DARUNAVIR/COBICISTAT

MODULE 2.6.6

TOXICOLOGY WRITTEN SUMMARY

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ABBREVIATIONS

A:G ratio	albumin to globulin ratio
ALP	alkaline phosphatase
ALT	alanine aminotransferase
API	active pharmaceutical ingredient
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATV	atazanavir
AUC	area under the plasma concentration-time curve
BCOP	bovine corneal opacity-permeability
b.i.d.	twice daily
BUN	blood urea nitrogen
CAC	carcinogenicity assessment committee
COBI	cobicistat
C_{max}	maximum plasma concentration
CPN	chronic progressive nephropathy
CYP	cytochrome P450 enzyme
DAVP	Division of Antiviral Products
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DRV	darunavir
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EtOH	ethanol
F	Female
FDA	Food and Drug Administration
F_1	first generation
GD	gestation day
GLP	good laboratory practice
GGT	gamma glutamyltransferase
HCl	hydrochloric acid
HIV	human immunodeficiency virus
HP-β-CD	hydroxypropyl-β-cyclodextrin
IHC	Immunohistochemical
Ig	immunoglobulin
KLH	keyhole limpet hemocyanin
LLNA	local lymph node assay
M	Male
MFD	maximum feasible dose
MTD	Maximum Tolerated Dose
NOAEL	no observed adverse effect level
NOEL	no observed effect level

PEG	polyethylene glycol
PG	propylene glycol
PK	pharmacokinetic(s)
PNA	peanut agglutinin
PND	postnatal day
PR	interval between the peak of the P wave and the peak of the R wave on ECG
PT	prothrombin time
PXR	pregnane X receptor
QT	interval between the start of the Q wave and the end of the T wave on ECG
RBC	red blood cell
RR	interval between the peak of R waves of 2 consecutive ECG complexes
RTV	ritonavir
SEDDS	Self-Emulsifying Drug Delivery System
SI	Stimulation Index
TDAR	T-cell dependent IgG antibody response
T_3	Triiodothyronine
T_4	tetraiodothyronine/thyroxine
TK	toxicokinetic(s)
TSH	thyroid stimulating hormone
UDPGT	uridine 5'-diphosphate glucuronosyltransferase

1. BRIEF SUMMARY

The darunavir/cobicistat (DRV/COBI) fixed dose combination tablet contains 800 mg DRV and 150 mg COBI and is developed by the Applicant in collaboration with Gilead Sciences Inc (Gilead). Darunavir is a registered human immunodeficiency virus type 1 (HIV-1) protease inhibitor and COBI is a pharmacokinetic enhancer that has recently been approved. No nonclinical studies have been conducted with DRV in combination with COBI since the combined use of DRV and COBI is not expected to induce clinically relevant additive or synergistic effects.

In this summary the toxicology of DRV (also named TMC114 or Prezista®) and COBI (a structural analog of ritonavir [RTV]) is described. Both compounds have been investigated separately and therefore the individual DRV and COBI data are described in this document and they can be found in the Toxicology Tabulated Summary 2.6.7. The toxicology studies of both compounds are listed in the Overview Table (Tabulated Summary 2.6.7.1). Details concerning studies containing toxicokinetic data can be found in Tabulated Summaries 2.6.7.2 and 2.6.7.3.

The nonclinical toxicity program and design of the studies was consistent with the best scientific principals and international guidelines. The pivotal studies have been conducted according to Good Laboratory Practice standards (OECD Principals of GLP, which conform to FDA GLP regulations).

Darunavir

Darunavir was studied in single and repeated dose toxicity studies up to and including 6 months in rats and 12 months in Beagle dogs, a series of genetic toxicity studies, reproductive and developmental toxicity studies covering fertility to postnatal development (mouse, rat, rabbit, or minipig), juvenile studies (rat) and carcinogenicity studies (mouse and rat). Furthermore several local tolerance studies were performed as well as studies with impurities. Ritonavir is used as a pharmacokinetic enhancer for DRV in the clinic. To evaluate the potential effects of DRV in combination with RTV, this combination has been studied in repeated dose studies in rats for up to 6 months and in dogs for up to 2 weeks duration. In addition, embryo-fetal development studies in mice, rats and minipigs and a pre- and postnatal development study in rats were conducted using the combination of DRV

and RTV. Also 2 juvenile DRV/RTV studies were conducted in rats. In all pivotal repeated dose toxicity studies plasma levels of DRV have been determined and, where appropriate, RTV plasma levels.

The drug substance is a solvate (ethanolate) (Tabulated Summary Drug Substance 2.6.7.4). The dose levels used in all studies in this document are expressed as the nominal dose.

Single dose oral gavage studies were conducted in mice, rats and dogs, with DRV, and single dose intravenous studies in rats and dogs with DRV. There were no toxicologically relevant clinical signs, which were related to orally administered DRV up to 100, 2000 and 80 mg/kg in mice, rats and dogs, respectively. Intravenous dosing was well tolerated up to 40 and 10 mg/kg in rats and dogs, respectively.

Repeated dose studies evaluated the effects of DRV alone, formulated in polyethylene glycol (PEG) 400, in rats and dogs following oral gavage of up to 6 months and 12 months duration, respectively. In all studies, control animals were administered the vehicle and toxicokinetic parameters evaluated (using satellite animals in the rat studies). Darunavir (in PEG400) was also administered in combination with RTV, as two separate formulations given orally (RTV given first, immediately or within 1 hour, followed by DRV). Various dose level combinations and dose regimens were evaluated in studies of 2 weeks duration (rats and dogs) and 6 months duration (rats only). In addition, intravenous studies of 2 weeks duration with a hydroxypropyl-!-cyclodextrin (HP-β-CD) formulation of DRV alone have also been conducted in rats and dogs. The use of the intravenous route was limited by the poor solubility of DRV in !-cyclodextrin which prevented assessment of toxicity at higher dose levels.

In the **rat oral studies with DRV alone**, no deaths associated with DRV occurred, but there was a number of accidental gavage deaths that were considered related to the viscous and irritant nature of the vehicle. Effects on the hematopoietic system were most apparent at doses of 500 mg/kg/day but changes also occurred at 100 mg/kg/day. These changes included a variable, but slight decrease (about 10%) in some or all of the red blood cell counts, hemoglobin, hematocrit and derived red blood cell (RBC) parameters (mean corpuscular hemoglobin and mean corpuscular volume). There was also an increase in reticulocytes and bilirubin levels. These changes are all indicative

of an increased red blood cell turnover, which was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen, sometimes accompanied by an increase in spleen weight. An increase in the activated partial thromboplastin time (APTT) was observed at 100 and 500 mg/kg/day but was not associated with any gross or microscopic evidence of bleeding. This was accompanied by an increase in platelets. Liver weight increases correlated with hepatocellular hypertrophy and sometimes with vacuolation found microscopically at doses of 100 mg/kg/day (and above) in rats given DRV alone. The no observed adverse effect level (NOAEL) was set at 20 mg/kg/day in the 3- and 6-month studies. Exposure at these doses, expressed as mean C_{max} and $AUC_{0\text{-}24h}$ was in the range 1 to 2 µg/mL and 3 to 4 µg.h/mL, respectively, at the end of the 6-month study.

In **rat oral studies of DRV and RTV in combination**, broadly similar changes to those with DRV alone were observed. In the 6-month combination study, 33 animals were found dead or sacrificed before scheduled necropsy and of these, all but 12, were considered accidental deaths owing to twice daily (b.i.d.) gavage dosing of irritant formulations (vehicles). The cause of death of the 12 animals was not evident and, as the majority of animals had been given 75 mg/kg/day RTV, a relationship between mortality and RTV cannot be excluded. A reduction of body weight gain was evident in male animals given 500/75 and 1000/75 mg/kg/day, but not in animals given RTV alone, indicating an effect of DRV and that a maximum tolerated dose had been achieved. Effects on RBC parameters in the presence of extramedullary hematopoiesis were also seen with RTV co-administration to a similar extent when compared to DRV alone. In the 6-month study with DRV co-administered with RTV, initial increases in APTT occurred but were not found in animals with the greatest exposure to DRV, and were decreased at the end of the study. There was also a decrease in prothrombin time (PT). In rat oral studies of DRV and RTV in combination, effects on the liver were similar to those seen in the 6-month study with DRV alone but were exacerbated owing to increased DRV exposure and the presence of RTV. In the combination study increases in liver weight and centrilobular hypertrophy were accompanied by multinucleated hepatocytes and some single cell necrosis. The latter findings were also noted in groups treated with RTV only. Increases in liver transaminase enzyme activities

were noted in males after treatment with RTV only and to a lesser extent in some of the DRV/RTV treated groups. Lipid biochemistry was altered in this combination study. Cholesterol was increased in all groups but mainly in females, similarly, phospholipids (but males only at higher exposure), while triglycerides were increased in females and reduced in males. Histopathological changes also included an increase in thyroid hypertrophy and hyperplasia with increasing exposure to DRV, but a contribution of RTV to this effect cannot be ruled out. The combination of DRV with RTV resulted in increased exposure to DRV and the exacerbation of changes related to DRV was dose related. In rat oral studies of DRV and RTV in combination, it was not possible to establish a NOAEL and given the complex nature of these studies and the potential effects of both compounds these results should be considered in comparison with other studies. Exposure, expressed as mean C_{max} and $AUC_{0\text{-}24h}$ was in the range 2 to 27 µg/mL and 11 to 318 µg.h/mL, respectively, at the end of the 6-month study.

In **dogs**, the **oral (gavage) toxicity** of **DRV alone** in PEG400 was evaluated in studies of 2 weeks, 3, 6 and 12 months duration at doses of 0 (vehicle), 30, 60, 120 and 360 mg/kg/day. Various **combinations of DRV with RTV** were evaluated in two pilot studies of 2-week duration to increase exposure to DRV. Efforts to increase exposure were limited by adverse clinical effects, such as vomiting, which would be unacceptable in a chronic study. Consequently, no further studies with DRV/RTV in dogs were conducted.

In the **dog oral** studies with **DRV alone**, 360 mg/kg/day was not tolerated and there were only limited effects of treatment with DRV up to the highest tolerated dose of 120 mg/kg/day. The major change was limited liver enlargement in some animals, which occurred generally in the absence of histopathological observations. Only after 12 months treatment was increased hepatocellular pigment and vacuolation observed with a limited increase in alkaline phosphatase (ALP) levels in males and females at 60 and 120 mg/kg/day. In the 12-month dog study, the NOAEL dose was 30 mg/kg/day, based on the absence of any relevant liver enzyme and histopathological changes. Exposure at NOAEL dose, expressed as mean C_{max} and AUC_{last} for males and females, was 12 and 9 µg/mL and 32 and 21 µg.h/mL, respectively, at the end of the 12-month study. However, in dogs 6 months treatment at 120 mg/kg was without any significant

toxicological effect and only after 12 months of treatment there was a limited response in the liver. At 120 mg/kg after 12 months treatment exposure (AUC_{last}) was 130 and 100 µg.h/mL, for males and females, respectively.

Genotoxicity tests, in vitro and in vivo, have shown DRV to be free of genotoxic potential. These studies included in vitro mutation assays (Ames test), an in vitro clastogenicity or chromosome aberration assay (human lymphocytes) and an in vivo clastogenicity assay (mouse bone marrow micronucleus test up to a dose of 2000 mg/kg).

