4	4
AUC (µg.h/mL)	11.8	10.9	156	70.3	199	360
(Time for calculation –h)	(0-#)	(0-#)	(0-#)	(0-#)	(0-#)	(0-8)
$t_{1/2}$ (h)	0.7	1.4[2]	0.6[2]	0.3[2]	2.2[2]	NC
(Time for calculation –h)	NS	NS	NS	NS	NS	-

[1] : doses expressed as TMC114 base were 93.3, 280 and 933 mg/kg
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified
NC: not calculated

2.6.5.3.D Pharmacokinetics: Absorption after a Single Dose in Rats

Test article: darunavir

Location in CTD	**4.2.3.1**					
Study No.	**TMC114-NC101**					
Species	**Rat (Wistar)**					
Feeding Condition	fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	M/2	F/2	M/2	F/2	M/2	F/2
Dose[1] (mg/kg)	20	20	100	100	500	500
Concentration (mg/mL)	4	4	20	20	100	100
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} [2] (µg/mL)	0.622	1.38	4.92	10.1	12.4	10.7
t_{max} [3] (h)	1	1	1	1	1.5	1
AUC[2] (µg.h/mL)	1.07	2.09	17.1	30.3	64.9[4]	60.2[5]
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ [2] (h)	0.4[6]	0.3[6]	0.9[6]	1.1[6]	0.4[6]	0.2[6]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base were 18.8, 94.2 and 471 mg/kg
[2] : mean value
[3] : median value
[4] : not accurately determined (% of extrapolation > 15%): $AUC_{0\text{-}8h}$ = 51.8 µg.h/mL
[5] : not accurately determined (% of extrapolation > 15%): $AUC_{0\text{-}8h}$ = 50.5 µg.h/mL
[6] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.3.E Pharmacokinetics: Absorption after a Single Dose in Rats

Test article: darunavir

Location in CTD	**4.2.3.1**			
Study No.	**TMC114-NC104**			
Species	**Rat (Wistar)**			
Feeding Condition	fasted			
Vehicle/Formulation	PEG400			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	M/2 + F/2	M/2 + F/1	M/2 + F/2	M/2 + F/2
Dose[1] (mg/kg)	500	1000	1500	2000
Concentration (mg/mL)	100	200	300	400
Sample	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	11.0	8.90	9.47	11.9
t_{max} (h)	1	1	1	2
AUC (µg.h/mL)	91.3[2]	49.7	48.6	66.8[3]
(Time for calculation –h)	(0-∞)	(0-∞)	(0-8)	(0-∞)
$t_{1/2}$ (h)	2.9[4]	2.0[4]	NC	2.6[4]
(Time for calculation –h)	NS	NS	-	NS

[1] : doses expressed as TMC114 base were 483, 965, 1448 and 1884 mg/kg
[2] : not accurately determined (% of extrapolation > 15%) : $AUC_{0\text{-}8h}$ = 68.5 µg.h/mL
[3] : not accurately determined (% of extrapolation > 15%) : $AUC_{0\text{-}8h}$ = 56.6 µg.h/mL
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified
NC : not calculated

2.6.5.3.F Pharmacokinetics: Absorption after a Single Dose in Rats

Test article: darunavir

Location in CTD	**4.2.3.1**		
Study No.	**TMC114-NC110**		
Species	**Rat (Wistar)**		
Feeding Condition	not fasted		
Vehicle/Formulation	PEG400/EtOH/NaCl (50/10/40 % v/v)		
Route	Intravenous (slow infusion)		
Gender (M/F)/Number of Animals	M/5 + F/5	M/5 + F/5	M/5 + F/5
Dose[1] (mg/kg)	10	20	40
Concentration (mg/mL)	1	2	4
Sample	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
AUC (µg.h/mL)	3.10	7.23	15.2
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	0.4	1.0	0.4[2]
(Time for calculation –h)	NS	NS	NS

[1] : doses expressed as TMC114 base were 9.3, 18.7 and 37.3 mg/kg
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
EtOH : Ethanol
NaCl : Sodium Chloride
NS: not specified

2.6.5.3.G Pharmacokinetics: Absorption after a Single Dose in Dogs

Test article: darunavir

Location in CTD	**4.2.3.1**			
Study No.	**TMC114-NC102**			
Species	**Dog (Beagle)**			
Feeding Condition	fasted			
Vehicle/Formulation	PEG400			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	M/2 + F/2	M/2 + F/2	M/1 + F/1	M/1 + F/1
Dose[1] (mg/kg)	20	80	320	80
Concentration (mg/mL)	20	80	320	80
Duration of Dosing (day)	1	1	1	3
Sample	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max}[2] (µg/mL)	7.49	19.1	16.7	22.4
t_{max}[3] (h)	1.0	1.0	1.3	1.3
AUC[2] (µg.h/mL)	17.9	74.6	62.6	68.4
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-9)
$t_{1/2}$[2] (h)	0.6[4]	0.8[4]	0.9[4]	0.6
(Time for calculation –h)	NS	NS	NS	NS
Additional information				
Placebo and escalating doses of TMC114 were administered once daily on 4 consecutive days. The maximum tolerated dose (80 mg/kg) was given once daily on 3 consecutive days.				

[1] : doses expressed as TMC114 base were 18.8, 75.4 and 301 mg/kg
[2] : mean value
[3] : median value
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.3.H Pharmacokinetics: Absorption after a Single Dose in Rat Pups

Test article: darunavir

Location in CTD	**4.2.3.5.4**								
Study No.	**TMC114-NC240**								
Species	**Rat (Sprague-Dawley pup)**								
Feeding Condition	not fasted								
Vehicle/Formulation	PEG400								
Route	Oral (gavage)								
Gender (M/F)/Number of Animals	M + F/ >14								
Age (Postnatal day)	5			8			11		
Dose (mg base eq./kg/day)	20	40	80	40	80	120	40	80	160
Concentrations (mg/mL)	2	4	8	4	8	12	4	8	16
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters									
C_{max} (µg/mL)	2.70	3.50	14.9	5.14	17.6	55.9	4.11	8.14	22.5
t_{max} (h)	1	2	4	2	1	1	2	2	2
AUC (µg.h/mL)	11.0	22.9	89.2	34.4	122	242	16.4	50.6	232
(Time for calculation –h)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	1.1	2.3	2.8	3.0	2.2	2.3	4.4	2.7	2.5
(Time for calculation –h)	(4-8)	(8-24)	(8-24)	(4-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)
Additional Information									
The AUC_{0-8h} values ranged between 2.24 to 54.4 µg.h/g in the brain and between 68.2 to 917 µg.h/g in the liver. At all doses, TMC114 AUC_{0-8h} were 4 to 8 times lower in brain than in plasma but were 5 to 7 times higher in liver than in plasma.									

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.3.I Pharmacokinetics: Caco-2 Permeability of COBI (In Vitro)

Report Title	Study Type	Test Article	Report Number Location in CTD
Bi-directional Permeability of GS-9350 and Ritonavir in Caco-2 Cell Monolayers	Absorption study (in vitro)	COBI	AD-216-2023 4.2.2.2

Bi-directional Permeability of COBI Through Caco-2 Cells

Direction	Target Conc. (µM)	Initial Conc. (µM)	Recovery (%)	P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
Cell-Free	1	1.2	ND	9.45	--	9.45	
Forward		1.4	73.8	7.28	7.95	7.61	1.1
Reverse		1.3	55.0	5.26	11.8	8.51	

Caco-2 = human colonic adenocarcinoma cell line; COBI = cobicistat; Conc. = concentration; ND = not determined due to missing donor well concentration at 120 minutes; P_{app} = apparent permeability

2.6.5.3.J Pharmacokinetics: Absorption after a Single Dose in Mice

Report Title	Study Type	Test Article	Report Number Location in CTD
Determination of the Pharmacokinetics of GS-9350 Following a Single Oral Gavage Dose to Male and Female 001178-W (wild-type) Mice	Single-Dose Pharmacokinetics	COBI	PC-216-2013-PK 4.2.2.2

Mean Pharmacokinetic Parameters of COBI Following Single Oral Doses in Female and Male CByB6F1-Tg(HRAS)2Jic (001178-W) Mice

Species/Strain Number of Animals/Group Sex	Administration Route	Dosage (mg/kg)	Gender	Feeding Condition	AUC_{0-24} (ng•h/mL)	C_{max} (ng/mL)	t_{max} (h)	C_{24h} (ng/mL)
01178-W (wild), CByB6F1-Tg(HRAS)2Jic mice 24M and 24F/group	Oral (10% Propylene glycol in 40 mM acetate buffer, pH 4.0)	30	Female	Nonfasted	46,306	10,158	1.0	1.28
			Male		35,535	5940	2.0	1.67
		100	Female		128,930	16,205	1.0	1532
			Male		108,796	11,130	2.0	1418
		300	Female		NC	23,464	2.0	NC
			Male		NC	29,392	4.0	NC

AUC = area under the plasma concentration-time curve; C_{24h} = plasma concentration at 24 hours after administration; C_{max} = maximum plasma concentration; COBI = cobicistat; NC = not calculated due to insufficient data (animals euthanized at 4 hour time point); t_{max} = time to reach the maximum plasma concentration

2.6.5.3.K Pharmacokinetics: Absorption after a Single Dose in Rats

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics of GS-9350 in Sprague-Dawley Rats	Single-Dose Pharmacokinetics	COBI	AD-216-2020 4.2.2.2

Pharmacokinetic Parameters of COBI in Male and Female Sprague-Dawley Rats (mean ± SD, n = 3)

Species/Strain Number of Animals/Group Sex	Administration Route	Dose Level (mg/kg)	Dose Volume (mL/kg)	Feeding Condition	t_{max} (h)	C_{max} (nM)	$t_{½}$ (h)	$AUC_{0-\infty}$ (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	F (%)
Sprague-Dawley Rat 3 Male or 3 Female animals/group	IV Infusion (M)[a]	1	5	Fasted	0.48 ± 0.0	664 ± 31.4	0.40 ± 0.02	351 ± 12.6	3.59 ± 0.14	0.76 ± 0.14	—
	IV Infusion (F)[a]	1	5		0.48 ± 0.0	890 ± 74.3	0.35 ± 0.01	566 ± 50.1	2.37 ± 0.18	0.70 ± 0.09	—
	Oral (M)[b]	5	10		0.50 ± 0.0	764 ± 506	0.92 ± 0.22	594 ± 42.6	—	—	33 ± 3

AUC = area under the plasma concentration-time curve; CL = clearance; C_{max} = maximum plasma concentration; COBI = cobicistat; F = bioavailability; IV = intravenous; SD = standard deviation; t½ = elimination half-life; t_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Intravenous dosing vehicle was 5% ethanol, 10% propylene glycol, and 85% water.

b Oral dosing vehicle was 5% ethanol, 15% propylene glycol, 80% water (pH 3.5, HCl) for the male rat groups and 10% ethanol, 30% propylene glycol, and 60% water for the female rat groups.

2.6.5.3.K Pharmacokinetics: Absorption after a Single Dose in Rats (Continued)

Test Article: COBI

Pharmacokinetic Parameters of COBI Following Escalating Oral Doses of COBI in Male and Female Sprague-Dawley Rats (mean ± SD, n = 3)

Species/Strain Number of Animals/Group Sex	Administration Route	Dosage (mg/kg)	Dose Volume (mL/kg)	Gender	Feeding Condition	PK Parameter				
						AUC_{0-t} (nM•h)	C_{max} (nM)	t_{max} (h)	C_{last} (nM)	t_{last} (h)
Sprague-Dawley Rat 3 Male or 3 Female animals/group	Oral[a]	25	10	M	Fasted	13,233 ± 1942	4000 ± 684	1.08 ± 0.88	6.58 ± 2.02	8
		25	10	F		26,087 ± 6923	4506 ± 237	0.83 ± 1.01	1.24 ± 0.16	24
		100	10	M		65,185 ± 21,658	6895 ± 933	1.42 ± 1.01	14.0 ± 8.70	24
		110	10	F		170,525 ± 20,189	12,784 ± 956	6.67 ± 2.31	2708 ± 1510	24

AUC = area under the plasma concentration-time curve; C_{last} = concentration of last measurable sample; C_{max} = maximum plasma concentration; COBI = cobicistat; F = female; M = male; SD = standard deviation; t_{last} = the last time point at which a quantifiable drug concentration can be measured; t_{max} = time to reach the maximum plasma concentration

a Oral dosing vehicle was 5% ethanol, 15% propylene glycol, 80% water (pH 3.5, HCl) for the male rat groups and 10% ethanol, 30% propylene glycol, and 60% water for the female rat groups

2.6.5.3.L Pharmacokinetics: Absorption after a Single Dose in Dogs

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics of GS-9350 in Male Beagle Dogs	Single-Dose Pharmacokinetics	COBI	AD-216-2021 4.2.2.2

Pharmacokinetic Parameters of COBI in Male Beagle Dogs (mean ± SD, n = 3)

Species/Strain Number of Animals/Group Sex	Administration Route	Dose Level (mg/kg)	Dose Volume (mL/kg)	Feeding Condition	t_{max} (h)	C_{max} (nM)	$t_{½}$ (h)	$AUC_{0-\infty}$ (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	F (%)
Beagle Dog 3 animals/group	IV Infusion[a]	1	1	Fasted	0.48 ± 0.0	924 ± 267	1.02 ± 0.04	565 ± 155	2.18 ± 0.69	1.33 ± 0.69	—
	Oral[b,c]	5	2		1.00 ± 0.43	313 ± 186	1.12 ± 0.14	331 ± 130	—	—	11 ± 4

AUC = area under the plasma concentration-time curve; CL = clearance; C_{max} = maximum plasma concentration; COBI = cobicistat; F = bioavailability; IV = intravenous; SD = standard deviation; t½ = elimination half-life; t_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Intravenous (via 30-minute infusion) dosing vehicle was 5% ethanol, 15% propylene glycol, and 80% water

b Oral dosing vehicle was 5% ethanol, 30% propylene glycol, 65% water

c Elvitegravir was coadministered with COBI in this group of animals

2.6.5.3.L Pharmacokinetics: Absorption after a Single Dose in Dogs (Continued)

Test Article: COBI

Pharmacokinetic Parameters of COBI Following Escalating Oral Doses of COBI in Beagle Dogs (mean +/- SD, n = 3)

Species/Strain Number of Animals/Group Sex	Adminis-tration Route	Dosage (mg/kg)	Dose Volume (mL/kg)	Feeding Condition	PK Parameter				
					AUC_{0-t} (nM•h)	C_{max} (nM)	t_{max} (h)	C_{last} (nM)	t_{last} (h)
Beagle Dog 3 animals/group	Oral[a]	10	5	Fasted	355 ± 435	118 ± 57.6	1.50 ± 2.17	2.90 ± 2.73	9.33 ± 2.31
		30			34,538 ± 13,033	4373 ± 2307	2.33 ± 1.53	13.6 ± 14.3	24.0 ± 0.0
		100			102,223 ± 23,511	9640 ± 572	1.67 ± 2.02	872 ± 752	24.0 ± 0.0

AUC = area under the plasma concentration-time curve; C_{last} = concentration of last measurable sample; C_{max} = maximum plasma concentration; COBI = cobicistat; SD = standard deviation; t_{last} = the last time point at which a quantifiable drug concentration can be measured; t_{max} = time to reach the maximum plasma concentration

a Oral dosing vehicle (by volume) was 1.5% ethanol, 1.5% propylene glycol, 6% Labrasol, 6% Solutol, and 85% water

2.6.5.3.M Pharmacokinetics: Absorption after a Single Dose in Cynomolgus Monkeys

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics of GS-9350 in Cynomolgus Monkeys	Single-Dose Pharmacokinetics	COBI	AD-216-2022 4.2.2.2

Pharmacokinetic Parameters of COBI in Male Cynomolgus Monkeys (mean ± SD, n = 3)

Species/Strain Number of Animals/Group Sex	Administration Route	Dose Level (mg/kg)	Dose Volume (mL/kg)	Feeding Condition	t_{max} (h)	C_{max} (nM)	$t_{½}$ (h)	$AUC_{0-\infty}$ (nM•h)	CL (L/h/kg)	V_{ss} (L/kg)	F (%)
Cynomolgus Monkey 3 animals/group Male	IV Infusion[a]	1	1	Fasted	0.48 ± 0.0	1222 ± 41.1	1.42 ± 0.07	977 ± 83.7	1.36 ± 0.14	1.31 ± 0.12	—
	Oral[b]	6	2.5		2.17 ± 1.76	161 ± 102	1.36 ± 0.21	445 ± 280	—	—	7.3 ± 4.6

AUC = area under the plasma concentration-time curve; CL = clearance; C_{max} = maximum plasma concentration; COBI = cobicistat; F = bioavailability; IV = intravenous; SD = standard deviation; t½ = elimination half-life; t_{max} = time to reach the maximum plasma concentration; V_{ss} = volume of distribution at steady state

a Intravenous (via 30-minute infusion) dosing vehicle was 5% ethanol, 10% propylene glycol, and 85% water (pH 3.0)

b Oral dosing vehicle was 5% ethanol, 10% propylene glycol, 85% water

2.6.5.4.A Pharmacokinetics: Absorption and Plasma Kinetics after Repeated Oral Doses in Mice

Test article: darunavir

Location in CTD		**4.2.2.2**	
Study No.		**TMC114-NC200**	
Species		**Mouse (CD1)**	
Feeding Condition		Fed	
Vehicle/Formulation		Dietary admixture	
Route		Oral	
Gender (M/F)/Number of Animals	M/18	M/18	M/18
Dose (mg.base eq./kg/day)	150	1000	5000
Duration of Dosing (day)	7	7	7
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	0.052	0.318	0.908
t_{max} [1] (hh:min)	23:00	07:00	23:00
AUC (µg.h/mL)	0.734	5.60	15.0
(Time for calculation –h)	(0-24)	(0-24)	(0-24)
Additional information, In the control group, 2 out 18 samples were slightly higher than the lower limit of quantification (5 ng/mL).			

[1] : o'clock time

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4.B Pharmacokinetics: Absorption after Repeated Oral Doses in Mice

Test article: darunavir

Location in CTD	**4.2.3.4.3**											
Study No.	**TMC114-NC194**											
Species	**Mouse (CB6F1-non TgrasH2)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/15		F/15		M/15		F/15		M/15		F/15	
Dose (mg.base eq./kg/day)	150		150		450		450		1000		1000	
Concentrations (mg/mL)	15		15		45		45		100		100	
Duration of Dosing (day)	1	28	1	28	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters												
C_{max} (µg/mL)	5.95	4.20	6.49	3.06	8.10	5.96	12.7	7.01	23.5	8.77	25.8	14.4
t_{max} (h)	1	1	1	1	1	1	1	1	1	1	2	1
AUC (µg.h/mL)	11.3	9.12	13.1	8.73	49.6	19.1[1]	57.3	30.4	128	38.2	126	46.5
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	1.4	NC	1.6	6.8	1.6	2.6	2.2	3.4	9.2	3.0	2.6	2.3
(Time for calculation –h)	(6-12)	-	(6-12)	(12-24)	(12-24)	(6-12)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)

Additional information

TMC114 levels above the lower limit of quantification (LLOQ) were detected in the control vehicle group. These levels occurred frequently (4/9 to 6/9 samples per sex and per sampling day had TMC114 levels above LLOQ) but in trace amounts (the plasma levels were less than 2 % of the corresponding C_{max} value at the low dose-level).

[1] : extrapolated AUC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC: not calculated

2.6.5.4.C Pharmacokinetics: Absorption after Repeated Oral Doses in Mice

Test article: darunavir

Location in CTD	**4.2.3.4.1**											
Study No.	**TMC114-NC157**											
Species	**Mouse (CD1)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/30		F/30		M/30		F/30		M/30		F/30	
Dose (mg.base eq./kg/day)	150		150		450		450		1000		1000	
Concentrations (mg.base eq./mL)	15		15		45		45		100		100	
Duration of Dosing (day)	1	87	1	87	1	87	1	87	1	87	1	87
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	5.02	1.78	7.92	2.89	9.46	4.79	14.3	8.45	11.9	4.18	29.0	6.80
t_{max} (h)	1	2	1	2	1	1	2	2	1	2	1	1
AUC (µg.h/mL)	10.7	6.03[1]	21.3	8.48[1]	28.4	22.4[1]	79.0	45.8[1]	59.8	33.0	143	50.3
(Time for calculation –h)	(0-!)	(0-24)	(0-8)	(0-24)	(0-!)	(0-24)	(0-8)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	1.0	1.6	5.9	1.6	0.6	1.7	6.2	2.3	2.8	2.1	1.6	1.7
(Time for calculation –h)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(8-24)	(8-24)	(8-24)	(8-24)

[1] : extrapolated AUC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.4.D Pharmacokinetics: Absorption after Repeated Oral Doses in Mice (TMC114 in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.2.2**							
Study No.	**TMC114-NC223**							
Species	**Mouse (Swiss mice)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400 (TMC114)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/9		F/9		M/9		F/9	
Dose (mg.base eq./kg/day) of TMC114	1000		1000		1000		1000	
Concentrations (mg.base eq./mL) of TMC114	100		100		100		100	
Dose[1] (mg/kg/day) of ritonavir	0		0		50		50	
Concentrations (mg/mL) of ritonavir	0		0		8		8	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	18.9	9.41	22.0	13.6	13.7	16.0	19.3	15.2
t_{max} (h)	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AUC (µg.h/mL)	65.8	34.9	109	55.9	83.3	65.3	137	82.1
(Time for calculation –h)	(0-∞)	(0-8)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.7	NC	2.3	1.8	4.3	1.7	3.2	1.7
(Time for calculation –h)	(8-24)	-	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

[1] : ritonavir was administered just before the administration of TMC114

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.4.D Pharmacokinetics: Absorption after Repeated Oral Doses in Mice (TMC114 in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.2.2**			
Study No.	**TMC114-NC223**			
Species	**Mice (Swiss mice)**			
Feeding Condition	not fasted			
Vehicle/Formulation	Propylene glycol			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	M/9		F/9	
Dose (mg.base eq./kg/day) of TMC114	1000		1000	
Concentrations (mg.base eq./mL/kg) of TMC114	100		100	
Dose[1] (mg/kg/day) of ritonavir	50		50	
Concentrations (mg/mL) of ritonavir	8		8	
Duration of Dosing (day)	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	0.850	0.942	2.55	1.31
t_{max} (h)	1	0.5	0.5	0.5
AUC (µg.h/mL)	2.56	1.63	4.06	3.74
(Time for calculation –h)	(0-∞)	(0-8)	(0-∞)	(0-8)
$t_{1/2}$ (h)	4.5	NC	4.3	NC
(Time for calculation –h)	(4-24)	-	(4-24)	-

[1]: ritonavir was administered just before the administration of TMC114

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NC : not calculated

2.6.5.4.E Pharmacokinetics: Absorption after Repeated Oral Doses in Rats

Test article: darunavir

Location in CTD		**4.2.2.2**	
Study No.		**TMC114-NC201**	
Species		**Rat (Sprague Dawley)**	
Feeding Condition		Fed	
Vehicle/Formulation		Dietary admixture	
Route		Oral	
Gender (M/F)/Number of Animals	M/6	M/6	M/6
Dose (mg.base eq./kg/day)	100	500	2500
Duration of Dosing (day)	7	7	7
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	0.608	1.63	3.35
t_{max} [1] (hh:min)	7:00	7:00	7:00
AUC (µg.h/mL)	9.05	30.5	52.2
(Time for calculation –h)	(0-24)	(0-24)	(0-24)

[1] : o'clock time

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4.F Pharmacokinetics: Absorption after Repeated Oral Doses in Rats

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC107**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5		F/5		M/5		F/5		M/5		F/5	
Dose[1] **(mg/kg/day)**	40		40		200		200		1000		1000	
Concentrations (mg/mL)	8		8		40		40		200		200	
Duration of Dosing (day)	1	14	1	14	1	14	1	14	1	14	1	14
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	1.47	2.20	2.73	2.29	9.36	5.88	9.93	5.56	13.1	8.31	15.5	7.98
t_{max} (h)	0.5	0.5	0.5	1	1	2	1	1	2	2	2	0.5
AUC (µg.h/mL)	6.27	7.30	10.6	7.86	62.4	38.7	74.2	33.2	109	73.2	177[2]	65.1
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	2.0[3]	2.7[3]	2.1	2.2	1.9[3]	0.6[3]	1.7[3]	5.7[3]	1.9[3]	6.3[3]	6.7[3]	56[3]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base were 37.3, 187 and 933 mg/kg/day
[2] : not accurately determined (% of extrapolation > 15%) : $AUC_{0\text{-}12h}$ = 128 µg.h/mL
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.G Pharmacokinetics: Absorption after Repeated Oral Doses in Rats

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC130**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5			F/5			M/5			F/5		
Dose[1] (mg/kg/day)	20			20			100			100		
Concentrations (mg/mL)	4			4			20			20		
Duration of Dosing (day)	1	48	91	1	48	91	1	48	91	1	48	91
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	TMC114			TMC114			TMC114			TMC114		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	0.900	0.207	0.776	2.22	1.54	1.66	7.80	8.11	5.51	8.26	4.87	8.16
t_{max} (h)	1	4	1	1	1	1	1	1	1	2	1	2
AUC (µg.h/mL)	3.90[2]	1.87	2.10	6.98	5.43	3.66	38.1	22.1	27.4	42.9	29.6	28.2
(Time for calculation –h)	(0-!)	(0-24)	(0-8)	(0-!)	(0-24)	(0-8)	(0-!)	(0-8)	(0-24)	(0-!)	(0-24)	(0-8)
$t_{1/2}$ (h)	2.9[3]	4.6	1.3	3.4[3]	4.3[3]	1.3	2.2	1.7	3.1[3]	3.0	2.6	1.6
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bioavailability[4]	37			58								

[1] : doses expressed as TMC114 base were 19 and 95 mg/kg/day
[2] : not accurately determined (% of extrapolation >15 %) : AUC_{0-8h} = 3.17 µg.h/mL
[3] : not accurately determined
[4] : calculated relative to intravenous data obtained after single dose at 25 mg/kg from TMC114-NC160 (2.6.5.4O)

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.G Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**					
Study No.	**TMC114-NC130**					
Species	**Rat (Sprague Dawley)**					
Feeding Condition	not fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals		M/5			F/5	
Dose[1] (mg/kg/day)		500			500	
Concentrations (mg/mL)		100			100	
Duration of Dosing (day)	1	48	91	1	48	91
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	11.8	6.52	9.00	14.2	14.3	9.68
t_{max} (h)	8	1	1	2	1	2
AUC (µg.h/mL)	184	83.8	67.6	166	83.0	71.8
(Time for calculation –h)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)
$t_{1/2}$ (h)	4.9[2]	4.5	4.2	3.1[2]	4.1	6.5
(Time for calculation –h)	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base were 475 mg/kg/day
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.H Pharmacokinetics: Absorption after Repeated Oral Doses in Rats

Test article: darunavir

Location in CTD	**4.2.3.4.3**											
Study No.	**TMC114-NC196**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	Fed											
Vehicle/Formulation	dietary admixture											
Route	Oral (diet)											
Gender (M/F)/Number of Animals	M/6			F/6			M/6			F/6		
Dose[1] (mg.base eq./kg/day)	260			260			1040			1040		
Duration of Dosing (Week)	1	4	13	1	4	13	1	4	13	1	4	13
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	TMC114			TMC114			TMC114			TMC114		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	1.43	1.68	1.42	1.24	1.32	1.89	2.71	2.81	3.03	3.16	3.78	3.47
t_{max}[2] (hh:mm)	03:00	03:00	03:00	19:00	03:00	03:00	03:00	03:00	03:00	03:00	03:00	07:00
AUC (µg.h/mL)	24.8	26.9	23.9	21.9	23.5	30.8	53.2	54.4	47.4	62.4	60.8	66.4
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
Additional information												
In the control group (received no TMC114 treatment) TMC114-plasma concentrations above the lower limit of quantitation (5 ng/mL) were found in 17 out of 30 control samples taken in weeks 1 to 13 (concentrations ranged between 0.005 and 0.024 µg/mL).												

[1] : Nominal doses
[2] : o'clock time

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4.H Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (continued)

Test article: darunavir

Location in CTD	**4.2.3.4.3**						
Study No.	**TMC114-NC196**						
Species	**Rat (Sprague Dawley)**						
Feeding Condition	Fed						
Vehicle/Formulation	dietary admixture						
Route	Oral (diet)						
Gender (M/F)/Number of Animals		M/6			F/6		
Dose[1] (mg.base eq./kg/day)		2600			2600		
Duration of Dosing (Week)	1	4	13	1	4	13	
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters							
C_{max} (µg/mL)	4.36	3.76	4.56	4.44	5.22	6.30	
t_{max}[2] (hh:mm)	03:00	03:00	07:00	19:00	19:00	19:00	
AUC (µg.h/mL)	65.1	76.1	72.2	95.2	113	123	
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	

[1]: Nominal doses
[2] : o'clock time

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4.I Pharmacokinetics: Absorption after Repeated Oral Doses in Rats

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC132 (FK4848)[1]**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5			F/5			M/5			F/5		
Dose (mg/kg/day)	20			20			100			100		
Concentration (mg/mL)	4			4			20			20		
Duration of Dosing (Day)	1	86	177	1	86	177	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)	Plasma			Plasma			Plasma			Plasma		
Analyte	TMC114			TMC114			TMC114			TMC114		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	1.48	1.14	1.03	2.03	0.761[3]	1.77[4]	6.67	6.04[4]	7.11	7.62	9.45	12.8
t_{max} (h)	1	1	1	2	2[3]	1[4]	2	1[4]	1	2	2	1
AUC (µg.h/mL)	3.34	2.92[5]	2.92	6.46	1.79[3,5]	4.38[4]	28.1	24.6[4]	31.6	44.0	31.3	35.8
(Time for calculation –h)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)	(0-8)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)
$t_{1/2}$ (h)	2.2	2.3	4.6	1.1	1.5	6.2	6.8	2.9	5.6	2.4	3.5	3.0
(Time for calculation –h)	(4-8)	(4-8)	(4-24)	(4-8)	(4-8)	(4-24)	(4-8)	(4-24)	(8-24)	(4-24)	(4-24)	(4-24)

[1] : Data after interpretation at Johnson and Johnson in GLP context
[2] : doses expressed as TMC114 base were 19 and 95 mg/kg/day. From day 43, doses expressed as TMC114 base were 18.8 and 94 mg/kg/day
[3] : inaccurate determination : sampling time 1h missing
[4] : inaccurate determination : sampling time 2h missing
[5] : extrapolated AUC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.4.I Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**					
Study No.	**TMC114-NC132 (FK4848)[1]**					
Species	**Rat (Sprague Dawley)**					
Feeding Condition	not fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals		M/5			F/5	
Dose[2] (mg/kg/day)		500			500	
Concentration (mg/mL)		100			100	
Duration of Dosing (Day)	1	86	177	1	86	177
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	12.1	4.51	10.3	11.6	21.9	24.5
t_{max} (h)	4	2	1	1	2	1
AUC (µg.h/mL)	164	58.0	63.8	140	110	121
(Time for calculation –h)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)
$t_{1/2}$ (h)	3.7	3.8	5.9	4.9	NC	5.3
(Time for calculation –h)	(8-24)	(4-24)	(4-24)	(4-24)	-	(4-24)

[1] : Data after interpretation at Johnson and Johnson in GLP context
[2] : dose expressed as TMC114 base was 475 mg/kg/day . From day 43, dose expressed as TMC114 base was 470 mg/kg/day

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.4.J Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.2.2**					
Study No.	**TMC114-NC139**					
Species	**Rat (Wistar)**					
Feeding Condition	not fasted					
Vehicle/Formulation	PEG400 (TMC114) – Propylene glycol (ritonavir)					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	M/4		M/4		M/4	
Dose[1] (mg/kg/day) of TMC114	150		500		500	
Concentrations (mg/mL) of TMC114	37.5		125		125	
Dose[2] (mg/kg/day) of ritonavir	25		15		25	
Concentrations (mg/mL) of ritonavir	40		40		40	
Duration of Dosing (day)	1	7	1	7	1	7
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max}[3] (µg/mL)	8.81	7.48	10.4	7.47	12.7	10.5
t_{max}[4] (h)	1	2	4	6	2	1
AUC[3] (µg.h/mL)	100	76.3	151	92.8	176	108
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$[3] (h)	2.7[5]	1.7[5]	2.9[5]	2.5[5]	3.2[5]	2.8[5]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base were139 and 464 mg/kg/day
[2] : ritonavir was administered just before the administration of TMC114
[3] : mean value
[4] : median value
[5] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400; NS : not specified

2.6.5.4.K Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC141**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5		F/5		M/5		F/5		M/5		F/5	
Dose[1] (mg/kg/day) of TMC114	150		150		500		500		1000		1000	
Concentrations (mg/mL) of TMC114	30		30		100		100		200		200	
Dose[2] (mg/kg/day) of ritonavir	25		25		25		25		50		50	
Concentrations (mg/mL) of ritonavir	20		20		20		20		40		40	
Duration of Dosing (day)	1	14	1	14	1	14	1	14	1	14	1	14
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	9.95	9.09	9.26	7.95	12.4	12.9	13.0	11.0	14.0	14.9	11.0	13.6
t_{max} (h)	2	2	6	1	6	2	2	0.5	2	2	0.5	0.5
AUC (µg.h/mL)	144	68.3	127	98.9	193	102	213	117	219	111	189	126
(Time for calculation –h)	(0-!)	(0-12)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	2.0[3]	2.8[3]	5.0	1.9[3]	5.5	1.9[3]	5.8	3.3	7.2	3.3	5.5[3]	2.8[3]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base were 156, 518 and 1022 mg/kg/day
[2] : ritonavir was administered just before the administration of TMC114
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.K Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article : darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC141**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	Propylene glycol											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5		F/5		M/5		F/5		M/5		F/5	
Dose[1] (mg/kg/day) of TMC114	150		150		500		500		1000		1000	
Concentrations (mg/mL) of TMC114	30		30		100		100		200		200	
Dose[2] (mg/kg/day) of ritonavir	25		25		25		25		50		50	
Concentrations (mg/mL) of ritonavir	20		20		20		20		40		40	
Duration of Dosing (day)	1	14	1	14	1	14	1	14	1	14	1	14
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	ritonavir		ritonavir		ritonavir		ritonavir		ritonavir		ritonavir	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	0.934	0.335	0.767	0.246	0.256	0.271	0.219	0.166	0.666	0.287	0.325	0.631
t_{max} (h)	2	2	2	0.5	2	1	2	0.5	1	0.5	2	0.5
AUC (µg.h/mL)	9.38[3]	2.25	6.36	1.50	1.98	0.790	1.80[4]	0.570	5.84	1.47	3.03	1.38
(Time for calculation –h)	(0-!)	(0-12)	(0-!)	(0-12)	(0-!)	(0-12)	(0-!)	(0-12)	(0-!)	(0-12)	(0-!)	(0-12)
$t_{1/2}$ (h)	6.6[5]	2.2[5]	3.3	NC	4.6	14[5]	5.0	4.8	2.9	2.3	3.6	NC
(Time for calculation –h)	NS	NS	NS	-	NS	NS	NS	NS	NS	NS	NS	-

[1] : doses expressed as TMC114 base were 156, 518 and 1022 mg/kg/day
[2] : ritonavir was administered just before the administration of TMC114
[3] : not accurately determined (% of extrapolation>15%) : $AUC_{0\text{-}12h}$ = 6.36 µg.h/mL
[4] : not accurately determined (% of extrapolation>15%): $AUC_{0\text{-}12h}$ = 1.41 µg.h/mL
[5] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS : not specified
NC : not calculated

2.6.5.4.K Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**			
Study No.	**TMC114-NC141**			
Species	**Rat (Wistar)**			
Feeding Condition	not fasted			
Vehicle/Formulation	Propylene glycol			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	M/5		F/5	
Dose (mg/kg/day) of TMC114	0		0	
Concentrations (mg/mL) of TMC114	0		0	
Dose[1] (mg/kg/day) of ritonavir	50		50	
Concentrations (mg/mL) of ritonavir	40		40	
Duration of Dosing (day)	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	5.20	4.72	4.45	3.62
t_{max} (h)	2	2	0.5	0.5
AUC (µg.h/mL)	531[2]	35.3	57.9	38.7
(Time for calculation –h)	(0-!)	(0-12)	(0-!)	(0-24)
$t_{1/2}$ (h)	105[3]	4.2	2.4[3]	2.4[3]
(Time for calculation –h)	NS	NS	NS	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined (% of extrapolation > 15 %): $AUC_{0\text{-}12h}$ = 44.7 µg.h/mL
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS : not specified

2.6.5.4.L Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.3.2**							
Study No.	**TMC114-NC143**							
Species	**Rat (Wistar)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/5		F/5		M/5		F/5	
Dose (mg.base eq./kg/day) of TMC114	2000		2000		2000		2000	
Concentrations (mg.base eq./mL) of TMC114	333		333		333		333	
Dose[1] (mg/kg/day) of ritonavir	0		0		75		75	
Concentrations (mg/mL) of ritonavir	0		0		60		60	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	15.8	8.52	14.3	8.98	13.3	16.8	15.6	21.5
t_{max} (h)	2	2	2	2	2	0.5	2	0.5
AUC (µg.h/mL)	193	74.0	154	86.9	198	193	741[2]	357
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	7.1	8.6	5.6	28[3]	5.8[3]	5.0	46[3]	17[3]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined (% of extrapolation > 15%) : AUC_{0-24h} = 235 µg.h/mL
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.L Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**							
Study No.	**TMC114-NC143**							
Species	**Rat (Wistar)**							
Feeding Condition	not fasted							
Vehicle/Formulation	Propylene glycol							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/5		F/5		M/5		F/5	
Dose (mg/kg/day) of TMC114	0		0		2000		2000	
Concentrations (mg/mL) of TMC114	0		0		333		333	
Dose[1] (mg/kg/day) of ritonavir	75		75		75		75	
Concentrations (mg/mL) of ritonavir	60		60		60		60	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	5.78	7.71	6.88	3.25	0.547	1.16	0.998	1.17
t_{max} (h)	6	2	1	12	1	1	2	2
AUC (µg.h/mL)	83.0	63.8	157[2]	62.3	4.21	6.75	9.02	14.9
(Time for calculation –h)	(0-!)	(0-12)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	2.2[3]	3.8[3]	23[3]	15[3]	3.2	2.8	4.0[3]	4.9[3]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined (% of extrapolation > 15%): AUC_{0-24h} = 87.7 µg.h/mL
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS : not specified

2.6.5.4.M Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.3.7.2**							
Study No.	**TMC114-NC187**							
Species	**Rat (Sprague-Dawley)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6	M/6	F/6	M/6	F/6
Dose (mg.base eq./kg/day) of TMC114	20	20	100	100	500	500	100	100
Concentrations (mg.base eq./mL) of TMC114	4	4	20	20	100	100	20	20
Dose[1] (mg/kg/day) of ritonavir	0	0	0	0	0	0	50	50
Concentrations (mg/mL) of ritonavir	0	0	0	0	0	0	20	20
Duration of Dosing (day)	26	25	26	25	26	25	26	25
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.748	1.71	3.87	6.54	13.6	9.96	6.62	6.63
t_{max} (h)	2	1	1	1	4	4	2	4
AUC (µg.h/mL)	4.20	4.68	25.2	30.0	102	84.4	59.0	57.7
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NC	10	3.6	16	5.3	3.3	1.7	3.4
(Time for calculation –h)	-	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)	(12-24)

[1] : ritonavir was administered just before the administration of TMC114

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.4.M Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD			**4.2.3.7.2**	
Study No.			**TMC114-NC187**	
Species			**Rat (Sprague-Dawley)**	
Feeding Condition			not fasted	
Vehicle/Formulation			Propylene glycol	
Route			Oral (gavage)	
Gender (M/F)/Number of Animals	M/6	F/6	M/6	F/6
Dose (mg.base eq./kg/day) of TMC114	0	0	100	100
Concentrations (mg.base eq./mL) of TMC114	0	0	20	20
Dose[1] (mg/kg/day) of ritonavir	50	50	50	50
Concentrations (mg/mL) of ritonavir	20	20	20	20
Duration of Dosing (day)	26	25	26	25
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	3.43	5.45	1.92	2.70
t_{max} (h)	1	1	4	4
AUC (µg.h/mL)	21.8[2]	37.3	14.4	20.1
(Time for calculation –h)	(0-24)	(0-12)	(0-12)	(0-24)
$t_{1/2}$ (h)	1.5	NC	NC	1.7
(Time for calculation –h)	(8-12)	-	-	(12-24)
Additional Information				
In the group receiving only ritonavir, TMC114 values above the lower limit of quantification (5 ng/mL) were found in 3 out of 18 samples in the males (range 0.011-0.016 µg/mL) and in 7 out of 18 samples in the females (range : 0.006- 0.031 µg/mL)				

[1] : ritonavir was administered just before the administration of TMC114
[2] : extrapolated value

LC-MS/MS = liquid chromatography with tandem mass spectroscopy; NC : not calculated

2.6.5.4.N Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC146**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5			F/5			M/5			F/5		
Dose (mg.base eq./kg/day) of TMC114	20			20			100			100		
Concentrations (mg.base eq./mL) of TMC114	5			5			25			25		
Dose[1] (mg/kg/day) of ritonavir	50			50			50			50		
Concentrations (mg/mL) of ritonavir	40			40			40			40		
Duration of Dosing (Day)	1	85	176	1	85	176	1	85	176	1	85	176
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	TMC114			TMC114			TMC114			TMC114		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	2.0	1.62	1.66	1.54	2.21	1.95	4.87	7.43	5.30	4.29	9.43	6.42
t_{max} (h)	1	2	2	1	1	1	1	2	1	1	1	1
AUC (µg.h/mL)	17.1[2]	10.0	11.2	826[3]	20.5	13.4	66.3	70.9	69.0	60.5	67.2	70.4
(Time for calculation –h)	(0-!)	(0-8)	(0-8)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)
$t_{1/2}$ (h)	5.2[4]	6.4[4]	9.5[4]	628[4]	5.6	7.2	4.2	2.5	2.4	7.0	3.1	6.0
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined (% of extrapolation > 15%) : $AUC_{0\text{-}8h}$ = 10.9 µg.h/mL
[3] : not accurately determined (% of extrapolation > 15%) : $AUC_{0\text{-}8h}$ = 7.79 µg.h/mL
[4] : not accurately determined
LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.N Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC146**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5			F/5			M/5			F/5		
Dose (mg.base eq./kg/day) of TMC114	500			500			1000			1000		
Concentrations (mg.base eq./mL) of TMC114	125			125			250			250		
Dose[1] (mg/kg/day) of ritonavir	75			75			75			75		
Concentrations (mg/mL) of ritonavir	60			60			60			60		
Duration of Dosing (Day)	1	85	176	1	85	176	1	85	176	1	85	176
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	TMC114			TMC114			TMC114			TMC114		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	11.4	16.1	11.5	7.81	22.0	26.6	9.74	12.7	14.4	10.8	16.4	24.8
t_{max} (h)	1	1	1	8	1	1	8	1	2	1	1	1
AUC (µg.h/mL)	296[2]	191	183	778[3]	294	318	282[4]	164	198	192	210	297
(Time for calculation –h)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	14[5]	12[5]	13[5]	65[5]	176[5]	8.5[5]	15[5]	10[5]	5.9	NC	NC	41[5]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	-	-	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined (% of extrapolation > 15%) : AUC_{0-24h} = 194 µg.h/mL
[3] : not accurately determined (% of extrapolation > 15%) : AUC_{0-24h} = 163 µg.h/mL
[4] : not accurately determined (% of extrapolation > 15%) : AUC_{0-24h} = 179 µg.h/mL
[5] : not accurately determined
LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC : not calculated ; NS : not specified

2.6.5.4.N Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC146**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	Propylene glycol											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5			F/5			M/5			F/5		
Dose (mg.base eq./kg/day) of TMC114	20			20			100			100		
Concentrations (mg.base eq./mL) of TMC114	5			5			25			25		
Dose[1] (mg/kg/day) of ritonavir	50			50			50			50		
Concentrations (mg/mL) of ritonavir	40			40			40			40		
Duration of Dosing (Day)	1	85	176	1	85	176	1	85	176	1	85	176
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	ritonavir			ritonavir			ritonavir			ritonavir		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	2.32	3.70	4.84	2.98	5.97	7.09	2.10	2.37	3.68	2.96	3.23	6.45
t_{max} (h)	8	1	2	8	1	2	4	1	2	1	2	1
AUC (µg.h/mL)	16.1	43.2	52.9	19.6	28.0	33.9	27.6	10.6	15.4	31.8	13.6	35.2
(Time for calculation –h)	(0-8)	(0-24)	(0-24)	(0-#)	(0-24)	(0-24)	(0-!)	(0-8)	(0-8)	(0-!)	(0-8)	(0-24)
$t_{1/2}$ (h)	NC	2.1	2.2	NC	23[2]	4.3	2.4	7.0[2]	3.0[2]	2.5	NC	2.7
(Time for calculation –h)	-	NS	NS	-	NS	NS	NS	NS	NS	NS	-	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS: not specified
NC : not calculated

2.6.5.4.N Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC146**											
Species	**Rat (Wistar)**											
Feeding Condition	not fasted											
Vehicle/Formulation	Propylene glycol											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/5			F/5			M/5			F/5		
Dose (mg.base eq./kg/day) of TMC114	500			500			1000			1000		
Concentrations (mg/mL) of TMC114	125			125			250			250		
Dose[1] (mg/kg/day) of ritonavir	75			75			75			75		
Concentrations (mg/mL) of ritonavir	60			60			60			60		
Duration of Dosing (Day)	1	85	176	1	85	176	1	85	176	1	85	176
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	ritonavir			ritonavir			ritonavir			ritonavir		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max} (µg/mL)	1.28	1.54	1.30	1.58	2.37	5.34	1.53	2.22	1.48	1.65	1.21	3.56
t_{max} (h)	1	1	2	2	8	1	1	2	1	1	1	1
AUC (µg.h/mL)	12.5	9.53	10.1	15.3	31.5	25.2	12.2	9.20	10.3	18.3	8.89	15.3
(Time for calculation –h)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)
$t_{1/2}$ (h)	4.2	5.5	4.6	5.6	5.9[2]	3.8	4.4	4.3	3.3	6.4	12[2]	9.6[2]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS: not specified

2.6.5.4.N Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD				**4.2.3.2**		
Study No.				**TMC114-NC146**		
Species				**Rat (Wistar)**		
Feeding Condition				not fasted		
Vehicle/Formulation				Propylene glycol		
Route				Oral (gavage)		
Gender (M/F)/Number of Animals		M/5			F/5	
Dose (mg.base eq./kg/day)		0			0	
Concentrations (mg/mL) of TMC114		0			0	
Dose (mg/kg/day) of ritonavir[1]		75			75	
Concentrations (mg/mL) of ritonavir		60			60	
Duration of Dosing (Day)	1	85	176	1	85	176
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	6.61	4.83	9.60	7.76	10.4	12.1
t_{max} (h)	1	2	1	2	1	1
AUC (µg.h/mL)	77.0	51.8	80.9	73.1	85.5	70.2
(Time for calculation –h)	(0-!)	(0-24)	(0-24)	(0-!)	(0-24)	(0-24)
$t_{1/2}$ (h)	3.7[2]	6.7	4.6	4.8	2.2[2]	5.5
(Time for calculation –h)	NS	NS	NS	NS	NS	NS
Additional information						
Some contaminations by TMC114 were found in this group and TMC114 concentrations were measured in males on Day 85 (4 out of 18 samples ranged between 0.020 and 0.044 µg/mL), in females on Day 85 (3 out of 18 samples ranged between 0.021 and 0.035 µg/mL), in males on Day 176 (2 out of 18 samples: 0.030 and 0.042 µg/mL) and in females on Day 176 (1 out of 18 samples: 0.027 µg/mL)						

[1] : ritonavir was administered just before the administration of TMC114
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS: not specified

2.6.5.4.O Pharmacokinetics: Absorption after Repeated Intravenous Doses in Rats

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC160**											
Species	**Rat (Sprague Dawley)**											
Feeding Condition	not fasted											
Vehicle/Formulation	15 % hydroxypropyl-β–cyclodextrin in pyrogen free water											
Route	Intravenous (bolus)											
Gender (M/F)/Number of Animals	M/6		F/6		M/6		F/6		M/6		F/6	
Dose (mg.base eq./kg/day)	2.5		2.5		10		10		25		25	
Concentrations of TMC114 (mg.base eq./mL)	5		5		5		5		5		5	
Duration of Dosing (day)	1	15	1	15	1	15	1	15	1	15	1	15
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
AUC (µg.h/mL)	0.992	1.03[1]	1.18	0.872[1]	3.31	3.79[1]	5.16	4.56[1]	13.8	12.9[1]	15.9	13.7[1]
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	NS	NS	NS	NS	NS	NS	1.5	2.4	1.3	1.1	1.0	1.4
(Time for calculation –h)							(4-8)	(4-8)	(4-8)	(4-8)	(4-8)	(4-8)
Cl (L/h/kg)	2.52	2.43	2.12	2.87	3.02	2.64	1.94	2.20	1.81	1.93	1.58	1.82
Vd_{ss} (L/kg)	0.951	1.58	1.11	1.52	1.75	1.73	1.96	2.11	2.05	1.92	1.99	1.76

[1] : extrapolated AUC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS : not specified

2.6.5.4.P Pharmacokinetics: Absorption after a Single or Repeated Oral Dose in Rabbits

Test article: darunavir

Location in CTD	**4.2.3.5.2**							
Study No.	**TMC114-NC124**							
Species	**Rabbit (New Zealand White)**							
Feeding Condition	Not Fasted							
Vehicle/Formulation	1 % CMC/0.2%Tween 80 in Water							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	F/2		F/2		F/2			
Dose[1] (mg/kg/day)	250		500		1000			
Concentration (mg/mL)	25		50		100			
Duration of Dosing (day)	1		1		1		7	
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters[2]								
C_{max} (µg/mL)	0.135	0.183	0.326	0.399	0.281	0.274	0.343	0.419
t_{max} (h)	4	2	1	2	2	0.5	0.5	0.5
AUC (µg.h/mL)	0.536	0.646[3]	1.03	1.37	1.09	0.691	0.782	0.686
(Time for calculation –h)	(0-∞)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-8)	(0-4)
$t_{1/2}$ (h)	0.7[3]	1.5[3]	NC	1.2	NC	5.0	4.3[3]	0.6
(Time for calculation –h)	NS	NS	-	NS	-	NS	NS	NS

[1] : doses expressed as TMC114 base were 235, 470 and 939 mg/kg/day
[2] : individual results
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
CMC : carboxymethyl cellulose
NS: not specified
NC : not calculated

2.6.5.4.Q Pharmacokinetics: Absorption after a Repeated Oral Dose in Rabbits (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.3.5.2**		
Study No.	**TMC114-NC189**		
Species	**Rabbit (New Zealand White)**		
Feeding Condition	Not Fasted		
Vehicle/Formulation	0.5 % HPMC/0.2%Tween 20 in Water		
Route	Oral (gavage)		
Gender (M/F)/Number of Animals	F/4	F/4	F/3
Dose[1] (mg.base eq./kg/day) of TMC114	200	1000	1000
Concentration (mg.base eq./mL) of TMC114	20	100	100
Dose (mg/kg/day) of ritonavir	40	40	0
Concentration (mg/mL) of ritonavir	80	80	0
Duration of Dosing (day)	5	5	5
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} [1] (µg/mL)	3.82	6.16	0.411
t_{max} [1] (h)	2.8	1.5	1.5
AUC [1] (µg.h/mL)	16.8[2]	24.4	1.63
(Time for calculation –h)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ [1] (h)	1.7	3.7	4.3
(Time for calculation –h)	(4-8 or 24)	(8-24)	(4-8 or 24)

[1] : mean value
[2] : extrapolated value

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
HPMC : hydroxypropyl methyl cellulose

2.6.5.4.R Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs

Test article: darunavir

Location in CTD	**4.2.2.2**											
Study No.	**TMC114-NC106**											
Species	**Dog (Beagle)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose[1] (mg/kg/day)	30		30		60		60		120		120	
Concentrations (mg/mL)	30		30		60		60		120		120	
Duration of Dosing (day)	1	14	1	14	1	14	1	14	1	14	1	14
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max}[2] (µg/mL)	5.96	4.23	13.3	9.57	11.4	8.83	18.9	17.4	16.7	12.2	16.5	10.5
t_{max}[3] (h)	1	1	0.5	0.5	1	0.5	1	1	2	2	1	1
AUC (µg.h/mL)	13.0	9.23	21.8	14.3	33.7	20.1	52.1	39.6	82.0	52.7	73.7	25.5
(Time for calculation –h)	(0-∞)	(0-6)	(0-∞)	(0-4 or 10)	(0-∞)	(0-6 or 10)	(0-∞)	(0-10)	(0-∞)	(0-10)	(0-∞)	(0-10)
$t_{1/2}$[2] (h)	0.6[4]	0.7[4]	1.3[4]	0.9[4]	1.0[4]	1.3[4]	4.8[4]	5.9[4]	1.1[4]	1.1	1.0[4]	1.0[4]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Bioavailability (%)	60[5]		122[5]									

[1] : doses expressed as TMC114 base were 28, 56 and 112 mg/kg/day
[2] : mean value
[3] : median value
[4] : not accurately determined
[5] : calculated relative to intravenous data obtained after single dose at 12.5 mg/kg from TMC114-NC199 (2.6.5.4AB)

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.S Pharmacokinetics: Absorption after a Repeated Oral Dose in Dogs

Test article: darunavir

Location in CTD	**4.2.3.2**					
Study No.	**TMC114-NC173**					
Species	**Dog (Beagle)**					
Feeding Condition	Not Fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	M/1		F/1		M/1	F/1
Dose (mg.base eq./kg/day) of TMC114	360		360		120	120
Concentration (mg.base eq./mL) of TMC114	180		180		60	60
Duration of Dosing (day)	1	14	1	14	1	1
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	19.2	31.8	23.8	49.9	8.36	29.4
t_{max} (h)	2	4	4	4	1	4
AUC (µg.h/mL)	116	195	185	445	79.1	202
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-∞)
$t_{1/2}$ (h)	1.4	7.7[1]	2.3	1.8	2.2	2.0
(Time for calculation –h)	NS	NS	NS	NS	NS	NS
Additional information						
Same dogs received all the dose levels: 120 mg/kg/day doses were administered after a wash out period of 2 weeks						

[1] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.T Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs

Test article: darunavir

Location in CTD	**4.2.3.2**							
Study No.	**TMC114-NC131**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose[1] **(mg/kg/day)**	30				30			
Concentrations (mg/mL)	15				15			
Duration of Dosing (day)	1	29	58	86	1	29	58	86
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max}[2] **(µg/mL)**	3.81	4.47	3.94	4.34	5.95	2.41	2.27	3.59
t_{max}[3] **(h)**	1.5	1	1	1	1	1	1	1
AUC[2] **(µg.h/mL)**	12.3	10.1	9.36	10.8	14.5[4]	6.55	6.57	8.43
(Time for calculation –h)	(0-!)	(0-4 or 8 or 12)	(0- 8 or 12)	(0-8)	(0-!)	(0-8 or 12)	(0-8 or 24)	(0-8 or 12)
$t_{1/2}$[2] **(h)**	1.4[5]	1.2[5]	0.8[5]	0.8	110[5]	1.6	24[5]	1.2
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1]: dose expressed as TMC114 base was 28.5 mg/kg/day
[2] : mean value
[3] : median value
[4] : not accurately determined (% of extrapolation for at least one dog > 15 %): Mean $AUC_{0\text{-}8 \text{ or } 24h}$ = 13.7 µg.h/mL
[5] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.T Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**							
Study No.	**TMC114-NC131**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose[1] (mg/kg/day)	60				60			
Concentrations (mg/mL)	30				30			
Duration of Dosing (day)	1	29	58	86	1	29	58	86
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} [2](µg/mL)	12.2	8.01	8.58	9.71	12.5	5.77	7.41	8.09
t_{max} [3] (h)	1.5	1.5	1.5	1.0	1.5	2.0	1.0	1.5
AUC [2] (µg.h/mL)	48.3	25.8	27.0	33.3	48.6	20.7	26.0	24.3
(Time for calculation –h)	(0-!)	(0- 12 or 24)	(0- 8 or 12)	(0-12 or 24)	(0-!)	(0- 12)	(0-8 or 12 or 24)	(0-8 or 12)
$t_{1/2}$ [2] (h)	15[4]	4.0[4]	1.0	1.8[4]	7.4[4]	1.4	6.1[4]	1.5[4]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1]: dose expressed as TMC114 base was 57 mg/kg/day
[2] : mean value
[3] : median value
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.T Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**							
Study No.	**TMC114-NC131**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/4				F/4			
Dose[1] (mg/kg/day)	120				120			
Concentrations (mg/mL)	60				60			
Duration of Dosing (day)	1	29	58	86	1	29	58	86
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} [2](µg/mL)	22.9	11.7	17.3	20.9	27.7	10.1	19.4	16.9
t_{max} [3] (h)	2	2	2	2	2	2	2	2
AUC [2] (µg.h/mL)	135	55.4	93.6	109	161	53.9	99.3	96.2
(Time for calculation –h)	(0-!)	(0- 12 or 24)	(0- 12 or 24)	(0-12 or 24)	(0-!)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ [2] (h)	3.3[4]	1.2[4]	2.0	2.2[4]	3.6[4]	3.8[4]	2.8	4.3[4]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1]: dose expressed as TMC114 base was 114 mg/kg/day
[2] : mean value
[3] : median value
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

NS : not specified

2.6.5.4.U Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC133**											
Species	**Dog (Beagle)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/4			F/4			M/4			F/4		
Dose[1] (mg/kg/day)	30			30			60			60		
Concentrations (mg/mL)	15			15			30			30		
Duration of Dosing (day)	1	86	177	1	86	177	1	86[2]	177[2]	1	86	177
Sample	Plasma			Plasma			Plasma			Plasma		
Analyte	TMC114			TMC114			TMC114			TMC114		
Assay	LC-MS/MS			LC-MS/MS			LC-MS/MS			LC-MS/MS		
Pharmacokinetic Parameters												
C_{max}[3] (µg/mL)	9.69	7.76	7.91	10.9	8.39	11.4	18.1	12.7	15.0	17.6	16.0	16.5
t_{max}[4] (h)	1	1	1	1	1	1	1.5	2	1	1	2	1
AUC[3] (µg.h/mL)	22.0	20.0	22.4	23.2	19.9	21.8	60.1	44.3	44.0	54.9	51.8	53.1
(Time for calculation –h)	(0-!)	(0-12)	(0-12 or 24)	(0-!)	(0-8 or 12 or 24)	(0-8 or 12)	(0-!)	(0-12 or 24)	(0-12)	(0-!)	(0-8 or 12)	(0-8 or 12)
$t_{1/2}$[3] (h)	1.9[5]	1.7	4.4[5]	3.0[5]	1.5	1.6	8.5[5]	1.7[5]	1.2	3.1[5]	1.5[5]	1.2
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base were 27.8 and 55.7 mg/kg/day
[2] : n=3
[3] : mean value
[4] : median value
[5] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.U Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (continued)

Test article: darunavir

Location in CTD						
Study No.			**4.2.3.2 TMC114-NC133**			
Species			**Dog (Beagle)**			
Feeding Condition			not fasted			
Vehicle/Formulation			PEG400			
Route			Oral (gavage)			
Gender (M/F)/Number of Animals		M/4			F/4	
Dose[1] (mg/kg/day)		120			120	
Concentrations (mg/mL)		60			60	
Duration of Dosing (day)	1	86[2]	177[2]	1	86	177
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max}[3] (µg/mL)	23.4	24.4	19.8	29.0	19.6	26.0
t_{max}[4] (h)	1.5	1	1	1.5	1.5	1.5
AUC[3] (µg.h/mL)	107	84.8	70.3	133	78.2	97.3
(Time for calculation –h)	(0-!)	(0-12 or 24)	(0-12 or 24)	(0-!)	(0- 12 or 24)	(0-24)
$t_{1/2}$[3] (h)	7.5[5]	2.2	1.5[5]	2.9[5]	2.7	2.8[5]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS

[1] : doses expressed as TMC114 base was 111 mg/kg/day
[2] : n=3
[3] : mean value
[4] : median value
[5] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.V Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs

Test article: darunavir

Location in CTD	**4.2.3.2**									
Study No.	**TMC114-NC145**									
Species	**Dog (Beagle)**									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals			M/4					F/4		
Dose (mg.base eq./kg/day)			30					30		
Concentrations (mg.base eq./mL)			22.6					22.6		
Duration of Dosing (day)	1	85	176	267	358	1	85	176	267	358
Sample	Plasma							Plasma		
Analyte	TMC114							TMC114		
Assay	LC-MS/MS							LC-MS/MS		
Pharmacokinetic Parameters										
C_{max}[1] (µg/mL)	10.0	7.54	12.5	8.67	11.5	9.89	7.33	8.02	5.85	9.11
t_{max}[2] (h)	1	1	1	1	1	1	1.5	1	1	1
AUC[1] (µg.h/mL)	23.0[3]	22.1	37.9	29.2	31.6	17.9	17.3	20.3	16.2	21.2
(Time for calculation –h)	(0- !)	(0-8 or 12)	(0-12 or 24)	(0-12)	(0-12)	(0- !)	(0-8 or 12)	(0-4 or 8 or 24)	(0-8 or 12)	(0-8 or 12)
$t_{1/2}$[1] (h)	1.0[4]	1.5	3.9[4]	1.6	1.6	0.7	3.0[4]	2.5[4]	1.2	2.2[4]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : mean value
[2] : median value
[3] : not accurately determined (% of extrapolation for at least one dog > 15 %)
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.V Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**									
Study No.	**TMC114-NC145**									
Species	**Dog (Beagle)**									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals			M/4					F/4		
Dose (mg.base eq./kg/day)			60					60		
Concentrations (mg.base eq./mL)			45.1					45.1		
Duration of Dosing (day)	1	85	176[1]	267[1]	358[1]	1	85	176	267	358
Sample (whole blood, plasma, serum, etc.)			Plasma					Plasma		
Analyte			TMC114					TMC114		
Assay			LC-MS/MS					LC-MS/MS		
Pharmacokinetic Parameters										
C_{max}[2] (µg/mL)	14.1	18.6	17.8	19.1	15.4	12.3	16.8	12.8	10.0	13.5
t_{max}[3] (h)	1	1	2	2	2	1	1.5	1	2	1.5
AUC[2] (µg.h/mL)	59.4[4]	70.0	72.8	80.2	71.4	41.1[5]	63.0	43.2	37.2	50.3
(Time for calculation –h)	(0-!)	(0-12)	(0-12 or 24)	(0-12)	(0-12 or 24)	(0-!)	(0-12 or 24)	(0-12)	(0-12)	(0-12 or 24)
$t_{1/2}$[2] (h)	1.2[6]	11[6]	2.4[6]	1.3[6]	2.2	1.2[6]	3.3[6]	1.4	1.2	4.6[6]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]: n=3
[2] : mean value
[3] : median value
[4] : not accurately determined (% of extrapolation for at least one dog > 15%) : Mean $AUC_{0\text{-}8 \text{ or} 12 \text{ h}}$ = 57.8 µg.h/mL
[5] : not accurately determined (% of extrapolation for at least one dog > 15%) : Mean $AUC_{0\text{-}8 \text{ or} 12 \text{ h}}$ = 38.2 µg.h/mL
[6] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.V Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**									
Study No.	**TMC114-NC145**									
Species	**Dog (Beagle)**									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals			M/4					F/4		
Dose (mg.base eq./kg/day)			120					120		
Concentrations (mg.base eq./mL)			90.2					90.2		
Duration of Dosing (day)	1	85	176	267	358	1	85	176	267	358
Sample			Plasma					Plasma		
Analyte			TMC114					TMC114		
Assay			LC-MS/MS					LC-MS/MS		
Pharmacokinetic Parameters										
C_{max}[1] (µg/mL)	16.6	22.9	13.3	24.8	27.8	15.0	17.4	17.0	14.4	23.9
t_{max}[2] (h)	1.0	2.0	1.5	2.0	2.0	1.0	1.0	2.0	2.0	2.0
AUC[1] (µg.h/mL)	69.4	90.7	74.3	111	130	53.4	59.3	79.2	55.0	100
(Time for calculation –h)	(0-!)	(0-12 or 24)	(0-8 or 12 or 24)	(0-12)	(0-8 or 12 or 24)	(0-!)	(0-12)	(0-12)	(0-12 or 24)	(0-12 or 24)
$t_{1/2}$[1] (h)	1.2	1.7	4.7[3]	2.9[3]	2.8[3]	1.0	2.0	1.3	1.5[3]	2.4[3]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] : mean value
[2] : median value
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS: not specified

2.6.5.4.W Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.2.2**							
Study No.	**TMC114-NC147**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400 (TMC114) –Propylene glycol (ritonavir)							
Route	Oral (gavage or capsule)							
Gender (M/F)/Number of Animals	M/3	M/3	M/3	M/3	M/3	M/3	M/3	M/3
Dose (mg.base eq./kg/day) of TMC114	120 (sol.)	120 (caps.)	120 (caps.)	240 (caps.)	120 (sol.)	120 (caps.)	120 (caps.)	240 (caps.)
Concentrations (mg.base eq./mL) of TMC114	120	-	-	-	120	-	-	-
Dose[1] (mg/kg/day) of ritonavir	10 (sol.)	10 (sol.)	16 (caps)	16 (caps)	10 (sol.)	10 (sol.)	16 (caps)	16 (caps)
Concentrations (mg/mL) of ritonavir	80	80	-	-	80	80	-	-
Duration of Dosing (day)	4	4	4	4	4	4	4	4
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max}[2](µg/mL)	18.0	6.16	9.11	4.98	0.465	13.4	23.2	13.7
t_{max}[3] (h)	2.0	1.0	1.0	2.0	4.0	1.0	1.0	4.0
AUC[2] (µg.h/mL)	111	20.8	48.3	23.0	2.25	63.4	141	43.6
(Time for calculation –h)	(0-12)	(0- 12)	(0- 12 or 24)	(0-12)	(0-8 or 12)	(0-12)	(0-12 or 24)	(0-12)
$t_{1/2}$[2] (h)	1.8[4]	2.9[4]	11[4]	3.0[4]	1.1	0.8[4]	1.1[4]	0.9
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1] : ritonavir was administered just before the administration of TMC114
[2] : mean value
[3] : median value
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.X Pharmacokinetics: Absorption after a Repeated Oral Dose in Dogs (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.2.2**					
Study No.	**TMC114-NC138**					
Species	**Dog (Beagle)**					
Feeding Condition	Not Fasted					
Vehicle/Formulation	PEG400 (TMC114) –Propylene glycol (ritonavir)					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	M/3		M/3		M/2	
Dose[1] (mg/kg/day) of TMC114	120		120		300	
Concentration (mg/mL) of TMC114	120		120		300	
Dose[2] (mg/kg/day) of ritonavir	10	2	50	10	50	10
Concentration (mg/mL) of ritonavir	80	20	80	20	80	20
Duration of Dosing (day)	1	7	1	7	1	7
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max}[3] (µg/mL)	13.8	10.7	13.2	16.0	18.7	26.0
t_{max}[4] (h)	3.0	2.0	1.0	2.0	1.0	2.0
AUC[3] (µg.h/mL)	80.4	46.0	36.3	68.1	91.2	130
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$[3] (h)	3.5[5]	3.5[5]	35[5]	10[5]	2.7[5]	2.9[5]
(Time for calculation –h)	NS	NS	NS	NS	NS	NS
Additional information						
The dose levels of TMC114 were combined with doses of 10 and 50 mg/kg/day of ritonavir on the first 2 days of dosing and with 2 and 10 mg/kg/day of ritonavir from day 3 onwards						

[1]: doses expressed as TMC114 base were 111 and 277 mg/kg/day
[2] : ritonavir was administered just before the administration of TMC114
[3]: mean value; [4]: median value; [5] : not accurately determined
LC-MS/MS = liquid chromatography with tandem mass spectroscopy; PEG400 : polyethylene glycol 400; NS : not specified

2.6.5.4.Y Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC140 (FK4847)** [1]											
Species	**Dog (Beagle)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose[2] (mg/kg/day) of TMC114	40		40		120		120		360		360	
Concentrations (mg/mL) of TMC114	40		40		120		120		360		360	
Dose (mg/kg/day) of ritonavir	10		10		10		10		10		10	
Concentrations (mg/mL) of ritonavir	50		50		50		50		50		50	
Duration of Dosing (day)	1	12	1	12	1	12	1	12	1	12	1	12
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max}[3] (µg/mL)	13.8	12.5	13.1	14.4	20.1	23.9	25.1	22.3	17.2	19.4	16.4	20.3
t_{max}[3] (h)	1.3	1.2	1.2	0.8	2	1.7	2	1.3	1.7	1	1.3	1.3
AUC[3] (µg.h/mL)	61.2	48.9[4]	71.2[5]	46.3[4]	108	103[4]	131	105	77.2	84.7	67.2	64.1
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0- 24)	(0-!)	(0- 24)	(0-!)	(0-24)
$t_{1/2}$[6] (h)	1.0-3.6	1.0-1.7	1.0-4.6	1.2-2.7	0.9-3.9	0.9-1.2	0.9-3.5	0.8-5.3	0.9-5.7	0.9-4.2	0.7-2.6	0.6-4.0
(Time for calculation –h)	(6-12, 8-12 ,12-24)	(4-8, 6-12)	(4-8, 6-12)	(6-12)	(6-12, 8-12)	(6-12)	(6-12, 8-24, 12-24)	(6-12, 8-24, 12-24)	(6-12, 8-24)	(6-12, 8-24)	(4-8, 6-12, 8-24)	(3-6, 8-12, 8-24)

[1]: Data after interpretation at Johnson and Johnson in GLP context
[2] : doses expressed as TMC114 base were 37.2, 112 and 335 mg/kg/day
[3] : mean value
[4] : extrapolated AUC
[5] : n=1 : Mean AUC_{0-t} = 51.6 µg.h/mL
[6] : range ;
LC-MS/MS = liquid chromatography with tandem mass spectroscopy; PEG400 : polyethylene glycol 400

2.6.5.4.Y Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC140 (FK4847)[1]**											
Species	**Dog (Beagle)**											
Feeding Condition	not fasted											
Vehicle/Formulation	Propylene glycol											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose[2] (mg/kg/day) of TMC114	40		40		120		120		360		360	
Concentrations (mg/mL) of TMC114	40		40		120		120		360		360	
Dose (mg/kg/day) of ritonavir	10		10		10		10		10		10	
Concentrations (mg/mL) of ritonavir	50		50		50		50		50		50	
Duration of Dosing (day)	1	12	1	12	1	12	1	12	1	12	1	12
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	ritonavir		ritonavir		ritonavir		ritonavir		ritonavir		ritonavir	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max}[3] (µg/mL)	6.68	9.95	5.61	5.03	1.93	3.01	2.85	3.89	1.85	2.62	4.38	1.68
t_{max}[3] (h)	2.0	2.3	4.0	1.3	2.3	2.0	2.3	1.0	1.7	1.7	1.8	1.5
AUC[3] (µg.h/mL)	27.8	41.9[4]	28.1[4]	15.3[4]	6.07	9.13[4]	10.5	13.1[4]	3.86	7.91[4]	8.92	3.29
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0-24)	(0-!)	(0- 24)	(0-!)	(0-24)
$t_{1/2}$[5] (h)	0.7-1.3	0.9-3.0	0.9-3.2	0.5-2.1	0.8-2.6	1.1-2.5	0.9-1.5	1.5-2.0	0.9-1.1	1.0-1.9	0.8-2.4	1.3-2.7
(Time for calculation –h)	(6-8, 8-12)	(8-12)	(6-8, 8-12)	(6-8, 8-12)	(6-8, 8-12)	(8-12)	(8-12)	(8-12)	(4-6, 6-8)	(6-8, 8-12)	(4-6, 6-8, 8-12)	(4-6, 6-8, 8-12)

[1]: Data after interpretation at Johnson and Johnson in GLP context
[2] : doses expressed as TMC114 base were 37.2, 112 and 335 mg/kg
[3] : mean value
[4] : extrapolated value
[5] : range

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4Y Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.3.2**			
Study No.	**TMC114-NC140 (FK4847)** [1]			
Species	**Dog (Beagle)**			
Feeding Condition	not fasted			
Vehicle/Formulation	Propylene glycol			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	M/3		F/3	
Dose[2] (mg/kg/day) of TMC114	0		0	
Concentrations (mg/mL) of TMC114	0		0	
Dose (mg/kg/day) of ritonavir	10		10	
Concentrations (mg/mL) of ritonavir	50		50	
Duration of Dosing (day)	1	12	1	12
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max}[2] (µg/mL)	5.65	7.00	10.5	9.13
t_{max}[2] (h)	2.3	2.0	1.5	2.2
AUC [2] (µg.h/mL)	18.4	25.1[3]	44.1	30.9[3]
(Time for calculation –h)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$[2] (h)	3.6	1.3	0.79	1.5
(Time for calculation –h)	(8-12)	(8-12)	(8-12)	(8-12)

[1]: Data after interpretation at Johnson and Johnson in GLP context
[2] : mean value
[3] : calculated with extrapolated values

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4.Z Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir)

Test article: darunavir

Location in CTD	**4.2.2.2**			
Study No.	**TMC114-NC144**			
Species	**Dog (Beagle)**			
Feeding Condition	not fasted			
Vehicle/Formulation	capsule			
Route	Oral			
Gender (M/F)/Number of Animals	M/3		F/3	
Dose[1] (mg/kg/day) of TMC114	120 b.i.d		120 b.i.d	
Dose (mg/dog/day) of ritonavir	100 b.i. d		100 b.i. d	
Duration of Dosing (day)	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max1}[2] (µg/mL)	5.10	5.05	4.99	3.54
t_{max1}[3] (h)	2.0	3.0	1.0	3.0
C_{max2}[2] (µg/mL)	4.66	5.72	5.26	3.44
t_{max2}[3] (h)	3.0	3.0	2.0	2.0
AUC [2] (µg.h/mL)	71.3[4]	71.4	55.6[5]	51.6
(Time for calculation –h)	(0-!)	(0- 24)	(0-!)	(0-24)
$t_{1/2}$ [2] (h)	42[6]	14[6]	3.8[6]	7.6[6]
(Time for calculation –h)	NS	NS	NS	NS
Additional information				
C_{max1} and t_{max1} : after the first dosing; C_{max2} and t_{max2} : after the second dosing				

[1] : Daily dose of 240 mg/kg/d ; interval between doses :approximately 7 hours; [2] : mean value; [3] : median value
[4] : not accurately determined : Mean AUC_{0-24h} =59.9 µg.h/mL
[5] : not accurately determined: Mean AUC_{0-24h} = 46.7 µg.h/mL; [6] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy; NS : not specified; b.i.d : twice daily

2.6.5.4.Z Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.2.2**							
Study No.	**TMC114-NC144**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg.base eq./kg/day) of TMC114	120		120		120		120	
Concentrations (mg.base eq./mL) of TMC114	120		120		120		120	
Dose (mg/kg/day) of ritonavir	10		10		20		20	
Concentrations (mg/mL) of ritonavir	80		80		80		80	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} [1] (µg/mL)	9.77	16.9	14.8	12.7	6.44	12.4	5.27	14.7
t_{max} [2] (h)	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0
AUC [1] (µg.h/mL)	27.0	54.0	64.4	54.2	15.9	30.4	23.7	72.0
(Time for calculation –h)	(0-!)	(0-8 or 12)	(0-!)	(0-12 or 24)	(0-!)	(0-12 or 24)	(0-!)	(0-12 or 24)
$t_{1/2}$ [1] (h)	3.7[3]	1.1[3]	1.1[3]	NC	1.1[3]	3.6[3]	2.3[3]	1.8[4]
(Time for calculation –h)	NS	NS	NS	-	NS	NS	NS	NS

[1] : mean value
[2] : median value
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified
NC : not calculated

2.6.5.4.Z Pharmacokinetics: Absorption after Repeated Oral Doses in Dogs (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.2.2**							
Study No.	**TMC114-NC144**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	capsule							
Route	Oral							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg.base eq./kg/day) of TMC114	0		0		120 b.i.d		120 b.i.d	
Dose (mg/dog/day) of ritonavir	100 b.i. d		100 b.i. d		100 b.i.d		100 b.i. d	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters [1]								
C_{max1} [2] (µg/mL)	13.1	14.4	20.2	36.5	23.8	18.6	23.1	19.8
t_{max1} [3] (h)	1.5	1.5	1.5	1.5	1.5	3.0	1.5	4.0
AUC [2] (µg.h/mL)	39.0	48.7	63.7	149	70.1	75.1	80.0	77.7
(Time for calculation –h)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)	(0-6)
$t_{1/2}$ [2] (h)	2.2[4]	1.3[4]	1.1[4]	NC	1.1[4]	2.4[4]	1.4[4]	2.7[4]
(Time for calculation –h)	NS	NS	NS	-	NS	NS	NS	NS

[1] : Parameters after the first dosing
[2] : mean value
[3] : median value
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NS : not specified
NC : not calculated
b.i.d : twice daily

2.6.5.4.Z Pharmacokinetics: Absorption after Repeated Oral Doses in Dog (in combination with ritonavir) (continued)

Test article: darunavir

Location in CTD	**4.2.2.2**							
Study No.	**TMC114-NC144**							
Species	**Dog (Beagle)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3	
Dose (mg.base eq./kg/day) of TMC114	120		120		120		120	
Concentrations (mg/mL) of TMC114	120		120		120		120	
Dose (mg/kg/day) of ritonavir	10		10		20		20	
Concentrations (mg/mL) of ritonavir	80		80		80		80	
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} [1] (µg/mL)	8.13	4.49	14.3	4.23	12.0	6.94	17.4	17.0
t_{max} [2] (h)	1.5	2.0	2.0	3.0	2.0	1.5	1.5	2.0
AUC [1] (µg.h/mL)	21.8	15.5	44.1	10.2	33.6	18.3	110	60.9
(Time for calculation –h)	(0-!)	(0-13)	(0-!)	(0-9 or 13)	(0-!)	(0-9 or 13)	(0-!)	(0-13)
$t_{1/2}$ [1] (h)	1.3[3]	2.3[3]	1.2[3]	2.0[3]	0.9[4]	1.6	1.2[3]	1.0
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1] : mean value
[2] : median value
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.AA Pharmacokinetics: Absorption after a Single or Repeated Intravenous Dose in Dogs

Test article: darunavir

Location in CTD	**4.2.3.1**			
Study No.	**TMC114-NC109**			
Species	**Dog (Beagle)**			
Feeding Condition	Not fasted			
Vehicle/Formulation	PEG400/EtOH/NaCl (50/10/40 % v/v)			
Route	Intravenous (short infusion)			
Gender (M/F)/Number of Animals	M/2 + F/2	M/2 + F/2	M/1 + F/2	M/1 + F/1
Dose[1] (mg/kg/day)	10	20	40	10
Concentration (mg/mL)	4	16	16	4
Duration of dosing	1	1	1	3
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
AUC[2] (µg.h/mL)	3.4	12.7	39.5	4.6
(Time for calculation –h)	(0-!)	(0-!)	(0-!)	(0-3 or 6)
$t_{1/2}$[2] (h)	1.1[4]	1.2[4]	0.9[4]	1.7[4]
(Time for calculation –h)	NS	NS	NS	NS
Additional information				
Escalating doses of TMC114 were administered once daily for 4 consecutive days. Subsequently, the maximum tolerated dose (10 mg/kg/day) was administered once daily for 3 consecutives days.				