In the **mouse and rat carcinogenicity** study, DRV was well tolerated. Mortalities related to DRV exposure occurred in high-dose male mice only and were due to atrial thrombosis (5/65) and liver tumors (10/65). In rats, test-article related mortality was seen in 1 high-dose male (hepatocellular carcinoma), and 10 females given 150 (3) or 500 mg/kg/day (7) (chronic progressive nephropathy (CPN)). In mice and rats, dose-related increases in the incidence of hepatocellular adenomas and carcinomas, hypertrophy, foci of cellular alteration, pigmentation and oval cell hyperplasia in the liver were observed and correlated with presence of masses/nodules/discoloration, organ weight changes and clinical chemistry changes. These findings are considered to be related to liver enzyme induction and/or alterations in lipid metabolism. Other treatment-related changes in mice were nephrosis and/or tubular pigmentation that linked to the increase in kidney weight. The atrial thrombosis seen in the heart is considered mouse-specific age-related change. Vascular mineralization was observed in the heart, pancreas, tongue, seminal vesicles, epididymes and kidney; mineralization was also present in the vibrissae and in the sclera of the eyes in high-dose male and/or female mice and correlated to the increases in inorganic phosphorus. The spleen and the mesenteric lymph nodes showed minimal increases in hemopoiesis that correlated with the erythron parameter changes. Given the hepatocellular changes seen at the lowest tested dose (150 mg/kg/day), an NOAEL was not established in the mouse study. At 150 mg/kg/day $AUC_{0\text{-}24h}$ was 6.23 and 7.79 µg.h/mL in male and female mice, respectively. In the rat the slight increase in bone marrow cellularity in males dosed from 150 mg/kg/day and the slight increase in splenic extramedullary hematopoiesis in males at 500 mg/kg/day correlated with hematology changes. The NOAEL in rats was 50 mg/kg/day corresponding to an $AUC_{0\text{-}24h}$ of 17.1 and 24.9 µg.h/mL in males and females, respectively.

In a **fertility and early embryonic development** study, there was an effect on body weights at 1000 mg/kg/day in males and females. In females treated with 1000 mg/kg/day there were fewer ovulations and fewer live fetuses than control females, possibly as a result of the lower body weights. It was concluded that there was no effect on male or female fertility at doses up to a dose producing effects on body weight gain (1000 mg/kg/day). The NOAEL was 200 mg/kg/day based on the absence of an adverse body weight effect in males and females.

In the **rat embryo-fetal development** study, the only adverse effects were lower mean body weight and food consumption in dams given 1000 mg/kg/day. There was no effect on dams at 200 mg/kg and there were no effects on embryo-fetal development at any of the doses administered, including the maternally toxic dose. There was no evidence that DRV is teratogenic in rats at dose levels up to 1000 mg/kg. The maternal NOAEL in this study was 200 mg/kg, based on the body weight effect, whereas the fetal NOAEL was 1000 mg/kg, based on the absence of any effects.

In the DRV/RTV combination study in the rat, DRV, alone or in combination with RTV, was well tolerated and was not associated with mortality related to treatment in pregnant rats throughout the period of organogenesis (gestation day [GD] 6 to GD 17). There was a dosage related reduction in dam body weight gain and food consumption in all groups receiving DRV with an associated decrease in the fetal weight. The minor visceral and skeletal abnormalities in fetuses were considered to be related to the maternal and subsequent fetal body weight decreases. The NOAEL of DRV when co-administered with RTV, for maternal toxicity was considered to be less than 300/50 mg/kg b.i.d and the NOAEL for embryo-fetal development was considered to be 300/50 mg/kg b.i.d.

In the **rabbit embryo-fetal development** study, high dose group animals showed body weight gain decreases accompanied by an effect on food consumption. There were no other effects of treatment on any maternal or fetal parameters examined. The maternal and fetal NOAEL was considered to be 1000 mg/kg/day. Darunavir was considered not to be teratogenic in rabbits at dose levels up to 1000 mg/kg/day, but from the limited toxicokinetic evaluation in the dose range finding study, the exposure (AUC_{last}) was not higher than 6 µg.h/mL. RTV combination substantially

increased DRV exposure in the rabbit. However DRV exposure remained significantly low in the rabbit relative to other animal species (mouse and rat) and, as a consequence, the mouse was selected as a third species for embryo-fetal development studies.

In the **mouse embryo-fetal development** study, DRV was given with and without RTV. There were no adverse effects in the dams at any of the doses administered. The maternal NOAEL was considered to be 1000 mg/kg/day with DRV alone administration based on the absence of any effects. When DRV was given in combination with RTV or when RTV was administered alone, only a slight reduction in food consumption was recorded. There were no effects of DRV treatment on embryo-fetal development at any of the doses administered. The embryo-fetal NOAEL was, therefore, considered to be 1000 mg/kg/day. There was no evidence that DRV is teratogenic in mice.

A dose range finding developmental study was conducted in minipigs. Due to the poor tolerability and low exposures obtained in minipigs compared to patients, this species was not considered suitable for further assessment of DRV effect on embryo-fetal development.

A **pre- and postnatal development** dose range finding study including assessment of juvenile toxicity was conducted in rats. Dams received DRV at doses up to 1000 mg/kg/day from gestation day 6 until lactation day 7. In addition pups were directly dosed from Day 12 to 25 of age. Body weight gain of pups up to day 6 of lactation was lower in groups given 200 and 1000 mg/kg/day. The maternal NOAEL in this study was 200 mg/kg/day and in pups not directly dosed the NOAEL was 40 mg/kg/day. For pups directly dosed at 40 and 200 mg/kg/day from day 12 until day 25 of age, a NOAEL could not be established owing to the deaths and adverse clinical signs. A dose of 500 or 1000 mg/kg/day was not tolerated. From dams dosed at 40, 200 and 1000 mg/kg/day from GD6 to 7 of lactation, toxicokinetic data indicated that the pups were indirectly exposed to DRV via the dam’s milk. At 1000 mg/kg/day, exposure was higher after a single direct dose to juvenile rats on Day 12 than on Day 25. Exposure in juvenile animals directly dosed was dose and age dependent and influenced by the maturation of the liver enzymes involved in the elimination of DRV. Darunavir distribution to the brain was very likely influenced by the maturation of the blood brain barrier.

In the definitive pre- and postnatal development study conducted in rats, DRV was given alone or in combination with RTV and the F1 generation was allowed to mature untreated. The maternal NOAEL for DRV was considered to be 40 mg/kg/day based on the effects seen in dams at higher doses. For pups, not directly dosed, the NOAEL was 40 mg/kg/day based on an effect on body weight gain and resulting effects on developmental parameters at higher dose levels. When DRV was administered with RTV at 1000/75(50) mg/kg/day, the effects on pup development were more pronounced than with DRV alone. In addition pup survival during lactation was reduced. The reduced bodyweight gain observed in F1 pups during lactation was directly related to DRV/RTV administration to the mother during lactation and exposure of the pups via the milk and not resulting from *in utero* exposure. RTV alone was associated with an increase in pups deaths, poor body weight gains and delayed development. Sexual development, fertility or mating performance of F1 animals was not affected by maternal treatment with DRV, alone or in combination with RTV, or RTV alone.

Local tolerance studies, including skin irritation and sensitization and eye irritation, indicated that there was no irritation or sensitization potential.

Immunotoxicity was evaluated in rats in a 4-week toxicity study. Changes in the hematopoietic system and liver as previously seen in the repeated dose toxicity studies were present but the immune response of treated animals, as measured by immunoglobulin (Ig) M production, was not affected by treatment at mean exposures ($AUC_{0\text{-}24h}$) of up to 102 µg.h/mL. The NOAEL was 20 mg/kg/day, and mean C_{max} and $AUC_{0\text{-}24h}$ values for males and females, were 0.75 and 1.7 µg/mL and 4.2 and 4.68 µg.h/mL, respectively, at the end of the study.

Drug substance impurities of DRV were investigated and Batch qualification studies as necessary were conducted and no differences in toxicity were found.

Cobicistat

The general toxicity (single and repeat dose), genotoxicity, carcinogenicity, reproductive toxicity, local tolerance, and immunotoxicity of COBI, as well

as the potential for hypersensitivity to COBI have been characterized in a variety of in vitro and in vivo studies. Combination toxicity studies with atazanavir (ATV), compound that is boosted by COBI, were also conducted. Specific endpoints were incorporated into study designs to help interpret effects of COBI on various target organs. Toxicological profiles, NOAELs, and the species specific mechanisms of action were used in the assessment of safety margins (presented in Module 2.4 Nonclinical Overview). Notwithstanding the different mechanism of action on cytochrome P450 (CYP) inhibition between humans and rats and dogs, the rat and dog were considered appropriate species for the toxicology studies because of the similar metabolism of COBI in humans and the ability to achieve high systemic exposures in these species.

All in vivo studies utilized oral administration, the clinical route of administration, with the exception of the sensitization and eye and dermal irritation studies. For oral dosing, COBI was generally administered as a solution in a vehicle of 95% propylene glycol (PG) and 5% ethanol (EtOH), acidified to pH 2.3 with hydrochloric acid (HCl). In the 28-day mouse study and the pre/postnatal developmental toxicity study, the vehicle was 10% PG in 40 mM acetate buffer, pH 4. In the 14-day range-finding and 28-day rat studies, COBI was administered in a 15% Self-Emulsifying Drug Delivery System (SEDDS; 6% Labrasol, 6% Solutol HS-15, 1.5% EtOH, 1.5% PG) in reverse osmosis water, pH adjusted to 2.3 (Tabulated Summary Drug Substance 2.6.7.4).

In a **single-dose** pharmacokinetic (PK) study in male and female mice, mortality was observed at 300 mg/kg; the maximum tolerated dose (MTD) was considered to be 100 mg/kg. In a GLP single-dose toxicity study in rats, no adverse effects were observed at 500 mg/kg, the highest dose tested. At higher doses, as part of the preliminary toxicity test for the bone marrow micronucleus test, mortality was noted at 750 mg/kg in female rats.

Repeat-dose toxicity studies were conducted in mice for up to 13 weeks; in rats for up to 26 weeks; and in dogs for up to 39 weeks. Juvenile rats were used in a 4-week oral toxicity study starting on postnatal Day 22 (PND 22). The dogs in studies up to 39 weeks duration were also considered immature, since the animals were approximately 6 to 8 months old at study initiation. Reversibility (after 1-month or 3-month recovery periods) was investigated

in all pivotal rat and dog toxicity studies with COBI, and in the combination toxicity studies in rats with COBI administered with ATV.