[1] : doses expressed as TMC114 base were 9.3, 18.7 and 37.3 mg/kg/day
[2] : mean value
[3] : median value
[4] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : Polyethylene glycol 400
EtOH : Ethanol
NaCl : Sodium chloride
NS : not specified

2.6.5.4.AB Pharmacokinetics: Absorption after Repeated Intravenous Doses in Dogs

Test article: darunavir

Location in CTD	**4.2.3.2**											
Study No.	**TMC114-NC199**											
Species	**Dog (Beagle)**											
Feeding Condition	not fasted											
Vehicle/Formulation	15 % hydroxypropyl-β–cyclodextrin in pyrogen free water											
Route	Intravenous (bolus)											
Gender (M/F)/Number of Animals	M/3		F/3		M/3		F/3		M/3		F/3	
Dose(mg.base eq./kg/day) of TMC114	3.12		3.12		6.25		6.25		12.5		12.5	
Concentrations (mg.base eq./mL) of TMC114	5		5		5		5		5		5	
Duration of Dosing (day)	1	15	1	15	1	15	1	15	1	15	1	15
Sample	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
AUC (µg.h/mL)	1.71	1.80[1]	1.44	1.49[1]	3.76	3.37[1]	3.27	3.22[1]	9.63	9.77[1]	7.97	6.92[1]
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.5	0.5	0.5
(Time for calculation –h)	(0.083-2)	(0.083-2)	(0.083-2)	(0.083-2)	(0.083-4)	(0.083-4)	(0.083-4)	(0.083-4)	(0.083-4)	(0.083-4)	(0.083-4)	(0.083-4)
Cl (L/h/kg)	1.86	1.77	2.23	2.16	1.77	1.91	1.92	1.99	1.32	1.30	1.60	1.85
Vd_{ss} (L/kg)	0.677	0.716	0.824	0.838	0.787	0.901	7.42	1.92	0.870	1.29	1.03	1.75

Additional information

In all the dogs, the plasma concentrations declined in a monophasical manner except for 4 dogs where two phases were seen (the t1/2 corresponding to the second phase ranged between 3.6 to 57 hours calculated between 4 to 8 hours).

[1]: extrapolated AUC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.4.AC Pharmacokinetics: Absorption after Repeated Oral Doses in Mice (Carcinogenicity Study)

Test article: darunavir

Location in CTD	**4.2.3.4.1**											
Study No.	**TMC114-NC159**											
Species	**Mice (CD1)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/15		F/15		M/15		F/15		M/15		F/15	
Dose (mg base eq./kg/day)	150		150		450		450		1000		1000	
Concentrations (mg/mL)	15		15		45		45		100		100	
Duration of Dosing (day)	32	188	32	188	32	188	32	188	32	188	32	188
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	2.33	3.36	3.74	3.72	4.94	3.12	7.89	5.97	5.88	10.1	8.52	10.7
t_{max} (h)	1	1	1	1	1	1	1	2	2	2	1	1
AUC (µg.h/mL)	4.33	6.23	7.47	7.79	22.0	14.8	41.8	40.8	34.4	48.1	56.9	63.8
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-8)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	19	1.2	1.4	1.1	2.4	1.8	4.0	3.4	2.1	1.9	1.6	1.9
(Time for calculation –h)	(8-24)	(4-8)	(4-8)	(4-8)	(8-24)	(4-8)	(4-8)	(4-8)	(8-24)	(8-24)	(8-24)	(8-24)

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.4.AD Pharmacokinetics: Absorption after Repeated Oral Doses in Juvenile Rats

Test article: darunavir

Location in CTD	**4.2.3.5.4**							
Study No.	**TMC114-NC248**							
Species	**Rat (Sprague-Dawley pups)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/12		F/12		M/12		F/12	
Dose (mg base eq./kg/day) of TMC114	20		20		100		100	
Concentrations (mg/mL) of TMC114	2		2		10		10	
Dose (mg/kg/day) of ritonavir	0		0		0		0	
Concentrations (mg/mL) of ritonavir	0		0		0		0	
Age of Pups (day)	23	36	23	36	23	36	23	36
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	0.376	0.657	0.484	1.00	4.29	2.87	5.10	3.04
t_{max} (h)	2	2	2	1	2	2	2	2
AUC (µg.h/mL)	1.07	2.46	1.43	2.67	14.1	10.0	15.0	12.0
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.3	1.7	1.3	1.5	1.4	1.4	1.3	1.7
(Time for calculation –h)	(2-8)	(2-8)	(2-8)	(2-8)	(2-8)	(2-8)	(2-8)	(2-8)

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.4.AD Pharmacokinetics: Absorption after Repeated Oral Doses in Juvenile Rats (Continued)

Test article: darunavir

Location in CTD	**4.2.3.5.4**							
Study No.	**TMC114-NC248**							
Species	**Rat (Sprague-Dawley pups)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/12		F/12		M/12		F/12	
Dose (mg base eq./kg/day) of TMC114	500		500		500		500	
Concentrations (mg/mL) of TMC114	50		50		50		50	
Dose (mg/kg/day) of ritonavir	0		0		50		50	
Concentrations (mg/mL) of ritonavir	0		0		40		40	
Age of pups (day)	23	36	23	36	23	36	23	36
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	8.74	9.03	10.8	10.5	10.7	14.1	12.0	12.6
t_{max} (h)	2	2	2	2	8	2	8	2
AUC (µg.h/mL)	87.7	79.1	122	81.6	228[1]	134	284[2]	105
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	3.6	1.9	5.0	1.7	11	2.6	12	2.2
(Time for calculation –h)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

[1] : not accurately determined (27.3 % of extrapolation) : $AUC_{0\text{-}24h}$ = 166 µg.h/mL
[2] : not accurately determined (29 % of extrapolation) : $AUC_{0\text{-}24h}$ = 202 µg.h/mL

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.4.AD Pharmacokinetics: Absorption after Repeated Oral Doses in Juvenile Rats (Continued)

Test article: darunavir

Location in CTD	**4.2.3.5.4**							
Study No.	**TMC114-NC248**							
Species	**Rat (Sprague-Dawley pups)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/12		F/12		M/12		F/12	
Dose (mg base eq./kg/day) of TMC114	0		0		500		500	
Concentrations (mg/mL) of TMC114	0		0		50		50	
Dose (mg/kg/day) of Ritonavir	50		50		50		50	
Concentrations (mg/mL) of Ritonavir	40		40		40		40	
Age of Pups (day)	23	36	23	36	23	36	23	36
Duration of Dosing (day)	1	14	1	14	1	14	1	14
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.60	2.78	3.30	2.43	1.33	0.326	0.965	0.433
t_{max} (h)	1	2	1	8	2	2	1	2
AUC (µg.h/mL)	15.0	13.8	13.4	13.9	10.5	1.75	11.0	1.54
(Time for calculation –h)	(0-8)	(0-8)	(0-8)	(0-8)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	NC	4.3	18	NC	4.0	3.0	6.2	1.9
(Time for calculation –h)	-	(2-8)	(2-8)	-	(8-24)	(2-8)	(8-24)	(2-8)

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.4.AE Pharmacokinetics: Absorption after Repeated Oral Doses in Juvenile Rats

Test article: darunavir

Location in CTD	**4.2.3.5.4**							
Study No.	**TMC114-NC241**							
Species	**Rat (Sprague-Dawley pups)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/12		F/12		M/12		F/12	
Dose (mg base eq./kg/day) of TMC114	40		40		200		200	
Concentrations (mg/mL) of TMC114	4		4		20		20	
Dose (mg/kg/day) of Ritonavir	0		0		0		0	
Concentrations (mg/mL) of Ritonavir	0		0		0		0	
Age of Pups (day)	23	50	23	50	23	50	23	50
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.34	1.40	2.71	1.66	9.87	7.15	9.73	6.81
t_{max} (h)	2	2	2	1	2	2	1	2
AUC (µg.h/mL)	7.50	6.38	8.51	10.8	61.5	26.5	73.9	39.8
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	1.4	3.2	1.3	2.2	1.7	3.7	1.6	2.3
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

Additional Information

In the vehicle group, 4 out of 12 samples on Day 50 were higher than the limit of quantification (TMC114 concentration range was 6.38-423 ng/mL)
In the group receiving 200 mg/kg/day of TMC114, concentrations of ritonavir were observed in 2 out of 12 samples on Day 50.

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.AE Pharmacokinetics: Absorption after Repeated Oral Doses in Juvenile Rats (Continued)

Test article: darunavir

Location in CTD	**4.2.3.5.4**							
Study No.	**TMC114-NC241**							
Species	**Rat (Sprague-Dawley pups)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/12		F/12		M/12		F/12	
Dose (mg base eq./kg/day) of TMC114	500		500		500		500	
Concentrations (mg/mL) of TMC114	50		50		50		50	
Dose (mg/kg/day) of Ritonavir	0		0		75		75	
Concentrations (mg/mL) of Ritonavir	0		0		60		60	
Age of Pups (day)	23	50	23	50	23	50	23	50
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	10.2	8.28	12.4	8.72	12.3	12.1	12.8	13.1
t_{max} (h)	2	2	2	2	8	2	8	8
AUC (µg.h/mL)	144	71.3	173	73.9	254	135	228	171
(Time for calculation –h)	(0-∞)	(0-24)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	8.2	1.8	6.1	2.4	48	3.4	16	5.1
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS

[1] : not accurately determined (27.3 % of extrapolation) : $AUC_{0\text{-}24h}$ = 166 µg.h/mL
[2] : not accurately determined (29 % of extrapolation) : $AUC_{0\text{-}24h}$ = 202 µg.h/mL

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.AE Pharmacokinetics: Absorption after Repeated Oral Doses in Juvenile Rats (Continued)

Test article: darunavir

Location in CTD	**4.2.3.5.4**							
Study No.	**TMC114-NC241**							
Species	**Rat (Sprague-Dawley pups)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/12		F/12		M/12		F/12	
Dose (mg base eq./kg/day) of TMC114	0		0		500		500	
Concentrations (mg/mL) of TMC114	0		0		10		10	
Dose (mg/kg/day) of Ritonavir	75		75		75		75	
Concentrations (mg/mL) of Ritonavir	60		60		60		60	
Age of Pups (day)	23	50	23	50	23	50	23	50
Duration of Dosing (day)	1	28	1	28	1	28	1	28
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	2.62	5.80	3.51	5.85	0.854	1.13	1.51	1.36
t_{max} (h)	2	1	1	2	1	2	2	2
AUC (µg.h/mL)	29.3	25.8	30.1	40.7	11.9	4.96	7.88	7.31
(Time for calculation –h)	(0-∞)	(0-8)	(0-∞)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	3	7.6	4.5	1.9	140	2.5	38	2.7
(Time for calculation –h)	NS	NS	NS	NS	NS	NS	NS	NS
Additional Information								
In the group receiving ritonavir alone, concentrations of TMC114 (between 5.26 and 54.7 ng/mL) were observed in 27 out of 47 samples								

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NS : not specified

2.6.5.4.AF Pharmacokinetics: Absorption after Repeated Oral Doses in Rats

Test article: darunavir

Location in CTD	**4.2.3.7.3**							
Study No.	**TMC114-NC162**							
Species	**Rat (Sprague-Dawley)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	M/6		F/6		M/6		F/6	
Dose (mg base eq./kg/day) of TMC114	50		50		500		500	
Concentrations (mg/mL) of TMC114	10		10		100		100	
Duration of Dosing (day)	1	29	1	29	1	29	1	29
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	3.52	3.32	4.14	7.06	11.5	8.93	15.2	8.86
t_{max} (h)	1	2	2	4	2	2	2	1
AUC (µg.h/mL)	20.3	12.7	22.6	23.9	119	56.3	139	83.8
(Time for calculation –h)	(0-∞)	(0-8)	(0-∞)	(0-8)	(0-∞)	(0-24)	(0-∞)	(0-24)
$t_{1/2}$ (h)	2.6	NC	1.8	NC	4.2	NC	3.8	NC
(Time for calculation –h)	(4-8)	-	(4-8)	-	(8-24)	-	(8-24)	-

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.4.AG Pharmacokinetics: Absorption after Repeated Oral Doses in Rats (Carcinogenicity Study)

Test article: darunavir

Location in CTD	**4.2.3.4.1**											
Study No.	**TMC114-NC158**											
Species	**Rat (Sprague-Dawley)**											
Feeding Condition	not fasted											
Vehicle/Formulation	PEG400											
Route	Oral (gavage)											
Gender (M/F)/Number of Animals	M/6		F/6		M/6		F/6		M/6		F/6	
Dose (mg base eq./kg/day)	50		50		150		150		500		500	
Concentrations (mg/mL)	10		10		30		30		100		100	
Duration of Dosing (day)	31	184	31	184	31	184	31	184	31	184	31	184
Sample (whole blood, plasma, serum, etc.)	Plasma		Plasma		Plasma		Plasma		Plasma		Plasma	
Analyte	TMC114		TMC114		TMC114		TMC114		TMC114		TMC114	
Assay	LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters												
C_{max} (µg/mL)	3.03	3.72	3.86	7.19	7.53	7.02	8.10	10.1	14.9	12.6	13.0	12.8
t_{max} (h)	2	2	1	1	4	2	2	2	4	2	2	1
AUC (µg.h/mL)	15.9	17.1	16.8	24.9	50.1	50.4	39.4	51.7	133	90.3	102	89.5
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	1.5	2.1	2.9	3.3	1.9	2.1	2.2	2.3	2.7	2.7	2.5	3.7
(Time for calculation –h)	(4-8)	(4-8)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)	(8-24)

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
PEG400 : polyethylene glycol 400

2.6.5.4.AH Pharmacokinetics: Absorption after Single/Repeated Oral Doses in Minipigs

Test article: darunavir

Location in CTD	**4.2.3.5.2**								
Study No.	**TMC114-NC399**								
Species	**Minipig (Göttingen SPF)**								
Feeding Condition	not fasted								
Route	Oral (gavage)								
Gender (M/F)/Number of Animals		F/3		F/3		F/3		F/3	
Dose (mg.base eq./kg/day) of darunavir	1000 (2 x 500; 6 hours apart)	2000 (2 x 1000; 6 hours apart)	1000 (2 x 500; 6 hours apart)	500 (2 x 250; 9 hours apart) for 6 days	250 for 7 days	1000 (2 x 500; 9 hours apart) for 6 days	500 for 7 days	500 (2 x 250; 9 hours apart) for 6 days	250 for 7 days
Concentrations (mg.base eq./mL) of darunavir	100	200	100	50	50	100	100	50	50
Vehicle/Formulation of darunavir	PEG400	PEG400	PEG400	PEG400	PEG400	PEG400	PEG400	PEG400	PEG400
Dose (mg/kg/day) of ritonavir	-	-	100 (2 x 50 ; 6 hours apart)	-	-	-	-	100 (2 x 50; 9 hours apart)	50
Concentrations (mg/mL) of ritonavir	-	-	40	-	-	-	-	40	40
Vehicle/Formulation of ritonavir	-	-	Propylene Glycol	-	-	-	-	Propylene Glycol	
Duration of Dosing (day)	1	1	1	1	13	1	13	1	13
Sample (whole blood, plasma, serum, etc.)	Plasma			Plasma		Plasma		Plasma	
Analyte	TMC114			TMC114		TMC114		TMC114	
Assay	LC-MS/MS			LC-MS/MS		LC-MS/MS		LC-MS/MS	
Pharmacokinetic Parameters									
C_{max1} (µg/mL)	6.9	9.6	7.2	3.7	6.0	3.9	8.1	4.4	5.6
t_{max1}(h)	4.7	3.3	3.3	6.3	4	9	4	4	5.3
C_{max2} (µg/mL)	12	15	11	8.0		14		8.7	
t_{max2} (h)	8.7	9.3	13	10		11		12	
AUC (µg.h/mL)	190	238	181	109	59	159	82	115	71
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
AUC (µg.h/mL)	344	358	345						
(Time for calculation –h)	(0-48)	(0-48)	(0-48)						
$t_{1/2}$ (h)	NC	NC	NC	NC	NC	NC	NC	NC	NC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy; PEG400 : polyethylene glycol 400; NC : not calculated

2.6.5.4.AH Pharmacokinetics: Absorption after Single/Repeated Oral Doses in Minipigs (to be continued)

Test article: darunavir

Location in CTD	**4.2.3.5.2**		
Study No.	**TMC114-NC399**		
Species	**Minipig (Göttingen SPF)**		
Feeding Condition	not fasted		
Route	Oral (gavage)		
Gender (M/F)/Number of Animals	F/3	F/3	
Dose (mg.base eq./kg/day) of darunavir	1000 (2 x 500; 6 hours apart)	500 (2 x 250; 9 hours apart) for 6 days	250 for 7 days
Concentrations (mg.base eq./mL) of darunavir	100	50	50
Vehicle/Formulation of darunavir	PEG400	PEG400	PEG400
Dose (mg/kg/day) of ritonavir	100 (2 x 50 ; 6 hours apart)	100 (2 x 50; 9 hours apart)	50
Concentrations (mg/mL) of ritonavir	40	40	40
Vehicle/Formulation of ritonavir	Propylene Glycol	Propylene Glycol	Propylene Glycol
Duration of Dosing (day)	1	1	13
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma
Analyte	Ritonavir	Ritonavir	Ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max1} (µg/mL)	0.095	0.080	0.076
t_{max1}(h)	3	1.3	11
C_{max2} (µg/mL)	0.31	0.14	
t_{max2} (h)	21	10	
AUC (µg.h/mL)	1.6	0.92	0.95
(Time for calculation –h)	(0-24)	(0-24)	(0-24)
AUC (µg.h/mL)	2.3		
(Time for calculation –h)	(0-48)		
$t_{1/2}$ (h)	NC	NC	NC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy;PEG400 : polyethylene glycol 400; NC : not calculated

2.6.5.4 Pharmacokinetics: Absorption and Plasma Kinetics after Repeated Doses

No pharmacokinetic studies of absorption of COBI after repeated doses have been conducted to date. Repeat-dose studies were performed in support of safety evaluation and toxicokinetics are presented in Module 2.6.7.

2.6.5.5.A Pharmacokinetics: Tissue/Organ Distribution-Darunavir

Test article: darunavir

Location in CTD	**4.2.2.3**									
Study No.	**TMC114-NC192**									
Species	Rat (Long Evans pigmented)									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/5									
Dose (mg.base eq./kg) of TMC114	36.7									
Radionuclide	^{14}C-TMC114									
Specific activity (kBq/mg)	151									
Sampling times (h)	1		4		24		96		336	
Tissues/organs	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B
Adrenal gland	25.2	8.7	30.4	10.0	1.62	2.0	0.527	2.6	LLOQ	-
Blood (RLG)	2.89	1.0	3.03	1.0	0.800	1.0	LLOQ	-	LLOQ	-
Blood (LSC)	2.01	0.7	2.24	0.7	0.502	0.6	0.200	1.0	0.104	1.0
Bone	LLOQ	-	0.427	0.1	LLOQ	-	LLOQ	-	LLOQ	-
Bone marrow	2.99	1.0	5.12	1.7	0.587	0.7	LLOQ	-	LLOQ	-
Brain	LLOQ	-	LLOQ	-	LLOQ	-	LLOQ	-	LLOQ	-
Brain ventricle (CSF)	LLOQ	-	LLOQ	-	LLOQ	-	LLOQ	-	LLOQ	-
Brown fat	3.80	1.3	3.94	1.3	0.840	1.1	LLOQ	-	LLOQ	-
Eye: total (LSC)	0.77	0.3	3.70	1.2	4.95	6.2	3.01	15.1	2.35	22.6
Eye: ciliary body	2.60	0.9	9.77	3.2	33.9	42.4	8.34	41.7	7.50	72.1
Eye: choroids	2.57	0.9	8.61	2.8	39.1	48.9	9.22	46.1	28.6	275
Heart (muscle)	4.05	1.4	5.09	1.7	LLOQ[1]	-	LLOQ	-	LLOQ	-
Kidney	6.73	2.3	10.6	3.5	1.64	2.1	0.763	3.8	LLOQ	-
Liver	43.5	15.1	48.5	16.0	13.3[2]	16.7	4.09	20.5	0.520	5.0
Lung	4.63	1.6	9.98	3.3	0.665	0.8	LLOQ[1]	-	LLOQ	-
Meninges	1.13	0.4	2.63	0.9	1.57	2.0	LLOQ	-	LLOQ	-

[1] : median value; [2] : n=2

LLOQ: lower limit of quantitation (< 0.295 µg-eq/g); RLG: radioluminography; LSC : liquid scintillation counting; CSF : cerebrospinal fluid

T/B : [Tissue]/[Blood] ratios (they were calculated preferably with blood total radioactivity levels as determined with RLG till 24 h after dosing and as determined with LSC from 96 h onwards); PEG400 : polyethylene glycol 400

2.6.5.5.A Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**									
Study No.	**TMC114-NC192**									
Species	Rat (Long Evans pigmented)									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/5									
Dose (mg.base eq/kg) of TMC114	36.7									
Radionuclide	^{14}C-TMC114									
Specific activity (kBq/mg)	151									
Sampling times (h)	1		4		24		96		336	
Tissues/organs	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B
Muscle	2.22	0.8	2.90	1.0	LLOQ	-	LLOQ	-	LLOQ	-
Omentum	ULOQ[1]	-	LLOQ	-	LLOQ	-	LLOQ	-	LLOQ	-
Pancreas	5.68	2.0	8.61	2.8	1.03	1.3	0.688	3.4	LLOQ	-
Prostate gland	2.32[2]	0.8	3.03	1.0	LLOQ[3]	-	LLOQ	-	LLOQ	-
Skin pigmented	3.50	1.2	2.16	0.7	2.21	2.8	1.74	8.7	LLOQ	-
Skin white	1.37	0.5	1.62	0.5	0.360[3]	0.5	LLOQ	-	LLOQ	-
Spleen	5.27[2]	1.8	10.9[2]	3.6	0.803	1.0	LLOQ[3]	-	LLOQ	-
Testicle	0.830	0.3	0.867	0.3	0.682	0.9	LLOQ	-	LLOQ	-
Thyroid	4.34	1.5	5.31	1.8	0.515	0.6	LLOQ	-	LLOQ	-
White fat	0.670[3]	0.2	1.32	0.4	LLOQ	-	LLOQ	-	LLOQ	-
Additional Information: Quantification achieved by radioluminography of whole-body section										

[1] : markedly higher than the upper limit of quantification (ULOQ) of 63.2 µg-eq./g, estimated by extrapolation at 463 µg-eq/g.
[2] : n=2
[3] : median value

LLOQ: lower limit of quantitation (< 0.295 µg-eq/g)
T/B : [Tissue]/[Blood] ratios (they were calculated preferably with blood total radioactivity levels as determined with RLG till 24 h after dosing and as determined with LSC from 96 h onwards).
PEG400 : polyethylene glycol 400

2.6.5.5.A Pharmacokinetics: Tissue/Organ Distribution -Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**									
Study No.	**TMC114-NC192**									
Species	Rat (Long Evans pigmented)									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/5									
Dose of TMC114 (mg.base eq./kg) and ritonavir (mg/kg/day)	36.7 (TMC114 on 3rd day)-25 (ritonavir for 3 days)									
Radionuclide	^{14}C-TMC114									
Specific activity (kBq/mg)	151									
Sampling times (h)	1		4		24		96		336	
Tissues/organs	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B
Adrenal gland	68.9[1]	10.3	92.2[1]	7.9	1.75	8.4	0.953	7.7	LLOQ	-
Blood (RLG)	6.68	1.0	11.7	1.0	LLOQ[2]	-	LLOQ	-	LLOQ	-
Blood (LSC)	5.23	0.8	8.63	0.7	0.209	1.0	0.123	1.0	0.021	1.0
Bone	0.513	0.1	1.85	0.2	LLOQ	-	LLOQ	-	LLOQ	-
Bone marrow	10.1	1.5	16.6	1.4	LLOQ	-	LLOQ	-	LLOQ	-
Brain	LLOQ	-	0.300	0.03	LLOQ	-	LLOQ	-	LLOQ	-
Brain – ventricle (CSF)	LLOQ	-	3.20	0.3	LLOQ	-	LLOQ	-	LLOQ	-
Brown fat	13.4	2.0	24.5	2.1	LLOQ	-	LLOQ	-	LLOQ	-
Eye: total (LSC)	1.96	0.3	5.51	0.5	2.44	12	1.83	15	0.445	21
Eye: ciliary body	6.44	1.0	19.6	1.7	10.3	49	11.4	93	2.87	137
Eye: choroids	4.65	0.7	17.0	1.5	17.2	82	13.1	107	1.33	63
Heart (muscle)	16.3	2.4	25.3	2.2	LLOQ	-	LLOQ	-	LLOQ	-
Kidney	21.4	3.2	33.4	2.9	0.897	4.3	0.590	4.8	LLOQ	-
Liver	64.6	9.7	78.2[1,2]	6.7	7.02	34	2.49	20	LLOQ	-
Lung	9.49	1.4	20.2	1.7	0.470	2.2	LLOQ	-	LLOQ	-

[1] : extrapolated value, slightly above the upper limit of quantification (ULOQ) of 63.2 µg-eq./g; [2] : median value

LLOQ: lower limit of quantitation (< 0.295 µg-eq/g); RLG: radioluminography; LSC : liquid scintillation counting; CSF : cerebrospinal fluid; PEG400 : polyethylene glycol 400

T/B : [Tissue]/[Blood] ratios (they were calculated preferably with blood total radioactivity levels as determined with RLG till 24 h after dosing and as determined with LSC from 96 h onwards).

2.6.5.5.A Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**									
Study No.	**TMC114-NC192**									
Species	Rat (Long Evans pigmented)									
Feeding Condition	not fasted									
Vehicle/Formulation	PEG400									
Route	Oral (gavage)									
Gender (M/F)/Number of Animals	M/5									
Dose of TMC114 (mg.base eq./kg) and ritonavir (mg/kg/day)	36.7 (TMC114 on 3rd day)-25 (ritonavir for 3 days)									
Radionuclide	^{14}C-TMC114									
Specific activity (kBq/mg)	151									
Sampling time (h)	1		4		24		96		336	
Tissues/organs	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B	Conc (µg-eq/g)	T/B
Meninges	1.77	0.3	3.59	0.3	LLOQ[1]	-	LLOQ[1]	-	LLOQ	-
Muscle	8.39	1.3	15.5	1.3	LLOQ	-	LLOQ	-	LLOQ	-
Omentum	ULOQ[2]	-	ULOQ[3]	-	LLOQ	-	LLOQ	-	LLOQ	-
Pancreas	21.7	3.2	34.4	2.9	0.745	3.6	LLOQ	-	LLOQ	-
Prostate gland	6.92	1.0	18.6	1.6	LLOQ	-	LLOQ	-	LLOQ	-
Skin pigmented	5.91	0.9	11.2	1.0	2.35	11	2.16	18	LLOQ	-
Skin white	5.67	0.8	11.3	1.0	LLOQ	-	LLOQ	-	LLOQ	-
Spleen	12.5[4]	1.9	31.0[5]	2.6	0.400	1.9	LLOQ	-	LLOQ	-
Testicle	1.65	0.2	4.52	0.4	LLOQ[1]	-	LLOQ	-	LLOQ	-
Thyroid	12.4	1.9	21.5	1.8	LLOQ	-	LLOQ	-	LLOQ	-
White fat	6.20	0.9	6.50	0.6	LLOQ	-	LLOQ	-	LLOQ	-
Additional Information: Quantification achieved by radioluminography of whole-body section										

[1] : median value; [2] : markedly higher than the upper limit of quantification (ULOQ) of 63.2 µg-eq./g, estimated by extrapolation at 693 µg-eq/g.
[3] : markedly higher than the upper limit of quantification (ULOQ) of 63.2 µg-eq./g, estimated by extrapolation at 364 µg-eq/g.
[4] : as quantified by RLG, spleen could not be appropriately dissected; [5] : as quantified by RLG, estimated at 76.6 µg-eq./g by oxidation and LSC
LLOQ: lower limit of quantitation (< 0.295 µg-eq/g); RLG: radioluminography; LSC : liquid scintillation counting; T/B : [Tissue]/[Blood] ratios (they were calculated preferably with blood total radioactivity levels as determined with RLG till 24 h after dosing and as determined with LSC from 96 h onwards). PEG400 : polyethylene glycol 400

2.6.5.5.A Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD			**4.2.2.3**	
Study No.			**TMC114-NC192**	
Species	Rat (Long Evans pigmented)		Rat (Long Evans pigmented)	
Feeding Condition	not fasted		not fasted	
Vehicle/Formulation	PEG400		PEG400	
Route	Oral (gavage)		Oral (gavage)	
Gender (M/F)/Number of Animals	M/5		M/5	
Dose (mg.base eq./kg)	36.7		36.7 (TMC114 on 3rd day)-25 (ritonavir for 3 days)	
Radionuclide	^{14}C-TMC114		^{14}C-TMC114	
Specific activity (kBq/mg)	151		151	
Sampling times (h)	1, 4, 24, 96 and 336		1, 4, 24, 96 and 336	
Tissues/organs	$AUC_{0\text{-}24h}$ (µg-eq.h/g)	T/B	$AUC_{0\text{-}24h}$ (µg-eq.h/g)	T/B
Adrenal gland	292 (362[1])	6.67 (6.68[2])	733 (827[1])	10.7 (10.3[2])
Blood (RLG)	43.8	1.00	30.9[5]	1.00
Blood (LSC)	30.6 (54.2[1], 89.5[3])	0.70	68.7(80.4[1], 94.2[3])	0.76[6]
Bone	NC	NC	3.80[5]	0.12[6]
Bone marrow	55.5	1.27	45.3[5]	1.46[6]
Brain	NC	NC	NC	NC
Brain ventricle (CSF)	NC	NC	NC	NC
Brown fat	53.6	1.22	63.5[5]	2.06[6]
Eye: total (LSC)	93.6 (1014[3])	2.14 (11.3[4])	87.6 (475[3])	1.28 (5.05[4])
Eye: ciliary body	457 (3668[3])	10.4 (41.0[4])	331 (2597[3])	4.83 (27.6[4])
Eye: choroids	495 (6523[3])	11.3 (72.9[4])	377 (2696[3])	5.49 (28.6[4])
Heart (muscle)	15.7[5]	1.52[6]	70.4[5]	2.28[6]
Kidney	125 (208[1])	2.86 (3.83[2])	273 (325[1])	3.97 (4.05[2])
Liver	704 (1682[3])	16.1 (18.8[4])	837 (1151[1])	12.2 (14.3[2])
Lung	93.0	2.12	154	2.25
Meninges	47.3	1.08	8.90[5]	0.29[6]

[1] : $AUC_{0\text{-}96h}$; [2] : calculated as $AUC_{0\text{-}96h}$ tissue/ $AUC_{0\text{-}96h}$ blood; [3] : $AUC_{0\text{-}336h}$, [4] : calculated as $AUC_{0\text{-}336h}$ tissue/ $AUC_{0\text{-}336h}$ blood; [5] : $AUC_{0\text{-}4h}$,
[6] : calculated as $AUC_{0\text{-}4h}$ tissue/ $AUC_{0\text{-}4h}$ blood
NC : not calculated; RLG : radioluminography; LSC : liquid scintillation counting; CSF : cerebrospinal fluid; T/B : [Tissue]/[Blood] ratios (they were calculated preferably with blood total radioactivity levels as determined with RLG till 24 h after dosing and as determined with LSC from 96 h onwards).; PEG400 : polyethylene glycol 400

2.6.5.5.A Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD			**4.2.2.3**	
Study No.			**TMC114-NC192**	
Species	Rat (Long Evans pigmented)		Rat (Long Evans pigmented)	
Feeding Condition	not fasted		not fasted	
Vehicle/Formulation	PEG400		PEG400	
Route	Oral (gavage)		Oral (gavage)	
Gender (M/F)/Number of Animals	M/5		M/5	
Dose (mg.base eq./kg)	36.7		36.7 (TMC114 on 3rd day)-25 (ritonavir for 3 days)	
Radionuclide	^{14}C-TMC114		^{14}C-TMC114	
Specific activity (kBq/mg)	151		151	
Sampling times (h)	1, 4, 24, 96 and 336		1, 4, 24, 96 and 336	
Tissues/organs	$AUC_{0\text{-}24h}$ (µg-eq.h/g)	T/B	$AUC_{0\text{-}24h}$ (µg-eq.h/g)	T/B
Muscle	8.79[1]	0.85[2]	40.1[1]	1.30 [2]
Omentum	NC	NC	NC	NC
Pancreas	95.7 (157[3])	2.18 (2.89[4])	271	3.94
Prostate gland	9.19[1]	0.89[2]	41.8[1]	1.35[2]
Skin pigmented	53.8 (196[3])	1.23 (3.60[4])	142 (305[3])	2.07 (3.79[4])
Skin white	21.9	0.50	28.3[1]	0.92[2]
Spleen	104	2.38	212	3.09
Testicle	18.4	0.42	10.1[1]	0.33[2]
Thyroid	57.7	1.32	57.0[1]	1.84[2]
White fat	3.32[1]	0.32[2]	22.2[1]	0.72[2]

[1] : $AUC_{0\text{-}4h}$, [2] : calculated as $AUC_{0\text{-}4h}$ tissue/ $AUC_{0\text{-}4h}$ blood
[3] : $AUC_{0\text{-}96h}$; [4] : calculated as $AUC_{0\text{-}96h}$ tissue/ $AUC_{0\text{-}96h}$ blood

NC: not calculated; RLG : radioluminography; LSC : liquid scintillation counting; PEG400 : polyethylene glycol 400
T/B : [Tissue]/[Blood] ratios (they were calculated preferably with blood total radioactivity levels as determined with RLG till 24 h after dosing and as determined with LSC from 96 h onwards).

2.6.5.5.B Pharmacokinetics: Tissue/Organ Distribution-Darunavir

Test article: darunavir

Location in CTD	**4.2.2.3**			
Study No.	**TMC114-NC205**			
Species	Rat (Sprague-Dawley)			
Feeding Condition	not fasted			
Vehicle/Formulation	PEG400			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	pregnant F/4			
Dose (mg.base eq./kg) of TMC114	40			
Radionuclide	^{14}C-TMC114			
Specific activity (kBq/mg)	151			
Sampling times (h)	1	4	8	24
Tissues/organs	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)
Adrenal gland	50.1	68.3	10.9	1.84
Blood (RLG)	3.48	4.80	1.18	LLOQ
Blood (LSC)	2.39	3.59	0.673	0.185
Brain	LLOQ	LLOQ	LLOQ	LLOQ
Brown fat	7.21	10.9	2.19	1.04
Foetus (RLG)	0.538	0.994	0.522[1]	LLOQ
Foetus (LSC)	0.659	0.929	0.198	0.061
Heart	6.70	9.57	1.31	LLOQ
Kidney	10.7	15.8	3.68	1.25
Lacrimal gland	9.48	16.6	3.10	1.99
Liver	52.4	57.6	12.5	4.8
Lung	5.63	10.6	4.61	0.950
Mammary gland	3.83	6.43	1.56	0.426[1]
Muscle	3.43	5.75	0.74	LLOQ
Ovary	3.43	4.21	0.91	LLOQ

[1] : median value

LLOQ: lower limit of quantitation (< 0.399 µg-eq/g); RLG: radioluminography; LSC : liquid scintillation counting; PEG400 : polyethylene glycol 400

2.6.5.5.B Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**			
Study No.	**TMC114-NC205**			
Species	Rat (Sprague-Dawley)			
Feeding Condition	not fasted			
Vehicle/Formulation	PEG400			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	pregnant F/4			
Dose (mg.base eq./kg) of TMC114	40			
Radionuclide	^{14}C-TMC114			
Specific activity (kBq/mg)	151			
Sampling times (h)	1	4	8	24
Tissues/organs	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)
Pancreas	11.7	13.2	2.8	0.706
Placenta	3.06	5.10	1.18	LLOQ
Salivary gland	8.20	12.1	2.32	0.712
Spleen	5.50	9.56	2.99	0.841
Uterine epithelium	17.7	35.9	15.3	4.41
Uterus	3.34	5.16	1.53	LLOQ
Vagina	3.47	5.51	2.03	LLOQ
White fat	1.37	3.04	0.615	LLOQ

LLOQ: lower limit of quantitation (< 0.399 µg-eq/g); PEG400 : polyethylene glycol 400

2.6.5.5.B Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**			
Study No.	**TMC114-NC205**			
Species	Rat (Sprague-Dawley)			
Feeding Condition	not fasted			
Vehicle/Formulation	PEG400			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	pregnant F/4			
Dose (mg.base eq./kg)	40 (TMC114 on 3rd day)-50 (ritonavir for 3 days)			
Radionuclide	^{14}C-TMC114			
Specific activity (kBq/mg)	151			
Sampling times (h)	1	4	8	24
Tissues/organs	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)
Adrenal gland	27.4	20.0	18.2	2.25
Blood (RLG)	1.66	1.34	1.18	LLOQ[1)]
Blood (LSC)	1.43	1.03	0.945	0.115
Brain	LLOQ	LLOQ	LLOQ	LLOQ
Brown fat	3.74	1.77	2.01	LLOQ
Foetus (RLG)	LLOQ	LLOQ	0.553[1]	LLOQ
Foetus (LSC)	0.079	0.223	0.095	0.0276
Heart	3.54	2.13	2.08	LLOQ
Kidney	4.61	3.10	3.07	0.452[1]
Lacrimal gland	3.93	3.29	2.69	0.425[1]
Liver	13.3	12.1	14.7	2.87
Lung	2.59	1.89	1.96	0.446[1]
Mammary gland	1.60	1.46	1.32	LLOQ
Muscle	1.60	1.38	1.52	LLOQ
Ovary	2.61	1.19	3.06	LLOQ

[1] : median value

LLOQ: lower limit of quantitation (< 0.399 µg-eq/g); RLG: radioluminography; LSC : liquid scintillation counting; PEG400 : polyethylene glycol 400

2.6.5.5.B Pharmacokinetics: Tissue/Organ Distribution -Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**			
Study No.	**TMC114-NC205**			
Species	Rat (Sprague-Dawley)			
Feeding Condition	not fasted			
Vehicle/Formulation	PEG400			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	pregnant F/4			
Dose (mg.base eq./kg)	40 (TMC114 on 3rd day)-50 (ritonavir for 3 days)			
Radionuclide	^{14}C-TMC114			
Specific activity (kBq/mg)	151			
Sampling times (h)	1	4	8	24
Tissues/organs	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)	Conc (µg-eq/g)
Pancreas	5.13	2.77	3.34	0.866
Placenta	1.85	1.43	1.55	LLOQ
Salivary gland	3.62	2.44	1.94	LLOQ
Spleen	2.90	3.19	1.90	0.420[1]
Uterine epithelium	11.3	15.0	8.43	3.31
Uterus	1.59	1.14	1.11	LLOQ
Vagina	1.49	2.99	0.964	0.600[1]
White fat	0.583	0.868	0.552	LLOQ

[1] : median value

LLOQ: lower limit of quantitation (< 0.399 µg-eq/g); PEG400 : polyethylene glycol 400

2.6.5.5.B Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**			
Study No.	**TMC114-NC205**			
Species	Rat (Sprague-Dawley)		Rat (Sprague-Dawley)	
Feeding Condition	not fasted		not fasted	
Vehicle/Formulation	PEG400		PEG400	
Route	Oral (gavage)		Oral (gavage)	
Gender (M/F)/Number of Animals	pregnant F/4		pregnant F/4	
Dose (mg.base eq./kg)	40		40 (TMC114 on 3rd day)-50 (ritonavir for 3 days)	
Radionuclide	^{14}C-TMC114		^{14}C-TMC114	
Specific activity (kBq/mg)	151		151	
Sampling times (h)	1, 4, 8 and 24		1, 4, 8 and 24	
Tissues/organs	AUC_{0-8h} (µg-eq.h/g)	T/B	AUC_{0-8h} (µg-eq./g)	T/B
Adrenal gland	328	13.4	161	15.5
Blood (RLG)	24.5	1.00	10.3	1.00
Blood (LSC)	17.1	0.701	8.33	0.80
Brain	LLOQ	NC	LLOQ	NC
Brown fat	52.4	2.14	17.3	1.67
Foetus (RLG)	5.50	0.225	LLOQ	NC
Foetus (LSC)	4.60	0.270	1.09	0.131
Heart	44.4	1.81	18.5	1.79
Kidney	78.2	3.20	26.0	2.52
Lacrimal gland	76.0	3.11	24.7	2.39
Liver	309	12.6	98.3	9.51
Lung	55.8	2.28	15.7	1.51
Mammary gland	31.1	1.27	10.9	1.06
Muscle	25.3	1.03	11.1	1.07
Ovary	21.8	0.892	15.2	1.47
Pancreas	70.0	2.86	26.3	2.54
Placenta	24.5	1.00	11.8	1.14

LLOQ: lower limit of quantitation (< 0.399 µg-eq/g); NC : not calculated; RLG: radioluminography; LSC : liquid scintillation counting; T/B : [Tissue]/[Blood] ratios
PEG400 : polyethylene glycol 400

2.6.5.5.B Pharmacokinetics: Tissue/Organ Distribution-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.3**			
Study No.	**TMC114-NC205**			
Species	Rat (Sprague-Dawley)		Rat (Sprague-Dawley)	
Feeding Condition	not fasted		not fasted	
Vehicle/Formulation	PEG400		PEG400	
Route	Oral (gavage)		Oral (gavage)	
Gender (M/F)/Number of Animals	pregnant F/4		pregnant F/4	
Dose (mg.base eq./kg)	40		40 (TMC114 on 3rd day)-50 (ritonavir for 3 days)	
Radionuclide	^{14}C-TMC114		^{14}C-TMC114	
Specific activity (kBq/mg)	151		151	
Sampling times (h)	1, 4, 8 and 24		1, 4, 8 and 24	
Tissues/organs	$AUC_{0\text{-}8h}$ (µg-eq.h/g)	T/B	$AUC_{0\text{-}8h}$ (µg-eq./g)	T/B
Salivary gland	58.3	2.38	19.5	1.89
Spleen	48.0	1.96	20.5	1.98
Uterine epithelium	186	7.6	90.5	8.75
Uterus	26.4	1.08	9.34	0.903
Vagina	29.1	1.19	14.6	1.41
White fat	13.4	0.547	5.26	0.509

T/B : [Tissue]/[Blood] ratios
PEG400 : polyethylene glycol 400

2.6.5.5.C Pharmacokinetics: Tissue/Organ Distribution-Cobicistat

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-9350 Following Oral Administration to Rats	Distribution	[^{14}C]COBI	AD-216-2034 4.2.2.3
Species:	Sprague Dawley Rat (H1a:[SD]CVF)		
Gender (M/F) / No. of Animals:	Group 3: 6 male (one per time point)		
Feeding Condition:	Fasted		
Vehicle / Formulation:	5% (v/v) ethanol, 15% (v/v) propylene glycol adjusted to pH 3.60 with HCl		
Method of Administration:	Oral		
Dose (mg/kg):	1 dose of [^{14}C]COBI 10 mg/kg		
Radionuclide:	Carbon-14		
Specific Activity:	21.5 !Ci/mg (in dose)		
Sampling Time:	0.25, 1, 4, 8, 12, and 24 hours postdose		
Analyte/Assay:	[^{14}C]/Quantitative whole body autoradiography		

2.6.5.5.C Pharmacokinetics: Tissue/Organ Distribution-Cobicistat (Continued)

Test Article: [^{14}C]COBI

	ng Equivalents [^{14}C]COBI/g					
	Animal Number (Sacrifice Time)					
	B07114	B07115	B07116	B07117	B07118	B07119
Tissue	(0.25 Hours)	(1 Hour)	(4 Hours)	(8 Hours)	(12 Hours)	(24 Hours)
Adipose (brown)	1320	2870	1330	1030	1480	1280
Adipose (white)	205	748	142	128	117	87.8
Adrenal gland	5090	28,800	4290	1390	1480	1740
Bile	353,000[a]	214,000	89,300	14,400	11,500	11,000
Blood	347	723	200	119	117	77.7
Bone	74.0	142	54.6	94.1	52.7	BLQ
Bone marrow	1020	3710	1170	1210	1260	650
Brain	BLQ	47.7	49.0	55.4	49.1	BLQ
Cecum	890	1210	1840	2540	1820	1030
Cecum contents	BLQ	58.5	398,000[a]	229,000	89,200	6660
Choroid plexus	1620	2580	824	1210	1350	1550
Diaphragm	870	2050	787	451	556	357
Epididymis	59.5	267	165	214	263	208
Esophageal contents	33,600	53.0	641	115	BLQ	BLQ
Esophagus	557	1230	613	487	300	278
Exorbital lacrimal gland	515	3530	2290	1960	1870	1410
Eye	87.0	144	85.0	54.5	98.3	62.0
Eye (lens)	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eye (uveal tract)	349	979	545	557	318	197
Harderian gland	367	2650	1670	2270	3410	1720
Heart	1070	2260	707	775	763	634

2.6.5.5.C Pharmacokinetics: Tissue/Organ Distribution-Cobicistat (Continued)

Test Article: [^{14}C]COBI

Tissue	ng Equivalents [^{14}C]COBI/g					
	Animal Number (Sacrifice Time)					
	B07114 (0.25 Hours)	B07115 (1 Hour)	B07116 (4 Hours)	B07117 (8 Hours)	B07118 (12 Hours)	B07119 (24 Hours)
Intra-orbital lacrimal gland	368	3650	2020	2150	1730	1370
Kidney	3630	6350	1810	1390	1550	1030
Large intestinal contents	NR	51.2	BLQ	487,000[a]	330,000	11,600
Large intestine	582	2350	1620	1380	2540	1900
Liver	49,800	33,900	16,500	6280	5570	2620
Lung	865	2230	579	508	540	486
Lymph nodes	279	2860	708	567	650	477
Muscle (skeletal)	424	1150	231	215	349	149
Nasal turbinates	111	395	186	277	324	220
Pancreas	1280	2960	1370	1090	1010	725
Pituitary gland	1550	6720	2820	1820	3500	1550
Preputial gland	461	1760	795	1080	NR	NR
Prostate	242	1100	751	443	720	342
Renal cortex	3550	6160	1920	1480	1660	1080
Renal medulla	3690	6900	1740	1190	1340	941
Salivary gland	1430	5190	1950	1540	1170	691
Seminal vesicles	BLQ	137	133	189	212	146
Skin	191	521	232	188	236	186
Small intestinal contents	318,000[a]	608,000[a]	803,000[a]	6410	3910	1910
Small intestine	1440	4110	2670	1920	961	623
Spinal cord	BLQ	BLQ	BLQ	52.6	BLQ	BLQ
Spleen	1990	6060	1470	1400	1630	785

2.6.5.5.C Pharmacokinetics: Tissue/Organ Distribution-Cobicistat (Continued)

Test Article: [^{14}C]COBI

Tissue	ng Equivalents [^{14}C]COBI/g					
	Animal Number (Sacrifice Time)					
	B07114 (0.25 Hours)	B07115 (1 Hour)	B07116 (4 Hours)	B07117 (8 Hours)	B07118 (12 Hours)	B07119 (24 Hours)
Stomach	1340	2550	1550	1820	1470	1230
Stomach contents	198,000	147,000	3610	183	BLQ	5840
Testis	BLQ	128	120	166	282	136
Thymus	247	1360	602	552	462	366
Thyroid	2520	6140	536	1270	1200	748
Urinary bladder	630	1830	1660	360	NR	NR
Urine	2300	5680	5990	319	NR	NR

BLQ = below limit of quantitation (<43.5 ng equivalents [^{14}C]COBI/g); COBI = cobicistat; NR = not represented (tissue not present in section)

a One or more samples were above the upper limit of quantitation (ULOQ, >424,000 ng equivalents [^{14}C]COBI/g)

2.6.5.5.D Pharmacokinetics: Tissue/Organ Distribution-Cobicistat

Report Title	Study Type	Test Article	Report Number Location in CTD
Whole-Body Autoradiography (WBA) of Rats Following Oral Administration of [^{14}C]GS-9350	Distribution	[^{14}C]COBI	AD-216-2060 4.2.2.3
Species:	Long Evans rats (HsdBlu:LE)		
Gender (M/F) / No. of Animals:	Group 1: 7 male (one per time point)		
Feeding Condition:	Fasted		
Vehicle / Formulation:	5% (v/v) ethanol, 15% (v/v) propylene glycol adjusted to pH 3.61 with HCl		
Method of Administration:	Oral		
Dose (mg/kg):	1 dose of [^{14}C]COBI 10 mg/kg		
Radionuclide:	Carbon-14		
Specific Activity:	250 !Ci/kg (in dose)		
Sampling Time:	0.25, 1, 4, 12, 24, 48, and 72 hours postdose		
Analyte/Assay:	[^{14}C]/Quantitative whole body autoradiography		

2.6.5.5.D Pharmacokinetics: Tissue/Organ Distribution-Cobicistat (Continued)

Test Article: [^{14}C]COBI

Tissue	ng Equivalents [^{14}C]COBI/g						
	Animal Number (Sacrifice Time)						
	B10527 (0.25 Hours)	B10528 (1 Hour)	B10529 (4 Hours)	B10530 (12 Hours)	B10531 (24 Hours)	B10532 (48 Hours)	B10533 (72 Hours)
Adipose (brown)	1430	7610	1180	1080	1270	711	806
Adipose (white)	297	2380	BLQ	82.5	113	69.2	BLQ
Adrenal gland	8530	35,400	3280	1850	2600	812	1330
Bile	175,000	94,100	30,000	ND	8170	3860	5070
Blood	960	1700	192	132	127	BLQ	BLQ
Bone	51.8	255	128	65.6	52.7	BLQ	63.5
Bone marrow	1070	5000	902	817	735	263	251
Brain	BLQ	BLQ	53.4	BLQ	BLQ	BLQ	BLQ
Cecum	1070	3210	1150	2040	809	225	99.6
Cecum contents	BLQ	3430	281,000	259,000	3450	595	276
Diaphragm	1860	3940	604	672	540	250	245
Epididymis	109	674	254	207	216	74.8	91.6
Esophageal contents	148,000	483	55.1	60.2	BLQ	BLQ	BLQ
Esophagus	2770	3300	443	338	386	193	200
Exorbital lacrimal gland	942	6930	2750	1660	1300	357	698
Eye	63.7	587	480	709	678	324	566
Eye (lens)	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
Eye (uveal tract)	1030	5820	4400	6620	6530	3760	4500
Harderian gland	374	3790	2870	2440	1670	482	476
Heart	2010	4440	815	643	669	183	242

2.6.5.5.D Pharmacokinetics: Tissue/Organ Distribution-Cobicistat (Continued)

Test Article: [^{14}C]COBI

Tissue	ng Equivalents [^{14}C]COBI/g						
	Animal Number (Sacrifice Time)						
	B10527 (0.25 Hours)	B10528 (1 Hour)	B10529 (4 Hours)	B10530 (12 Hours)	B10531 (24 Hours)	B10532 (48 Hours)	B10533 (72 Hours)
Intra-orbital lacrimal gland	623	5730	2650	1770	1170	391	582
Kidney	5290	11,500	1530	1260	1230	578	570
Large intestinal contents	BLQ	4520	9030	145,000	11,000	1730	405
Large intestine	594	3250	2060	947	709	460	203
Liver	77,400	48,000	12,700	6350	3850	2070	1560
Lung	2160	6090	642	523	522	222	205
Lymph nodes	496	3880	726	664	397	225	245
Muscle (skeletal)	465	2190	246	260	249	101	137
Nasal turbinates	154	836	147	559	159	BLQ	120
Pancreas	2150	5010	1510	1280	820	240	293
Pituitary gland	2600	14,200	3130	3520	4570	1120	1030
Preputial gland	407	3630	884	1010	1060	534	593
Prostate	279	2030	605	552	537	93.5	194
Renal cortex	5230	9490	1550	1320	1260	644	617
Renal medulla	5350	13,200	1530	1220	1180	550	531
Salivary gland	2410	7660	1410	895	564	208	190
Seminal vesicles	72.1	438	98.5	159	240	136	222
Skin (nonpigmented)	244	1280	233	238	224	73.2	88.8
Skin (pigmented)	292	1440	396	502	315	197	147
Small intestinal contents	205,000	310,000	316,000	2870	613	190	107

2.6.5.5.D Pharmacokinetics: Tissue/Organ Distribution-Cobicistat (Continued)

Test Article: [^{14}C]COBI

Tissue	ng Equivalents [^{14}C]COBI/g						
	Animal Number (Sacrifice Time)						
	B10527 (0.25 Hours)	B10528 (1 Hour)	B10529 (4 Hours)	B10530 (12 Hours)	B10531 (24 Hours)	B10532 (48 Hours)	B10533 (72 Hours)
Small intestine	1150	10,600	2440	1730	961	269	160
Spinal cord	BLQ	46.6	51.1	BLQ	BLQ	BLQ	BLQ
Spleen	2330	8220	1620	1060	726	323	311
Stomach	1220	3150	1070	1040	829	335	377
Stomach contents	291,000	74,300	1400	BLQ	BLQ	BLQ	BLQ
Testis	BLQ	174	125	122	122	55.5	68.7
Thymus	274	2340	524	305	297	167	189
Thyroid	3510	6350	1120	756	1110	254	398
Urinary bladder	2240	8350	10,200	281	477	279	187
Urine	2020	1870	4770	494	1240	88.6	108

BLQ = below the limit of quantitation (< 46.2 ng equivalents [^{14}C]COBI/g); ND = not detectable (sample shape not discernible from background or surrounding tissue)

Note: ng Equivalent/g values are reported to 3 significant figures with a maximum of 3 decimal places

2.6.5.6.A Pharmacokinetics: Plasma Protein Binding-Darunavir

Test Article: Darunavir

Location in CTD	**4.2.2.3**								
Study No.	**TMC114-NC113**								
Methodology:	Plasma protein binding was assessed by equilibrium dialysis in which plasma was fortified with TMC114. Plasma was dialyzed against phosphate buffer, pH 7.35 at 36.7-38.1 !C for 8 hours. Concentration of TMC114 (n=2) in dialysis compartments was determined by HPLC-MS/MS.								
Parameters Measured	**Rat (Male Wistar)**			**Dog (Male Beagle)**			**Human (Male)**		
Concentration Tested in Plasma (µg base-eq/mL)	0.064	0.801	10.2	0.088	1.17	25.5	0.088	0.746	7.11
Plasma Bound %	92.8	97.1[1]	78.3	71.4	80.8	79.5[1]	95.8	96.1	92.3
Additional Information Methodological study evaluated the length of time needed to reach equilibrium in the dialysis cells (8 hours was deemed sufficient)									

[1] : n=1

2.6.5.6.B Pharmacokinetics: Plasma Protein Binding-Darunavir

Test Article: Darunavir

Location in CTD	**4.2.2.3**													
Study No.	**TMC114-NC215**													
Methodology:	Plasma protein binding was assessed by equilibrium dialysis in which plasma was fortified with ^{14}C-TMC114. Plasma was dialyzed against 0.067 M phosphate buffer, pH 7.17 at 37 °C for 4 hours. Concentration of ^{14}C- TMC114 (n=2) in dialysis compartments was evaluated by radioactivity levels determined by liquid scintillation counting. In blood distribution studies, samples of whole blood were combusted in an oxidizer and $^{14}CO_2$ captured and counted by liquid scintillation.													
Parameters Measured	**Mouse (Male Swiss CD-1)**		**Mouse (Female Swiss CD-1)**		**Rat (Male Sprague-Dawley)**		**Rat (Female Sprague-Dawley)**		**Rabbit (Female New Zealand White)**		**Dog (Male Beagle)**		**Human (Male)**	
Concentration Tested in Plasma (µg base-eq/mL)	0.500		0.500		0.500		0.500		0.500		0.346		0.500	
Plasma Bound %	89.6		90.4		94.6		95.0		62.5		80.7		95.3	
Plasma Free %	10.5		9.6		5.4		5.0		37.5		19.3		4.7	
Concentration Tested in Blood (µg. base eq./mL)	0.500	5.00	0.500	5.00	0.500	5.00	0.500	5.00	0.500	5.00	0.500	3.46	0.500	5.00
Blood-to-Plasma Ratio	0.79	0.83	0.77	0.80	0.70	1.12	0.70	0.85	1.11	1.14	0.85	0.91	0.64	0.74
Distribution to: Plasma Water (%)	8.0	5.6	7.4	5.3	4.7	11.9	4.6	15.7	21.2	20.8	10.7	6.8	3.8	5.2
Plasma Proteins (%)	68.4	67.7	69.9	69.3	82.9	43.2	85.9	59.4	35.4	34.3	45.1	44.7	77.4	65.4
Blood Cells (%)	23.6	26.7	22.7	25.5	12.4	44.9	9.5	24.9	43.4	45.0	44.2	48.5	18.8	29.4
Hematocrit (%)	40		41		39		37		37		54		48	

Additional Information

Plasma was obtained from blood of five individual female rabbits, four individual male dogs, and five individual male humans, from two pools of male and female rat blood, and from two pools of male and female mouse blood.

Methodological studies evaluated: (a) the length of time needed to reach equilibrium in the dialysis cells (4 hours was deemed sufficient); (b) the effect of pH on the plasma protein binding in human plasma (in the pH-range 6.8 to 8.1, the percentage bound TMC114 amounted to about 94%); (c) the plasma concentration dependence; no plasma concentration dependence was seen in female rabbit in all the range tested (0.052 to 4.69 µg base eq./ml); no plasma concentration dependence in mouse, dog and human in the range 0.052 to 4.69 µg base eq./ml , plasma free % was around 17.2 at 37.5 µg base eq./ml , 35.3 at 37.5 µg base eq./ml and 24.5 at 18.7 µg base eq./ml in mouse, in male dog and male human, respectively; no plasma concentration dependence were seen in rats in the range 0.052 to 0.520 µg base eq./ml, plasma free % was around 21.3 and 41.7 at 4.69 and 37.5 µg base eq./ml , respectively; and (d) binding to purified human plasma protein (at physiological concentrations, TMC114 bound to human serum albumin, 43.4%, and α_1-acid glycoprotein, between 83.6% and 93.4%).

2.6.5.6.C Pharmacokinetics: Plasma Protein Binding-Cobicistat

Report Title	Study Type	Test Article	Report Number Location in CTD
Plasma Protein Binding of GS-9350 in CD-1 Mice	Plasma protein binding	Cobicistat	AD-216-2076 4.2.2.3
Plasma Protein Binding of GS-9350			AD-216-2026 4.2.2.3

Study System: Plasma from Mouse, Rat, Dog, Monkey, and Human

Target Entry, Test System, and Method: Equilibrium dialysis for 3 hours at 37°C against 0.133 M phosphate buffer, pH 7.4. Analysis by LC/MS/MS.

	Fraction Unbound (%)			
	Concentration			
Matrix	**1 µM**	**10 µM**	**30 µM**	**Mean**
Mouse Plasma	3.31 ± 0.14	4.78 ± 0.27	6.15 ± 0.48	4.75
Rat plasma	2.33 ± 0.06	5.34 ± 0.24	8.51 ± 0.48	5.40
Dog plasma	5.68 ±0.60	6.46 ± 0.60	6.33 ± 0.40	6.16
Cynomolgus monkey plasma	4.31 ± 0.50	6.17 ± 0.50	9.13 ± 0.30	6.54
Human plasma	6.33 ± 0.80	8.92 ± 0.9	7.54 ± 0.60	7.60

LC/MS/MS = liquid chromatography coupled to tandem mass spectrometry

2.6.5.7.A Pharmacokinetics: Study Absorption and Plasma Kinetics in Pregnant or Nursing Animals - Mice

Test article: darunavir

Location in CTD	**4.2.3.5.2**							
Study No.	**TMC114-NC172**							
Species	**Mouse (Pregnant CD1)**							
Feeding Condition	not fasted							
Vehicle/Formulation	PEG400 (TMC114) –Propylene Glycol (ritonavir)							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	F/10		F/10		F/10		F/10	
Dose (mg.base eq./kg/day) of TMC114	150		450		1000		1000	
Concentrations (mg/mL) of TMC114	15		45		100		100	
Dose (mg/kg/day) of ritonavir	-	-	-	-	-	-	50	50
Concentrations (mg/mL) of ritonavir	-	-	-	-	-	-	6.25	6.25
Duration of Dosing (day)	1(GD6)	10(GD15)	1(GD6)	10(GD15)	1(GD6)	10(GD15)	1(GD6)	10(GD15)
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max} (µg/mL)	8.38	5.99	15.7	8.00	19.7	7.73	25.3	16.1
t_{max} (h)	1	1	1	1	1	1	1	1
AUC (µg.h/mL)	24.2	12.5	69.8	27.6	122	63.9	158	81.6
(Time for calculation –h)	(0-!)	(0-24)	(0-8)	(0-8)	(0-!)	(0-24)	(0-!)	(0-24)
$t_{1/2}$ (h)	0.7	NC	NC	NC	2.5	5.5	3.7	NC
(Time for calculation –h)	(2-8)	-	-	-	(4-24)	(4-24)	(4-24)	-

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
GD : day of gestation
NC : not calculated
PEG400 : polyethylene glycol 400

2.6.5.7.A Pharmacokinetics: Study Absorption and Plasma Kinetics in Pregnant or Nursing Animals - Mice (continued)

Test article: darunavir

Location in CTD	**4.2.3.5.2**			
Study No.	**TMC114-NC172**			
Species	**Mice (Pregnant CD1)**			
Feeding Condition	not fasted			
Vehicle/Formulation	PEG400 (TMC114) –Propylene Glycol (ritonavir)			
Route	Oral (gavage)			
Gender (M/F)/Number of Animals	F/10		F/10	
Dose (mg.base eq./kg/day) of TMC114	-		1000	
Concentrations (mg/mL) of TMC114	-		100	
Dose (mg/kg/day) of ritonavir	50		50	
Concentrations (mg/mL) of ritonavir	6.25		6.25	
Duration of Dosing (day)	1(GD6)	10(GD15)	1(GD6)	10(GD15)
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma
Analyte	ritonavir	ritonavir	ritonavir	ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters				
C_{max} (µg/mL)	13.7	9.36	4.61	5.90
t_{max} (h)	1	1	24	1
AUC (µg.h/mL)	37.0	42.4	41.6[1]	13.1
(Time for calculation –h)	(0-8)	(0-8)	(0-24)	(0-24)
$t_{1/2}$ (h)	NC	NC	NC	3.3
(Time for calculation –h)	-	-	-	(4-24)

[1] : due to high value (9.21 µg/mL) for one animal at 24h

LC-MS/MS = liquid chromatography with tandem mass spectroscopy; GD : day of gestation; NC : not calculated; PEG400 : polyethylene glycol 400

2.6.5.7.B Pharmacokinetics: Study Absorption and Plasma Kinetics in Pregnant or Nursing Animals - Rats

Test article: darunavir

Location in CTD	**4.2.3.5.2**					
Study No.	**TMC114-NC127**					
Species	**Rat (Pregnant Sprague Dawley)**					
Feeding Condition	not fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	F/6		F/6		F/6	
Dose[1] (mg/kg/day)	40		200		1000	
Concentrations (mg/mL)	4		20		100	
Duration of Dosing (day)	1 (GD7)	13 (GD19)	1 (GD7)	13 (GD19)	1 (GD7)	13 (GD19)
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	1.80	1.70	11.1	6.13	12.4	11.3
t_{max} (h)	0.5	1	2	4	8	2
AUC (µg.h/mL)	12.5	7.59	73.8	36.8	84.5	66.2
(Time for calculation –h)	(0-!)	(0-8)	(0-8)	(0-8)	(0-8)	(0-8)
$t_{1/2}$ (h)	1.5[2]	12[2]	NC	3.0[2]	NC	4.0[2]
(Time for calculation –h)	NS	NS	-	NS	-	NS

[1] : doses expressed as TMC114 base were 37.6 or 38.6 , 188 or 193, 939 or 965 mg/kg/day depending on the batch used
[2] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
GD : day of gestation
NS: not specified
NC : not calculated
PEG400 : polyethylene glycol 400

2.6.5.7.C Pharmacokinetics: Study Absorption and Plasma Kinetics in Pregnant or Nursing Animals - Rats

Test article: darunavir

Location in CTD	**4.2.3.5.3**						
Study No.	**TMC114-NC178**						
Species	**Rat (Sprague-Dawley)**						
Feeding Condition	not fasted						
Vehicle/Formulation	PEG400						
Route	Oral (gavage)						
Gender (M/F)/Number of Animals	F/5	M/4	F/4	M/5[1]	F/6[1]	M/9[1]	F/10[1]
Dose (mg.base eq./kg/day)	40	200	200	1000	1000	1000	1000
Concentrations (mg/mL)	4	20	20	100	100	100	100
Age (day)	25	25	25	12	12	26	26
Duration of Dosing (day)	24	24	24	1	1	1	1
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters							
C_{max} (µg/mL)	1.53	7.21	5.63	75.4	78.4	18.3-29.7	19.4-31.9
t_{max} (h)	1	4	4	5	5	3	1-3
AUC (µg.h/mL)	6.31	29.8	28.6	215	222-249	66.4-249	133-149
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-5)	(0-5)	(0-5 or 24)	(0-24)
$t_{1/2}$ (h)	1.1	0.9	1.2	NC	NC	NC	NC
(Time for calculation –h)	(4-8)	(4-8)	(4-8)	-	-	-	-

Additional Information

AUC_{0-5h} values in rats on day 25 of age were 5.37 µg.h/mL in females at 40 mg/kg/day and 25.2 µg.h/mL and 23.2 µg.h/mL in males and females at 200 mg/kg/day, respectively.
On day 7 of lactation, TMC114 concentrations were detected in plasma of male and female pups whose mothers received 40, 20 and 1000 mg/kg/day from day 6 of gestation to day 7 of lactation.
The distribution of TMC114 in limited tissues (brain and liver) was investigated after 1000 mg/kg. On day 12 of age, AUC_{0-5h} was 1200 to 1790 µg.h/g in liver and 111 to 133 µg.h/g in brain. On day 26, AUC_{0-5h} was 59.6 to 102 µg.h/mL in plasma , 371 to 604 µg.h/g in liver and 4.7 to 7.1 µg.h/g in brain. These values were lower by up to 4.8-fold in the liver and 27-fold in the brain, when compared to those obtained at day 12 of age.

[1] : groups 2 and 5 together

LC-MS/MS = liquid chromatography with tandem mass spectroscopy; NC : not calculated; PEG400 : polyethylene glycol 400

2.6.5.7.D Pharmacokinetics: Study Absorption and Plasma Kinetics in Pregnant or Nursing Animals - Rabbits

Test article: darunavir

Location in CTD	**4.2.3.5.2**					
Study No.	**TMC114-NC125**					
Species	**Rabbit (New Zealand White)**					
Feeding Condition	Not Fasted					
Vehicle/Formulation	1 % CMC/0.2% Tween 80 in Water					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	F/6		F/6		F/6	
Dose[1] (mg/kg/day)	40		200		1000	
Concentration (mg/mL)	4		20		100	
Duration of Dosing (day)	1 (GD8)	13 (GD20)	1 (GD8)	13 (GD20)	1 (GD8)	13 (GD20)
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	0.029	0.151	0.147	0.601	1.92	1.74
t_{max} (h)	0.5	1.0	1.0	0.5	2.0	0.5
AUC (µg.h/mL)	0.128[2]	0.465	0.517	1.81	6.26	5.99
(Time for calculation –h)	(0-∞)	(0-8)	(0-∞)	(0-8)	(0-8)	(0-8)
$t_{1/2}$ (h)	3.0[3]	1.8[3]	1.4	3.2[3]	NC	2.0
(Time for calculation –h)	NS	NS	NS	NS	-	NS

[1] : doses expressed as TMC114 base were 39, 193 and 965 mg/kg/day
[2] : not accurately determined (% of extrapolation > 15 %): AUC_{0-2h} = 0.042 µg.h/mL
[3] : not accurately determined

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
CMC : carboxymethyl cellulose
GD: day of gestation
NC : not calculated

2.6.5.7.E Pharmacokinetics in Pregnant or Nursing Rats

Test article: darunavir

Location in CTD	**4.2.3.5.2**							
Study No.	**TMC114-NC397**							
Species	**Pregnant Rat (Sprague-Dawley)**							
Feeding Condition	not fasted							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	F/6				F/6			
Dose (mg.base eq./kg/day) of darunavir	1000 (2 x 500; 6 hours apart)				2000 (2 x 1000; 6 hours apart)			
Concentrations (mg.base eq./mL) of darunavir	100				200			
Vehicle/Formulation of darunavir	PEG400				PEG400			
Day of gestation	6	11	12	17	6	11	12	17
Duration of Dosing (day)	1	6	1	6	1	6	1	6
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max1} (µg/mL)	12	8.5	13	7.9	11	5.8	12	5.4
t_{max1}(h)	1	1	2	1	2	6	1	6
C_{max2} (µg/mL)	23	9.6	25	8.7	26	7.7	15	7.2
t_{max2} (h)	7	7	7	7	8	7	8	7
AUC (µg.h/mL)	228	104	222	97	291	107	198	103
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NC	NC	NC	NC	NC	NC	NC	NC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy;
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5. 7.E Pharmacokinetics in Pregnant or Nursing Rats (continued)

Test article: darunavir

Location in CTD	**4.2.3.5.2**				
Study No.	**TMC114-NC397**				
Species	**Pregnant Rat (Sprague-Dawley)**				
Feeding Condition	not fasted				
Route	Oral (gavage)				
Gender (M/F)/Number of Animals	F/6				
Dose (mg.base eq./kg/day) of darunavir	1000 (2 x 500; 6 hours apart)				
Concentrations (mg.base eq./mL) of darunavir	100				
Vehicle/Formulation of darunavir	PEG400				
Dose (mg/kg/day) of ritonavir	100 (2 x 50; 6 hours apart)				
Concentrations (mg/mL) of ritonavir	40				
Vehicle/Formulation of ritonavir	Propylene Glycol				
Day of gestation	6	11	12	17	
Duration of Dosing (day)	1	6	1	6	
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	
Analyte	TMC114	TMC114	TMC114	TMC114	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters					
C_{max1} (µg/mL)	9.5	24	11	20	
t_{max1}(h)	6	2	1	1	
C_{max2} (µg/mL)	16	22	24	31	
t_{max2} (h)	7	7	7	7	
AUC (µg.h/mL)	284	419	275	397	
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	
$t_{1/2}$ (h)	NC	NC	NC	NC	

LC-MS/MS = liquid chromatography with tandem mass spectroscopy;
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5. 7.E Pharmacokinetics in Pregnant or Nursing Rats (continued)

Test article: darunavir

Location in CTD	**4.2.3.5.2**				
Study No.	**TMC114-NC397**				
Species	**Pregnant Rat (Sprague-Dawley)**				
Feeding Condition	not fasted				
Route	Oral (gavage)				
Gender (M/F)/Number of Animals	F/6				
Dose (mg.base eq./kg/day) of darunavir	1000 (2 x 500; 6 hours apart)				
Concentrations (mg.base eq./mL) of darunavir	100				
Vehicle/Formulation of darunavir	PEG400				
Dose (mg/kg/day) of ritonavir	100 (2 x 50; 6 hours apart)				
Concentrations (mg/mL) of ritonavir	40				
Vehicle/Formulation of ritonavir	Propylene Glycol				
Day of gestation	6	11	12	17	
Duration of Dosing (day)	1	6	1	6	
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	
Analyte	Ritonavir	Ritonavir	Ritonavir	Ritonavir	
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	
Pharmacokinetic Parameters					
C_{max1} (µg/mL)	1.7	2.0	0.63	0.78	
t_{max1}(h)	2	1	1	2	
C_{max2} (µg/mL)	1.6	1.3	3.3	2.7	
t_{max2} (h)	8	7	7	7	
AUC (µg.h/mL)	18	9.6	14	12	
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	
$t_{1/2}$ (h)	NC	NC	NC	NC	

LC-MS/MS = liquid chromatography with tandem mass spectroscopy;
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.7.F Pharmacokinetics in Pregnant or Nursing rats

Test article: darunavir

Location in CTD	**4.2.3.5.2**							
Study No.	**TMC114-NC398**							
Species	**Pregnant Rat (Sprague-Dawley)**							
Feeding Condition	not fasted							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	F/6				F/6			
Dose (mg.base eq./kg/day) of darunavir	600 (2 x 300; 6 hours apart)				1000 (2 x 500; 6 hours apart)			
Concentrations (mg.base eq./mL) of darunavir	60				100			
Vehicle/Formulation of darunavir	PEG400				PEG400			
Dose (mg/kg/day) of ritonavir	100 (2 x 50 ; 6 hours apart)				100 (2 x 50 ; 6 hours apart)			
Concentrations (mg/mL) of ritonavir	40				40			
Vehicle/Formulation of ritonavir	Propylene Glycol				Propylene Glycol			
Day of gestation	6	11	12	17	6	11	12	17
Duration of Dosing (day)	1	6	1	6	1	6	1	6
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max1} (µg/mL)	9.8	11	9.7	17	12	13	14	13
t_{max1}(h)	1	6	2	1	2	2	1	2
C_{max2} (µg/mL)	22	14	20	14	35	23	24	24
t_{max2} (h)	8	7	7	8	7	7	8	7
AUC (µg.h/mL)	280	202	240	261	423	283	312	219
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NC	NC	NC	NC	NC	NC	NC	NC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy;
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5. 7.F Pharmacokinetics in Pregnant or Nursing rats (continued)

Test article: darunavir

Location in CTD	**4.2.3.5.2**							
Study No.	**TMC114-NC398**							
Species	**Pregnant Rat (Sprague-Dawley)**							
Feeding Condition	not fasted							
Route	Oral (gavage)							
Gender (M/F)/Number of Animals	F/6				F/6			
Dose (mg.base eq./kg/day) of darunavir	600 (2 x 300; 6 hours apart)				1000 (2 x 500; 6 hours apart)			
Concentrations (mg.base eq./mL) of darunavir	60				100			
Vehicle/Formulation of darunavir	PEG400				PEG400			
Dose (mg/kg/day) of ritonavir	100 (2 x 50; 6 hours apart)				100 (2 x 50; 6 hours apart)			
Concentrations (mg/mL) of ritonavir	40				40			
Vehicle/Formulation of ritonavir	Propylene Glycol				Propylene Glycol			
Day of gestation	6	11	12	17	6	11	12	17
Duration of Dosing (day)	1	6	1	6	1	6	1	6
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Analyte	Ritonavir	Ritonavir	Ritonavir	Ritonavir	Ritonavir	Ritonavir	Ritonavir	Ritonavir
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters								
C_{max1} (µg/mL)	2.1	0.35	1.5	1.6	0.75	0.43	0.70	0.44
t_{max1}(h)	2	1	1	1	2	2	2	1
C_{max2} (µg/mL)	3.5	0.73	2.2	1.1	3.3	0.33	1.1	1.1
t_{max2} (h)	8	7	8	8	7	7	7	7
AUC (µg.h/mL)	35	4.0	18	18	34	3.9	8.4	5.5
(Time for calculation –h)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)	(0-24)
$t_{1/2}$ (h)	NC	NC	NC	NC	NC	NC	NC	NC

LC-MS/MS = liquid chromatography with tandem mass spectroscopy;
PEG400 : polyethylene glycol 400
NC : not calculated

2.6.5.7 Pharmacokinetics: Study Absorption and Plasma Kinetics in Pregnant or Nursing Animals

Test Article: Cobicistat

Studies of COBI in pregnant or nursing animals are presented in Module 2.6.7, Sections 2.6.7.11, 2.6.7.12, 2.6.7.13 and 2.6.7.14.

2.6.5.8 Pharmacokinetics: Other Distribution Study – Pregnant Rabbit

Test article: darunavir

Location in CTD		**4.2.3.5.2**	
Study No.		**TMC114-NC126**	
Species		**Rabbit (New Zealand White)**	
Feeding Condition		Not Fasted	
Vehicle/Formulation		1 % CMC/0.2%Tween 80 in Water	
Route		Oral (gavage)	
Gender (M/F)/Number of Animals		F/3	
Dose[1] (mg/kg)		1000	
Concentration (mg/ml)		100	
Duration of Dosing (day)		13 (GD20)	
Analyte	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters[2]			
C (µg/mL) in maternal plasma at approximately 2 hours after dosing	1.52	0.616	0.089
C (µg/mL) in fetal plasma at approximately 2 hours after dosing	0.0442	0.065	NQ
C (µg/mL) in Amnion fluid at approximately 2 hours after dosing	0.010	NQ	NQ

[1] : dose expressed as TMC114 base was 940 mg/kg
[2] : individual data

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
CMC : carboxymethyl cellulose
GD: day of gestation
NQ: not quantifiable (<10 ng/ml)

2.6.5.8 Pharmacokinetics: Other Distribution Study

No other distribution study has been performed for Cobicistat.