Target organs identified for COBI were the liver (mouse, rat, and dog) and thyroid (rat). Slight, nonadverse hematological changes were noted in rats and slight clinical chemistry changes were observed in mice, rats, and dogs, with urinalysis changes noted primarily at high doses in rats and dogs. In rats, the thyroid changes are considered rodent-specific, secondary to microsomal enzyme induction and thyroid hormone imbalance, and it is unlikely that COBI presents a risk to the human thyroid[1,2,3,4,5,6]. Liver changes in mice, rats, and dogs included microsomal enzyme induction, increased weights, and hepatocellular hypertrophy and/or vacuolation. All effects appeared to be completely reversible after a 1- or 3-month recovery period and are considered to be adaptive responses. Urinalysis changes (higher urine volume, lower urine specific gravity, increases in electrolyte excretion) showed no progression after long term dosing, were not associated with remarkable serum chemistry or histopathological correlates, and were reversible. Other potential toxicities related to COBI that were observed in nonclinical studies include PR interval prolongation in the 4-week dog toxicity study and decreases in left ventricular function in isolated rabbit hearts (Module2.6.2/Section 4.2.1).

Combination toxicity studies of COBI with ATV did not result in unexpected or additive toxicity.

Cobicistat was negative for mutagenic potential in a bacterial reverse mutation test, negative in a forward mutation test in mouse lymphoma cells, and negative in a rat micronucleus assay. The overall potential for genetic toxicity is considered to be low.

In a **2-year carcinogenicity** study in mice, no drug-related increase in tumor incidence was observed. In a 2-year carcinogenicity study in rats, increases in thyroid follicular cell adenomas and/or carcinomas were observed in males and females. The follicular cell findings are considered to be rat-specific, secondary to a species-specific activation of the pregnane X receptor (PXR), hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans[3,5,6].

No adverse effects on fertility or embryo-fetal viability were observed in a fertility study in male and female rats, and no teratogenic effects were observed in rat and rabbit developmental toxicity studies. In rats at 125 mg/kg/day, increases in postimplantation loss and decreased fetal weights were associated with significant maternal toxicity. There were no significant effects on peri- or postnatal development in rats. Cobicistat was also well tolerated in juvenile rats at dose levels and exposures similar to those used in the repeat-dose studies with older rats.

Cobicistat was a mild irritant to rabbit skin, was not a strong eye irritant, and was negative for delayed-type hypersensitivity.

Results from a 4-week **immunotoxicity** study in rats showed a decrease in the T-cell dependent IgG antibody response (TDAR) in females only. In standard 13-week mouse, 26-week rat, and 39-week dog toxicity studies, microscopic changes suggestive of immunotoxicity have not been observed in lymphoid organs, and immunophenotyping of peripheral blood cells in the chronic rat and dog studies did not reveal any adverse effects.

The **impurities** and degradation products in the COBI drug substance and in COBI tablets have been identified and qualified in toxicology studies.

2. SINGLE-DOSE TOXICITY

2.1. Darunavir

Single dose oral gavage studies were conducted in mice, rats and dogs, with DRV formulated in PEG400, and single dose intravenous studies in rats and dogs with DRV formulated in PEG400/EtOH/saline.

Five CD-1 mice per group received DRV once at 0 (vehicle), 100, 300 and1000 mg/kg at a dose volume of 20 mL/kg. The NOAEL was considered to be 100 mg/kg, based on the mortalities at higher doses[1,2] (see Tabulated Summary 2.6.7.5).

Two acute toxicity studies were performed in the Wistar rat (5/sex/group) at 0 (vehicle), 20, 100 and500 mg/kg and at 0 (vehicle), 500, 1000, 1500 and 2000 mg/kg at a dose volume of 5 mL/kg. In the first study (low doses) the NOAEL was considered to be 500 mg/kg and in the second study it was 2000 mg/kg, based on the observations in the rats[3,4,5,6] (see Tabulated Summary 2.6.7.5). In addition, a single dose intravenous study at 0 (vehicle),

10, 20 and 40 mg/kg at a dose volume of 10 mL/kg, was performed. Based on the absence of any direct effects of DRV in this study the NOAEL was set at 40 mg/kg[7,8] (see Tabulated Summary 2.6.7.5).

Single escalating doses of DRV at 0 (vehicle), 20, 80 and 320 mg/kg (dose volume = 1 mL/kg) were given to Beagle dogs (2/sex) to determine the MTD. Due to salivation and vomiting, 320 mg/kg was considered to be above the MTD. The NOAEL was set at 80 mg/kg. After this phase, one male and one female received DRV at 80 mg/kg/day for 3 consecutive days[9,10.]. An intravenous toxicity study was also performed, with escalating doses (2 dogs/sex) at 0 (vehicle), 10, 20 and40 mg/kg of DRV. The NOAEL was considered to be 10 mg/kg, based on the absence of any relevant findings. This dose was also considered to be the maximum feasible dose (MFD), as dogs suffered from retching, vomiting, shaking of the head and abnormal posture at 40 mg/kg and one animal was comatose at 20 mg/kg. The dose-escalation phase was followed by a 3-day intravenous dosing phase at 10 mg/kg (1/sex) [11,12] .Detailed findings of these studies are summarized in Tabulated Summary 2.6.7.5.

In general, there were no toxicologically relevant clinical signs that were related to DRV in any of these studies. However, clinical signs related to the use of PEG400 as vehicle in the oral formulation and the poor solubility of the DRV in the intravenous formulation constituted a limitation to explore toxicity at higher doses.

2.2. Cobicistat

A single dose pharmacokinetics study of COBI was conducted in in CByB6F1-Tg(HRAS)2Jic (Model 001178-W; wild type for rasH2 transgenic mice) mice[7,8]. Male and female mice (24/sex/group) were administered COBI at dose levels of 30, 100, and 300 mg/kg in a dose volume of 10 mL/kg in vehicle (10% PG in 40 mM acetate buffer, pH 4). The MTD in this single dose study was considered to be 100 mg/kg based on the severe clinical signs and moribund euthanasia in males and females administered 300 mg/kg COBI. Details can be found in Tabulated Summary 2.6.7.5.

In Sprague-Dawley rats, a single dose study followed by a 14-day observation period was conducted with COBI doses of 0 (vehicle; 95% PG, 5% EtOH, pH adjusted to 2.3 with HCl), 125, 250 or 500 mg/kg. The NOAEL was considered to be 500 mg/kg[9] (Tabulated Summary 2.6.7.5). A

single dose of COBI was also given to rats in a bone marrow micronucleus study[10]. More details are given in Section 4.2.3 and Tabulated Summary 2.6.7.9.B.

3. REPEAT-DOSE TOXICITY

3.1. Darunavir

Repeated dose studies evaluated the effects of DRV alone, formulated in PEG400, in rats and dogs following oral gavage of up to 6 months and 12 months duration, respectively. Darunavir (in PEG400) was also administered in combination with RTV, as two separate formulations given orally (RTV given first, immediately or within 1 hour, followed by DRV). Various dose level combinations were evaluated in studies of 2 weeks duration (rats and dogs) and 6 months duration (rats only). In addition, intravenous studies of 2 weeks duration with HP-β-CD formulation of DRV alone have also been conducted in rats and dogs. The use of the intravenous route was limited by the poor solubility of DRV in β-cyclodextrin, which prevented assessment of toxicity at higher dose levels.

3.1.1. Rat

3.1.1.1. Darunavir Alone

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for **14 days**[13,14]. One control group and three treated groups were given 0 (vehicle), 40, 200 and 1000 mg/kg/day at a dose volume of 5 mL/kg. Each group consisted of 10 male and 10 female Wistar rats (principal animals) together with 5 male and 5 female satellite animals for toxicokinetic analysis. Due to hepatocellular hypertrophy and thyroid changes seen at 200 and 1000 mg/kg/day, the NOAEL was considered to be 40 mg/kg/day (see details in Tabulated Summary 2.6.7.6).

A **3-month** study was conducted in which DRV, formulated in PEG400, was administered once daily, by oral gavage.[15,16] One control group and three treated groups were given, 0 (vehicle), 20, 100 and 500 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 10 male and 10 female Sprague-Dawley rats (principal animals) together with 5 male and 5 female satellite animals (treated groups only) for toxicokinetic analysis. The NOAEL was considered to be 20 mg/kg/day based on the absence of hepatocellular hypertrophy and thyroid changes at this dose level (See Tabulated Summary 2.6.7.7.A).

Darunavir formulated in PEG400 was also administered once daily, by oral gavage, for **6 months**.[17,18] One control group and three treated groups were given, 0 (vehicle), 20, 100 and 500 mg/kg/day in a dose volume of 5 mL/kg. Each group consisted of 20 male and 20 female Sprague-Dawley rats (principal animals) together with 5 male and 5 female satellite animals (treated groups only) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis, week 6, 13 and 26). Blood samples were collected on day 1 and during weeks 13 and 26 during the 24 hours after dosing for toxicokinetic analysis. All principal animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy, and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from principal animals in the control and high dose group and a limited list of organs in the low and intermediate groups, as well as from all principal animals that died or were sacrificed early, and all gross lesions, were examined microscopically.

There were no mortalities associated with DRV, but there were a number of accidental deaths associated with the dosing or blood collection procedures: principal animals, 5 in the vehicle group, 6, 3 and 5 in groups given 20, 100 and 500 mg/kg/day, respectively (satellite animals, 1 in each group given 20, 100 and 500 mg/kg/day). There were no relevant clinical signs, or effects on body weight or food consumption.

Effects on the hematopoietic system were observed with decreases (up to 11%) in RBC count, hemoglobin, and hematocrit and an increase in reticulocyte count occurring in animals given 500 mg/kg/day. The reticulocyte increase was mostly greater at week 6 than at week 26 (up to 79% and 38%, respectively. Similar, but generally less marked changes in reticulocyte count occurred in animals given 100 mg/kg/day, at week 26 and also at week 6. Reticulocyte count was increased (19%) in males given 20 mg/kg/day at week 26. These changes (with increased bilirubin) indicate an increase in RBC turnover, which was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen. Platelet count increased (up to 37%) in animals given 500 mg/kg/day and was broadly comparable on each occasion. Similar, but generally less marked changes in

platelet count occurred in animals given 100 mg/kg/day and platelet counts were increased (14%) in males given 20 mg/kg/day at week 26. An increase in APTT occurred in animals given 100 and 500 mg/kg/day (up to 20% and 44%, respectively).

Bilirubin and cholesterol were increased (up to 7-fold and 74%, respectively) and triglycerides decreased (up to 67%) in animals given 500 mg/kg/day. These parameters were less markedly affected in animals given 100 mg/kg/day at week 26, although triglycerides were affected on all occasions. Bilirubin increased (2-fold) in males given 20 mg/kg/day at week 26. A number of other serum chemistry parameters changed in response to treatment with DRV, but either did not show a dose response or were decreases and/or were not associated with histopathological changes, are not considered further.

Urine volume was lower and osmolality higher in animals given 500 mg/kg/day.

Macroscopic examination and organs weights at necropsy indicated an increase in male kidney and spleen weight (about 13% and 13%, respectively, for relative weight) and male and female liver weight in animals given 100 and 500 mg/kg/day. The relative liver weights increased 8 to 14% and 27 to 71%, respectively. Microscopically this was confirmed as hepatocellular hypertrophy. Lipofuscin was present in the liver and kidney. The hepatocellular hypertrophy was considered to reflect an adaptive response to enzyme induction after administration of a xenobiotic rather than an adverse effect of DRV. In addition, minimal hypertrophy and hyperplasia of the bile ducts was noted in most animals treated at 500 mg/kg/day.