2.6.5.9.A Pharmacokinetics: Metabolism In Vivo

Test article: darunavir

Location in CTD	**4.2.2.4**	
Study No.	**TMC114-NC191**	
Species	**Rat (Sprague Dawley)**	
Gender (M/F)/ Number of Animals	M/2	
Feeding Condition	not fasted	
Vehicle/Formulation	PEG400	
Route	Oral (gavage)	
Dose (mg.base eq./kg) of TMC114	40	
Concentration (mg.base eq./mL)	16	
Radionuclide	^{14}C-TMC114	
Specific Activity (MBq/mg-eq)	0.051	
Sample (time interval)	**% of dose recovered**	
	Rat (K1)	**Rat (K2)**
I. Expired air (0-26 h)	0.060	0.044
II. Urine (0-26 h)	4.45	3.09
III. Faeces (methanolic extract + residue, 0-26 h)	82.73	85.14
Total recovery (I + II + III)	**87.24**	**88.27**
Additional information		
^{14}C-radiolabel position in ^{14}C-TMC114 was metabolically stable and is suitable for use in metabolism and pharmacokinetic studies		

PEG400 : polyethylene glycol 400

2.6.5.9.B Pharmacokinetics: Metabolism In Vivo

Test article: darunavir

Location in CTD	**4.2.2.4**									
Study No.	**TMC114-NC152**									
Species	Rat (Sprague-Dawley)									
Gender (M/F)/Number of Animals	M/5-F/5									
Feeding condition	Fed									
Vehicle/Formulation	PEG400 (16 mg/mL)									
Route	Oral (gavage)									
Dose (mg.base eq/kg) of TMC114	40									
Radionuclide	^{14}C-TMC114									
Specific Activity (MBq/mg)	0.05									
Sample	**Urine**		**Methanolic Fecal extract**		**Plasma**					
	Male	**Female**	**Male**	**Female**		**Male**			**Female**	
Time (h)	**0-24**	**0-24**	**0-48**	**0-48**	**1**	**4**	**8**	**1**	**4**	**8**
% of Administered Radioactivity in excreta or µg-eq/.mL in plasma	3.52[a]	4.20[a]	90.70[a]	92.62[a]	3.3	2.8	1.8	3.3	2.4	0.78
Parent drug	0.19	0.71	6.34	12.30	62.3[b]	78.6[b]	70.9[b]	77.7[b]	73.9[b]	56.6[b]
M2 Carbamate hydrolysis and aliphatic hydroxylation	0.07	0.06	ND	ND	ND	ND	ND	ND	ND	ND
M6 Carbamate hydrolysis and aliphatic hydroxylation (R426855)	0.17	0.11	ND	ND	ND	ND	ND	ND	ND	ND
M12 Oxidative ring opening	ND	ND	3.10	1.47	ND	ND	ND	ND	ND	ND
M15 Carbamate hydrolysis and aromatic hydroxylation	0.07	ND	3.21	1.42	ND	ND	ND	ND	ND	ND
M18 N-glucuronidation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M19 Carbamate hydrolysis (R374699)	0.10	0.17	2.81	4.46	3.9[b]	4.5[b]	7.2[b]	0.9[b]	2.1[b]	5.2[b]
M21 Aliphatic hydroxylation	0.08	0.19	2.65	6.28	ND	ND	ND	ND	ND	ND
M33 Aromatic hydroxylation (R330689)	ND	ND	3.75	4.88	ND	ND	ND	ND	ND	ND
M23 Aliphatic hydroxylation (R426857)	0.27	0.59	4.00	13.83	2.8[b]	2.9[b]	1.8[b]	2.7[b]	1.6[b]	7.3[b]
M25 Di-hydroxylation (alicyclic + aromatic)	ND	ND	0.96	0.79	ND	ND	ND	ND	ND	ND
M27 Alicyclic hydroxylation	ND	ND	2.58	1.81	ND	ND	ND	ND	ND	ND
M28 Alicyclic hydroxylation	ND	ND	1.13	0.55	ND	ND	ND	ND	ND	ND
M29 Aromatic hydroxylation (R330326)	ND	0.13	3.55	4.04	2.6[b]	2.5[b]	3.1[b]	1.5[b]	2.3[b]	5.5[b]
M30 N-acetylation	NA	0.17	1.16	1.96	ND	ND	ND	ND	ND	ND

Additional Information: By 96 hours after administration of TMC114, 92.61 and 93.72 % of the dose had been excreted in faeces, 3.87 and 4.48 % in urine, in males and females, respectively; [a] : Percentage of administered dose; [b] : Percentage of injected sample radioactivity; NA: Not analyzed; ND : Not detected in sample or under the limit of quantification; PEG400 : polyethylene glycol 400

2.6.5.9.B Pharmacokinetics: Metabolism In Vivo (continued)

Test article: darunavir

Location in CTD	**4.2.2.4**									
Study No.	**TMC114-NC152**									
Sample	**Urine**		**Methanolic Fecal extract**		**Plasma**					
	Male	**Female**	**Male**	**Female**	**Male**			**Female**		
Time (h)	**0-24**	**0-24**	**0-48**	**0-48**	**1**	**4**	**8**	**1**	**4**	**8**
% of Administered Radioactivity in excreta or µg-eq/.mL in plasma	5.48[a]	6.20[a]	93.93[a]	86.65[a]	3.5	4.7	3.0	3.1	3.2	2.8
Parent drug	0.84	1.23	20.00	15.53	83.0[b]	69.3[b]	67.3[b]	88.5[b]	72.7[b]	66.0[b]
M2 Carbamate hydrolysis and aliphatic hydroxylation	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
M6 Carbamate hydrolysis and aliphatic hydroxylation (R426855)	0.08	0.15	ND	ND	ND	ND	ND	ND	ND	ND
M12 Oxidative ring opening	ND	ND	0.63	1.02	ND	ND	ND	ND	ND	ND
M15 Carbamate hydrolysis and aromatic hydroxylation	ND	ND	1.21	1.22	ND	ND	ND	ND	ND	ND
M18 N-glucuronidation	ND	0.14	ND	ND	ND	ND	ND	ND	ND	ND
M19 Carbamate hydrolysis (R374699)	ND	ND	1.40	0.92	ND	ND	ND	ND	ND	ND
M21 Aliphatic hydroxylation	0.24	0.34	6.15	5.17	ND	ND	ND	ND	ND	ND
M33 Aromatic hydroxylation (R330689)	ND	ND	2.87	2.15	ND	ND	ND	ND	ND	ND
M23 Aliphatic hydroxylation (R426857)	2.29	2.31	17.21	16.11	6.7[b]	5.2[b]	4.9[b]	3.6[b]	6.1[b]	6.0[b]
M25 Di-hydroxylation (alicyclic + aromatic)	ND	ND	2.66	2.27	ND	ND	ND	ND	ND	ND
M27 Alicyclic hydroxylation	ND	ND	1.71	1.40	ND	ND	ND	ND	ND	ND
M28 Alicyclic hydroxylation	ND	ND	0.47	0.38	ND	ND	ND	ND	ND	ND
M29 Aromatic hydroxylation (R330326)	0.02	0.08	1.90	1.98	ND	ND	ND	ND	ND	ND
M30 N-acetylation	0.18	0.61	3.63	3.88	ND	5.6[b]	7.0[b]	ND	4.5[b]	8.8[b]
Additional Information By 96 hours after administration of TMC114, 94.75 and 88.54 % of the dose had been excreted in faeces, 5.76 and 6.47 % in urine, in males and females, respectively.										

[a] : Percentage of administered dose.
[b] : Percentage of injected sample radioactivity.
NA : Not analyzed
ND : Not detected in sample or under the limit of quantification
PEG400 : polyethylene glycol 400

2.6.5.9.C Pharmacokinetics: Metabolism In Vivo

Test article: darunavir

Location in CTD			**4.2.2.4**		
Study No.			**TMC114-NC164**		
Species			Rat[a] (Sprague-Dawley)		
Gender (M/F)/Number of Animals			M/3		
Feeding Condition			Overnight fasted		
Vehicle/Formulation			PEG400 (16 mg/mL)		
Route			Oral (gavage)		
Dose (mg.base eq/kg)			40		
Radionuclide			^{14}C		
Specific Activity of formulation (MBq/mg)			0.05		
Sample			**Bile**		
Time (0-24 h)	**Rat-1**	**Rat-2**	**Rat-3**	**Mean**	**S.D**
% of Administered Radioactivity in bile	45.38	52.66	63.30	53.78	9.01

Metabolite codes	**Metabolite profile - % dose radioactivity in 0- 24 h collected bile**			
	Rat-1	**Rat-2**	**Rat-3**	**Mean ± S.D**
Parent drug	0.9	0.9	1.3	1.0 ± 0.2
M2	1.2	2.1	1.9	1.7 ± 0.5
M6	2.3	2.7	3.2	2.7 ± 0.4
M11	3.6	4.0	3.6	3.7 ± 0.2
M12	3.3	3.4	3.5	3.4 ± 0.1
M17/M18[b]	8.5	9.4	7.5	8.5 ± 1.0
M19 (R374699)	4.5	1.9	5.3	3.9 ± 1.7
M20/M21/M22[b]	1.5	3.9	10.9	5.4 ± 4.9
M23 (R426857)	1.9	2.5	2.0	2.1 ± 0.4
M29 (R330326)	0.9	0.7	0.6	0.7 ± 0.2
M32	1.2	1.3	0.9	1.2 ± 0.2
Sum	**29.7**	**32.9**	**40.5**	**34.4 ± 5.6**

[a] : Bile duct cannulated rat; [b] :Metabolites were coeluting and measured as a single peak. Confirmation of their presence by LC-MS/MS; PEG400 : polyethylene glycol 400

2.6.5.9.D Pharmacokinetics: Metabolism in Vivo

Test article: darunavir

Location in CTD		**4.2.2.4**					
Study No.		**TMC114-NC153**					
Species		**Dog (Beagle)**					
Gender (M/F)/Number of Animals		M/3					
Feeding Condition		Dosing about 1 h after feeding					
Vehicle/Formulation		PEG 400					
Route		Oral (gavage)					
Dose (mg.base eq./kg)		30					
Radionuclide		^{14}C					
Specific Activity (MBq/mg)		0.013					
Sample		**Urine**	**Feces Extract**	**Plasma**			
Time (h)		**0 – 48**	**0 - 72**	**1**	**4**	**8**	**24**
% of Administered Radioactivity in excreta or µg-eq/mL in plasma		3.5[a]	63.9[a]	0.21	1.67	1.31	1.73
Parent	**TMC114**	0.27	25.28	40.5[b]	39.9[b]	6.5[b]	ND
M3	**hydroxylation + glucuronidation of M19**	0.22	ND	ND	ND	ND	ND
M6	**hydroxylation of M19 at isobutyl**	0.49	3.20	ND	ND	ND	ND
M11	**glucuronidation of M15**	0.27	ND	ND	ND	ND	ND
M15	**hydroxylation of M19 at aniline**	0.17	2.38	ND	21.0[b]	16.7[b]	46.7[b]
M19	**R374699**	0.60[c]	3.58	ND	28.5[b]	21.6[b]	25.6[b]
M20	**glucuronidation of parent**	-[c]	3.53	ND	ND	ND	ND
M23	**hydroxylation of parent at isobutyl**	0.19	4.47	ND	ND	ND	ND
M24	**hydroxylation of parent at aniline**	ND	2.22[d]	ND	ND	ND	ND
M25	**di-hydroxylation of parent**	ND	-[d]	ND	ND	ND	ND
M29	**R330326**	0.08	3.15	ND	5.7[b]	2.8[b]	ND

Additional Information

By 168 hours after administration of TMC114, 85.86 % of the dose had been excreted in faeces, 3.93 % in urine. The C_{max} of total radioactivity in plasma was reached between 4 and 8h after dosing and amounted to 3.17 ± 0.48 µg-eq/mL. The C_{max} of unchanged compound in plasma was reached at 2 or 6 h after dosing and amounted to 0.86 ± 0.51 µg -eq/mL.

2.6.5.9.E Pharmacokinetics: Metabolism in Vivo

Test article: darunavir

Location in CTD		**4.2.2.4**				
Study No.		**TMC114-NC213**				
Species/Study system		**Human**				
Gender (M/F)/Number of Subjects		M/4				
Feeding Condition		Fasted				
Vehicle/Formulation		TMC114 in PEG400: the formulation contained per millilitre: 20 mg base-eq. TMC114, 448 mg PEG400, 120 mg Vit E TPGS, 55 mg Aurantii Cortici Tinctura Fortis, 5 mg sodium saccharinate and 425 mg purified water				
Route		Oral				
Intended dose		400 mg base-eq.; 1500 kBq (40.5 µCi)				
Radionuclide		^{14}C-TMC114				
Specific Activity		3.75 kBq/mg base-eq. (0.1 !Ci/mg base-eq.)				
Sample		**Urine**	**Methanolic Fecal extract**	**Plasma**		
Time (h)		**0-48**	[b]	**1**	**2**	**4**
% of Administered Radioactivity in excreta or µg-eq/.mL in plasma		10.94[a]	81.68[a]	2.36	1.46	0.232
Parent drug		1.15	6.83	48.5[c]	50.0[c]	25.9[c]
M6	**Carbamate hydrolysis + aliphatic hydroxylation (R426855)**	1.02	4.38	4.8[c]	8.4[c]	n.d.
M11	**Glucuronidation of metabolite 15**	0.45	n.d.	n.d.	n.d.	n.d.
M15	**Carbamate hydrolysis + aromatic hydroxylation**	in combined peak	3.62	n.d.	n.d.	n.d.
M15 + M17 + M18 [d]	**M17: hydroxylation + glucuronidation M18: N-glucuronidation**	0.98	n.d.	n.d.	n.d.	n.d.
M19	**Carbamate hydrolysis (R374699)**	0.56	2.14	n.d.	n.d.	n.d.
Metabolites eluting between 53 and 56 min in plasma		-	-	6.0[c]	14.5[c]	n.d.
M20	**Glucuronidation**	0.12	n.d.	n.d.	n.d.	n.d.
M33	**Aromatic hydroxylation (R330689)**	n.d.	1.60	n.d.	n.d.	n.d.
M23	**Aliphatic hydroxylation (R426857)**	0.47	3.92	7.7[c]	9.2[c]	n.d.
M29	**Aromatic hydroxylation (R330326)**	0.28	4.17	2.4[c]	n.d.	n.d.

[a]: Percentage of administered dose; [b]: In pooled methanolic faeces extracts, representative for the principle part of radioactivity excreted via the faeces
[c]: Percentage of injected sample radioactivity; [d]: Combined peak of metabolites 15, 17 and 18.
n.d. – Not detected in sample or under the limit of quantification

2.6.5.9.E Pharmacokinetics: Metabolism in Vivo (continued)

Test article: darunavir

Location in CTD		**4.2.2.4**				
Study No.		**TMC114-NC213**				
Species/Study system		**Human**				
Gender (M/F)/Number of Subjects		M/4				
Feeding Condition		Fasted				
Vehicle/Formulation		TMC114 in PEG400: the formulation contained per millilitre: 20 mg base-eq. TMC114, 448 mg PEG400, 120 mg Vit E TPGS, 55 mg Aurantii Cortici Tinctura Fortis, 5 mg sodium saccharinate and 425 mg purified water Ritonavir (Norvir®): formulated as a capsule containing 100 mg ritonavir and the inactive ingredients butylated hydroxytoluene, ethanol, gelatin, iron oxide, oleic acid, polyoxyl 35 castor oil and titanium dioxide				
Route		Oral				
Intended dose		Single dose of 400 mg base-eq. ^{14}C-TMC114 (1500 kBq, 40.5 µCi) + 100 mg Ritonavir b.i.d. (2 days before until 6 days after TMC114 administration				
Radionuclide		^{14}C-TMC114				
Specific Activity		3.75 kBq/mg base-eq. (0.1 !Ci/mg base-eq.)				
Sample		**Urine**	**Methanolic Fecal extract**	**Plasma**		
Time (h)		**0-48**	[b]	**1**	**2**	**4**
% of Administered Radioactivity in excreta or µg-eq/.mL in plasma		12.77[a]	79.54[a]	5.45	3.52	2.37
Parent drug		7.65	41.16	79.4[c]	79.7[c]	86.3[c]
M6	**Carbamate hydrolysis + aliphatic hydroxylation (R426855)**	n.d.	n.d.	n.d.	n.d.	n.d.
M11	**Glucuronidation of metabolite 15**	n.d.	n.d.	n.d.	n.d.	n.d.
M15	**Carbamate hydrolysis + aromatic hydroxylation**	n.d.	n.d.	n.d.	n.d.	n.d.
M17 + M18 + M19 [d]	**M17: hydroxylation + glucuronidation M18: N-glucuronidation**	1.57	n.d.	n.d.	n.d.	n.d.
M19	**Carbamate hydrolysis (R374699)**	n.d.	0.67	n.d.	n.d.	n.d.
Metabolites eluting between 53 and 56 min in plasma		-	-	7.9 [c]	5.0 [c]	n.d.
M20	**Glucuronidation**	0.66	n.d.	n.d.	n.d.	n.d.
M33	**Aromatic hydroxylation (R330689)**	n.d.	1.70	n.d.	n.d.	n.d.
M23	**Aliphatic hydroxylation (R426857)**	0.25	2.01	n.d.	n.d.	n.d.
M29	**Aromatic hydroxylation (R330326)**	0.42	1.00	n.d.	n.d.	n.d.
Additional information: Over the 7-day collection period, 12.2 and 13.9 % in the urine in non-boosted (TMC114 alone) and boosted (TMC114 with RTV) were excreted.						

[a]: Percentage of administered dose; [b]: In pooled methanolic feces extracts, representative for the principle part of radioactivity excreted via the feces
[c]: Percentage of injected sample radioactivity; [d]: Combined peak of metabolites 17, 18 and 19; n.d. – Not detected in sample or under the limit of quantification

2.6.5.9.F Pharmacokinetics: Metabolism in Vivo

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics, Metabolism, and Excretion of [^{14}C]GS-9350 Following Oral Administration to Mice	Metabolism study	[^{14}C]COBI	AD-216-2073 4.2.2.4

Species/Strain Number of Animals/Group Sex	Administration Route (Dose level)	Sample	Time (h)	Concentration in plasma; ng eq. [^{14}C]COBI /g						
				Total (LSC)[a]	Total	COBI	M55	M21	M31	M69
ICR mice [Hsd:ICR(CD-1)] 15 animals/Group 2 male	Oral (30 mg/kg)	Plasma	1	6670 ± 1690	6240 (96.3)	5900 (91.02)	ND	158 (2.44)	141 (2.18)	43.4 (0.67)
			2	4270 ± 2270	3870 (96.2)	3530 (87.78)	ND	154 (3.83)	146 (3.62)	39.8 (0.99)
			4	4310 ± 320	3810 (96.4)	3440 (87.06)	ND	165 (4.18)	146 (3.68)	56.5 (1.43)
			8	1890 ± 1210	1470 (94.1)	1340 (85.97)	22.0 (1.41)	54.4 (3.48)	35.6 (2.28)	14.5 (0.93)

COBI = cobicistat; LSC = liquid scintillation counting; ND = peak not detected or below the established limit of quantitation (1% of run)

a Total concentration of radioactivity determined by liquid scintillation counting (mean ± SD, n = 3)

Note: The values in parentheses are the percent of radioactivity injected (% of run).

2.6.5.9.F Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Mouse Urine After Oral Administration of [^{14}C]COBI (Group 1; n = 4)

Final Metabolite Designation	Collection Interval (0-24 hours)	
	Percent of Radioactivity Injected (% of Run)	Percent of Radioactive Dose
M55	6.19	0.11
M56	1.19	0.02
M57	1.52	0.03
M10	7.30	0.13
M14	3.88	0.07
M21 (GS-343006)	38.21	0.66
M26 (GS-341842)	1.68	0.03
M31 (GS-364751)	6.35	0.11
M32	1.66	0.03
COBI	3.02	0.05
Total	71.0	1.23

2.6.5.9.F Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Mouse Feces After Oral Administration of [^{14}C]COBI (n = 4)			
	Collection Interval (hours)		
	0-24	24-48	Total
Final Metabolite Designation	Percent of Radioactive Dose		
M57	ND	0.06	0.06
M10	0.93	0.19	1.12
M58	ND	0.06	0.06
M14	3.29	0.39	3.68
M59	0.57	0.06	0.63
M60	ND	0.06	0.06
M61	ND	0.06	0.06
M62	0.49	0.05	0.54
M21 (GS-342006)	11.9	1.48	13.4
M48	0.83	0.04	0.86
M49	0.73	0.14	0.87
M26 (GS-341842)	1.99	0.07	2.06
M50	1.04	0.04	1.09
M29	2.35	0.15	2.50
M63	0.68	0.05	0.74
M31 (GS-364751)	5.04	0.16	5.21
M64	0.38	0.06	0.45
M65	2.37	0.07	2.44
M66	0.51	0.13	0.64
M67	0.74	ND	0.74
M68	0.97	0.07	1.04
M69	3.98	0.17	4.15
M39	0.54	0.06	0.60
COBI	14.3	0.22	14.5

COBI = cobicistat; ND = peak not detected or below the established limit of quantitation (1% of run); NA = not applicable

Note regarding data from pooled fecal samples: If at least one interval had a reportable value above the limit of quantitation, then other intervals may include a value below the limit of quantitation.

2.6.5.9.G Pharmacokinetics: Metabolism in Vivo

Report Title	Study Type	Test Article	Report Number Location in CTD
Metabolite Profiling and Identification of Rat Plasma, Bile, Urine, and Feces Following Oral Administration of [^{14}C]GS-9350	Metabolism study	[^{14}C]COBI	AD-216-2082 4.2.2.4

Species/Strain Number of Animals/Group Sex	Administration Route (Dose level)	Sample	Time (h)	Concentration (plasma; ng eq. [^{14}C]COBI /g)						
				Total (LSC)[a]	Total	COBI	M1	M9	M21	M31
Sprague Dawley Rat (H1a:[SD]CVF) Pooled samples from 3 rats per time point	Oral (10 mg/kg)	Plasma	0.083	160 ± 89	125 (81.0)	111 (71.57)	ND	ND	9.78 (6.32)	4.74 (3.06)
			0.25	1170 ± 580	933 (85.7)	815 (74.83)	14.1 (1.29)	16.3 (1.50)	73.2 (6.72)	14.5 (1.33)
			1	2170 ± 210	1710 (85.8)	1490 (75.00)	54.4 (2.73)	ND	116 (5.81)	44.8 (2.25)
			2	844 ± 480	618 (92.3)	526 (78.51)	29.7 (4.43)	ND	35.2 (5.25)	27.3 (4.08)
			4	567 ± 194	288 (87.3)	201 (60.70)	37.7 (11.40)	ND	38.3 (11.58)	12.0 (3.62)

COBI = cobicistat; LSC = liquid scintillation counting; ND = peak not detected or below the established limit of quantitation (1% of run)

a Total concentration of radioactivity determined by liquid scintillation counting (mean ± SD, n = 3) from AD-216-2034

Note: The values in parentheses are the percent of radioactivity injected (% of run).

2.6.5.9.G Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Rat Urine After Oral Administration of [^{14}C]COBI

	Percent of Radioactive Dose					
	Male Rats			Male Bile Duct-Cannulated Rats		
	Collection Interval (hours)			Collection Interval (hours)		
Final Metabolite Designation	0-12	12-24	Total	0-12	12-24	Total
# **M1**	# 0.16	# 0.05	# 0.21	0.10	0.02	0.12
# **M2**	# ND	# ND	# 0.00	ND	0.01	0.01
# **M3**	# ND	# ND	# 0.00	ND	0.00	0.00
# **M4**	# 0.04	# 0.00	# 0.04	ND	0.00	0.00
# **M5**	# 0.02	# 0.01	# 0.03	ND	ND	0.00
# **M6**	# 0.07	# 0.02	# 0.10	0.06	0.00	0.07
# **M7**	# 0.03	# ND	# 0.03	0.04	ND	0.04
# **M8**	# 0.02	# ND	# 0.02	ND	0.00	0.00
# **M10**	# 0.03	# ND	# 0.03	0.04	0.00	0.05
# **M11**	# 0.05	# 0.01	# 0.05	0.17	0.01	0.18
# **M12**	# 0.02	# ND	# 0.02	0.04	ND	0.04
# **M14**	# 0.02	# ND	# 0.02	0.10	0.01	0.12
# **M17**	# ND	# ND	# 0.00	0.04	ND	0.04
# **M21 (GS-342006)**	# 0.40	# 0.03	# 0.43	1.10	0.09	1.19
# **M26 (GS-341842)**	# 0.02	# ND	# 0.02	0.06	ND	0.06
# **M28**	# 0.02	# ND	# 0.02	0.06	0.01	0.06
# **M31 (GS-364751)**	# 0.21	# ND	# 0.21	0.74	0.02	0.76
# **M34**	# 0.03	# ND	# 0.03	0.06	ND	0.06
# **M39**	# 0.02	# ND	# 0.02	0.06	0.00	0.06
# **COBI**	# 0.05	# ND	# 0.05	0.19	0.01	0.19
# **M41**	# ND	# ND	# 0.00	ND	0.00	0.00

2.6.5.9.G Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Rat Bile Samples After Oral Administration of [^{14}C]COBI

Final Metabolite Designation	Percent of Radioactive Dose									
	Collection Interval (hours)									
	0-2	2-4	4-6	6-8	8-12	12-24	24-48	48-72	72-96	Total
M10	0.39	ND	0.33	ND	0.08	0.22	0.03	ND	ND	1.05
M11	ND	ND	ND	ND	ND	0.11	0.04	ND	ND	0.15
M14	0.65	0.85	0.71	0.33	0.38	0.34	0.11	0.07	ND	3.44
M16	ND	ND	ND	ND	0.12	ND	ND	ND	0.02	0.14
M19	ND	ND	ND	0.22	0.35	ND	ND	ND	ND	0.57
M21 (GS-342006)	0.59	0.95	0.57	0.34	0.54	0.51	ND	ND	ND	3.50
M22	0.41	ND	0.68	ND	ND	ND	ND	ND	ND	1.09
M23	0.33	0.63	ND	ND	ND	ND	ND	ND	ND	0.96
M24	0.43	ND	ND	ND	ND	0.07	ND	ND	ND	0.50
M25	0.44	ND	ND	ND	ND	0.13	0.13	0.06	ND	0.76
M26 (GS-341842)	0.66	0.85	0.48	0.16	ND	ND	0.13	ND	0.07	2.35
M27	0.56	ND	0.39	0.14	ND	ND	ND	0.05	ND	1.14
M28	0.65	1.23	0.90	0.19	ND	ND	ND	ND	ND	2.97
M29	ND	ND	0.36	ND	0.17	ND	0.03	0.02	ND	0.58
M31 (GS-364751)	0.80	0.72	ND	0.20	ND	0.14	0.06	ND	ND	1.92
M32	0.38	0.61	0.30	0.11	ND	ND	ND	ND	ND	1.40
M33	0.61	0.71	0.50	0.13	ND	ND	ND	ND	ND	1.95
M34	0.74	0.85	0.53	ND	ND	ND	ND	ND	ND	2.12
M39	0.63	0.76	0.53	0.22	0.12	0.08	ND	ND	ND	2.34
M42	ND	0.42	ND	ND	ND	ND	ND	ND	ND	0.42

2.6.5.9.G Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Rat Feces After Oral Administration of [^{14}C]COBI

	Percent of Radioactive Dose							
	Male Rats					Male Bile Duct-Cannulated Rats		
	Collection Interval (hours)					Collection Interval (hours)		
Final Metabolite Designation	0-24	24-48	48-72	72-96	Total	0-12	12-24	Total
M9	ND	ND	0.01	0.00	0.01	ND	ND	0.00
M10	1.00	0.16	0.02	0.00	1.17	ND	ND	0.00
M12	ND	0.10	0.01	0.00	0.11	ND	ND	0.00
M13	ND	ND	0.01	0.00	0.01	ND	ND	0.00
M14	1.84	0.36	0.04	0.01	2.25	0.18	0.03	0.22
M15	ND	ND	ND	0.00	0.00	ND	ND	0.00
M16	ND	ND	ND	0.00	0.00	ND	ND	0.00
M17	ND	ND	0.01	0.00	0.01	ND	ND	0.00
M18	ND	ND	ND	0.00	0.00	ND	ND	0.00
M19	ND	0.11	0.01	0.00	0.13	ND	ND	0.00
M20	1.16	0.28	0.03	0.01	1.48	ND	0.02	0.02
M21 (GS-342006)	9.69	1.48	0.15	0.04	11.4	3.03	0.40	3.44
M23	ND	ND	0.01	0.00	0.01	ND	ND	0.00
M25	1.92	0.22	0.02	0.00	2.16	ND	0.02	0.02
M26 (GS-341842)	2.53	0.28	0.02	ND	2.83	ND	0.02	0.02
M28	2.95	0.34	0.02	ND	3.31	0.26	0.03	0.29
M29	2.14	0.19	0.02	0.00	2.35	0.19	0.03	0.22
M30	ND	0.09	ND	ND	0.09	ND	ND	0.00
M31 (GS-364751)	6.60	0.57	0.04	0.00	7.22	1.11	0.12	1.24
M33	0.67	0.20	0.01	0.00	0.88	ND	0.02	0.02
M34	1.11	0.11	0.01	0.00	1.23	ND	ND	0.00
M35	0.69	ND	0.01	ND	0.70	ND	ND	0.00
M36	0.76	0.11	ND	ND	0.88	ND	0.03	0.03
M37	ND	0.09	ND	ND	0.09	ND	ND	0.00

2.6.5.9.G Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Rat Feces After Oral Administration of [^{14}C]COBI

Final Metabolite Designation	Percent of Radioactive Dose							
	Male Rats					Male Bile Duct-Cannulated Rats		
	Collection Interval (hours)					Collection Interval (hours)		
	0-24	24-48	48-72	72-96	Total	0-12	12-24	Total
M38	0.68	ND	0.01	ND	0.69	ND	ND	0.00
M39	3.24	0.44	0.03	0.00	3.72	0.65	0.06	0.71
M40	0.68	0.12	ND	ND	0.80	0.18	ND	0.18
COBI	5.33	0.32	0.01	ND	5.67	7.14	0.55	7.69
M41	1.49	ND	ND	ND	1.49	ND	ND	0.00
M43	ND	0.12	ND	ND	0.12	ND	0.12	ND
M44	ND	ND	ND	0.00	0.00	0.00	0.00	ND

COBI = cobicistat; NA = not applicable; ND = peak not detected or below the established limit of quantitation (1% of run for plasma, urine, and fecal samples; 2% of run for bile samples)

2.6.5.9.H Pharmacokinetics: Metabolism in Vivo

Report Title	Study Type	Test Article	Report Number Location in CTD
Profiling and Identification of Metabolites in Selected Plasma, Urine, Bile, and Feces Samples from Intact and Bile Duct Cannulated Dogs after Oral Administration of [^{14}C]GS-9350	Metabolism study	[^{14}C]COBI	AD-216-2101 4.2.2.4

Species/Strain Number of Animals/Group Sex	Administration Route (Dose level)	Sample	Time (h)	Concentration (plasma; ng eq. [^{14}C]COBI /g)						
				Total (LSC)[a]	Total	COBI	M21 (GS-342006)	M22	M31 (GS-364751)	M37
Beagle Dog Pooled samples from 3M/group	Oral (5 mg/kg)	Plasma	0.5	519 ± 472	595 (93.4)	419 (65.76)	64.0 (10.04)	ND	47.9 (7.52)	64.0 (10.04)
			1	821 ± 447	544 (94.4)	423 (73.33)	59.0 (10.24)	ND	25.7 (4.46)	36.8 (6.39)
			2	796 ± 191	652 (91.2)	473 (66.17)	53.8 (7.53)	12.6 (1.76)	41.2 (5.77)	70.9 (9.92)
			4	456 ± 151	301 (89.2)	226 (66.99)	19.6 (5.82)	ND	23.6 (7.00)	31.6 (9.38)

COBI = cobicistat; LSC = liquid scintillation counting; ND = peak not detected or below the established limit of quantitation (1% of run)

a Total concentration of radioactivity determined by liquid scintillation counting (mean ± SD, n = 3) from AD-216-2067

Note: The values in parentheses are the percent of radioactivity injected (% of run).

2.6.5.9.H Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Pooled Dog Urine After Oral Administration of [^{14}C]COBI

Final Metabolite Designation	Percent of Radioactive Dose					
	Male Dogs			Male Bile Duct-Cannulated Dogs		
	Collection Interval (hours)			Collection Interval (hours)		
	0-24	24-48	Total	0-24	24-48	Total
M56	0.11	0.06	0.17	0.09	0.03	0.12
M4	ND	ND	ND	ND	0.01	0.01
M5	ND	0.00	0.00	0.06	0.01	0.07
M6	0.02	0.00	0.02	0.03	0.01	0.04
M7	0.04	0.01	0.05	0.02	0.01	0.03
M10	0.08	0.01	0.09	0.08	0.00	0.08
M57	ND	ND	ND	ND	0.01	0.01
M58	ND	0.00	0.00	ND	0.01	0.01
M14	0.03	0.01	0.04	0.05	0.01	0.06
M21 (GS-342006)	0.15	0.03	0.18	0.23	0.03	0.25
M26 (GS-341842)	0.02	ND	0.02	0.03	0.00	0.03
M31 (GS-364751)	0.35	0.01	0.37	0.35	0.02	0.36
M30	0.03	ND	0.03	0.04	0.00	0.04
M39	0.04	0.01	0.05	0.01	ND	0.01
COBI	0.03	0.01	0.04	0.05	ND	0.05

Note: For plasma and urine samples, if at least one analyzed time point has a value above the limit of quantitation, values below the limit of quantitation may be reported for other time points.

a Represents an individual sample (Animal No. 1001)

b Represents an individual sample (Animal No. 1001; 12-24-hour sample

2.6.5.9.H Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Dog Pooled Bile Samples After Oral Administration of [^{14}C]COBI

Final Metabolite Designation	Percent of Radioactive Dose				
	Collection Interval (hours)				
	0-4	4-12	12-24	24-48	Total
M10	1.09	0.26	ND	ND	1.35
M14	1.95	0.84	ND	ND	2.79
M79	0.84	ND	ND	ND	0.84
M21 (GS-342006)	4.75	0.38	0.14	ND	5.27
M19	ND	1.48	ND	ND	1.48
M48	1.32	0.86	ND	0.03	2.21
M22	1.16	0.35	ND	0.13	1.63
M26 (GS-341842)	1.69	0.45	0.14	0.04	2.31
M25	0.84	ND	0.17	ND	1.01
M50	1.33	0.38	0.08	ND	1.79
M27	ND	0.65	0.18	ND	0.84
M80	2.12	0.68	0.11	0.56	3.47
M29	1.41	0.96	0.11	0.12	2.60
M31 (GS-364751)	3.64	ND	0.07	0.03	3.74
M63	1.16	ND	ND	0.04	1.20
M30	1.22	2.24	ND	ND	3.46
M65	1.34	0.48	0.27	0.16	2.25
M81	ND	0.66	0.21	0.08	0.94
M82	0.96	0.32	0.10	0.07	1.45
M39	2.93	1.13	0.28	0.04	4.38
COBI	1.01	0.44	0.09	0.12	1.65

2.6.5.9.H Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Dog Pooled Bile Samples After Oral Administration of [^{14}C]COBI

Final Metabolite Designation	Percent of Radioactive Dose				
	Collection Interval (hours)				
	0-4	4-12	12-24	24-48	Total
M40	ND	ND	0.12	ND	0.12
M41	ND	ND	0.10	ND	0.10
M42	ND	ND	0.13	ND	0.13
M83	ND	ND	ND	0.05	0.05
M84	ND	ND	ND	0.04	0.04
M43	ND	ND	0.12	0.03	0.15

2.6.5.9.H Pharmacokinetics: Metabolism in Vivo (Continued)

Test Article: [^{14}C]COBI

Composition of Radioactivity in Dog Feces After Oral Administration of [^{14}C]COBI

	Percent of Radioactive Dose							
	Male Dogs				Male Bile Duct-Cannulated Dogs			
	Collection Interval (hours)				Collection Interval (hours)			
Final Metabolite Designation	0-24[b]	24-48	48-72	Total	0-24	24-48	48-72	Total
M10	0.53	1.04	0.05	1.62	0.01	0.18	0.19	0.38
M14	0.71	2.07	0.08	2.86	ND	ND	0.17	0.17
M21 (GS-342006)	4.10	7.86	0.46	12.4	0.08	1.01	0.76	1.85
M48	0.37	2.06	0.04	2.47	ND	ND	ND	ND
M49	0.60	1.33	0.03	1.96	ND	ND	0.20	0.20
M26 (GS-341842)	0.75	1.46	0.04	2.25	0.03	0.31	0.19	0.53
M25	0.27	0.79	0.02	1.08	ND	ND	ND	ND
M50	0.37	1.60	0.02	2.00	ND	ND	0.05	0.05
M29	1.25	3.38	0.10	4.72	0.01	0.14	0.19	0.34
M31 (GS-364751)	2.58	6.09	0.09	8.76	0.17	1.84	0.60	2.61
M63	0.61	1.42	0.05	2.08	ND	ND	ND	ND
M30	0.27	0.65	ND	0.92	ND	ND	0.08	0.08
M65	1.00	3.16	0.05	4.22	0.01	ND	0.06	0.07
M81	ND	ND	ND	ND	ND	ND	0.05	0.05
M76	0.30	0.79	0.02	1.12	ND	ND	ND	ND
M39	2.87	5.64	0.12	8.63	0.03	0.39	0.22	0.64
COBI	5.13	1.96	0.05	7.15	0.73	8.20	0.61	9.54

COBI = cobicistat; NA = not applicable; ND = peak not detected or below the established limit of quantitation (1% of run and 10 cpm peak height for plasma, urine, and fecal samples; 2% of run and 10 cpm peak height for bile samples)

2.6.5.10.A Pharmacokinetics: Metabolism In Vitro-Darunavir

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC154**

Methodology: ^{14}C-TMC114 (5 µM, 2.74 µg/ml) was incubated for two hours with microsomes (1mg/mL) and with hepatocytes (in suspension culture; 4 x 10^6 cells/mL). Samples were analysed by radio-HPLC. Co-chromatography, enzyme hydrolysis and LC-MS/MS techniques were used for identification of the metabolites.

Percentage of the injected sample radioactivity accounted for by unchanged compound and its metabolites

Study System	Mouse (male, Swiss albino)		Mouse, (female Swiss albino)		Mouse (male, black Agouti rasH2)		Mouse (female, black Agouti rasH2)		Rat (male, Sprague-Dawley)		Rat (female, Sprague-Dawley)		Rabbit (female, New Zealand)		Dog (male, Beagle)		Man	
Metabolites	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M
1	3.2	-	3.2	-	2.4	-	1.2	-	-	-	-	-	-	-	-	-	-	-
2	9.4	-	7.8	-	8.8	-	6.4	-	1.3	-	-	-	6.1	-	7.3	-	-	-
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	1.6	-	3.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	7.7	-	11.5	-	7.2	-	5.6	-	-	-	-	-	8.2	4.6	6.6	-	2.0	5.4
7	5.2	-	4.1	-	5.3	-	3.2	-	-	-	-	-	-	-	-	-	-	-
8	14.8	-	20.2	-	14.8	-	14.3	-	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-	-	-	3.9	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.7	-	-	-
11	3.9	-	4.3	-	3.2	-	4.9	-	-	-	-	-	14.4	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6.2	-	-	-
13	1.1	-	4.2	-	2.7	-	2.9	-	1.6	-	1.1	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	13.6	-	-	-	-
15	-	1.4	-	-	-	-	-	-	-	1.3	-	T	-	5.5	-	-	1.2	5.6
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0
17	-	-	-	-	-	-	-	-	-	-	-	-	8.2	-	18.3	-	-	-
17 + 18	6.4	-	6.4	-	8.0	-	8.8	-	2.0	-	-	-	-	-	-	-	4.6	-
18	-	-	-	-	-	-	-	-	-	-	-	-	6.9	-	-	-	-	-
19	-	6.8	-	4.6	-	5.4	-	6.2	6.2	4.4	2.3	2.0	-	9.9	9.0	7.3	6.5	16.7
20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.8	-	-	-
20 + 21	-	-	-	-	-	-	-	-	2.1	-	5.2	-	-	-	-	-	-	-

H: hepatocytes in suspension culture; M: Microsomes; T: ≤ 1%

HPLC: high performance liquid chromatography; LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.10.A Pharmacokinetics: Metabolism In Vitro-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.4**																	
Study No.	**TMC114-NC154**																	
Percentage of the injected sample radioactivity accounted for by unchanged compound and its metabolites																		
Study System	**Mouse (male, Swiss albino)**		**Mouse (female, Swiss albino)**		**Mouse (male, black Agouti rasH2)**		**Mouse (female, black Agouti rasH2)**		**Rat (male, Sprague-Dawley)**		**Rat (female, Sprague-Dawley)**		**Rabbit (female, New Zealand)**		**Dog (male, Beagle)**		**Man**	
Metabolites	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M
21	1.0	1.7	1.9	4.3	2.7	3.8	4.5	2.2	-	-	-	-	-	3.3	-	-	0.9	2.8
22	-	-	-	-	-	-	-	-	1.5	-	-	-	-	-	-	-	-	-
23	1.3	7.0	4.1	5.1	5.9	4.8	10.8	3.8	7.6	2.0	18.7	2.9	-	10.4	4.9	15.9	5.5	13.9
24	-	-	-	-	-	-	1.0	-	-	-	-	-	-	-	T	-	0.4	-
25	-	-	-	-	-	-	-	-	-	-	-	-	-	5.1	-	-	-	3.3
26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	-	4.5	-	1.8	-	2.9	-	2.7	-	2.6	-	2.5	-	3.6	-	4.9	-	8.0
28	-	3.7	-	T	-	2.2	-	2.5	-	1.6	-	T	-	3.8	-	2.6	-	4.3
29	T	6.1	-	4.1	-	10.8	-	6.8	4.2	4.2	5.1	1.7	-	7.4	T	4.8	3.0	7.8
30	-	-	-	-	-	-	-	-	-	-	4.7	-	-	-	-	-	-	-
UD	T	22.1	-	18.5	-	58.7	-	67.7	70.4	33.6	60.1	55.7	1.0	9.8	-	37.3	68.9	16.3
31	-	-	-	-	-	-	-	-	-	-	-	-	-	2.4	-	1.6	-	1.4
32	-	5.0	-	4.1	-	1.5	-	T	-	12.1	-	5.5	-	12.4	1.5	5.0	-	3.2

Additional Information

Samples were analysed by radio-HPLC. Co-chromatography, enzyme hydrolysis and LC-MS/MS techniques were used for identification of the metabolites. TMC114 was metabolised via different metabolic pathways, namely aliphatic hydroxylation, aromatic hydroxylation, alicyclic hydroxylation, carbamate hydrolysis, glucuronidation, *N*-dealkylation and *N*-acetylation.

H: hepatocytes in suspension culture
M: Microsomes
T: ≤ 1%

2.6.5.10.B Pharmacokinetics: Metabolism In Vitro-Darunavir

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC246**

Type of Study: The *in vitro* metabolism of ^{14}C-TMC114 was studied in liver sub-cellular fractions (microsomes and 12,000 *x g* supernatant fractions) of male and female juvenile (age groups day-13 and day-26) Sprague-Dawley rats in a toxicology study and compared with the metabolism of TMC114 in liver sub-cellular fractions of normal adult (~ 8 week's old) male and female Sprague-Dawley rats.

Method :TMC114 (5 !M, 2.74 !g/ml) was incubated in the above matrices at 37 °C for 60 and 120 mins. and the incubates were analysed by radio-HPLC. Co-chromatography along with comparison of relative retention time of radio-HPLC peaks in this study and a previous *in vitro* metabolism study were used in the identification of metabolites.

Study System	**Juvenile-Day13**				**Juvenile-Day26**				**Adult-Week-8**			
Test System : Liver S9 and microsomes	**Male**		**Female**		**Male**		**Female**		**Male**		**Female**	
TMC114 and its Metabolites	**S9**	**MIC**	**S9**	**MIC**	**S9**	**MIC**	**S9**	**MIC**	**S9**	**MIC**	**S9**	**MIC**
M6	--	--	--	--	--	--	--	--	2.1	1.2	0.6	--
M14 (R330576)	--	--	--	--	1.3	0.9	--	--	9.6	1.0	-	--
M15	--	--	--	--	2.6	2.9	--	--	16.1	4.2	-	--
M19 (R374699)	5.8	4.8	9.4	7.9	17.8	17.8	15.6	13.5	23.5	20.6	2.2	3.1
M21	--	--	--	--	--	0.7	1.3	--	2.1	1.2	3.5	2.1
M23 (R426857)	1.4	1.4	1.1	1.6	5.9	3.7	4.6	3.3	6.3	5.0	9.4	8.5
M25	--	--	--	--	1.7	--	--	--	2.5	2.1	-	--
M27	3.1	3.1	3.1	4.3	5.9	5.4	6.6	6.0	4.1	7.2	1.5	3.1
M28	2.0	2.2	2.6	1.9	4.2	3.4	4.1	2.5	1.6	3.4	0.8	1.2
M29 (R330326)	6.0	4.1	9.8	6.9	12.1	10.2	12.0	8.1	10.3	9.2	13.4	3.8
Parent (UD-TMC114)	**78.0**	**75.2**	**68.5**	**73.0**	**33.8**	**46.0**	**45.1**	**51.5**	**5.2**	**18.6**	**46.2**	**66.8**
M31	1.1	--	1.6	--	3.0	1.3	2.3	--	3.1	2.8	1.1	3.4
M32	1.6	4.9	2.1	0.7	1.7	3.1	--	3.7	3.1	9.6	12.6	5.3
Sum	99.0	95.7	98.2	96.3	90.0	95.4	91.6	88.6	89.6	86.1	91.3	97.3

1) M6, M14, M15, M19, M21, M23, M25, M27, M28 and M29 are the metabolites of TMC114 resulted from direct oxidation or hydrolysis or in combination of the both reactions. M31 and M32 are unknown. Available metabolite reference standards are indicated in the brackets.
2) Values expressed as percent of the injected sample radioactivity accounted for by UD and its metabolites.
3) All incubations were conducted at 5 !M concentration in the presence of NADPH regenerating system. The above results were obtained from 120 min incubated samples.

S9 = Liver 12,000 *x g* fractions; MIC = Liver microsomes; -- = not detected

2.6.5.10.C Pharmacokinetics: Metabolism In Vitro-Darunavir

Test article: darunavir

Location in CTD	**4.2.2.4**		
Study No.	**TMC114-NC112**		
Methodology:	TMC114 (2, 5, 10, 20 and 50 µM) was incubated for 15 minutes at 37° C with rat, dog and human liver microsomes containing 0.1M potassium phosphate buffer pH 7.4 and 3 mM NADPH. Samples were analysed by LC-MS/MS		
Study System	**Rat**	**Dog**	**Human**
Km (µM)	21 ± 9	3.5 ± 0.4	4.6 ± 2.3
Vmax (pmol/min/mg protein)	1288 ± 265	458 ± 16	1080 ± 155
Additional Information Incubation of TMC114 at 20 µM, 5 µM and 5 µM in absence or presence of chemical inhibitors (α-naphthoflavone, Coumarin, Sulfaphenazole, Quinidine/Quinine, Acetone and ketoconazole inhibitors of CYP1A, CYP2A6, CYP2C9, CYP2D6, CYP2E1 and CYP3A, respectively) were also performed in rat, dog and human liver microsomes, respectively. Rat liver microsomes showed an inhibition profile different from dog and human liver : TMC114 metabolism in rat was inhibited by α-naphthoflavone (66 %), Coumarin (57%), Sulfaphenazole (4%), Quinidine/Quinine (36%), Ketoconazole (56%). TMC114 metabolism in dog was inhibited by α-naphthoflavone (2 %), Coumarin (19%), Sulfaphenazole (31%), Ketoconazole (69%). TMC114 metabolism in human was inhibited by Sulfaphenazole (10%) and Ketoconazole (55%). Incubation with TMC114 (5 µM) and CYP450 were performed using the same conditions: CYP3A4 is the only human P450 involved in the metabolism of TMC114.			

LC-MS/MS = liquid chromatography with tandem mass spectroscopy

2.6.5.10.D Pharmacokinetics: Metabolism In Vitro-Darunavir

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC202**

Type of Study: CYP reaction phenotyping- Effect of diagnostic CYP inhibitors on the metabolism of TMC114

Method: Inhibition of the metabolism of TMC114 in human liver microsomes by diagnostic inhibitors was carried out with ^{14}C-TMC114 (3 !M) for 20 minutes at a protein concentration of 0.5 mg/ml. The amounts of unchanged TMC114 and its metabolites (M6, M19[1], M23[1], M27, M28 and M29[1]) were determined by radio-HPLC. The values represent the percentage of inhibition obtained for each inhibitor in comparison to a control incubate (without inhibitor). Each value represents mean of three observations.

Results:

		% Inhibition of Metabolism[2]						
Diagnostic Inhibitor	**CYP P450 Form**	**Overall[3,4]**	**M6**	**M19[1]**	**M23[1]**	**M27[3]**	**M28[3]**	**M29[1,3]**
Furafylline (10 µM)	CYP1A2	4.6	59.1	44.5	7.8	-25.6	-66.7	13.0
Coumarin (100 µM)	CYP2A6	-1.1	40.9	39.4	-2.8	-57.3	-61.1	13.5
Sulphaphenazole (10 µM	CYP2C8/9/10	-2.5	27.3	34.6	3.6	-50.0	-75.0	-1.3
Quinidine (10 µM)	CYP2D6	-2.0	34.8	31.5	4.2	-17.1	-69.4	-7.6
4-methylpyrazole (20 µM)	CYP2E1	-14.7	31.8	36.6	12.2	-4.9	-138.9	-1.3
Ticlopidine (5 µM)	CYP2C19/D6	4.4	27.3	19.4	5.8	2.4	-51.5	11.7
Ketoconazole (1 µM)	CYP3A4	47.2	69.7	65.8	61.4	42.7	30.6	58.3
Troleandomycin (200 µM)	CYP3A4	79.4	100.0	93.5	96.1	100.0	100.0	93.5
Clarithromycin (15 µM)	CYP3A	32.4	60.6	34.2	32.2	31.7	25.0	37.0
Ritonavir (0.15 !M)	CYP3A4	59.6	65.2	72.6	75.6	68.3	55.6	63.2
1-aminobenzotriazole (1000 µM)	CYP P450	-12.5[5]	100.0	99.3	99.2	100.0	100.0	97.4

Additional Information

[1] : Major metabolites in human liver microsomes (> 5 % of the sample radioactivity)

[2] : Calculated from control incubation (without inhibitor); higher the positive value and higher the extent of inhibition

[3] : Negative values indicates higher % product formation in test sample compared to the control. This was more prominent with the minor metabolites. For all qualitative purposes, all negative values were considered as no inhibition.

[4] : Overall % metabolism of TMC114 calculated from % drug that remained in the sample at the end of the incubation

[5] : Although complete inhibition of the metabolites was observed, there was an unusual peak observed in this sample. This was not observed any of the HLM samples.

2.6.5.10.D Pharmacokinetics: Metabolism In Vitro-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC202**

Type of Study: CYP reaction phenotyping- Metabolism of ^{14}C-TMC114 in *E. coli* expressed CYP isoforms

Method: The metabolism of TMC114 in *E. coli* expressed CYP systems was carried out with ^{14}C-TMC114 (30 !M) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged TMC114 and its metabolites (M6, M19[1], M23[1], M27, M28 and M29[1]) were determined by radio-HPLC. Each value represents mean ± Standard error of three observations.

Results:

Cytochrome P-450 Form (100 pmol/ml)	**Overall % Metabolism[2]**	**Product formation rate (pmol/min.100 pmol P450)**					
		M6	**M19[1]**	**M23[1]**	**M27**	**M28**	**M29[1]**
CYP1A2	0.70 ± 0.20	-	-	-	-	-	-
CYP2A6	0.73 ± 0.15	-	-	-	-	-	-
CYP2B6	2.47 ± 2.04	-	-	-	-	-	-
CYP2C8	0.43 ± 0.49	-	-	-	-	-	0.33 ± 0.58
CYP2C9	1.07 ± 0.15	-	-	-	-	-	-
CYP2C19	7.93 ± 0.81	-	-	0.33 ± 0.58	-	-	6.67 ± 0.29
CYP2D6	4.90 ± 0.30	-	0.50 ± 0.87	1.17 ± 1.04	-	-	5.67 ± 2.25
CYP2E1	0.33 ± 0.12	-	1.00 ± 0.00	0.50 ± 0.50	-	-	-
CYP3A4	10.40 ± 0.69	2.33 ± 0.29	11.33 ± 1.53	13.67 ± 0.76	2.17 ± 2.02	2.00 ± 0.50	7.83 ± 1.53
CYP3A5	6.77 ± 0.23	-	15.83 ± 1.26	2.50 ± 0.50	-	3.50 ± 0.50	3.00 ± 0.50

Additional Information

[1] : Major metabolites of human liver microsomes

[2] : Overall % metabolism of TMC114 calculated from % drug that remained in the sample at the end of the incubation

- : No measurable product observed in radio-HPLC profile (lower limit of quantitation= 200 dpm)

2.6.5.10.D Pharmacokinetics: Metabolism In Vitro-Darunavir (Continued)

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC202**

Type of Study: CYP reaction phenotyping- Metabolism of ^{14}C-TMC114 in CYP isoforms (Supersomes®)

Method: The metabolism of TMC114 in expressed CYP systems (Supersomes®) was carried out with ^{14}C-TMC114 (30 !M) for 60 minutes at a CYP P450 concentration of 100 pmol/ml of incubation. The amounts of unchanged TMC114 and its metabolites (M6, M19[1], M23[1], M27, M28 and M29[1]) were determined by radio-HPLC. Each value represents mean ± standard error of three observations.

Results:

Cytochrome P-450 Form (100 pmol/ml)	**Overall % Metabolism[2]**	**Product formation rate (pmol/min.100 pmol P450)**		
		M6	**M19[1]**	**M23[1]**
CYP1A2	0.00 ± 0.00	-	-	-
CYP2A6	0.00 ± 0.00	-	-	-
CYP2B6	0.00 ± 0.00	-	-	-
CYP2C8	0.00 ± 0.00	-	-	-
CYP2C9	0.00 ± 0.00	-	-	-
CYP2C19	38.63 ± 1.36	-	-	10.17 ± 0.29
CYP2D6	14.27 ± 0.81	-	3.50 ± 0.50	3.50 ± 1.50
CYP2E1	0.00 ± 0.00	-	-	-
CYP3A4	9.57 ± 1.62	3.33 ± 1.04	16.00 ± 0.61	18.83 ± 2.60
CYP3A5	33.47 ± 0.42	-	88.00 ± 2.60	10.50 ± 1.32
CYP3A7	4.30 ± 0.30	-	5.33 ± 1.15	8.50 ± 0.87

Additional Information

[1] : Major metabolites of human liver microsomes

[2] : Overall % metabolism of TMC114 calculated from % drug remained in the sample at the end of the incubation

- : No measurable product observed in radio-HPLC profile (lower limit of quantitation= 200 dpm)

2.6.5.10.E Pharmacokinetics: Metabolism In Vitro-Cobicistat

Report Title	Study Type	Test Article	Report Number Location in CTD
Identification of Major Metabolites of GS-9350 in CD-1 Mouse Microsomes In Vitro	Metabolism study	COBI	AD-216-2074 4.2.2.4
In Vitro Metabolism of GS-9350 in Hepatocytes and Hepatic Subcellular Fractions from Rat, Dog, Monkey, and Human			AD-216-2024 4.2.2.4

Type of Study: Determination of rates of metabolism of COBI by hepatic microsomal fraction

Method: Cobicistat (3 µM) was incubated with pooled hepatic microsomal fractions from CD-1 mice, Sprague-Dawley rats, beagle dogs, cynomolgus monkeys, and humans, with NADPH cofactor. Rates of metabolism (in vitro half-life values) were determined, and hepatic clearance and hepatic extraction were predicted using the well-stirred liver model. Analysis was by LC/MS/MS.

In Vitro Rate of Metabolism of COBI at 3 µM in Hepatic Microsomes (Mean Values, N=2–6)

Species	Half-Life (min)	Predicted Hepatic Clearance (L/h/kg)	Predicted Hepatic Extraction (%)
Mouse	137.0	0.99	19.1
Rat	82.1	1.50	35.6
Dog	43.7	0.88	48.8
Monkey	8.9	1.35	84.7
Human	154.9	0.37	28.3

COBI = cobicistat; LC/MS/MS = high performance liquid chromatography coupled to tandem mass spectrometry

2.6.5.10.F Pharmacokinetics: Metabolism In Vitro-Cytochrome P450 Phenotyping-Cobicistat

Report Title	Study Type	Test Article	Report Number Location in CTD
Cytochrome P450 Phenotyping for GS-9350	Metabolism study	COBI	AD-216-2025 4.2.2.4

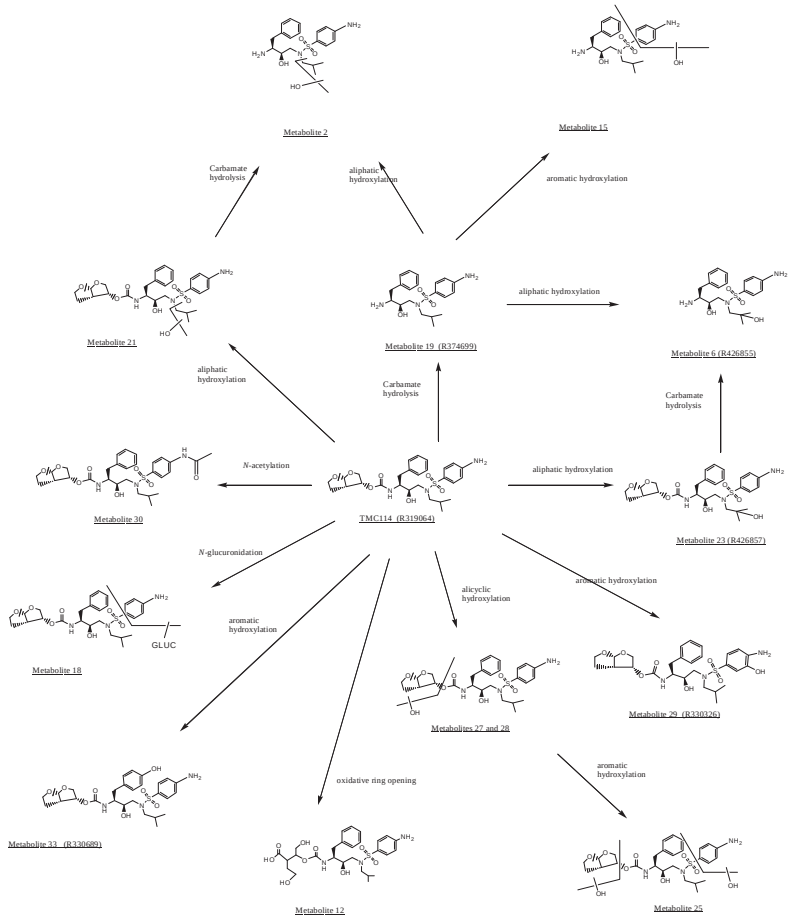
Study System: Rates of metabolism of COBI catalyzed by cDNA expressed major human cytochrome P450 enzyme preparations coexpressed with human NADPH CYP450 reductase ($min^{\#1}$ $pmol^{\#1}$)

Compound	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
COBI (% of Positive Control)	0.00 (0.0%)	0.00 (0.0%)	0.00 (0.0%)	0.105 (22.5%)	0.003 (4.5%)
Ethoxycoumarin	0.407	--	--	--	--
Diclofenac	--	0.467	--	--	--
Diazepam	--	--	0.035[a]	--	--
Dextromethorphan	--	--	--	0.467	--
Testosterone	--	--	--	--	0.066

cDNA = complementary deoxyribonucleic acid; COBI = cobicistat; CYP = cytochrome P450 enzyme(s)
a Diazepam is a selective substrate for CYP2C19, but is metabolized relatively slowly

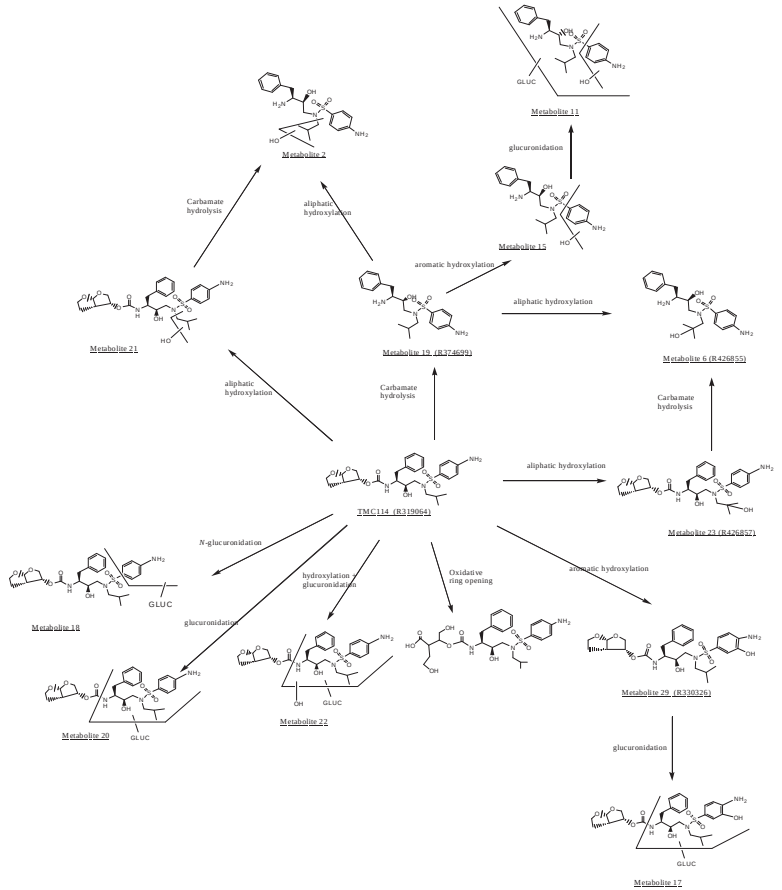
2.6.5.11.A Pharmacokinetics: Proposed In Vitro Metabolic Pathway-Adult Rats

Test article: darunavir
Location in CTD: 4.2.2.4
Study No.: TMC114-NC152



2.6.5.11.B Pharmacokinetics: Proposed In Vitro Metabolic Pathway-Adult Rats (Bile Study)

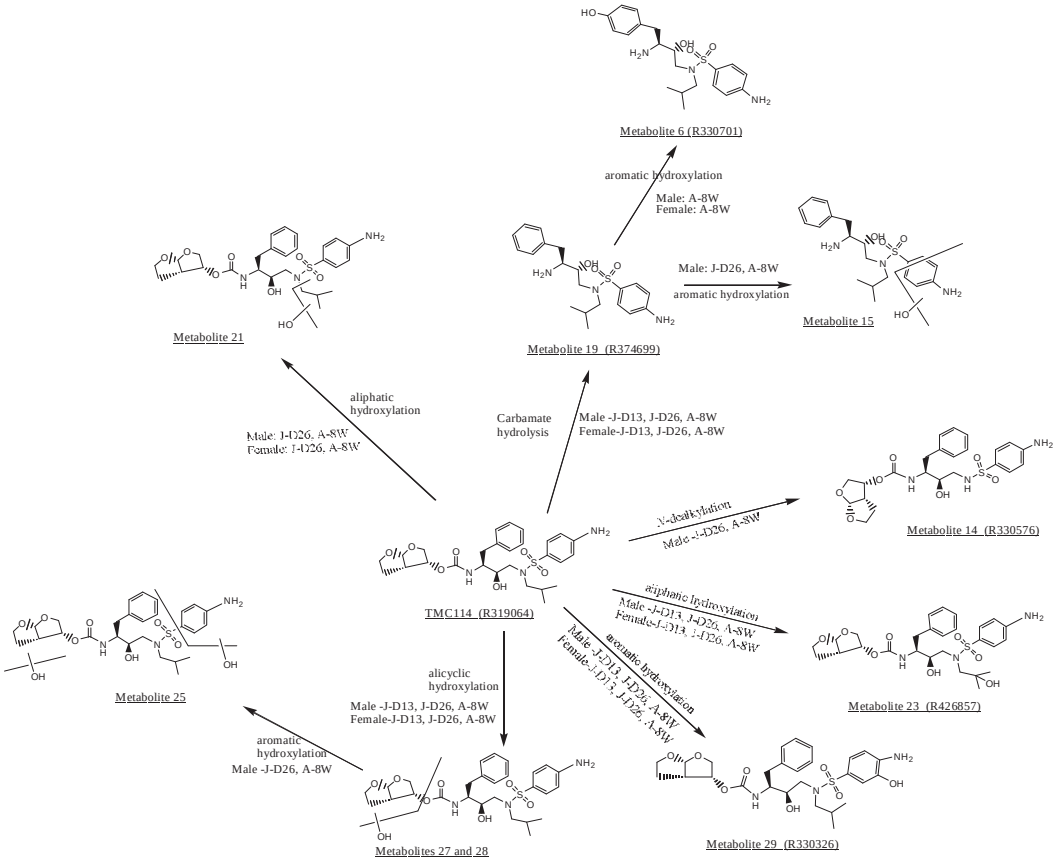
Test article: darunavir
Location in CTD: 4.2.2.4
Study No.: TMC114-NC164



2.6.5.11.C Pharmacokinetics: Proposed In Vitro Metabolic Pathway-Young Rats

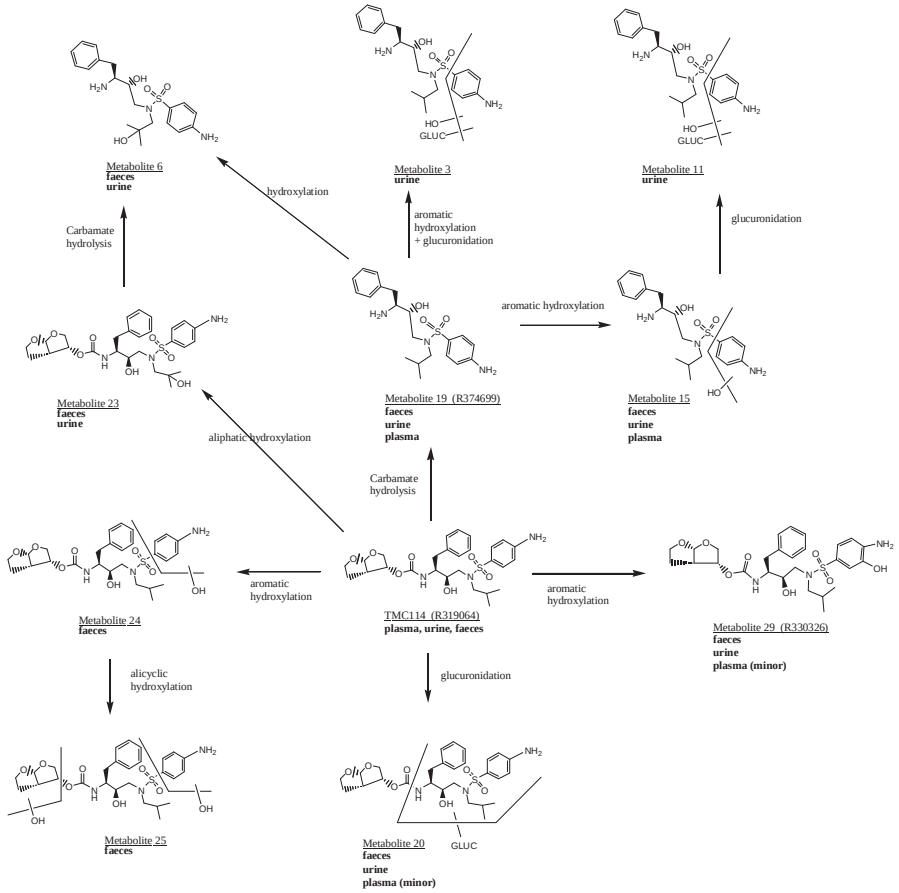
Test article: darunavir
Location in CTD: 4.2.2.4
Study No.: TMC114-NC246

Study System: Metabolism of male and female juvenile (age groups day-13 and day-26) and normal adult (~ 8 week's old) male and female Sprague-Dawley rats in liver sub-cellular fractions (microsomes and 12,000 *x g* supernatant fractions). The in vitro metabolic pathway is derivated from this study.



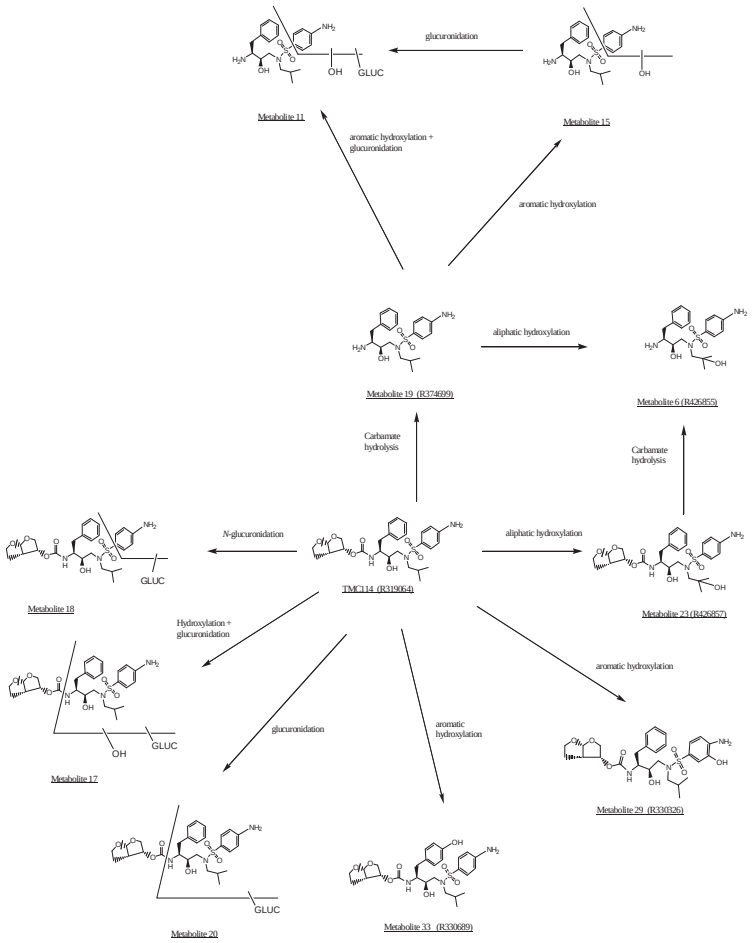
2.6.5.11.D Pharmacokinetics: Proposed In Vitro Metabolic Pathway-Dogs

Test article: darunavir
Location in CTD: 4.2.2.4
Study No.: TMC114-NC153



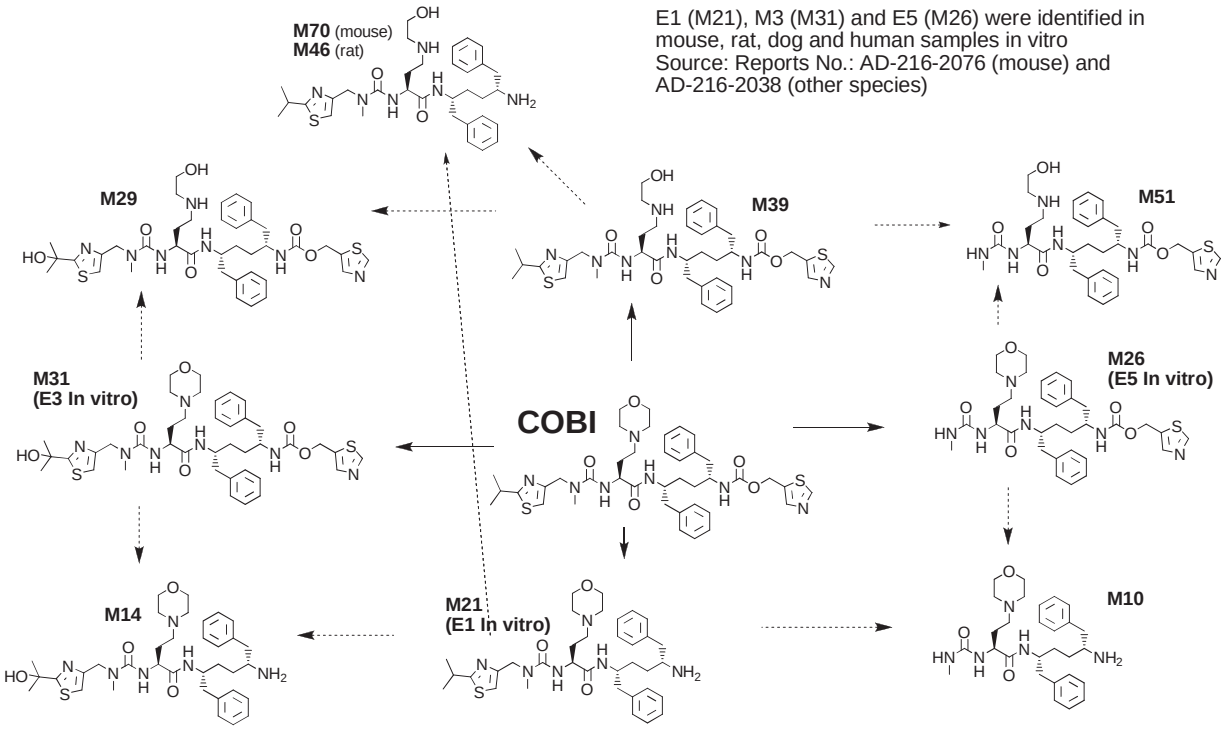
2.6.5.11.E Pharmacokinetics: Proposed In Vitro Metabolic Pathway-Human

Test article: darunavir
Location in CTD: 4.2.2.4
Study No.: TMC114-NC213



2.6.5.11.F Pharmacokinetics: Proposed In Vitro Metabolic Pathway

Common Primary and Secondary Routes of COBI Metabolism in Mouse, Rat, Dog, and Human In Vivo and In Vitro

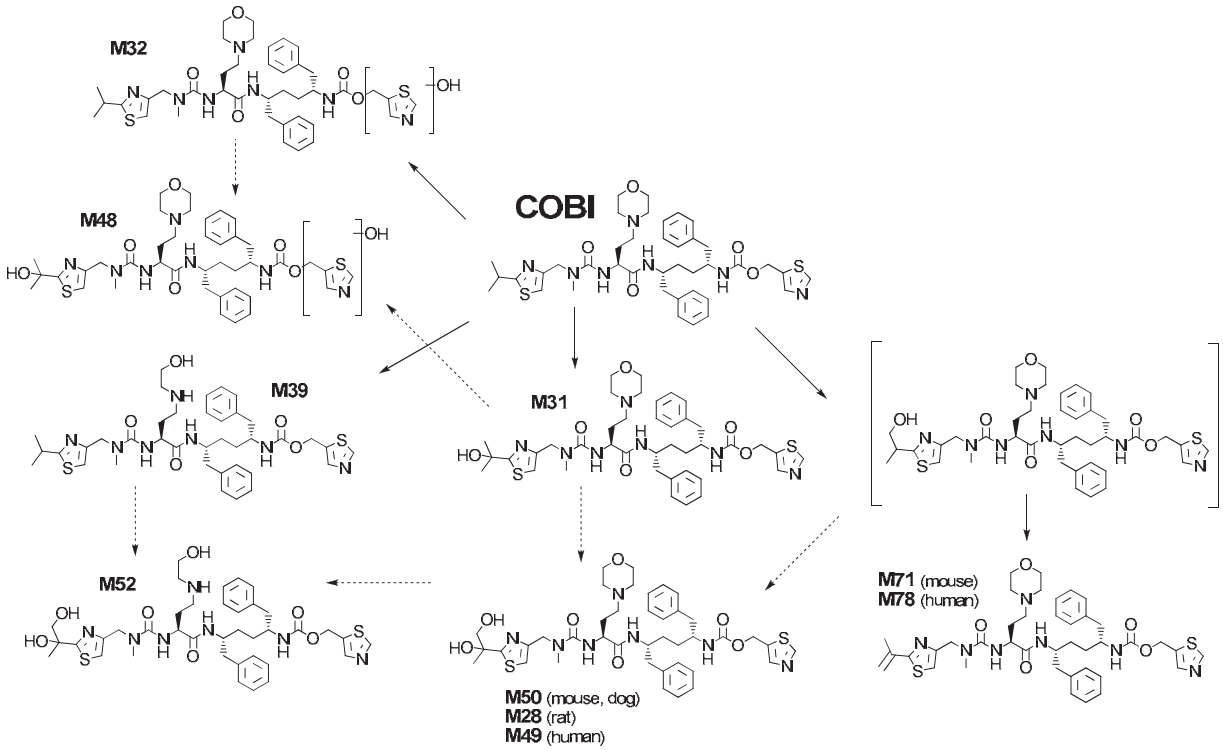


COBI and all metabolites were detected in samples from mouse, rat, dog, and human, except M29 (not in human), M51 (rat only) and M70/M46 (mouse and rat only).

Dashed arrows indicate combinations of primary metabolic pathways, not proven routes of metabolism.