The NOAEL was considered to be 20 mg/kg/day based on the absence of any relevant hematological and histopathological changes (See Tabulated Summary 2.6.7.7.B).

Toxicokinetic analysis indicated non-linear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values, which generally decreased after repeated dosing, are given in Table 1 below.

Table 1: Darunavir C_{max} and AUC values after repeated oral administration (6 months) in rats

TMC114 Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
20	Day 1	1.48	2.03	3.34	6.46
	Week 26	1.03	1.77	2.92	4.38
100	Day 1	6.67	7.62	28.1	44.0
	Week 26	7.11	12.8	31.6	35.8
500	Day 1	12.1	11.6	164	140
	Week 26	10.3	24.5	63.8	121

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose

In addition to the oral dosing studies with DRV, one **intravenous study** was conducted in which Sprague-Dawley rats received DRV (in 15% HP-β-CD in pyrogen free water) at 0 (vehicle), 2.5, 10 and 25 mg/kg/day for 14 days at a fixed concentration of 5 mg in dose volumes of 5, 0.5, 2 and 5 mL/kg, respectively.[19] Each group consisted of 10 male and 10 female rats (principal animals) together with 6 male and 6 female satellite animals (treated groups only) for toxicokinetic analysis. The NOAEL was considered to be 25 mg/kg/day in the absence of any changes (See Tabulated Summary 2.6.7.6).

3.1.1.2. Darunavir Co-Administered with Ritonavir

Two **14-day** repeated dose oral toxicity studies with DRV (in PEG400) co-administered with RTV (in PG) were conducted in Wistar rats. In the first study[20,21] the following combinations of DRV/RTV were given: 0/0 (vehicles), 0/50, 150/25, 500/25 and 1000/50 mg/kg/day in a dose volume of 5 mL/kg for DRV and 1.25 mL/kg for RTV. RTV was given first immediately followed by DRV. Each group consisted of 10 male and 10 female rats (principal animals) together with 5 male and 5 female satellite animals for toxicokinetic analysis in dosed groups only. In the second study[22,23] the following combinations of DRV/RTV were given: 0/0 (vehicles), 0/75, 2000/0 and 2000/75 mg/kg/day in a dose volume of 6 mL/kg for DRV and 1.25 mL/kg for RTV. The RTV was given first immediately followed by the DRV. Each group consisted of 5 male and 5 female Wistar rats (principal animals) together with 5 male and 5 female satellite animals for toxicokinetic analysis. No NOAEL was established in both studies (Tabulated Summary 2.6.7.6).

Darunavir formulated in PEG400 was co-administered with RTV formulated in PG, once daily, by oral gavage, for **6 months** in the following

combinations of DRV /RTV: 0/0 (vehicles), 0/75, 20/50, 100/50, 500/75 and 1000/75 mg/kg/day in a dose volume of 4 mL/kg for DRV and 1.25 mL/kg for RTV.[24,25] RTV was given first immediately followed by DRV. Each group consisted of 20 male and 20 female Wistar rats (principal animals) together with 5 male and 5 female satellite animals (except for the vehicle only group which was 2 male and 2 female) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination and clinical laboratory investigations (hematology, blood chemistry and urine analysis, week 6, 13 and 26). Blood samples were collected on day 1 and during weeks 13 and 26 during the 24 hours after dosing for toxicokinetic analysis. All principal animals and all satellite females given 0/75 mg/kg/day sacrificed at the end of the study and all animals, including satellite animals, found dead or sacrificed during the study were subject to macroscopic examination at necropsy and a number of organs were weighed from the principal animals. Samples of a range of tissues and organs were preserved for histological examination. Tissues and organs from principal animals in the vehicle control, the 0/75 and 1000/75 mg/kg/day dose groups and a limited list of organs in the other groups, as well as from all animals that died or were sacrificed early, and all gross lesions, were examined microscopically.

There were no mortalities associated with DRV, but 33 animals were found dead or were sacrificed prematurely during the study. In the principal animals these were 2, 10, 3, 4, 6 and 3 given vehicle, 0/75, 20/50, 100/50, 500/75 and 1000/75 mg/kg/day, respectively (in satellite animals, 2, 0, 1, 0, 1 and 1 given vehicle, 0/75, 20/50, 100/50, 500/75 and 1000/75 mg/kg/day, respectively). The majority of these deaths were associated with the dosing procedures and there were 3 incidental deaths. However, the cause of death was not evident in 12 animals; 1, 4, 1, 1, 4, and 1 in groups given vehicle, 0/75, 20/50, 100/50, 500/75 and 1000/75 mg/kg/day, respectively. A relationship between mortality and RTV treatment at 75 mg/kg/day cannot be excluded as 9/12 animals were dosed with RTV at 75 mg/kg/day. The high mortality in females dosed at 0/75 mg/kg/day was the reason why female satellites in this group were necropsied and examined macro- and microscopically. There were no relevant clinical signs. Body weight gain was reduced (up to 15%) in animals given 500/75 and 1000/75 mg/kg/day

and appears to be related to DRV exposure. Food consumption was not affected by treatment with DRV but was reduced in week 1 only following initial exposure to 75 mg/kg/day RTV.

Effects on the hematopoietic system were observed with decreases (up to 16%) in RBC count, hemoglobin and hematocrit and an increase in reticulocyte count (up to 2.6-fold) occurring in all treated groups, including RTV only group, at week 26. These disturbances of the hematological parameters occurred from the start of the study. These changes (with the increase in bilirubin) indicate increased RBC turnover, and this was confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen in DRV -dosed groups at 500 and 1000 mg/kg/day. The greatest change was noted in animals in the highest dose levels of the combined treatment of DRV and RTV. An increase in platelet count (up to 63% and mainly in males) occurred in all treated groups at week 26. In addition, there were changes in APTT and PT. After an initial increase in APTT (up to 15%) this subsequently decreased (up to 19%) at the end of the study and PT was lower in all female treated groups (by up to 11%). When compared to the RTV only group, DRV treatment resulted in a marginal increase in the effect on the hematopoietic system and PT.

Creatinine (males), bilirubin and cholesterol were increased (up to 18%, up to 3-fold and up to 3-fold, respectively) and triglycerides increased in females and decreased in males (up to 2.4-fold and 73%, respectively) in animals given 500/75 and 1000/75 mg/kg/day. Phospholipids were increased in both sexes at the higher dose levels. The observed deviations in serum chemistry parameters (mainly protein and lipid changes) were considered secondary effects to changes that occurred in the liver (see below). An increase in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was noted in males after treatment with RTV alone (up to 2.9-fold or 81%, respectively) and, to a lesser extent, in groups treated with RTV co-administered with DRV (up to 78%). With increasing doses of DRV the effect became less pronounced or near normal, which was consistent with the decrease in RTV-levels when co-administered with DRV. A number of other serum chemistry parameters changed in response to treatment with DRV and/or RTV but are not considered further, as they either did not show a dose response, were decreases and/or were not associated with histopathological changes.

Urine volume was higher as a result of treatment and sodium and chloride excretion was increased. This was also seen with RTV alone although to a lesser extent.

Macroscopic examination and organ weights at necropsy indicated an increase in liver weight (up to 3-fold in relative weight) in all treated groups, including RTV alone, and, similarly, the spleen and kidney weights were increased (up to 39% and up to 20% in relative weight, respectively). Higher adrenal and thyroid weights were noted at 500 and 1000 mg/kg/day. When compared to RTV only, DRV treatment resulted, in general, in higher liver weights in both sexes and higher adrenal weights in males given 500/75 and 1000/75 mg/kg/day.

Microscopically the liver changes consisted of hepatocellular hypertrophy, vacuolation, multinucleated hepatocytes and single cell necrosis (of multinucleated hepatocytes) observed after RTV alone or in combination with DRV. Nephropathy, especially in males occurred in all dose groups including the RTV only group but has not been previously reported with DRV in rats. In the thyroid, hypertrophy and/or hyperplasia of follicular cells was noted in all dose groups, including the RTV only group. In addition, findings were noted in the pancreas (minimal increased incidence/severity of islet fibrosis/siderocytes in males only) and adrenals (increased severity of cortical vacuolation) in DRV dosed animals only.

There was an increase in alveolar macrophages in lungs of treated animals, with the most severe reaction in animals given 75 mg/kg/day RTV alone. This finding was absent when animals were also given 500 or 1000 mg/kg/day DRV. It is noted that DRV co-administered with RTV appears to reduce the effects of RTV on tissue macrophages (lung, liver and mesenteric lymph nodes), and this is probably related to the lower exposure to RTV.

No NOAEL was established in this study. Given the complex design of this study and the potential effects of both compounds these results should be considered in comparison with other studies. This comparison shows a small additive effect of the combination of DRV and RTV on the liver (although serum liver transaminases decreased with increasing DRV dose), red blood cell parameters and thyroid. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV and were not of a

different order of magnitude observed in animals with each compound alone (See Tabulated Summary 2.6.7.7.C).

Toxicokinetic analysis indicated that combining DRV with RTV resulted in a dose proportional increase in exposure of DRV for the 20/50 and 100/50 mg/kg/day dosing regimens. Less than dose proportional (broadly comparable) increase in exposure of DRV occurred with the 500/75 and 1000/75 mg/kg/day dosing regimens. A decrease (up to 8-fold) in exposure of RTV occurred when RTV was combined with DRV compared to administration of RTV alone. Systemic exposure, expressed as C_{max} and AUC values are given in Table 2 and Table 3 below.

Table 2: Darunavir C_{max} and AUC values after repeated oral administration (6 months) in rats in combination with RTV

TMC114/RTV Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
20/50	Day 1	2.00	1.54	17.1	7.79[b]
	Week 26	1.66	1.95	11.2	13.4
100/50	Day 1	4.87	4.29	66.3	60.5
	Week 26	5.30	6.42	69.0	70.4
500/75	Day 1	11.4	7.81	194[c]	163[c]
	Week 26	11.5	26.6	183	318
1000/75	Day 1	9.74	10.8	179[c]	192
	Week 26	14.4	24.8	198	297

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose; [b] AUC_{0-8h}; [c] AUC_{0-24h}

Table 3: RTV C_{max} and AUC values after repeated oral administration (6 months) in rats

TMC114/RTV Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
0/75	Day 1	6.61	7.76	77.0	73.1
	Week 26	9.60	12.1	80.9	70.2
20/50	Day 1	2.32	2.98	16.1	19.6
	Week 26	4.84	7.09	52.9	33.9
100/50	Day 1	2.10	2.96	27.6	31.8
	Week 26	3.68	6.45	15.4	35.2
500/75	Day 1	1.28	1.58	12.5	15.3
	Week 26	1.30	5.34	10.1	25.2
1000/75	Day 1	1.53	1.65	12.2	18.3
	Week 26	1.48	3.56	10.3	15.3

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose.

3.1.2. Dog

3.1.2.1. Darunavir Alone

Fourteen day repeated dosing was conducted in 2 dog studies. In the first study DRV was given at 0 (vehicle; PEG400), 0, 60 and 120 mg/kg/day in a dose volume of 1 mL/kg.[26,27] Each group consisted of 3 male and 3 female Beagle dogs. The NOAEL was considered to be 120 mg/kg/day based on the absence of any relevant changes that could be attributable to treatment (See Tabulated Summary 2.6.7.6). In the second study a higher dose was applied: one male and one female Beagle dog were given 120 and 360 mg/kg/day in a dose volume of 2 mL/kg.[28] A dose of 120 mg/kg/day was well tolerated while 360 mg/kg/day was not considered tolerable for a long-term study (for details, see Tabulated Summary 2.6.7.6).