Source: Reports AD-216-2038, AD-216-2076 (in vitro), AD-216-2073 (mouse), AD-216-2082 (rat), AD-216-2101 (dog), and GS-US-216-0111 (human)

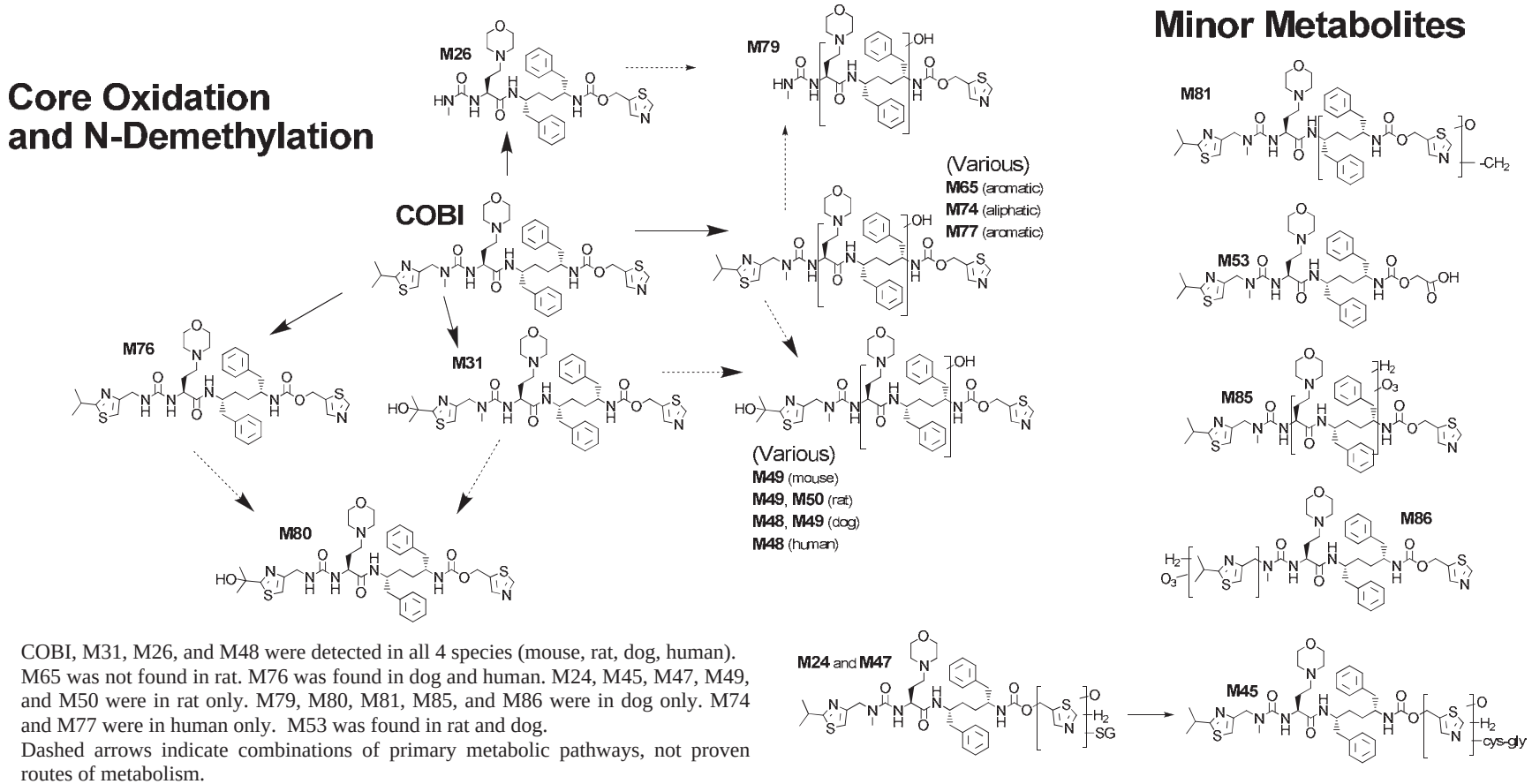
2.6.5.11.G Pharmacokinetics :Proposed In Vitro Metabolic Pathway- Routes of COBI Metabolism Involving 2-Isopropyl-5 Thiazole Oxidation and 4-Thiazole Oxidation



Bracketed metabolite was not detected, but its formation is implied by the presence of secondary metabolites.

COBI, M31, M39 and the dihydroxy-isopropyl metabolite (M50/M28/M49) were detected in all 4 species (mouse, rat, dog, and human). The dehydro isopropyl metabolite (M71/M78) was detected in mouse and human. M32 was not detected in dog. M48 and M52 were only detected in rat. Dashed arrows indicate combinations of primary metabolic pathways, not proven routes of metabolism. Source: Reports AD-216-2073 (mouse), AD-216-2082 (rat), AD-216-2101 (dog), and GS-US-216-0111 (human)

2.6.5.11 H. Pharmacokinetics :Proposed In Vitro Metabolic Pathway- Other Metabolic Routes of COBI



COBI, M31, M26, and M48 were detected in all 4 species (mouse, rat, dog, human). M65 was not found in rat. M76 was found in dog and human. M24, M45, M47, M49, and M50 were in rat only. M79, M80, M81, M85, and M86 were in dog only. M74 and M77 were in human only. M53 was found in rat and dog.

Dashed arrows indicate combinations of primary metabolic pathways, not proven routes of metabolism.

Source: Reports AD-216-2073 (mouse), AD-216-2082 (rat), AD-216-2101 (dog), and GS-US-216-0111 (human)

2.6.5.12.A Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes-Mice

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC226**

Type of Study: Possible induction and/or inhibition of drug metabolising enzymes by TMC114 evaluated *ex vivo* in liver mouse microsomes

Method

Microsomal fractions of livers from TMC114 treated animals were isolated. Swiss albino CD1 mice were treated with 0 (polyethylene glycol 400 vehicle), 150, 450 and 1000 mg.base eq/kg/day TMC114 for 3 months. Liver microsomes were analysed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Results are presented for groups of either 4 or 5 liver pools, each pool being prepared from the livers of 2 mice.

Tabulated Results

	Control (vehicle only)		TMC114					
			150 mg.base eq/kg/day		450 mg.base eq/kg/day		1000 mg.base eq/kg/day	
	M	F	M	F	M	F	M	F
Microsomal protein[a]	29.6	29.5	30.3	28.6	31.3	29.5	31.4	28.0
CYP content[b]	0.63	0.54	0.86**	0.55	0.96***	0.80***	1.05***	0.82*
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	182	143	215	166	242*	209*	294***	262***
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	42	84	58*	83	67**	104	82***	121**
4-Nitrophenol hydroxylase (CYP2E)[d]	0.78	0.92	1.07**	1.18*	1.41***	1.49***	1.72***	1.74***
Testosterone 6β-hydroxylase (CYP3A)[d]	0.91	1.04	2.08***	1.91***	3.09***	3.10***	3.69***	3.99***
Lauric acid 11-hydroxylase[d]	0.36	0.62	0.52**	0.72	0.58***	0.83**	0.50**	0.84**
Lauric acid 12-hydroxylase (CYP4A)[d]	0.24	0.48	0.44*	0.42	0.60***	0.35	0.47**	0.42
Thyroxine (T_4) UDPglucuronosyltransferase[c]	7.3	7.6	5.3*	7.2	5.5*	7.7	5.7*	7.7

Additional Information

Values significantly different from control are: *$p<0.05$; **$p<0.01$; ***$p<0.001$

[a] : Units : mg protein/g liver; [b] : Units : nmol/mg protein; [c] : Units : pmol/min/mg protein; [d] : Units : nmol/min/mg protein.

2.6.5.12.B Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes-Rats

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC208**

Type of Study: Possible induction and/or inhibition of drug metabolising enzymes by TMC114 and Ritonavir evaluated *ex vivo* in liver rat microsomes

Method

Microsomal fractions of livers from TMC114 and ritonavir treated animals were isolated. Sprague-Dawley rats were treated with demineralized water, 0 (polyethylene glycol 400 vehicle), 20, 100 and 500 mg/kg/day TMC114, 50 mg/kg/day ritonavir and both 100 mg/kg/day TMC114 and 50 mg/kg/day ritonavir for 4 weeks. The demineralized water group served as the control for the Ritonavir treated group, whereas the vehicle only group served as the control for the TMC114 treated groups. Liver microsomes were analyzed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Results are presented for groups of 5 rat livers.

Tabulated Results	Vehicle (polyethylene glycol 400)		TMC114						Demineralized water		Ritonavir 50 mg/kg/day		TMC114 100 mg.base eq/kg/day and Ritonavir 50 mg/kg/day	
			20 mg.base eq /kg/day		100 mg. base eq/kg/day		500 mg.base eq/kg/day							
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Microsomal protein[a]	32.0	30.6	37.9*	29.8	38.2*	32.9	43.5***	42.8***	34.5	29.9	41.8	40.7**	44.8	43.4
CYP content[b]	0.59	0.40	0.62	0.43	0.62	0.43	0.66	0.53***	0.66	0.45	0.74	0.53	0.84	0.53
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	20	21	15	17	14	15*	12*	11**	16	17	16	16	21	12
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	41	19	63*	17	44	20	58	30***	45	26	76*	28	93	28
4-Nitrophenol hydroxylase (CYP2E)[d]	0.53	0.48	0.57	0.52	0.73	0.68*	0.99**	0.88***	0.50	0.45	0.65	0.66*	0.82*	0.64
Testosterone 6β-hydroxylase (CYP3A)[d]	0.88	0.19	1.16*	0.18	2.07***	0.61***	5.28***	2.41***	0.96	0.57	2.51**	0.51	4.66***	0.67
Lauric acid 11-hydroxylase[d]	0.26	0.20	0.30	0.18	0.35	0.22	0.39*	0.36***	0.30	0.15	0.50***	0.22	0.49	0.28
Lauric acid 12-hydroxylase (CYP4A)[d]	0.37	0.39	0.48	0.40	0.56	0.36	0.61*	0.54*	0.43	0.33	0.73*	0.32	0.56	0.40
Thyroxine (T_4) UDPglucuronosyltransferase[c]	3.8	2.6	4.0	4.2	6.6**	5.7**	9.2***	7.5***	2.9	3.2	8.5***	5.5*	11.5	8.7**

Additional Information : Values significantly different from appropriate control (i.e. vehicle group for the TMC114 treated groups, demineralized water group for the Ritonavir 50 mg/kg/day group, and Ritonavir 50 mg/kg/day group for the TMC114 100 mg/kg/day and ritonavir 50 mg/kg/day group) are: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

[a] : Units : mg protein/g liver; [b] : Units : nmol/mg protein; [c] : Units : pmol/min/mg protein; [d] : Units : nmol/min/mg protein;

2.6.5.12.C Pharmacokinetics: Induction /Inhibition of Drug-Metabolizing Enzymes- in dogs

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC209**

Type of Study: Possible induction and/or inhibition of drug metabolising enzymes by TMC114 evaluated *ex vivo* in liver dog microsomes

Method

Microsomal fractions of livers from TMC114 treated animals were isolated. Beagle dogs were treated with 0 (polyethylene glycol 400 vehicle), 30, 60 and 120 mg.base eq/kg/day TMC114 for 12 months. Liver microsomes were analysed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below.

Tabulated Results

	Control (vehicle only)	TMC114		
		30 mg.base eq/kg/day	60 mg.base eq/kg/day	120 mg.base eq/kg/day
Microsomal protein[a]	33.2	38.5*	37.3	36.4
CYP content[b]	0.55	0.50	0.48	0.52
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	110	99	130	116
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	19	20	24	23
4-Nitrophenol hydroxylase (CYP2E)[d]	0.63	0.59	0.60	0.84
Testosterone 6β-hydroxylase (CYP3A)[d]	0.36	0.31	0.28*	0.34
Lauric acid 11-hydroxylase[d]	0.15	0.16	0.13	0.16
Lauric acid 12-hydroxylase (CYP4A)[d]	0.80	0.86	0.68	0.79
Thyroxine (T_4) UDPglucuronosyltransferase[c]	2.2	2.6	2.6	2.9

Additional Information :
Values significantly different from control are: *$p<0.05$.

[a] : Units : mg protein/g liver; [b] :Units : nmol/mg protein; [c] : Units : pmol/min/mg protein; [d] : Units : nmol/min/mg protein.

2.6.5.12.D Pharmacokinetics: Induction/inhibition of Drug-Metabolising Enzymes in human

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC171**

Type of Study: *In* vitro study on the potential of TMC114 to induce CYP mRNA in cryopreserved human hepatocytes

Method

After establishment of the hepatocyte cultures, human hepatocytes were treated either with vehicle, with various concentrations of TMC114 or with the positive control compounds, omeprazole, rifampicin, phenobarbital, or ritonavir for 24 h. At the end of the treatment period, induction of mRNA expression of CYP enzymes was measured with TaqMan quantitative RT-PCR. Mean fold induction of the different CYP-isoforms in cryopreserved human hepatocytes treated with TMC114 and positive controls was expressed against the vehicle control. In total, three different individual batches of cryopreserved human hepatocytes were used in this study. Each value is mean of three observations.

Results

Test Condition	**Mean fold induction**						
	CYP1A2	**CYP2B6**	**CYP2C8**	**CYP2C9**	**CYP2C18**	**CYP2C19**	**CYP3A4**
Control (Vehicle)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
TMC114 (2.5 !M)	1.3	2.3	3.3	2.0*	1.7*	1.7	3.4*
TMC114 (10 !M)	1.3	3.1	5.0	2.7	2.1*	2.3	4.8*
TMC114 (25 !M)	1.3	3.0	3.9	2.3	1.8	1.9	4.2*
TMC114 (50 !M)	2.2	5.9*	5.4*	3.0*	2.4*	2.6	5.5*
Rifampicin (25 !M)	0.5	7.6*	6.0	2.9	2.2	3.5	5.3*
Omeprazole (25 !M)	22.0	4.5*	2.3	1.7*	1.5	1.8	2.4*
Phenobarbital (100 !M)	1.3	5.6	4.7	2.1	1.8*	1.9	3.3
Ritonavir (10 !M)	0.9	3.7	3.1	2.4*	2.1*	2.3	4.9*

***** : $p<0.05$

2.6.5.12.E Pharmacokinetics: Induction/inhibition of Drug-Metabolising Enzymes in Human

Test article: darunavir

Location in CTD	**4.2.2.4**
Study No.	**TMC114-NC247**

Type of Study: An in vitro study to assess the potential of TMC114 to induce CYP enzyme activities in cryopreserved human hepatocytes

Method

After establishment of the hepatocyte cultures, human hepatocytes were treated either with DMSO, with various concentrations of TMC114 or with the positive control compounds, omeprazole, or rifampicin for 72 h. At the end of the treatment period, induction of CYP activities (CYP1A2, CYP2B6, CYP2C19, CYP3A4) was measured based on the probe substrate metabolism. Mean fold induction of the different CYP-isoforms in cryopreserved human hepatocytes treated with TMC114 and positive controls was expressed against the vehicle control. In total, three different individual batches (Lot 82, Lot NLR, Lot BDF, [redacted], USA) of cryopreserved human hepatocytes were used in this study. The results are tabulated in the table below and each value is mean of three observations.

Results

Test Condition	**Mean fold induction**			
	CYP1A2	**CYP2B6**	**CYP2C19**	**CYP3A4**
Control (DMSO)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
TMC114 (2.5 !M)	1.15 ± 0.04	1.57 ± 0.14	2.30 ± 2.27	2.21 ± 0.58
TMC114 (50 !M)	1.02 ± 0.18	1.83 ± 0.45	1.02 ± 0.39	6.76 ± 3.40
Rifampicin (50 !M)	1.06 ± 0.12	2.97 ± 0.46	4.33 ± 0.67	10.71 ± 4.67
Omeprazole (25 !M)	3.98 ± 2.44	1.42 ± 0.34	0.54 ± 0.20	3.57 ± 0.34

2.6.5.12.F Pharmacokinetics: Induction /Inhibition of Drug-Metabolising Enzymes in human

Test article: darunavir

Location in CTD			**4.2.2.4**		
Study No.			**TMC114-NC123**		
Type of Study: To investigate the interaction of TMC114 with human CYP450 in vitro					
Method Incubations were performed with P450 probe substrates, selective towards human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A, in the presence of absence TMC114. In preliminary experiments, the extent of inhibition was estimated with TMC114 concentrations of 0.5, 5, 50 and 100 µM. In case inhibition was found, one concentration of TMC114 was selected for each P450 enzyme in order to establish the inhibition constants (Ki). **Results**					
	CYP3A	CYP2B6	CYP2C9	CYP2C19	CYP2D6
Ki at 0.5 µM of TMC114	0.40 ± 0.09 (competitive)				
Ki at 50 µM of TMC114				25 ± 3 (competitive)	41 ± 4 (competitive)
Ki at 100 µM of TMC114		500 ± 91 (non-competitive)	52 ± 4 (competitive)		
Additional Information : In the preliminary experiments, a concentration-dependent inhibition was observed for CYP2C9, CYP2C19, CYP2D6 and CYP3A. CYP1A2 and CYP2B6 were only slightly inhibited at the highest TMC114 concentration, while no inhibition of CYP2A6 and CYP2E1 could be detected. Since for CYP1A2 less than 20 % inhibition was found at the highest TMC114 concentration, inhibition of CYP1A2 activity was not further investigated.					

The actual dose levels, expressed as TMC114 base were 6.7% lower than stated above.

2.6.5.12.G Pharmacokinetics: Induction/Inhibition of Drug-Metabolising Enzymes in Rats

Test article: darunavir

Location in CTD: 4.2.2.4
Study No.: TMC114-NC388

Type of Study: Possible induction and/or inhibition of drug metabolising enzymes by TMC114 evaluated *ex vivo* in liver rat microsomes

Method

Microsomal fractions of livers from TMC114 treated animals were isolated. Sprague-Dawley rats were treated with 0 (polyethylene glycol 400 vehicle), 50 and 500 mg base eq/kg/day TMC114, for 4 weeks. The vehicle group served as the control. Liver microsomes were analyzed for protein and total cytochrome P450 (CYP) content and for the enzyme activities shown below. Results are presented as mean for groups of 5 rat livers. Figures in parentheses are percentages of control values.

Tabulated Results

	Vehicle (polyethylene glycol 400)		TMC114			
			50 mg base eq/kg/day		500 mg base eq/kg/day	
	M	F	M	F	M	F
Microsomal protein content[a]	32 (100)	30 (100)	39 (120)**	31 (102)	49 (153)***	45 (148)***
CYP content[b]	0.79 (100)	0.46 (100)	0.76 (96)	0.37 (80)*	0.79 (100)	0.52 (113)
7-Ethoxyresorufin O-deethylase (CYP1A)[c]	33 (100)	26 (100)	26 (79)*	21 (81)*	17 (52)***	17 (65)***
7-Pentoxyresorufin O-depentylase (CYP2B)[c]	112 (100)	18 (100)	91 (81)	16 (89)	93 (83)	16 (89)
4-Nitrophenol hydroxylase (CYP2E)[d]	1.1 (100)	0.69 (100)	1.0 (92)	0.76 (110)	1.1 (100)	1.3 (186)***
Testosterone 6β-hydroxylase (CYP3A)[d]	0.75 (100)	0.05 (100)	1.5 (201)	0.24 (480)***	4.8 (643)***	2.6 (5100)***
Lauric acid 11-hydroxylase (CYP2E)[d]	0.57 (100)	0.35 (100)	0.56 (98)	0.30 (86)	0.43 (75)*	0.47 (134)**
Lauric acid 12-hydroxylase (CYP4A)[d]	0.69 (100)	0.41 (100)	0.51 (74)	0.29 (71)*	0.46 (67)*	0.51 (124)
Thyroxine (T_4) UDPglucuronosyltransferase[c]	6.7 (100)	6.5 (100)	8.7 (130)	7.2 (111)	18 (272)***	16 (248)***

Additional Information :
Values significantly different from control group are: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.

[a] : Units : mg protein/g liver; [b] : Units : nmol/mg protein; [c] : Units : pmol/min/mg protein; [d] : Units : nmol/min/mg protein.

2.6.5.12.H Pharmacokinetics: Induction/Inhibition of Drug-Metabolising Enzymes in Human

Test article: darunavir

Location in CTD	**4.2.2.4**	
Study No.	**TMC114-NC392**	
Type of Study:	To investigate in vitro the potential inhibition of CYP2C8 and of UGT1A1 by TMC114	
Method The potential inhibition of CYP2C8 mediated paclitaxel 6-α–hydroxylation and of UGT1A1-mediated bilirubin glucuronidation by TMC114 was investigated in a pooled batch of human liver microsomes. The inhibitory potency of TMC114 towards bilirubin glucuronidation was compared with that of ritonavir. The inhibition potential of the compounds was evaluated by determining the apparent inhibition constant (K_i) from inhibition experiments at different substrate concentrations (5-80 µM paclitaxel; 0.2-15 µM bilirubin) in the presence of different inhibitor concentrations (0-250 µM TMC114; 0-80 µM ritonavir). In addition, inhibition experiments were performed with the CYP2C8 selective inhibitor montelukast and the UGT1A1 specific substrate β-estradiol, in order to assess the response of the paclitaxel 6-α–hydroxylation and bilirubin glucuronidation assays. **Results**		
	CYP2C8	UGT1A1
K_i (µM) for TMC114	56 ± 40 (non competitive inhibition model)	201 ± 18 (uncompetitive inhibition model)
K_i (µM) for ritonavir		7.2 ± 0.8 (uncompetitive inhibition model)
IC_{50} (µM) of the positive control (Montelukast)	0.67 ± 0.03	

2.6.5.12.I Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Human CYP3A Mechanism-Based Inhibition Potential of COBI In Vitro

Report Title	Study Type	Test Article	Report Number Location in CTD
Inhibition of Human CYP3A Activity by GS-9350 In Vitro	Metabolism study	COBI	AD-216-2028 4.2.2.4

Type of Study: Mechanistic and kinetic study of human CYP3A mechanism-based inhibition potential of COBI

Method: The preincubation time and cofactor dependence of the CYP3A inhibitory potency were determined. Values refer to the fractional decrease in CYP3A activity due to preincubation with NADPH, compared to preincubation in the absence of cofactor. Mifepristone, a known mechanism-based inhibitor of human CYP3A enzymes, and mibefradil, another compound showing both direct and mechanism-based inhibition of human CYP3A enzymes, were run as positive controls. To confirm and quantify the potency of COBI as a mechanism-based inhibitor, the kinetics for the inactivation of human CYP3A enzymes were determined using midazolam 1'-hydroxylase as a selective activity and a 2-step incubation protocol (10 × dilution between steps). Ritonavir was tested as a comparator.

	Cofactor- and Preincubation-Dependent Inhibition of CYP3A Activities by COBI, RTV, and Positive Control Compounds					
	Cofactor-Dependent Inhibition (%, Mean ±SD, n=2)					
Compound	**Midazolam 1'Hydroxylase Activity**			**Testosterone 6β-Hydroxylase Activity**		
COBI	69.6	±	0.15	82.1	±	0.23
RTV	55.9	±	13.5	74.8	±	5.22
Mibefradil	67.3	±	4.87	80.3	±	1.12
Mifepristone	83.9	±	1.56	71.2	±	3.69

COBI = cobicistat; CYP = cytochrome P450 enzyme(s); RTV = ritonavir

2.6.5.12.I Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Human CYP3A Mechanism-Based Inhibition Potential of COBI In Vitro (Continued)

Test Article: COBI

Kinetics for Inactivation[a] of Human Hepatic Microsomal CYP3A Activity by COBI and RTV

Parameter	Inhibitor	
	COBI	RTV
K_I (μM)	1.07	0.26
K_{inact} ($min^{#1}$)	0.47	0.23

COBI = cobicistat; CYP = cytochrome P450 enzyme(s); RTV = ritonavir

a Inactivation kinetics were determined using midazolam 1'hydroxylase as the probe activity.

2.6.5.12.J Pharmacokinetics: Induction/Inhibition of Drug-Metabolising- Inhibition of CYP3A Activity in Rat, Dog, and Monkey by COBI In Vitro

Report Title	Study Type	Test Article	Report Number Location in CTD
Inhibition of CYP3A Activity in Rat, Dog and Monkey by GS-9350 In Vitro	Metabolism study	COBI	AD-216-2040 4.2.2.4

Method: The potential for COBI to inhibit the catalytic activity of CYP3A enzymes of rat, dog, and cynomolgus monkey was assessed in vitro and compared to RTV. The inhibitory effects of COBI and RTV on hepatic microsomal midazolam 1'-hydroxylase activity were measured, and IC_{50} values were determined. Inactivation kinetics were determined, where possible, using a 2-stage incubation protocol with a 10-fold dilution step.

Inactivation Kinetics for COBI and RTV for Hepatic Microsomal CYP3A Activity in Rat, Dog and Monkey				Effect of COBI and RTV on Hepatic Microsomal CYP3A Activity in Rat, Dog and Monkey	
				Calculated IC_{50} (µM)	
Species	**Parameter**	**COBI**	**RTV**	**COBI**	**RTV**
Sprague Dawley Rat	K_I (µM)	0.32	0.24	0.17	0.06
	k_{inact} ($min^{\#1}$)	0.045	0.028		
Beagle Dog	K_I (µM)	ND	ND	0.12	0.04
	k_{inact} ($min^{\#1}$)	ND	ND		
Cynomolgus Monkey	K_I (µM)	ND	ND	0.43	0.12
	k_{inact} ($min^{\#1}$)	ND	ND		

COBI = cobicistat; CYP = cytochrome P450 enzyme(s); IC_{50} = concentration at which 50% maximum inhibition is achieved; K_I = affinity constant for enzyme inactivation; K_{inac} = theoretical maximum enzyme inactivation rate; RTV = ritonavir

ND = Cannot be determined (curve fit does not converge)

2.6.5.12.K Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Cytochrome P450 Inhibition Potential of COBI

Report Title	Study Type	Test Article	Report Number Location in CTD
In Vitro Assessment of Human Liver Cytochrome P450 Inhibition Potential of GS-9350	Metabolism study	COBI	AD-216-2029 4.2.2.4
In Vitro Assessment of Human Liver CYP2B6 and CYP2C8 Inhibition Potential of GS-9350			AD-216-2070 4.2.2.4

Method: With human hepatic microsomal fraction as the catalyst the rates of enzyme-specific metabolite formation for each cytochrome P450 enzyme were determined in the presence or absence of increasing concentrations of the test compound (0.05, 0.25, 0.5, 2.5, 5, and 25 μM). Substrates concentrations were equal to, or less than, their respective Km values and reactions were linear with respect to protein and time. IC_{50} values were determined for COBI and positive control inhibitors.

Enzyme	Activity	Calculated IC_{50} Value (μM)		
		Control Inhibitor[a]	COBI	RTV
CYP1A2	Ethoxyresorufin O-deethylase	0.03	> 25	> 25
CYP2B6	Bupropion 4-hydroxylase	2.80	2.8	2.9
CYP2C8	Paclitaxel 6α-hydroxylase	0.06	30.1	5.5
CYP2C9	Tolbutamide 4-hydroxylase	1.58	> 25	3.9
CYP2C19	(S) Mephenytoin 4'-hydroxylase	10.8	> 25	> 25
CYP2D6	Dextromethorphan O-demethylase	0.04	9.17	3.4
CYP3A	Midazolam 1'-hydroxylase	0.07	0.154	0.10
	Testosterone 6β-hydroxylase	0.09	0.151	0.11

COBI = cobicistat; CYP = cytochrome P450 enzyme(s); IC_{50} = concentration at which 50% maximum inhibition is achieved; RTV = ritonavir

a Positive Control Inhibitors: CYP1A2, α-Naphthoflavone (0–100 μM); CYP2B6, Triethylenethiophosphoramide (0–30 μM); CYP2C8 Montelukast (0–30 μM); CYP2C9, Sulfaphenazole (0–10 μM); CYP2C19, Tranylcypromine (0–100 μM); CYP2D6, Quinidine (0–10 μM); CYP3A, Ketoconazole (0–10 μM)

2.6.5.12.L Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Drug Interaction Properties of Human Metabolites of COBI

Report Title	Study Type	Test Article	Report Number Location in CTD
Drug Interaction Properties of Putative Human Metabolites of GS-9350	Metabolism study	COBI, GS-342006, GS-364751, GS-341842	AD-216-2041 4.2.2.4

Method: The effects of 3 human metabolites of COBI on the activities of 5 major human drug metabolizing cytochromes P450 were assessed and compared to COBI. In addition PXR and AhR activation by COBI, putative metabolites, and positive controls was assessed. The metabolites of COBI were identified during incubations with both human hepatocytes and human hepatic microsomal fractions and were later identified in vivo.

Enzyme	Activity	Calculated IC_{50} Value (μM)			
		COBI[a]	GS-342006 (E1 or M21)	GS-364751 (E3 or M31)	GS-341842 (E5 or M26)
CYP1A2	Ethoxyresorufin O-deethylase	> 25	> 25	> 25	> 25
CYP2C9	Tolbutamide 4-hydroxylase	> 25	> 25	> 25	> 25
CYP2C19	(S) Mephenytoin 4'-hydroxylase	> 25	> 25	2.95	> 25
CYP2D6	Dextromethorphan O-demethylase	9.17	> 5	0.21	> 5
CYP3A	Midazolam 1'-hydroxylase	0.154	2.41	0.179	0.23
	Testosterone 6β-hydroxylase	0.151	> 5	0.287	0.71
	Terfenadine oxidase	0.25	> 25	1.85	> 25

COBI = cobicistat; CYP = cytochrome P450 enzyme(s); IC_{50} = concentration at which 50% maximum inhibition is achieved

a Data for COBI are provided for comparison (Studies AD-216-2028 and AD-216-2029).

2.6.5.12.L Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Drug Interaction Properties of Human Metabolites of COBI

Test Article: COBI

PXR Activation							
	Fold Induction Over 0.1% DMSO Control						
Concentration (µM)	**COBI[a]**	**GS-342006 (E1 or M21)**	**GS-364751 (E3 or M31)**	**GS-341842 (E5 or M26)**	**Rifampicin**	**Mifepristone**	**Androstanol**
1	1.57	0.85	0.84	1.38	—	—	—
3	1.61	1.50	0.92	1.17	—	—	—
10	2.24	1.62	1.24	1.42	12.49[a]	7.10[a]	3.67[a]
AhR Activation							
1	1.12	0.86	0.93	0.81	**Omeprazole[b]**		
3	1.28	0.83	0.84	0.75	—		
10	1.60	0.83	0.76	0.68	—		
25	—	—	—	—	5.94		
50	—	—	—	—	13.83		
100	—	—	—	—	32.74		
200	—	—	—	—	52.45		

AhR = aryl hydrocarbon receptor; COBI = cobicistat; PXR = pregnane X receptor

a Data for COBI are provided for comparison (Study AD-216-2027)

b Average of values from GIL-2007-107 and GIL-2007-108

2.6.5.12.M Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Drug Interaction Properties of Human Metabolites of COBI- In Vitro Assessment of Human UGT1A1 Inhibition Potential of COBI

Report Title	Study Type	Test Article	Report Number Location in CTD
In Vitro Assessment of Human UGT1A1 Inhibition Potential of GS-9350	Metabolism study	COBI	AD-216-2075 4.2.2.4

Method: The potential for COBI to inhibit the catalytic activity of human UGT1A1 was assessed. The rates of formation of β-estradiol-3-glucuronide from estradiol substrate by hepatic microsomal fractions were determined in the presence and absence of test compound, and, where possible, IC_{50} values were determined. Ritonavir and ATV were used as comparators.

Enzyme	Activity	Calculated IC_{50} (μM)[a]		
		ATV	RTV	COBI
UGT1A1	β-estradiol-3-glucuronidation	0.83	4.73	16.3

ATV = atazanavir; COBI = cobicistat; RTV = ritonavir; UGT = uridine diphosphate glucuronosyl transferase
a Mean, n = 3

2.6.5.12.N Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Induction of Metabolizing Enzymes by COBI In Vitro

Report Title	Study Type	Test Article	Report Number Location in CTD
Induction of Metabolizing Enzymes by GS-9350 In Vitro	Metabolism study	COBI	AD-216-2027 4.2.2.4

Method: Assessments of induction were done using hepatoma-derived cell lines. DRE12.6 cells are transformed with an expression vector for human AhR and the Dioxin Response Element (DRE) of the human CYP1A2 gene linked to a luciferase reporter. DPX2 express human PXR and have the promoter for CYP3A4 linked to the luciferase reporter. Following 24 hours of exposure to the test articles, the luciferase substrate was added and the luminescence was read in a luminometer. The average luminescent units for the three replicates were divided by the average for the DMSO solvent control to determine the fold-induction. Positive control inducers were tested in parallel.

	Fold Induction of Human AhR Over 0.1% DMSO Control (DRE12.6 cells)			
Concentration (µM)	**COBI**	**RTV**	**β-Naphthoflavone**	**Omeprazole**
0.1 µM	—	—	2.17	
1 µM	1.12	0.80	5.91	
3 µM	1.28	0.69	—	
5 µM	—	—	17.72	
10 µM	1.60	0.80	27.31	
25 µM	—	—	—	8.16
50 µM	—	—	—	13.46
100 µM	—	—	—	27.34
200 µM	—	—	—	67.33

AhR = aryl hydrocarbon receptor; COBI = cobicistat; CYP = cytochrome P450 enzyme; RTV = ritonavir

2.6.5.12.N Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Induction of Metabolizing Enzymes by COBI In Vitro (Continued)

Test Article: COBI

Concentration	Fold Induction of Human PXR Over 0.1% DMSO Control (DPX2 cells)				
	COBI	**RTV**	**Rifampicin**	**Mifepristone**	**Androstanol**
0.3 !M	—	—	3.15	—	—
1 !M	1.57	3.64	6.09	—	—
3 !M	1.61	7.62	9.90	—	—
10 !M	2.24	10.14	14.30	8.58	3.38

COBI = cobicistat; PXR = pregnane X receptor (NR1I2); RTV = ritonavir

2.6.5.12.O Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes

Report Title	Study Type	Test Article	Report Number Location in CTD
In Vitro Assessment of the Induction Potential of GS-9350 in Primary Cultures of Human Hepatocytes	Metabolism study	COBI	AD-216-2071 4.2.2.4

Method: Cobicistat (1, 3, 10, and 30 μM) and known CYP inducers, 3-methylcholanthrene (3-MC), phenobarbital (PB), and rifampicin (RIF) were incubated in cultures of human hepatocytes from 3 separate donors for 3 consecutive days. Microsomes were isolated and CYP1A2, CYP2B6, and CYP3A levels were determined using enzyme-selective activities. Messenger RNA (mRNA) content for each of these CYP enzymes, UGT1A1 (UDP-glucuronosyltransferase), and MDR1 (multi-drug resistance protein) was also analyzed using TaqMan-based quantitative real-time polymerase chain reaction (qRT-PCR). Western immunoblotting was performed to detect the immunoreactive CYP3A protein.

Enzyme	CYP 1A2			CYP2B6			CYP3A		
Activity	Phenacetin O-deethylase			Bupropion 4-hydroxylase			Testosterone 6β-hydroxylase		
Donor	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053	Hu790	Hu793	Hu8053
Summary of Enzyme Activity (Percent Adjusted Positive Control) after Treatment with COBI or Positive Control Inducers									
3-MC (2 μM)	100	100	100	4.8	0.10	4.7	0.01	#4.2	4.9
Phenobarbital (1000 μM)	3.3	6.1	#2.7	100	100	100	70.6	65.4	72.9
Rifampicin (10 μM)	2.5	3.7	#3.5	80.2	54.1	38.5	100	100	100
COBI (1 μM)	0.54	#0.38	#5.8	8.4	1.2	5.2	#1.4	#6.7	#0.23
COBI (3 μM)	0.26	#0.31	#5.1	3.5	#1.7	9.0	#1.7	#6.5	0.16
COBI (10 μM)	1.7	1.3	#1.5	3.7	4.4	12.8	#1.6	#6.0	0.04
COBI (30 μM)	0.61	4.2	#1.4	20.5	2.5	3.8	8.2	#6.5	#1.2

2.6.5.12.O Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes (Continued)

Test Article: COBI

Treatment / Donor	CYP 1A2			CYP2B6			CYP3A			UGT1A1			MDR1		
	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053
Summary of Enzyme Activity (Fold Induction over DMSO Vehicle Control) after Treatment with COBI or Positive Control Inducers															
3-MC (2 µM)	102.6	42.5	15.5	1.2	1.0	1.8	1.0	0.6	2.2	—	—	—	—	—	—
Phenobarbital (1000 µM)	4.3	3.5	0.6	5.5	9.0	17.4	16.0	7.3	19.1	—	—	—	—	—	—
Rifampicin (10 µM)	3.5	2.5	0.5	4.6	5.3	7.3	22.2	10.6	25.9	—	—	—	—	—	—
COBI (1 µM)	1.5	0.8	0.2	1.4	1.1	1.8	0.7	0.4	0.9	—	—	—	—	—	—
COBI (3 µM)	1.3	0.9	0.3	1.2	0.9	2.5	0.6	0.4	1.0	—	—	—	—	—	—
COBI (10 µM)	2.7	1.5	0.8	1.2	1.4	3.1	0.7	0.4	1.0	—	—	—	—	—	—
COBI (30 µM)	1.6	2.7	0.8	1.9	1.2	1.6	2.7	0.4	0.7	—	—	—	—	—	—

2.6.5.12.O Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes (Continued)

Test Article: COBI

Treatment	CYP 1A2			CYP2B6			CYP3A4			UGT1A1			MDR1		
Donor	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053	Hu-790	Hu-793	Hu-8053
Summary of mRNA Content (Percent Adjusted Positive Control) after Treatment with COBI or Positive Control Inducers															
3-MC (2 µM)	100	100	100	3.1	–0.53	0.65	–0.97	–1.3	#2.8	140	81.2	83.6	#25.5	#33.1	#31.2
Phenobarbital (1000 µM)	0.14	0.34	0.15	100	100	100	61.0	79.6	47.8	124	127	107	89.8	98.9	106
Rifampicin (10 µM)	0.07	0.01	0.16	32.1	40.9	78.1	100	100	100	100	100	100	100	100	100
COBI (1 µM)	0.08	0.05	–0.01	0.65	–0.67	6.2	7.0	14.4	10.0	3.4	7.6	8.4	#3.6	#35.8	#11.4
COBI (3 µM)	0.15	–0.14	0.07	0.65	–0.02	13.2	17.6	25.2	32.0	3.4	10.6	17.6	#4.5	#21.5	8.0
COBI (10 µM)	0.53	0.04	0.65	0.29	–0.12	8.9	22.9	27.9	31.3	0.37	10.2	18.4	14.8	#6.9	2.3
COBI (30 µM)	1.4	3.0	1.7	–1.2	–1.2	–6.4	2.7	19.0	4.0	#4.4	6.2	#1.7	28.2	8.4	49.4
Summary of mRNA Content (Relative-Fold Induction) after Treatment with COBI or Positive Control Inducers															
3-MC (2 µM)	400	406	565	3.10	0.825	1.07	0.270	0.528	0.227	12.0	9.63	3.94	0.693	0.573	0.687
Phenobarbital (1000 µM)	1.55	2.46	1.78	65.3	46.4	12.1	45.2	31.9	12.2	10.8	14.5	4.78	1.96	2.30	2.00
Rifampicin (10 µM)	1.26	1.15	1.85	21.7	19.6	9.64	73.5	39.7	24.6	8.85	11.6	4.53	2.07	2.31	1.94
COBI (1 µM)	1.30	1.28	0.864	1.55	0.763	1.69	6.08	6.58	3.26	1.22	1.85	1.25	0.933	0.538	0.875
COBI (3 µM)	1.58	0.522	1.33	1.54	1.06	2.46	13.8	10.8	8.48	1.22	2.16	1.58	0.923	0.724	1.06
COBI (10 µM)	3.10	1.27	4.60	1.32	1.01	1.99	17.6	11.8	8.32	0.980	2.12	1.61	1.14	0.914	1.01
COBI (30 µM)	6.62	13.3	10.4	0.377	0.501	0.293	2.91	8.36	1.85	0.603	1.70	0.890	1.28	1.11	1.45

2.6.5.12.O Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes (Continued)

Test Article: COBI

Summary of Changes in Enzyme Activity After Treatment of Primary Human Hepatocytes with COBI or Positive Controls (Mean ± SD, N = 3)

Treatment	Conc. (µM)	CYP1A2		CYP2B6		CYP3A	
		Phenacetin O-deethylase		Bupropion 4-hydroxylase		Testosterone 6β-hydroxylase	
		Fold change[a]	%Max[b]	Fold change[a]	%Max[b]	Fold change[a]	%Max[b]
3-Methylcholanthrene	2	53.5 ± 44.6[c]	100%	1.3 ± 0.4	3.2%	1.3 ± 0.8	0.2%
Phenobarbital	1000	2.8 ± 1.9	2.2%	10.6 ± 6.1[c]	100%	14.1 ± 6.1	69.6%
Rifampicin	10	2.2 ± 1.5	0.9%	5.7 ± 1.4	57.6%	19.6 ± 8[c]	100%
COBI	1	0.8 ± 0.7	-1.9%	1.4 ± 0.4	4.9%	0.7 ± 0.3	-2.8%
	3	0.8 ± 0.5	-1.7%	1.5 ± 0.9	3.6%	0.7 ± 0.3	-2.7%
	10	1.7 ± 1	0.5%	1.9 ± 1	7.0%	0.7 ± 0.3	-2.5%
	30	1.7 ± 1	1.1%	1.6 ± 0.4	8.9%	1.3 ± 1.3	0.2%

COBI = cobicistat; CYP = cytochrome P450 enzyme

a Fold increase in enzyme activity compared to cells treated with 0.1% (v/v) DMSO vehicle control

b Change in enzyme activity as a fraction of that achieved by the positive control = (Fold Change of Treatment – 1) / (Fold Change of Positive Control – 1) x 100%

c Positive control for this activity

2.6.5.12.O Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes (Continued)

Test Article: COBI

Summary of Changes in mRNA Content After Treatment of Primary Human Hepatocytes with COBI or Positive Controls (Mean ± SD, N = 3)

Treatment	Conc. (µM)	CYP1A2		CYP2B6		CYP3A4		UGT1A1		MDR1	
		Fold change[a]	%Max[b]	Fold change[a]	%Max[b]	Fold change[a]	%Max[b]	Fold change[a]	%Max[b]	Fold change[a]	%Max[b]
3-Methylcholanthrene	2	457 ± 93.6[c]	100%	1.7 ± 1.2	1.1%	0.3 ± 0.2	-1.7%	8.5 ± 4.1	101.6%	0.7 ± 0.1	-29.9%
Phenobarbital	1000	1.9 ± 0.5	0.2%	41.3 ± 27[c]	100%	29.8 ± 16.6	62.8%	10 ± 4.9	119.3%	2.1 ± 0.2	98.2%
Rifampicin	10	1.4 ± 0.4	0.1%	17 ± 6.4	50.4%	45.9 ± 25[c]	100%	8.3 ± 3.6[c]	100%	2.1 ± 0.2[c]	100%
COBI	1	1.1 ± 0.2	0%	1.3 ± 0.5	2.1%	5.3 ± 1.8	10.5%	1.4 ± 0.4	6.5%	0.8 ± 0.2	-16.9%
	3	1.1 ± 0.6	0%	1.7 ± 0.7	4.6%	11 ± 2.7	24.9%	1.7 ± 0.5	10.5%	0.9 ± 0.2	-6.0%
	10	3.0 ± 1.7	0.4%	1.4 ± 0.5	3.0%	12.6 ± 4.7	27.4%	1.6 ± 0.6	9.7%	1.0 ± 0.1	3.4%
	30	10.1 ± 3.3	2.0%	0.4 ± 0.1	-2.9%	4.4 ± 3.5	8.6%	1.1 ± 0.6	0%	1.3 ± 0.2	28.7%

COBI = cobicistat; CYP = cytochrome P450 enzyme; MDR1 = P-glycoprotein (multidrug resistance protein 1); UGT = uridine diphosphate glucuronosyl transferase

a Fold increase in mRNA expression compared to cells treated with 0.1% (v/v) DMSO vehicle control

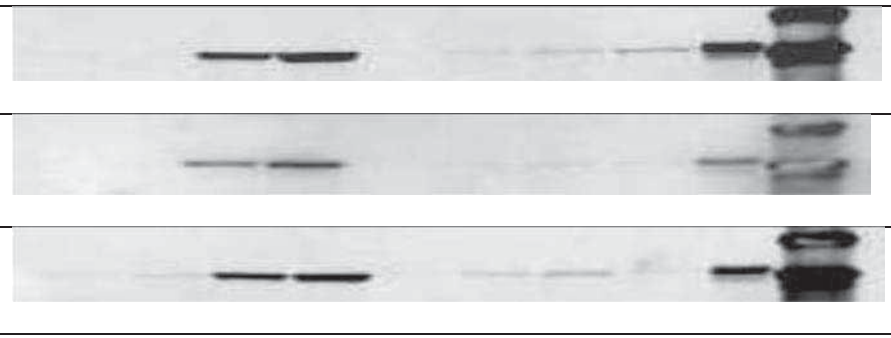
b Change in mRNA expression as a fraction of that achieved by the positive control = (Fold Change of Treatment – 1) / (Fold Change of Positive Control – 1) x 100%

c Positive control for this activity

2.6.5.12.O Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: In Vitro Assessment of the Induction Potential of COBI in Primary Cultures of Human Hepatocytes (Continued)

Test Article: COBI

CYP3A Western Immunoblotting of Primary Human Hepatocytes after Treatment with COBI or Positive Control Inducers



Treatments

1. Dimethylsulfoxide vehicle (0.1%)
2. 3-Methylcholanthrene (2 µM)
3 Phenobarbital (1000 µM)
4. Rifampicin (10 µM)
5. COBI (1 µM)
6. COBI (3 µM)
7. COBI (10 µM)
8. COBI (30 µM)
9. CYP3A4 standard
10. Electrophoresis standards

2.6.5.12.P Pharmacokinetics: Induction/Inhibition of Drug-Metabolising: Induction of Rat Metabolizing Enzymes By COBI In Vitro

Report Title	Study Type	Test Article	Report Number Location in CTD
Induction of Metabolizing Enzymes of Rat by GS-9350 In Vitro	Metabolism study	COBI	AD-216-2039 4.2.2.4

Method: The potential for induction of rat drug-metabolizing enzymes and transporters through the activation of the pregnane X receptor (PXR) by COBI was assessed in vitro, and the results were compared to those of ritonavir and positive control compounds (dexamethasone and miconazole). Assessments of induction were performed using a rat DAO hepatoma-derived cell line (rPXR) expressing rat PXR and with the CYP3A promoter linked to luciferase as a reporter. Positive control inducers were tested in parallel.

Rat PXR Activation by COBI, RTV, and Positive Controls

	Fold Induction Over DMSO Control			
Concentration	**COBI**	**RTV**	**Dexamethasone**	**Miconazole**
1 µM	1.25	1.36	—	—
3 µM	1.5	1.62	—	—
5 µM	—	—	6.54	—
10 µM	5.87	4.94	8.75	5.68
30 µM	5.14	6.53	—	—
100 µM	0.88	1.17	—	—

COBI = cobicistat; CYP = cytochrome P450 enzyme; PXR = pregnane X receptor (NR1I2); RTV = ritonavir

2.6.5.13.A Pharmacokinetics: Excretion (% of Dose) Into Milk

Test article: darunavir

Location in CTD	**4.2.2.5**					
Study No.	**TMC114-NC249**					
Species	**Rat (nursing Sprague-Dawley)**					
Feeding Condition	not fasted					
Vehicle/Formulation	PEG400					
Route	Oral (gavage)					
Gender (M/F)/Number of Animals	F/3		F/3		F/3	
Dose (mg base eq./kg/day)	1000		1000		1000	
Concentrations (mg/mL)	100		100		100	
Duration of Dosing (day)	4 (days 2-5 postpartum)		4 (days 5-8 postpartum)		4 (days 8-11 postpartum)	
Sample (whole blood, plasma, serum, etc.)	Plasma	Milk	Plasma	Milk	Plasma	Milk
Analyte	TMC114	TMC114	TMC114	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters						
C_{max} (µg/mL)	16.4	33.0	10.5	16.6	11.6	16.8
t_{max} (h)	2	2	2	2	2	2
AUC (µg.h/mL)	67.1	115	67.5	125	58.7	109
(Time for calculation –h)	(0-12)	(0-8)	(0-12)	(0-12)	(0-12)	(0-12)
$t_{1/2}$ (h)	3.6	NC	3.4	6.1	6	5.3
(Time for calculation –h)	(8-12)	-	(8-12)	(2-12)	(8-12)	(2-12)

Additional Information

The concentrations of TMC114 in milk from the vehicle-dosed dams were all above the limit of quantitation > 5 ng/mL and ranged from 0.064 to 0.901 µg/mL (corresponding to 0.7, 1.9 and 0.8% of the C_{max} values observed in dams dosed at 1000 mg/kg on days 5, 8 and 11 postpartum, respectively).
The milk to plasma AUC ratios were 2.3, 1.9 and 1.7, respectively, at days 5, 8 and 11 postpartum.

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NC : not calculated
PEG400 : polyethylene glycol 400

2.6.5.13.A Pharmacokinetics: Excretion (% of Dose) Into Milk (Continued)

Test article: darunavir

Location in CTD	**4.2.2.5**		
Study No.	**TMC114-NC249**		
Species	**Rat (Sprague-Dawley pups)**		
Feeding Condition	not fasted		
Vehicle/Formulation	PEG400		
Route	oral administration of dams (1000 mg/kg for 4 days)		
Gender (M/F)/Number of Animals	M + F/>21	M + F/>21	M + F/>21
Age (day postpartum)	4/5	7/8	10/11
Sample (whole blood, plasma, serum, etc.)	Plasma	Plasma	Plasma
Analyte	TMC114	TMC114	TMC114
Assay	LC-MS/MS	LC-MS/MS	LC-MS/MS
Pharmacokinetic Parameters			
C_{max} (µg/mL)	0.27	0.13	0.012
t_{max} (h)	11	3	7
AUC (µg.h/mL)	1.32	0.776	0.104
(Time for calculation –h)	(0-12)	(0-12)	(0-12)
$t_{1/2}$ (h)	NC	2.5	6.2
(Time for calculation –h)	-	(7-11)	(7-11)

Additional Information
Pups were dosed via the milk of dams.
The AUC_{0-12h} in the liver ranged between 0.87 and 1.02 µg.h/g. The pup-liver to pup-plasma AUC_{0-12h} ratios were about 0.8 at Day 5 postpartum and about 1.3 at Day 8 postpartum and about 8.3 on Day 11 postpartum. In most of cases, the concentration in pup brains was below the limit of quantification (< 0.040 ng/mL).

LC-MS/MS = liquid chromatography with tandem mass spectroscopy
NC : not calculated
PEG400 : polyethylene glycol 400

2.6.5.13 Pharmacokinetics: Excretion (% of Dose)

The other excretion study results of darunavir in rats, dogs and humans are described in the Tabulated Summaries 2.6.5.9.B, 2.6.5.9.D and 2.6.5.9.E, respectively.

2.6.5.13.B Pharmacokinetics: Excretion (% of Dose): Excretion of [^{14}C]COBI Following Single Oral Dose Administration to Mice

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics, Metabolism, and Excretion of [^{14}C]GS-9350 Following Oral Administration to Mice	Excretion	[^{14}C]COBI	AD-216-2073 4.2.2.4

Species/Strain Number of Animals/ Group Sex	Administration Route	Dose	Feeding Condition	% of Radioactive Dose				
				Time (h)	Urine	Time (h)	Feces	Cage Rinse
ICR mice [Hsd:ICR(CD-1)] 2 pairs of animals/ Group 1 male	Oral	[^{14}C]COBI 30 mg/kg	Nonfasting	0-6	1.00	0-24	79.1	0.06
				6-12	0.49			
				12-24	0.30			
				24-48	0.10	24-48	5.37	0.02
				48-72	0.05	48-72	0.94	0.01
				72-96	0.03	72-96	0.19	0.01
				96-120	0.02	96-120	0.12	0.01
				120-144	0.01	120-144	0.07	0.00
				144-168	0.01	144-168	0.05	!

COBI = cobicistat

Additional Information: Recovery of radioactivity from cage wash, cage wipe, and residual carcass totaled 0.7% of total dose.

2.6.5.13.C Pharmacokinetics: Excretion (% of Dose Excretion of [^{14}C]COBI Following Single Oral Dose Administration in the Rat

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-9350 Following Oral Administration to Rats	Excretion	[^{14}C]COBI	AD-216-2034 4.2.2.5

Species/Strain Number of Animals/ Group Sex	Administration Route	Dose	Feeding Condition	% of Radioactive Dose						
				Urine			Feces		Cage Rinse	
				Time (h)	Mean	SD	Time (h)	Mean	SD	Mean	SD	Mean	SD
Sprague Dawley Rat (H1a:[SD]CVF) Group 1: 3 male rats (bile duct-intact)	Oral	[^{14}C]COBI 10 mg/kg	Fasted until 4 h post-dose	0-12	1.57	0.22	0-24	77.8	15.2	0.05	0.02
				12-24	0.20	0.05					
				24-48	0.11	0.03	24-48	11.7	13.2	0.01	0.01
				48-72	0.06	0.01	48-72	0.94	0.70	0.01	0.01
				72-96	0.04	0.01	72-96	0.27	0.06	0.01	0.01
				96-120	0.03	0.01	96-120	0.33	0.11	0.00	0.01
				120-144	0.02	0.01	120-144	0.17	0.04	0.00	0.01
				144-168	0.02	0.00	144-168	0.11	0.02	!	!

COBI = cobicistat; SD = standard deviation

Additional Information: Further recovery of radioactivity from cage wash and cage wipe totaled 0.03% of total dose.

2.6.5.13.D Pharmacokinetics: Excretion (% of Dose: Excretion of [^{14}C]COBI After Oral Administration in the Dog

Report Title	Study Type	Test Article	Report Number Location in CTD
Mass Balance of Radioactivity after Oral Administration of [^{14}C]GS-9350 to Naive Male Beagle Dogs	Excretion	[^{14}C]COBI	AD-216-2067 4.2.2.5

Species/ Strain Number of Animals/ Group Sex	Adminis-tration Route	Dose (mg/kg)	Feeding Condition	Collection Period (h)	Cumulative Recovery of Total Radioactivity (% Dose) by Excretion Route (Mean ± SD, n = 3)					
					Urine		Feces		Cage Debris	
					Mean	SD	Mean	SD	Mean	SD
Beagle Dog 3M/group (bile duct-intact)	Oral	5	Fasted	0-12	1.37	0.56	0.04	0.08	0.07	0.04
				0-24	1.60	0.56	33.54	30.77	1.78	2.20
				0-48	1.81	0.59	76.84	4.27	2.05	2.27
				0-72	1.89	0.59	78.95	4.27	2.30	2.54
				0-96	1.96	0.59	79.56	4.14	2.46	2.75
				0-120	1.99	0.59	79.95	4.11	2.51	2.84
				0-144	2.04	0.58	80.30	4.17	2.56	2.93
				0-168	2.06	0.58	80.53	4.13	3.5[a]	—

COBI = cobicistat; SD = standard deviation

Note: Cage residue samples contained 3.52% of the radioactive dose.

a Includes hair, cage wash and cage wipe at 168 h post-dose

2.6.5.14 Pharmacokinetics: % of Dose Excreted in Bile

The excretion study results of darunavir in rats are described in the Tabulated Summaries 2.6.5.9.C.

2.6.5.14.A Pharmacokinetics: % of Dose Excreted in Bile- Excretion of [^{14}C]COBI Following Single Oral Dose Administration in the Rat

Report Title	Study Type	Test Article	Report Number Location in CTD
Pharmacokinetics, Distribution, Metabolism, and Excretion of [^{14}C]GS-9350 Following Oral Administration to Rats	Distribution	[^{14}C]COBI	AD-216-2034 4.2.2.5

Species/Strain Number of Animals/ Group Sex	Adminis-tration Route	Dose	Feeding Condition	% of Radioactive Dose			% of Radioactive Dose			% of Radioactive Dose				
				Urine			Bile			Feces		Cage Rinse		
				Time (h)	Mean	SD	Time (h)	Mean	SD	Time (h)	Mean	SD	Mean	SD
Sprague Dawley Rat (H1a:[SD]CVF) Group 4: 3 bile duct-cannulated male rats	Oral	[^{14}C]COBI 10 mg/kg	Fasted until 4 h post-dose	0-12	3.56	0.91	0-2	17.6	4.8	0-24	17.2	3.9	0.08	0.05
							2-4	19.9	4.8					
							4-6	14.7	1.3					
							6-8	5.27	2.87					
							8-12	4.22	1.97					
				12-24	0.24	0.12	12-24	3.47	0.37					
				24-48	0.16	0.06	24-48	1.52	0.19	24-48	2.06	1.19	0.01	0.01
				48-72	0.09	0.03	48-72	0.90	0.05	48-72	0.25	0.16	0.01	0.01
				72-96	0.05	0.02	72-96	0.62	0.06	72-96	0.08	0.06	0.01	0.01
				96-120	0.04	0.01	96-120	0.43	0.05	96-120	0.04	0.01	0.01	0.01
				120-144	0.03	0.00	120-144	0.31	0.03	120-144	0.02	0.01	0.00	0.01
				144-168	0.02	0.01	144-168	0.22	0.03	144-168	0.01	0.01	0.13	0.07

COBI = cobicistat; SD = standard deviation

Additional Information: Recovery of radioactivity from cage washes and cage wipes totaled 0.01% of total dose.

2.6.5.14.B Pharmacokinetics: % of Dose Excreted in Bile-: Mass Balance of Radioactivity after Oral Administration of [^{14}C]COBI to Naive Male Bile Duct-Cannulated Beagle Dogs

Report Title	Study Type	Test Article	Report Number Location in CTD
Mass Balance of Radioactivity after Oral Administration of [^{14}C]GS-9350 to Naive Male Bile Duct-Cannulated Beagle Dogs	Excretion	[^{14}C]COBI	AD-216-2068 4.2.2.5

Species/ Strain Number of Animals/ Group Sex	Adminis-tration Route	Dose (mg/kg)	Feeding Condition	Collection Period (h)	Cumulative Recovery of Total Radioactivity (% Dose) by Excretion Route (Mean ± SD, n = 2[a])			
					Bile	Urine	Feces	Mean Total
Beagle Dog 2M/group[a] (Bile duct cannulated)	Oral	5	Fasted	0–2	22.3 ± 0.01	—	—	22.3
				0–4	39.3 ± 0.33	—	—	39.3
				0–6	48.7 ± 1.51	—	—	48.7
				0–8	53.5 ± 2.97	—	—	53.5
				0–12	57.1 ± 3.25	1.07 ± 0.34	0.24 ± 0.33	58.5
				0–24	61.6 ± 3.38	1.40 ± 0.12	1.26 ± 1.12	64.3
				0–48	63.9 ± 3.70	1.56 ± 0.01	16.1 ± 1.43	81.7
				0–72	63.9 ± 3.70	1.72 ± 0.02	21.3 ± 3.41	87.1
				0–96	63.9 ± 3.70	1.78 ± 0.04	22.4 ± 3.07	88.3
				0–120	63.9 ± 3.70	1.82 ± 0.04	23.4 ± 2.78	89.4
				0–144	63.9 ± 3.70	1.85 ± 0.04	23.9 ± 2.68	89.9
				0–168	63.9 ± 3.70	1.88 ± 0.04	24.3 ± 2.73	90.3

COBI = cobicistat; SD = standard deviation Note: Cage residue samples contained 3.52% of the radioactive dose.

a One dog of original three excluded as an outlier (90.19% of dosed radioactivity recovered in urine 0 – 12 h postdose)

2.6.5.15.A Pharmacokinetics: Inhibition Potential towards OCT2 and MATE1

Report Title	Study Type	Test Article	Report Number Location in CTD
Potential of darunavir to inhibit the human OCT2 and MATE1.	Inhibition of transporters	darunavir	AD-216-2109 4.2.2.6

Method: Inhibition of the uptake of probe substrate TEA into the OCT2 and MATE-1 over-expressing cells was measured.

Compound	Transporter	IC_{50} (!M)	Maximal inhibition (%) at the highest tested concentrations
Darunavir	OCT2	>100	31
	MATE1	>100	44

Remark: maximum concentration tested was 100 !M

TEA = tetraethyl-ammonium

2.6.5.15.B Pharmacokinetics: Inhibition of P-glycoprotein-dependent Bidirectional Transport of Digoxin Through Caco-2 Cell Monolayers by COBI

Report Title	Study Type	Test Article	Report Number Location in CTD
Inhibition of P-glycoprotein-Dependent Bi-Directional Transport of Digoxin Through Monolayers of Caco-2 Cells by GS-9350	Drug-drug interaction study	COBI	AD-216-2072 4.2.2.6

Method: The potential for intestinal P-glycoprotein (MDR1) inhibition by COBI was assessed by measuring its effects on bidirectional transport of digoxin (a known MDR1 substrate) through Caco-2 monolayers. The known MDR1 inhibitors cyclosporin A and RTV were used for comparison.

Inhibitor	Inhibitor Conc. (µM)	Direction	Initial Digoxin Conc. (µM)	Digoxin P_{app} (10^{-6} cm/s)			Efflux Ratio
				Replicate 1	Replicate 2	Average	
None		Cell-Free	11.6	38.5	—	38.5	7.72
		Forward	13.6	1.07	1.53	1.30	
		Reverse	8.00	8.96	11.1	10.0	
Cyclosporin A	10	Cell-Free	9.30	47.0	—	47.0	1.68
		Forward	10.3	1.83	2.68	2.25	
		Reverse	10.0	3.56	4.00	3.78	
RTV	20	Cell-Free	8.50	45.6	—	45.6	1.84
		Forward	10.4	2.77	3.56	3.17	
		Reverse	8.60	5.57	6.05	5.81	
COBI	90	Cell-Free	10.8	51.1	—	51.1	1.69
		Forward	11.9	2.74	1.74	2.24	
		Reverse	11.6	5.33	2.26	3.80	

COBI = cobicistat; RTV = ritonavir

2.6.5.15.C Pharmacokinetics: Inhibition of Breast Cancer Resistance Protein-Dependent Bidirectional Transport of Prazosin through Monolayers of Caco-2 Cells by COBI

Report Title	Study Type	Test Article	Report Number Location in CTD
Inhibition of Breast Cancer Resistance Protein-Dependent Bidirectional Transport of Prazosin through Monolayers of Caco-2 Cells by Cobicistat	Drug-drug interaction study	COBI	AD-216-2104 4.2.2.6

Method: The potential for intestinal breast cancer resistance protein (BCRP) inhibition by COBI was assessed by measuring its effects on the bidirectional transport of prazosin, a known BCRP substrate, through Caco-2 monolayers. The known BCRP inhibitor, fumitremorgin C, was used for comparison. Ritonavir was also tested for its effect on prazosin.

Inhibitor	Inhibitor Conc. (µM)	Direction	Prazosin P_{app} (10^{-6} cm/s)					Efflux Ratio
			Replicate 1	Replicate 2	Replicate 3	Replicate 4	Average	
None	0	Cell-Free	49.84	—	24.04	—	36.94	5.1
		Forward	1.59	2.05	2.8	3.56	2.50	
		Reverse	9.12	13.91	12.92	15.08	12.78	
Fumitremorgin C	2	Cell-Free	58.80	—	34.70	—	46.75	2.6
		Forward	3.51	3.95	4.97	5.33	4.44	
		Reverse	11.55	12.45	11.18	11.14	11.58	
RTV	20	Cell-Free	51.30	—	28.44	—	39.87	2.8
		Forward	3.22	3.49	4.17	4.98	4.00	
		Reverse	9.98	12.62	9.93	12.49	11.26	
COBI	90	Cell-Free	41.75	—	34.90	—	38.33	2.4
		Forward	3.36	3.57	5.78	6.24	4.74	
		Reverse	11.34	12.50	9.68	11.29	11.20	

BCRP = breast cancer resistance protein; COBI = cobicistat; RTV = ritonavir

2.6.5.15.D Pharmacokinetics: Bidirectional Permeability of COBI Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells

Report Title	Study Type	Test Article	Report Number Location in CTD
Bidirectional Permeability of Cobicistat Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells	Excretion study (in vitro)	COBI	AD-216-2103 4.2.2.6

Method: The potential for COBI to act as a substrate for Pgp (MDR1) and BCRP was tested in monolayers of either wild type, MDR1 transfected or BCRP transfected Madin-Darby canine kidney (MDCK II) cells (MDCK II-WT, MDCK II-MDR1 and MDCK II-BCRP, respectively). The effects of transporter-selective inhibitors were also assessed.

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
Wild Type and MDR1 Transfected MDCK II Cells							
MDCK II-WT	Cell-Free	9.85	110.12	28.88	—	28.88	3.7
	Forward	10.18	81.81	3.26	3.27	3.26	
	Reverse	10.06	97.65	10.51	13.88	12.19	
MDCK II-MDR1	Cell-Free	10.19	103.94	33.68	—	33.68	60.9
	Forward	10.34	85.84	0.32	0.32	0.32	
	Reverse	9.72	102.22	17.56	21.20	19.38	
MDCK II-MDR1 (10 µM Cyclosporin A)	Cell-Free	11.2	102.90	26.84	—	26.84	3.8
	Forward	11.2	91.24	3.40	3.66	3.53	
	Reverse	10.1	94.85	12.41	14.00	13.21	

2.6.5.15.D Pharmacokinetics: Bidirectional Permeability of COBI Through Monolayers of P-glycoprotein and BCRP Overexpressing Cells (Continued)

Test Article: COBI

Cell Type	Direction	Initial Conc. (µM)	Recovery (%)	P_{app} (x 10^{-6} cm/s)			Efflux Ratio
				R1	R2	Average	
Wild Type and BCRP Transfected MDCK II Cells							
MDCK II-WT	Cell-Free	9.49	100.66	30.35	—	30.35	7.0
	Forward	9.35	78.66	1.71	2.27	1.99	
	Reverse	8.60	102.55	13.43	14.62	14.03	
MDCK II-BCRP	Cell-Free	9.15	96.97	26.81	—	26.81	13.4
	Forward	9.29	79.49	1.54	1.50	1.52	
	Reverse	8.43	110.12	19.15	21.50	20.33	
MDCK II-BCRP (10 µM Ko134)	Cell-Free	8.90	102.29	31.72	—	31.72	3.2
	Forward	9.12	76.97	3.54	4.04	3.79	
	Reverse	9.14	87.59	11.22	12.67	11.94	

2.6.5.15.E Pharmacokinetics: Interaction of COBI with Human MRP1, MRP2, and MDR1

Report Title	Study Type	Test Article	Report Number Location in CTD
Interaction of GS-9350 and Ritonavir with MRP1, MRP2, and Pgp	Drug-drug interaction study	COBI	AD-216-2030 4.2.2.6

Method: Cobicistat and RTV were incubated with Madin Darby canine kidney cells (MDCK II) transfected with ABCB1 (encodes Pgp/MDR1), ABCC1 (encodes MRP1), and ABCC2 (encodes MRP2). All incubations were carried out in cell culture medium (without FBS supplement) containing 10 !M calcein AM. Following removal of medium containing calcein AM and COBI, cells were lyzed at room temperature for 45 minutes in a buffer containing 20 mM Tris-HCl pH 9.0 and 0.4% Triton X-100. Each well was analyzed for calcein fluorescence.

	Percent Inhibition (%)		
COBI Concentration (µM)	**MRP1**	**MRP2**	**MDR1**
1.41	0	5.7	1.50
2.81	1.6	11.6	2.10
5.63	2.6	11.5	0.70
11.3	26.8	13.7	6.20
22.5	36.3	19.7	14.9
45.0	48.7	28.8	57.1
90.0	65.7	55.1	88.8
RTV Concentration (µM)			
0.31	1.6	0.4	3.50
0.63	11.1	2.4	4.90
1.25	11.3	1.5	5.90
2.50	20.2	3.3	3.70
5.00	29.4	2.9	12.0
10.0	23.2	2.4	28.8
20.0	52.9	5.5	69.3

COBI = cobicistat; MRP = multi-drug resistance-associated protein; MDR1 = P-glycoprotein (Pgp, ABCB1); RTV = ritonavir

2.6.5.15.F Pharmacokinetics: In Vitro Assessment of COBI and RTV Inhibition of Human Breast Cancer Resistance Protein

Report Title	Study Type	Test Article	Report Number Location in CTD
In Vitro Assessment of Cobicistat and Ritonavir Inhibition of Human Breast Cancer Resistance Protein	Drug-drug interaction study	COBI, RTV	AD-216-2099 4.2.2.6

Method: The inhibition of the ATP-Binding Cassette (ABC) efflux transporter Breast Cancer Resistance Protein (BCRP, ABCG2 gene product) by COBI and RTV was assessed in vitro using the Madin Darby Canine Kidney (MDCK II) cell line transfected with BCRP. Hoechst 33342 (10 µM) was the substrate, and fumitremorgin C (2 µM) was the positive control inhibitor. Fumitremorgin C treatment eliminated detectable transport activity.

Test compound	IC_{50} (µM)	Maximal inhibition (%)
COBI	59 # 28	~ 73
RTV	> 20[a]	~ 25

COBI = cobicistat; RTV = ritonavir

a Maximum concentration of RTV tested was 20 µM

2.6.5.15.G Pharmacokinetics: In Vitro Assessment of COBI and RTV Inhibition of Human OATP1B1 and OATP1B3

Report Title	Study Type	Test Article	Report Number Location in CTD
In Vitro Assessment of Cobicistat and Ritonavir Inhibition of Human OATP1B1 and OATP1B3	Drug-drug interaction study	COBI	AD-216-2100 4.2.2.6

Method: The inhibition of the Solute Carrier influx transporters organic anion transporting polypeptides 1B1 and 1B3 (OATP1B1 and OATP1B3) by COBI and RTV was assessed in vitro using Chinese hamster ovary cells transfected with the individual human transporters. Fluo3 (2 µM) was the substrate and rifampicin (50 µM) was the positive control inhibitor and reduced transport by ≥ 99%. Transport in transfected cells was ~10-fold (OATP1B1) or ~20-fold higher than in wild-type CHO cells.

Test Articles	Transporters	IC_{50} (µM)	Maximal inhibition (%)
COBI	OATP1B1	3.50 # 0.72	~ 98.5
	OATP1B3	1.88 # 0.76	~ 99.5
RTV	OATP1B1	2.05 # 1.33	~ 98.7
	OATP1B3	1.83 # 1.13	~ 99.1

COBI = cobicistat; OATP = organic anion transporting polypeptide; RTV = ritonavir

2.6.5.15.H Pharmacokinetics: In Vitro Interaction Studies of COBI with Human OCT2 Uptake Transporter

Report Title	Study Type	Test Article	Report Number Location in CTD
In vitro Interaction Studies of GS-9350 with human OCT2 Uptake Transporter	Drug-drug interaction study	COBI	AD-216-2093 4.2.2.6

Method: The inhibitory effect of COBI on recombinant expressed human organic cation transporter 2 (OCT2 or SLC22A2) was assessed using metformin (2 µM) as the substrate and an incubation period of 10 minutes. This study used Chinese hamster ovary cells expressing human OCT2. Ritonavir, an agent used for clinical pharmacokinetic enhancement of other drugs, was also tested. Cimetidine and trimethoprim, which have been shown to interact with human OCT2, were used for comparison. The positive control, verapamil (100 µM) inhibited transport by ≥ 91.5%.

Compound	IC_{50} (µM)	Inhibitory Efficacy (%)[a]
COBI	8.24	88
RTV	22.6	85
Cimetidine	44.5	70
Trimethoprim	29.4	90

COBI = cobicistat; IC_{50} = concentration at which 50% maximum inhibition is achieved; OCT2 = organic cation transporter 2; RTV = ritonavir

a Maximum concentrations tested were 100 µM (COBI and RTV) or 300 µM (cimetidine and trimethoprim).

2.6.5.15.I Pharmacokinetics: In Vitro Interaction Studies of COBI with Human MATE1 and MATE2-K Efflux Transporters

Report Title	Study Type	Test Article	Report Number Location in CTD
In vitro Interaction Studies of GS-9350 with human MATE1 and MATE2-K Efflux Transporters	Drug-drug interaction study	COBI	AD-216-2094 4.2.2.6

Method: The potential for COBI to inhibit the human multidrug and toxin extrusion (MATE) transporters MATE1 (SLC47A1 gene product) and MATE2 K (SLC47A2 gene isoform 2 product).was assessed using tetraethylammonium (TEA, 5 µM) as the substrate and an incubation time of 10 minutes. These studies employed the human embryonic kidney cell line, HEK293, transfected with expression vectors for human MATE1, human MATE2-K or empty vector. Cimetidine and trimethoprim were included as positive controls. Ritonavir was also included as a test article. Transport of TEA in MATE1-transfected cells was 27–52-fold higher than in vector control cells. Transport of TEA in MATE2-K-transfected cells was 9–18-fold higher than in vector control cells.

Compound	MATE1 IC_{50} (µM)	MATE2-K IC_{50} (µM)[a]
COBI	1.87	33.5
RTV	1.34	100
Cimetidine	1.64	43.4
Trimethoprim	6.35	1.38

COBI = cobicistat; MATE1 = multidrug and toxin extrusion protein 1 (SLC47A1); MATE2-K = multidrug and toxin extrusion protein 2-K (SLC47A2); RTV = ritonavir

a Maximum concentrations tested were 100 µM (COBI and RTV) or 300 µM (cimetidine and trimethoprim).

2.6.5.15.J Pharmacokinetics: In Vitro Interaction Studies of COBI and RTV With Human OCTN1 Transporter

Report Title	Study Type	Test Article	Report Number Location in CTD
In Vitro Interaction Studies of Cobicistat and Ritonavir with Human OCTN1 Transporter	Drug-drug interaction study	COBI	AD-216-2098 4.2.2.6

Method: The potential for COBI and RTV to inhibit the human organic cation transporter OCTN1 (SLC22A4) was assessed in vitro using Drosophila Schneider S_2 cells transfected with OCTN1. [^{14}C]Tetraethylammonium (5 µM) was the substrate and verapamil (100 µM) was the positive control inhibitor. Transport of the substrate was determined after 5 minutes, and was 7.6–9.0-fold higher than in untransfected S_2 cells. Verapamil inhibited substrate transport by 85.8%–90.2%.

Compound	IC_{50} (µM)	Maximal inhibition [a] (%)
COBI	2.49	~ 94
RTV	2.08	~ 91

COBI = cobicistat; IC_{50} = concentration at which 50% maximum inhibition is achieved; OCTN1 = organic cation transporter N1; RTV = ritonavir

a Maximum concentrations tested were 100 µM

2.6.5.15.K Pharmacokinetics: In Vitro Interaction Studies of COBI and RTV With Human OAT1 and OAT3 Transporters

Report Title	Study Type	Test Article	Report Number Location in CTD
In vitro Inhibition Studies of Cobicistat and Ritonavir with Human OAT1, OAT3 and MRP4 Transporters	Drug-drug interaction study	COBI	AD-216-2105 4.2.2.6

Method: The potential for COBI and RTV to inhibit the human organic anion transporters, OAT1 (SLC22A6) and OAT3 (SLC22A8) was assessed in vitro using transfected Chinese Hamster Ovary (CHO) cells (for OAT1) or transfected Human Embryonic Kidney HEK293 cells (for OAT3). Substrates were 0.5 µM [^{3}H]para-aminohippuric acid for OAT1 (3 min incubation) and 0.2 µM [^{3}H]estrone-3-sulfate for OAT3 (5 min incubation). Positive control inhibitors were 200 µM benzbromarone for OAT1 and 200 µM probenecid for OAT3, and inhibited transport by ≥ 98.9% and ≥ 96.4%, respectively.

Transporter	Compound	IC_{50} (µM)	Maximal inhibition [a] (%)
OAT1	COBI	> 100	140% activation
	RTV	> 20	ND
OAT3	COBI	> 100	ND
	RTV	8.46	~ 62

COBI = cobicistat; IC_{50} = concentration at which 50% maximum inhibition is achieved; OAT = organic anion transporter; RTV = ritonavir; ND = Not Determined

a Maximum concentrations tested were 100 µM

2.6.5.15.L Pharmacokinetics: In Vitro Interaction Studies of COBI and RTV with Human MRP4 Transporter

Report Title	Study Type	Test Article	Report Number Location in CTD
In vitro Inhibition Studies of Cobicistat and Ritonavir with Human OAT1, OAT3 and MRP4 Transporters	Drug-drug interaction study	COBI	AD-216-2105 4.2.2.6

Method: The potential for COBI and RTV to inhibit the multidrug resistance-associated protein MRP4 (ABCC4) was assessed in vitro using vesicles prepared from porcine kidney LLC-PK1 cells transfected with human MRP4. [^{3}H]5-Dehydroepiandrosterone sulfate (DHEAS, 0.02 µM) was the substrate and MK571 (150 µM) was the positive control inhibitor. ATP-Dependent transport of the substrate was determined after 8 minutes and was inhibited 80% by the positive control.

Compound	IC_{50} (µM)	Maximal inhibition [a] (%)
COBI	20.7	~ 92
RTV	> 20	~ 15

COBI = cobicistat; IC50 = concentration at which 50% maximum inhibition is achieved; MRP = multi-drug resistance-associated protein; RTV = ritonavir

a Maximum concentrations tested were 100 µM (COBI) and 20 µM (RTV).

2.6.5.15.M Pharmacokinetics: Assessment of the Potential for COBI and RTV to be Substrates of the Human OCT2 Uptake Transporter

Report Title	Study Type	Test Article	Report Number Location in CTD
Assessment of the Potential for GS-9350 and Ritonavir to be Substrates of the Human OCT2 Uptake Transporter	Excretion study (in vitro)	COBI	AD-216-2095 4.2.2.6

Method: The potential for COBI to be a substrate of the human organic cation uptake transporter OCT2 (SLC22A2) was assessed using a CHO cell line that stably expresses this protein. Time- and concentration-dependent accumulation of radioactivity was compared to that in wild type CHO cells and the effects of the OCT2 inhibitor, verapamil (100 µM), were tested. RTV and the OCT2 substrate, metformin, were tested as substrates in parallel.

Compound	Conc. (µM)	Time (min)	Verapamil (100 µM)	Accumulation (pmol/mg protein)		Ratio (OCT2/wt)
				CHO-OCT2	CHO-K (wt)	
[^{14}C]COBI	2	2	Without verapamil	401.9 ± 18.8	280.2 ± 1.7	1.43
			With verapamil	375.8 ± 17.8	235.4 ± 3.5	1.60
			Δ	6.5%	16.0%	—
		20	—	725.9 ± 22.9	508 ± 106.5	1.43
	20	2	—	3241.2 ± 184.2	2258.1 ± 330.9	1.44
		20	—	5872.5 ± 487.4	4614.6 ± 945.2	1.27
[^{3}H]RTV	10	2	Without verapamil	1872.8 ± 106.8	1119.7 ± 74.2	1.68
			With verapamil	1373.7 ± 155.1	798.1 ± 89.0	1.72
			Δ	26.6%	28.7%	—
		20	—	1834.8 ± 190.4	903.3 ± 352.3	2.03
	100	2	—	7682.8 ± 337	5684.0 ± 485.0	1.35
		20	—	11,454.7 ± 1380.7	6347.8 ± 1686.0	1.80
[^{14}C]Metformin	2	2	Without verapamil	215.2 ± 17.0	6.0 ± 1.3	35.9
			With verapamil	5.6 ± 1.1	6.6 ± 1.1	0.9
			Δ	97.4%	–10.0%	—

CHO = Chinese hamster ovary cell; COBI = cobicistat; OCT2 = organic cation transporter 2; RTV = ritonavir

2.6.5.16 Pharmacokinetics: Other

No additional studies for darunavir or cobicistat were performed.