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for **3 months**[29,30.] One control group and three treated groups were given, 0 (vehicle), 30, 60 and 120 mg/kg/day in a dose volume of 2 mL/kg. Each group consisted of 4 male and 4 female Beagle dogs. Based upon the findings in the study, the NOAEL was considered to be 120 mg/kg/day (see Tabulated Summary 2.6.7.7.D).

Six months dosing of DRV (in PEG400) occurred at 0 (vehicle), 30, 60 and 120 mg/kg/day.[31,32] One control group and three treated groups were given, 0 (vehicle), 30, 60 and 120 mg/kg/day in a dose volume of 2 mL/kg, reduced to 1.33 mL/kg from week 11 to reduce the incidence of loose or liquid feces reported in all groups. Each group consisted of 4 male and 4 female Beagle dogs. The NOAEL was also set at 120 mg/kg/day in the 6 month study, based on the absence of relevant changes at this dose (see Tabulated Summary 2.6.7.7.E).

Darunavir (in PEG400) was also dosed once daily via oral gavage for **12 months** to dogs at 0 (vehicle), 30, 60 and 120 mg/kg/day in a dose volume of 1.33 mL/kg.[33,34] Each group consisted of 4 male and 4 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmoscopic examination, electrocardiography and clinical laboratory investigations (hematology, blood chemistry and urine analysis, week 13, 26 and 52). Blood samples were collected on day 1 and during week 13, 26, 39 and 52 during the 24 hours after dosing for toxicokinetic analysis. All animals found dead or

sacrificed during or at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all dose groups, including decedent animals and all gross lesions, were examined microscopically.

There were no mortalities associated with DRV, but one male given 60 mg/kg/day was sacrificed during week 20 as a result of traumatic injury. One female given the vehicle control was sacrificed during week 41 owing to complications in the intubation procedure. There was an increase in the incidence of vomiting in treated groups, particularly in animals given 60 and 120 mg/kg/day. There was no effect on body weight, food consumption, ophthalmoscopy or electrocardiology. In the laboratory investigations there was an increase (up to 45% in means) in ALP in animals given 120 mg/kg/day (2 male and 2 females) and 60 mg/kg/day (2 females). Hematological and urinalysis parameters were unchanged with DRV treatment.

Macroscopic examination at necropsy indicated no changes that could be attributable to treatment. However, liver weight was slightly higher in animals given 120 mg/kg/day (13 to 22%) and in females given 60 mg/kg/day (16%). Histopathological examination revealed increased hepatocellular pigment indicative of altered metabolic activity and vacuolation, in males and females at 60 and 120 mg/kg/day. Spleen weight of females given 120 mg/kg/day was decreased (36% relative weight) in the absence of any histopathological change. The NOAEL was considered to be 30 mg/kg/day based on the absence of any relevant liver enzyme and histopathological changes (See Tabulated Summary 2.6.7.7.F).

Toxicokinetic analysis indicated non-linear pharmacokinetics. Systemic exposure expressed as C_{max} and AUC values are given in Table 4 below.

Table 4: Darunavir mean C_{max} and AUC values after repeated oral administration (12 months) in dogs

TMC114 Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC[a] (µg.h/mL)	
		M	F	M	F
30	Day 1	10.0	9.89	23.0	17.9
	Week 52	11.5	9.11	31.6	21.2
60	Day 1	14.1	12.3	59.4	41.1
	Week 52	15.4	13.5	71.4	50.3
120	Day 1	16.6	15.0	69.4	53.4
	Week 52	27.8	23.9	130	100

[a] AUC_{∞} after single dose (day 1) or AUC_{last} after repeated dose

Besides the oral toxicity studies, a **2-week intravenous** toxicity study was performed in Beagle dogs.[35] Darunavir formulated in 15% HP-β-CD in pyrogen free water was administered once daily, by bolus intravenous injection. One control group and three treated groups were given, 0 (vehicle), 3.12, 6.25 and 12.5 mg/kg/day at a fixed concentration of 5 mg in dose volumes of 2.5, 0.625, 1.25 and 2.5 mL/kg, respectively. Each group consisted of 3 male and 3 female Beagle dogs. Detailed results of this study can be found in Tabulated Summary 2.6.7.6. The NOAEL was considered to be 12.5 mg/kg/day.

3.1.2.2. Darunavir Co-Administered with Ritonavir

Two sub-acute 14-day repeated dose toxicity studies with DRV and RTV were conducted in Beagle dogs. In the first study, the following combinations of DRV (in PEG400)/RTV (in PG) were applied: 0/10 (vehicle/RTV), 40/10, 120/10 and 360/10 mg/kg/day in a dose volume of 1 mL/kg for DRV and 0.2 mL/kg for RTV.[36,37] Each group consisted of 3 male and 3 female Beagle dogs. In the second study, higher RTV doses were applied and both DRV and RTV were given in capsules: 0/200 and 240/200 mg/kg/day. [38,39] In both studies no NOAEL could be established (See Tabulated Summary 2.6.7.6).

3.2. Cobicistat

Pivotal studies (4 to 26 weeks in duration) with COBI in rats were preceded by a 2-week range-finding study that compared the toxicity and toxicokinetic profile of COBI to RTV. In the 2- and 4-week studies, COBI was administered in a 15% SEDDS vehicle. For the 6-month study, COBI was administered in a vehicle of 95% PG, 5% EtOH, pH adjusted to 2.3. In order to evaluate the potential for unexpected or additive toxicities between COBI

and ATV, a 13-week combination toxicity study were conducted; the COBI/ATV study was preceded by a 5-day dose range-finding study.

The pivotal studies with COBI in dogs, from 4- to 39-weeks in duration, were preceded by a 7-day range-finding study that compared the toxicity and toxicokinetics of COBI with RTV. Cobicistat was administered in a vehicle of 95% PG, 5% EtOH, pH 2.3 in the pivotal dog studies.

3.2.1. Rats

3.2.1.1. Cobicistat Alone

Cobicistat or **RTV** was administered once daily via oral gavage for **14 days** to male Sprague-Dawley rats (5/group)[11]. They received the vehicle (15% SEDDS) or COBI ad dose levels of 30, 100, 175, and 300 mg/kg/day, or RTV vehicle (50% PG, 5% EtOH in water) or RTV at a dose level of 100 mg/kg/day. Due to excessive toxicity noted at 300 mg/kg/day COBI, a group of animals was administered 175 mg/kg/day starting on Day 7, and dosed for the remaining 8 days of the study. Satellite groups of animals (3#6/group) were assigned for toxicokinetic assessment (Tabulated Summary 2.6.7.6)[11,12]. The NOAEL for oral gavage administration of COBI to rats is 30 mg/kg/day for 14 days due to focal erosion/ulceration and edema of the nonglandular stomach seen at 100 mg/kg/day, and 175 mg/kg/day for 8 days. The NOAEL for oral gavage administration of RTV was 100 mg/kg/day for 14 days (see Tabulated Summary 2.6.7.6). The results of the CYP activity measurements can also be found in Module 2.6.4/Section 5.5.2.1.

A **4-week** oral gavage toxicity study with a **4-week recovery period** was also conducted in Sprague-Dawley rats (10/sex/group main study, 5/sex/group for recovery, 3 to 9/sex/group for toxicokinetics)[13]. Each group received dose preparations containing 0 (vehicle: 15% SEDDS), 10, 20, 50, or 100 mg/kg/day of COBI in a dose volume of 10 mL/kg. Based upon the urinalysis findings at 100 mg/kg/day, the NOAEL for this study was set at 50 mg/kg/day (Tabulated Summary 2.6.7.7.G).

Cobicistat formulated in 95% PG, 5% EtOH, pH 2.3 was also administered to male and female Sprague-Dawley rats for **26 weeks followed by a 13-week recovery period**[14]. Rats were assigned to 4 toxicity groups (10/sex/main study groups, plus 5/sex in vehicle/control and high dose groups for recovery) and administered COBI by oral gavage at doses of 0

(vehicle), 10, 30, and 100 mg/kg/day at a dose volume of 2.5 mL/kg/day. Toxicokinetic analysis was performed on satellite groups of animals on Day 1, and during Weeks 13 and 26. Analysis of COBI metabolite, GS-9612, was conducted on Day 1 and during Week 26.

Assessment of toxicity was based on mortality, clinical signs, body weight, food consumption, ophthalmic evaluation, immunophenotyping, hepatic enzyme analysis, hematology, coagulation, thyroid function, clinical chemistry, urinalysis, urine chemistry, hormone analysis (vasopressin and aldosterone), organ weights, and macroscopic and microscopic pathology examination.

There were 5 unscheduled deaths on the study. Deaths of one 10 mg/kg/day female and two 30 mg/kg/day males were considered incidental and not attributable to treatment with COBI. Although the clinical signs (recumbency, labored respiration, cold to the touch, pale ears in 1 female, and thin appearance, and irregular/audible respiration in the second female) observed prior to death in the two 100 mg/kg/day females were isolated occurrences, the relationship of these deaths to treatment cannot be discounted.

All remaining animals survived until the scheduled termination during Week 27 or Recovery Week 13.

Treatment-related clinical signs included clear oral discharge in 30 mg/kg/day males, and in both sexes at 100 mg/kg/day, and rough haircoat in both sexes at 100 mg/kg/day. Clinical signs during the recovery phase were similar to controls.

There were no treatment-related ophthalmic findings.

Significant decreases in mean body weight and mean body weight gain were observed in 100 mg/kg/day males during Weeks 6#26 of the dosing phase. By Week 26, mean body weight in males was reduced by 16% versus controls. Decreases in mean body weight were associated with decreases in food consumption. Significant increases in mean body weight gain were noted in 100 mg/kg/day males during the 13-week recovery period. In 100 mg/kg/day females, there were slight, but not statistically significant,

decreases in mean body weights (reduced by < 7% versus controls) and body weight gain (reduced by 12% versus controls) during the dosing period.

Slight, but generally statistically significant, changes in the hematology parameters that were attributed to COBI administration included slightly lower mean values for erythrocyte volume, and mean corpuscular hemoglobin values in 100 mg/kg/day rats at Weeks 13 and 26 and slightly higher mean platelet counts in 30 mg/kg/day males at Week 13 and in 100 mg/kg/day males and females at Weeks 13 and 26. After 13 weeks of recovery, the values for the aforementioned parameters were generally unremarkable and comparable between control and 100 mg/kg/day rats, indicating reversibility. There were no treatment-related effects on coagulation values.

Subtle changes in the serum chemistry data at Week 13 and/or 26 were not considered adverse. These changes included slightly, but generally statistically significant, higher mean values for gamma glutamyltransferase (GGT) at 100 mg/kg/day (both sexes), cholesterol at 30 and 100 mg/kg/day (females), total protein at 30 mg/kg/day (females) and 100 mg/kg/day (both sexes), albumin at 100 mg/kg/day (males), globulin at 30 and 100 mg/kg/day (females), and calcium at 100 mg/kg/day (both sexes). After the 13-week recovery period, the cholesterol and total protein values remained slightly, but significantly, higher in 100 mg/kg/day females, whereas the values for the rest of the aforementioned parameters were generally comparable to control values, indicating reversibility.

Test article effects in thyroid parameters included decreased thyroxine (T4) in 100 mg/kg/day males and dose-dependent increases in thyroid-stimulating hormone (TSH) in males and females (Table 5). The T4 values were consistently lower in 100 mg/kg/day males at each collection interval throughout the dosing phase, but were similar to the control mean at the end of the recovery period. Mean TSH values were consistently higher at each collection interval throughout the dosing phase (Weeks 4, 13, and 27) in 30 mg/kg/day females (ranging from 1.6- to 2.4-fold control) and 100 mg/kg/day animals (ranging from 2- to 3.4-fold in males and 4.3- to 5.4-fold control in females), and slightly higher at Week 27 in 10 mg/kg/day females (1.8-fold control) and at Weeks 13 and 27 in 30 mg/kg/day males (ranging from 1.4- to 1.5-fold control). The TSH values decreased in

100 mg/kg/day rats after the 13-week recovery period at which time the mean values were slightly lower in males and slightly higher in females relative to controls.

Table 5: Mean Thyroid Hormone Levels in Rats Following Daily Oral Dosing of COBI for 26 Weeks

Dose Level (mg/kg/day)	Sex	T4 (µg/dL)			TSH (µIU/mL)		
		Week 13	Week 26	Recovery Week 13	Week 13	Week 26	Recovery Week 13
0	Male	10.2	6.4	4.4	0.189	0.342	0.181
	Female	5.4	3.6	4.0	0.113	0.178	0.157
10	Male	9.5	6.8	NA	0.169	0.235	NA
	Female	5.6	3.7	NA	0.130	0.314[a]	NA
30	Male	9.4	6.7	NA	0.279	0.495	NA
	Female	5.7	3.4	NA	0.266[a]	0.330	NA
100	Male	5.6[a]	4.0[a]	4.3	0.652[a]	0.708[a]	0.090
	Female	5.3	3.1	3.3	0.562[a]	0.971[a]	0.428

NA = not analyzed

a $p \leq 0.05$

Significantly higher urine volume and significantly lower urine osmolality (with lower urine specific gravity) were observed in 100 mg/kg/day rats throughout the dosing period; similar changes, but of smaller magnitude, were also observed for these parameters in 30 mg/kg/day rats at Week 26. Sodium excretion values were higher primarily in 100 mg/kg/day rats during the dosing phase. Slightly lower urinary inorganic phosphorus, calcium, and potassium concentrations noted primarily at 100 mg/kg/day and slightly lower urinary creatinine at 30 and 100 mg/kg/day were consistent with the lower urine osmolality and higher urine volume. After the 13-week recovery period, the mean values for the aforementioned parameters in 100 mg/kg/day rats were comparable to those of controls, indicating reversibility. There were no apparent test article-related changes in vasopressin or aldosterone at Weeks 13 and 27.

There were no adverse effects on mean immunophenotyping values at Week 26. Administration of COBI produced slightly to minimally higher absolute total lymphocyte, total T cell, and B cell values for animals given 100 mg/kg/day and slight to minimal changes (lower relative percent T cells;

higher relative percent B cells) in relative T cell subsets (total, helper, and cytotoxic T cells) and B cell values for animals given 100 mg/kg/day and females given 30 mg/kg/day, when compared with controls. None of these changes were considered adverse and most values were similar to controls at the end of the recovery period.

Exposure to COBI increased with the increase in COBI dose level from 10 to 100 mg/kg/day (Table 6). Exposures were higher in females as compared to males. In general, no accumulation of COBI was observed after multiple dosing.

Table 6: Mean Toxicokinetic Parameters of COBI in Rats Following Daily Oral Dosing for 26 Weeks

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)			C_{max} (µg/mL)		
		Day 1	Week 13	Week 26	Day 1	Week 13	Week 26
10	Male	0.4	0.7	2.1	0.1	0.2	0.8
	Female	2.1	2.0	3.5	0.9	0.8	1.3
30	Male	5.7	8.2	9.9	1.7	1.4	1.9
	Female	14.0	10.4	13.3	2.8	2.1	4.0
100	Male	32.7	29.9	47.2	3.7	3.8	4.4
	Female	42.9	51.6	71.4	5.0	5.7	7.7

Exposure to metabolite, GS-9612 (M31), generally increased with the increase in COBI dose level from 10 to 100 mg/kg/day. No consistent sex differences were observed in GS-9612 C_{max} and AUC_{0-t} values (Table 7). Potential accumulation of GS-9612 was observed after multiple dosing of COBI in rats. The percent C_{max} and AUC_{0-t} metabolite to parent ratios ranged from 1.6% to 4.1%, indicating that COBI is not extensively converted to GS-9612 in rats following oral gavage administration of COBI.

Table 7: Mean Toxicokinetic Parameters of Metabolite GS-9612 in Rats Following Daily Oral Dosing of COBI for 26 Weeks

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
		Day 1	Week 26	Day 1	Week 26
10	Male	0.014	0.075	0.005	0.022
	Female	0.042	0.081	0.022	0.021
30	Male	0.152	0.289	0.038	0.055
	Female	0.252	0.266	0.054	0.093
100	Male	0.698	1.405	0.151	0.143
	Female	0.678	2.251	0.098	0.269

The results of the CYP activity measurements can be found in Module 2.6.4/Section 5.5.2.1.

Test article-related increases in thyroid with parathyroid weights (relative to body weight) and liver weights (absolute and relative) were observed at 30 and 100 mg/kg/day.

At terminal necropsy, COBI-related histopathologic changes were present in the thyroid and liver (Table 8). In the thyroid, follicular cell hypertrophy was present in most animals at 100 mg/kg/day and in 1 female at 10 and 30 mg/kg/day. In addition, 1 male at 100 mg/kg/day had a follicular cell carcinoma in the thyroid. In the liver, a dose-related increase in centrilobular to midzonal hepatocellular hypertrophy was present in all groups given COBI. Following 13 weeks of post-treatment recovery, follicular cell hypertrophy in the thyroid and centrilobular to midzonal hepatocellular hypertrophy in the liver resolved fully. These liver and thyroid effects are considered to be nonadverse, adaptive changes, which have been frequently reported in rodents exposed to microsomal enzyme inducers and are considered secondary to microsomal enzyme induction and to the thyroid hormone changes[2,4,5,6]. These changes were not considered adverse under the conditions of this study.

Table 8: Notable Liver and Thyroid Microscopic Observations in Rats Following Daily Oral Dosing of COBI for 26 Weeks

	Male				Female			
Dose Level (mg/kg/day)	**0**	**10**	**30**	**100**	**0**	**10**	**30**	**100**
Thyroid Follicular Cell Hypertrophy								
Occurrence/Number Examined	0/10	0/10	0/9	10/10	0/10	1/8	1/10	7/8
Thyroid Follicular Cell Carcinoma								
Occurrence/Number Examined	0/10	0/10	0/9	1/10	0/10	0/8	0/10	0/8
Liver Hepatocellular Hypertrophy								
Occurrence/Number Examined	0/10	5/10	9/9	10/10	0/10	7/9	10/10	8/8

In conclusion, based on significant decreases in body weight and food consumption in males, slight changes in hematological parameters, and increases in urine volume at 100 mg/kg/day, the NOAEL for COBI when given daily via oral gavage to male and female rats for at least 26 weeks followed by a 13-week recovery period is 30 mg/kg/day (Week 26 AUC_{0-t} = 9.9 and 13.3 µg.h/mL, in males and females, respectively) (Tabulated Summary 2.6.7.7.H).

3.2.1.2. Cobicistat Co-Administered with Atazanavir

A **dose range finding study** of COBI alone or in combination with atazanavir (ATV) was conducted in female Sprague-Dawley rats[15,16]. Rats were dosed once daily during 5 days with COBI and ATV (co-formulated), at dose levels of 0 (vehicle: 95% PG, 5% EtOH), 50 mg/kg/day COBI, 150 mg/kg/day ATV, COBI/ATV 30/100 mg/kg/day, and COBI/ATV 50/150 mg/kg/day in a dose volume of 5 mL/kg. The results of this study are displayed in detail in Tabulated Summary 2.6.7.6.

The toxicity of COBI and ATV was evaluated when administered daily either alone or in combination by oral gavage to rats for **13 weeks** and assessed the reversibility, persistence, or delayed occurrence of any effects after a 4-week recovery phase[17] (Tabulated Summary 2.6.7.7.I).

Male and female Crl:CD(SD) rats were assigned to 6 toxicity groups (10/sex/group main study, 5/sex/group for recovery) and administered COBI at 0 and 30 mg/kg/day, ATV at 20 and 50 mg/kg/day, and COBI and ATV coformulated at 30/20 and 30/50 mg/kg/day COBI/ATV. Animals were dosed once daily via oral gavage in a dose volume of 2 mL/kg in a vehicle of 95% PG, 5% EtOH. Blood samples were collected from satellite animals for toxicokinetic analysis on Day 1 and Day 90.

Assessment of toxicity was based on survival, clinical observations, body weight, food consumption, ophthalmic examinations, clinical and anatomic pathology data, and hepatic enzyme activity.

One control male was found dead on Day 8 of the recovery phase. There were no previous clinical signs and the cause of death was undetermined. The remaining animals survived until their scheduled sacrifice.

There were no adverse treatment-related effects on body weight, body weight gain, food consumption, or ophthalmic examinations.

The clinical pathology data were generally unremarkable and similar among the groups. Slightly higher cholesterol (up to 1.7-fold control values) was observed on Day 93 primarily in females given COBI and ATV alone, and in combination at both dose levels. The urine volume was slightly higher in males given either 30 mg/kg/day COBI, 50 mg/kg/day ATV, or in combination at 30/20 or 30/50 mg/kg/day COBI/ATV and in females given both test articles in combination at 30/20 and 30/50 mg/kg/day COBI/ATV. The mean cholesterol value for females given 30/50 mg/kg/day COBI/ATV was essentially unchanged at the end of the recovery phase and the urine volume values were similar among the groups, indicating the reversibility with cessation of dosing.

There were no treatment-related macroscopic findings. Slight, though not generally statistically significant, increases in absolute and relative mean liver weight values were noted for each male and female group given 30 mg/kg/day of COBI, either alone or in combination with ATV. This finding, though not accompanied by a histomorphologic correlate, was consistent and likely occurred in direct response to COBI, possibly as a manifestation of liver microsomal P450 enzyme induction. After the 4-week recovery phase, liver weights in COBI/ATV male groups were comparable

to control, and remained slightly elevated in females administered 30/50 mg/kg/day COBI/ATV.

No remarkable accumulation of COBI was observed after multiple dosing at 30 mg/kg/day (Table 9). Coadministration of 30 mg/kg/day COBI with ATV at 20 and 50 mg/kg/day resulted in decreases in COBI exposures (AUC_{0-t}) of 40%–50% and 63%–71% on Day 1, and 12%–58% and 43%–50% on Day 90, respectively.

Table 9: Mean Toxicokinetic Parameters of COBI in Rats Following Oral Doses of COBI and ATV for 13 Weeks

Dose Level (mg/kg/day)			AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
COBI	**ATV**	**Sex**	**Day 1**	**Day 90**	**Day 1**	**Day 90**
30	0	Male	6.9	10.8	1.2	3.0
		Female	12.9	12.8	2.0	2.6
30	20	Male	4.2	4.6	0.8	0.9
		Female	6.4	11.2	0.8	2.1
30	50	Male	2.6	6.1	0.5	1.2
		Female	3.8	6.3	0.4	1.7

Exposure to ATV increased with the increase in ATV dose level from 20 to 50 mg/kg/day. Slight accumulation (2.3- to 2.7-fold) of ATV was observed after multiple dosing at 20 mg/kg/day (Table 10). Coadministration of ATV at 20 mg/kg/day with 30 mg/kg/day COBI, resulted in 5.4- to 11.3-fold and 2.6- to 3.9-fold increases in exposure (AUC_{0-t}) on Day 1 and Day 90, respectively, compared to ATV administration at 20 mg/kg/day alone. Coadministration of ATV at 50 mg/kg/day with 30 mg/kg/day COBI resulted in 2.7- to 4.2-fold and 1.5- to 2.9-fold increases in exposure on Day 1 and Day 90, respectively, compared to ATV administration at 50 mg/kg/day alone.

Table 10: Mean Toxicokinetic Parameters of ATV in Rats Following Oral Doses of COBI and ATV for 13 Weeks

Dose Level (mg/kg/day)		Sex	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
COBI	ATV	Day 1	Day 1	Day 90	Day 1	Day 90
0	20	Male	2.9	7.9	1.1	1.3
		Female	5.0	11.7	1.0	2.4
0	50	Male	12.0	15.4	2.5	3.3
		Female	17.6	31.6	3.2	5.2
30	20	Male	32.9	30.8	4.1	3.0
		Female	27.0	30.6	2.5	3.9
30	50	Male	50.4	44.3	5.8	6.8
		Female	48.0	45.9	3.6	7.8

The results of the hepatic enzyme activity measurements can be found in Module 2.6.4/Section 5.5.2.1.

In conclusion, the NOAEL following at least 90 days of once daily oral gavage dosing of COBI and ATV, either alone or in combination is considered to be 30 mg/kg/day COBI and 50 mg/kg/day ATV. At the 30/50 mg/kg/day COBI/ATV dose, systemic exposures on Day 90 were: COBI AUC_{0-t}: 6.1 and 6.3 µg.h/mL in males and females, respectively; ATV AUC_{0-t}: 44.3 and 45.9 µg.h/mL in males and females, respectively

3.2.2. Dogs

A **7-day repeated dose** toxicity and toxicokinetics study was conducted with COBI or RTV in male beagle dogs (3/group). Therefore dogs were dosed once daily via oral gavage with COBI at 0 ((94.4% PG, 5% EtOH, acidified with 1.5 molar equivalents methane sulfonic acid), 50, 125 and 250 mg/kg/day or with RTV at 125 mg/kg/day days[18,19]. No NOAEL could be determined due to the poor tolerance at all dose levels (see Tabulated Summary 2.6.7.6).

Daily oral administration of COBI via oral gavage was also performed in a **4-week study with 4 weeks recovery**[20]. Male and female dogs received COBI at 0 (vehicle), 5, 15, or 45 mg/kg at a dose volume of 2 mL/kg. On Day 11 due to body weight losses and thin appearance, treatment was stopped for females at 45 mg/kg/day, and re-initiated on Day 14 at

30 mg/kg/day for 14 days. The NOAEL was set at 15 mg/kg/day based on the observations in the study (see Tabulated Summary 2.6.7.7.J). The results of the CYP activity measurements can also be found in Module 2.6.4/Section 5.5.2.1.

Cobicistat has also been dosed during **39 weeks** (with **interim kill after 13 weeks**) followed by a **13-week recovery period**[21].

Male and female purebred beagles were assigned to 4 groups (9/sex/group in control and high dose groups; 7/sex/group in low and mid-dose groups), and administered the vehicle [95% PG and 5% EtOH with 1.5 eq. HCl (pH 2.3)], or 5, 10, and 20 mg/kg/day COBI in a dose volume of 2 mL/kg. Three animals/sex/group were designated for interim sacrifice and were terminated following at least 13 weeks of dose administration. The remaining animals were dosed for a total of 39 weeks, and 4/sex/group were designated for terminal sacrifice. Two animals/sex/group in control and high dose groups underwent 13 weeks of recovery after the last dose administration.

Assessment of toxicity was based on mortality, clinical signs, body weights, food consumption, ophthalmic and electrocardiogram (ECG) examinations, blood pressure measurements, immunophenotyping, hematology, coagulation, thyroid function, clinical chemistry, urinalysis, urine chemistry, organ weights, and macroscopic and microscopic pathology examination. Plasma samples for toxicokinetic analysis of COBI were collected on Day 1, and during Weeks 13 and 39, and on Day 1 and during Week 39 for analysis of metabolite, GS-9612.

For the first 9 weeks of study, dogs were housed individually for at least 6 hours each day and offered food and water ad libitum unless fasted for study procedures. Dogs were commingled overnight without access to food. There were no changes to these procedures for control, low-dose and mid-dose animals during the dosing period. Because of increased incidence of emesis and the significant decrease in body weights in the high-dose group, the commingling schedule was altered for high-dose animals to allow continuous access to food overnight beginning at Week 10 of study. High-dose dogs were housed individually overnight, then commingled, and fasted for at least 2 hours before dosing.

All animals survived to their scheduled sacrifice. Dose-related clinical signs included salivation, emesis, and fecal changes (discolored, liquid, mucoid and/or nonformed) in both sexes at 20 mg/kg/day. Emesis and salivation were primarily observed within 1 hour of dosing. The incidence of emesis and fecal changes was similar between controls and animals administered 5 and 10 mg/kg/day COBI. There were no notable clinical signs during the 13-week recovery period.

Decreases in mean body weight were observed beginning Week 3 at 20 mg/kg/day, and were generally statistically significant up to Week 14 for males and females, with corresponding decreases in food consumption. Slight, but not significant decreases in mean body weight and body weight gains were also observed in both sexes after Week 5 at 10 mg/kg/day. Mean body weight for 20 mg/kg/day dogs remained slightly lower than controls throughout the dosing phase. At Week 40, mean body weights were 88% and 90% of male controls, and 92% and 92% of female controls at 10 and 20 mg/kg/day, respectively. Mean body weights for 20 mg/kg/day dogs increased during the recovery phase compared to slight decreases noted in control animals, indicating reversibility of the body weight change.

No COBI-related effects were observed in blood pressure measurements, ophthalmic or ECG data, or in peripheral blood immunophenotyping.

No COBI-related adverse effects were observed in hematology, coagulation, clinical chemistry, thyroid function, urinalysis, or urine chemistry parameters. Subtle, nonadverse, reversible COBI-related changes included slightly higher mean platelet counts in 20 mg/kg/day dogs during Weeks 13, 26, and 39 of the dosing phase, and in 10 mg/kg/day females at Week 26. Platelet counts were similar between control and 20 mg/kg/day dogs at recovery sacrifice, indicating the reversibility of this change. Slightly higher ALP activity in both sexes, and slightly lower mean values for total protein and albumin (in males only) were noted at 20 mg/kg/day throughout the dosing phase (Weeks 13, 26, and 39). No meaningful differences were observed between control and 20 mg/kg/day dogs at recovery sacrifice.

Slightly higher urine volume, lower urine osmolality and lower urine specific gravity, observed in a few females at 20 mg/kg/day during the dosing phase (primarily at Weeks 13 and 26) resulted in slightly to moderately lower urinary sodium, potassium, chloride, calcium, and inorganic phosphorus

concentrations and excretion rates. The remaining urinalysis results were generally unremarkable, with the exception that there was a greater incidence of bilirubinuria in males at 20 mg/kg/day during the dosing phase. No meaningful differences were observed between control and 20 mg/kg/day dogs at recovery sacrifice.

No COBI-related gross pathologic findings were observed at any sacrifice interval. At the 13-week interim sacrifice, decreased thymus weights and minimally increased adrenal weights in 20 mg/kg/day males corresponded microscopically with minimal to moderate thymic involution and minimal cortical cell hypertrophy, respectively. These thymic and adrenal effects were considered indirect test article effects as they are consistent with a generalized high dose stress response; they were absent in terminal-sacrifice animals except for a slight, not statistically significant, reduction in thymus weights.

At the terminal sacrifice, dose-related minimal increases in liver-to-body weight and liver-to-brain weight values were noted in both sexes at 10 and 20 mg/kg/day. Mean absolute liver weights were also slightly increased in females at 10 and 20 mg/kg/day. Increases in liver weight parameters corresponded with minimal hepatocellular hypertrophy in males at 10 mg/kg/day, and in both sexes at 20 mg/kg/day. The hepatic changes were considered an adaptive response and not adverse based on the minimal severity, the absence of degeneration, and reversibility after cessation of dosing. Males at 10 and 20 mg/kg/day exhibited dose-dependent, but not statistically significant, decreases in thymus weight parameters; the decreased thymus weights were not considered adverse because they were of relatively small magnitude and lacked histologic correlates. There were no additional COBI-related microscopic findings, and no evidence of COBI-related effects was observed at recovery sacrifice.

Exposure to COBI increased with the increase in COBI dose level from 5 to 20 mg/kg/day (Table 11). Increases in mean C_{max} and AUC_{0-t} were generally greater than dose proportional. In general, no marked (> 2-fold) sex differences were observed in COBI C_{max} and AUC_{0-t} values. Accumulation of COBI was observed in dog plasma after multiple dosing of COBI. Exposure to metabolite GS-9612 (M31) generally increased with the increase in COBI dose level from 5 to 20 mg/kg/day (Table 12). No consistent sex

differences were observed in GS-9612 C_{max} and AUC_{0-t} values. Accumulation of GS-9612 was observed in dog plasma after multiple dosing of COBI. The percent metabolite to parent ratios for C_{max} and AUC_{0-t} ranged from 1.3% to 9.7%, indicating that COBI is not extensively converted to GS-9612 in dogs following oral gavage administration of COBI.

Table 11: Mean Toxicokinetic Parameters of COBI in Dogs Following Daily Oral Doses of COBI for up to 39 Weeks

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)			C_{max} (µg/mL)		
		Day 1	Week 13	Week 39	Day 1	Week 13	Week 39
5	Male	0.3	5.9	5.3	0.1	1.3	1.0
	Female	0.1	3.0	9.1	0.04	0.7	1.7
10	Male	5.3	22.2	19.6	1.1	3.4	2.6
	Female	1.1	15.9	16.8	0.3	2.5	2.4
20	Male	26.4	39.7	71.3	3.7	4.5	7.1
	Female	30.8	75.9	99.7	3.7	6.5	8.4

Table 12: Mean Toxicokinetic Parameters of Metabolite GS-9612 in Dogs Following Daily Oral Doses of COBI for up to 39 Weeks

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
		Day 1	Week 39	Day 1	Week 39
5	Male	0.021	0.118	0.009	0.025
	Female	0.009	0.239	0.004	0.043
10	Male	0.181	0.257	0.045	0.033
	Female	0.052	0.296	0.015	0.042
20	Male	1.084	1.534	0.174	0.114
	Female	0.822	2.796	0.101	0.285

In conclusion, COBI was well tolerated when administered daily by oral gavage to dogs at dose levels up to 10 mg/kg/day for up to 39 weeks. In males and females administered 20 mg/kg/day for 39 weeks, clinical signs (salivation, emesis, fecal changes), decreases in body weight and food consumption, nonadverse changes in hematology, clinical chemistry, urinalysis, and urine chemistry, and minimal, adaptive changes in the liver (increased weights, hypertrophy) were noted. Based on these findings, the

NOAEL for COBI when administered daily by oral gavage to dogs for up to 39 weeks is 10 mg/kg/day (Week 39 AUC_{0-t}: 19.6 and 16.8 µg.h/mL in males and females, respectively) (Tabulated Summary 2.6.7.7.K).

4. GENOTOXICITY

4.1. Darunavir

Genotoxicity tests included in vitro mutation assays (Ames test), an in vitro clastogenicity or chromosome aberration assay (human lymphocytes) both with and without a liver metabolic activation system and an in vivo clastogenicity assay (mouse bone marrow micronucleus test up to a dose of 2000 mg/kg).

4.1.1. In Vitro Nonmammalian Cell System

Darunavir formulated in dimethylsulfoxide (DMSO), was tested in a classical bacterial reverse mutation assay (Ames test), with 4 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and in the *Escherichia coli* reverse mutation assay with the tryptophan-requiring strain WP_2uvrA[40]. Following a dose-range finding test, DRV was tested in 2 independent assays, in the 4 tester strains of *Salmonella typhimurium* and in tester strain WP_2uvrA of *Escherichia coli*, in the absence or presence of S9-metabolic activation (Aroclor-induced rat liver S9-mix) up to 3330 µg/plate. Darunavir precipitated on the plates at the highest dose level tested but the bacterial background lawn was not reduced at any of the concentrations tested and no decrease in the number of revertants was observed. Darunavir did not induce a dose-related, more than two-fold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9-metabolic activation. These observations were confirmed in 2 independently repeated experiments. The vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. It was concluded that DRV was not mutagenic under the conditions of this assay (See Tabulated Summary 2.6.7.8.A).

4.1.2. In Vitro Mammalian Cell System

In the in vitro clastogenicity assay, the effect of DRV formulated in DMSO was tested on the induction of chromosome aberrations in cultured peripheral human lymphocytes, in the absence or presence of S9-metabolic activation system (Aroclor-induced rat liver S9-mix)[41]. In the absence of S9-mix DRV was tested at concentrations up to 333 µg/mL for a 3h treatment time with a

24h fixation time, up to 180 µg/mL for a 24h treatment time with a 24h fixation time, and up to 240 µg/mL for a 48h treatment time with a 48h fixation time. In the presence of S9-mix DRV was tested at concentrations up to 333 µg/mL for a 3h treatment time with a 24h fixation time and in a second experiment, with a 48h fixation time. Darunavir did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations in the absence or presence of S9-mix, in 2 independently repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was concluded that DRV was not clastogenic in human lymphocytes under the conditions of this assay (See Tabulated Summary 2.6.7.8.B).

4.1.3. In Vivo Mammalian System

To evaluate the genotoxic effect on erythrocytes in bone marrow, DRV formulated in PEG400, was administered to mice in an in vivo micronucleus test[42]. Single oral (gavage) doses of 0 (vehicle), 2000 mg/kg DRV and 50 mg/kg cyclophosphamide (positive reference article) were administered to NMRI BR mice (5 males/time point) and bone marrow was sampled 24h (vehicle control and DRV) or 48h (DRV and positive control) after dosing. No toxicokinetic evaluations were performed in this study. However, based on the toxicokinetic data from another study[1] where mice were dosed at 1000 mg/kg, an $AUC_{0-\infty}$ of approximately 200 µg.h/mL can be expected. No increase in the frequency of micronucleated cells was observed in the polychromatic erythrocytes of the bone marrow of animals tested with DRV. There was no effect on the ratio of polychromatic to normochromatic erythrocytes compared to vehicle controls. The vehicle control and the positive reference articles confirmed the adequacy of the test system. It was concluded that DRV was not genotoxic under the conditions of this assay (See Tabulated Summary 2.6.7.9.A).

4.2. Cobicistat

4.2.1. In Vitro Nonmammalian Cell System

The genotoxicity of COBI has been tested using a bacterial mutation test (Tabulated Summary 2.6.7.8.C)[22]. *Salmonella typhimurium* strains (TA1535, TA1537, TA98, TA100) and *Escherichia coli* strain WP2 *uvrA* were treated with the test article at a range of concentrations up to 5000 µg/plate (the standard limit dose for this assay) in the presence and absence of a

supplemented liver fraction (S9 mix) using the plate incorporation and preincubation versions of the bacterial mutation test.

Bacteria were incubated with standard positive control agents, and the response of the various bacterial strains to these agents confirmed the sensitivity of the test system and the activity of the S9 mix.

No substantial increases in the revertant colony counts were obtained with any strain following exposure to the test article in either the plate incorporation or preincubation assay in the absence or presence of S9 mix. It is concluded that COBI did not show evidence of genotoxic activity in this in vitro mutagenicity assay.

4.2.2. In Vitro Mammalian Cell System

A mouse lymphoma forward mutation assay has been conducted to evaluate the genotoxicity of COBI[23] (Tabulated Summary 2.6.7.8.D).

A preliminary toxicity test (concentrations of 0.980–3100 μg/mL) was used to determine concentrations for the main test (6.87–80 μg/mL). In the main test, mouse lymphoma L5178Y $TK^{+/-}$ cells were incubated with the vehicle, test article or positive control for 3 hours (with and without metabolic activation) or 24 hours (without metabolic activation, only). Following a subsequent 48-hour growth recovery period, the cells were transferred to selective medium which allowed only the growth of mutant cells into colonies.

After a suitable growth period in selective medium, the number of wells containing colonies was evaluated. The mutation frequency for each treatment was calculated taking into account the viability of the treated cells. Responses to the positive controls confirmed the sensitivity of the assay and the activity of the S9 mix.

No substantial increases in mutation frequency (neither in small or large colonies) were observed after treatment of cells with COBI at dose levels up to the limit of toxicity. It is concluded that COBI did not show evidence of genotoxicity at the thymidine kinase locus in L5178Y mouse lymphoma cells.

4.2.3. In Vivo Mammalian System

The purpose of this study was to evaluate COBI for in vivo clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in immature erythrocytes in Sprague-Dawley (Hsd:SD$^{®}$) rat bone marrow[10] (Tabulated Summary 2.6.7.9.B).

In the dose range-finding assay, COBI was administered by oral gavage to male rats at 750 and 850 mg/kg (3/group) and to female rats at 500 and 750 mg/kg (3/group) in a vehicle [95% PG and 5% EtOH with 1.5 eq HCl] at a dose volume of 10 mL/kg. Animals were observed for up to 2 days for toxic signs and/or mortality. Based on toxicity observed in the range-finding study, the definitive micronucleus assay was conducted in males and females with a high dose of 850 and 500 mg/kg for males and females, respectively.

In the definitive micronucleus test, COBI was administered once orally to male rats at 0, 212.5, 425, and 850 mg/kg, and to female rats at 0, 125, 250, and 500 mg/kg. The positive control, cyclophosphamide, was administered orally to 3 males and 3 females at 20 mg/kg. One high dose 850 mg/kg male was found dead prior to scheduled sacrifice on Day 3. Animals were sacrificed at 24h (all dose groups; 5 animals/group) and at 48h (vehicle and high dose groups; 5 animals/group). Bone marrow smears were fixed, stained with acridine orange and examined under code using fluorescence microscopy. A total of 2000 immature erythrocytes per animal were analyzed for the frequency of micronuclei, indicative of chromosome damage. In addition, the proportion of immature erythrocytes was assessed for each animal as a measure of bone marrow toxicity.

Clinical signs of toxicity were observed in mid- and high-dose COBI treated animals, including decreased activity, partly closed eyes, soft feces, staining of fur, piloerection, and shallow breathing.

Cobicistat did not induce statistically significant increases in micronucleated immature erythrocytes at any dose level. A significant decrease in the proportion of immature erythrocytes was obtained at the 24h sampling time; however, since no related decrease was seen at the 48h time point and all values were well within the historical control range, the apparent decrease at 24 hours was probably the result of normal experimental variation. Based on the mean incidence of micronucleated immature erythrocytes in the vehicle control and the significant increase in micronucleated immature erythrocytes

in the positive control, and that the highest test article dose approximated the MTD, the criteria for a valid assay was achieved.

Analysis of plasma samples from satellite animals confirmed exposure to COBI. Plasma levels at 1h postdose (approximately T_{max}) were in the range of 4.4 to 8.3 μg/mL (for males), and 4.2 to 9.1 μg/mL (for females).

It is concluded that a single oral administration of COBI up to a dose of 850 mg/kg in males and 500 mg/kg in females did not show evidence of genotoxic activity in this in vivo test for induction of chromosome damage. Cobicistat was concluded to be negative in the rat bone marrow micronucleus assay under the conditions of this study.

5. CARCINOGENICITY

5.1. Darunavir

5.1.1. Long Term Studies

5.1.1.1. Mouse Studies

In **a 2-week** study[43], DRV (in PEG400) was given once daily by oral gavage at 0 (vehicle), 50, 150, 450 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 5 male and 5 female CD-1 mice. The NOAEL was not established in this preliminary study but at 50 or 150 mg/kg/day, the changes were minimal (See Tabulated Summary 2.6.7.10).

Furthermore a **3-month** repeated dose study[44] was conducted in which mice received once daily oral doses of DRV (in PEG400) at 0 (vehicle), 150, 450 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 10 male and 10 female CD-1 mice (principal animals) together with 30 male and 30 female satellite animals (except for the vehicle group). No NOAEL was established in this study, and detailed data can be found in Tabulated Summary 2.6.7.10.

Based on these studies, the dose levels selected for the **2 year oral (gavage) carcinogenicity study in mice** were selected. Therefore DRV formulated in PEG400 was administered once daily by oral gavage for 24 months at 0 (vehicle 1), 0 (vehicle 2), 150, 450 or 1000 mg/kg/day[45]. Each group consisted of 65 male and 65 female CD1 mice (main animals) along with 15 male and 15 female satellite animals (treated groups only) or 9 male and 9 female animals (vehicle groups) for toxicokinetic analysis. Regular

observations were made for mortality, clinical signs, body weight and food consumption. Ophthalmic examinations in the first 20 surviving males and females (at predose, 13 and 24 months postdose) and clinical laboratory investigations in all mice (hematology and blood chemistry at the end of the study except for preterminated animals) were performed. A full necropsy was performed on all main animals, including those found dead or sacrificed moribund during the study, and all macroscopic changes were recorded. A number of organs were weighed and several tissues and organs were sampled and preserved for histological examination. Blood samples were collected after dosing for toxicokinetic analysis on Days 32 and 188.

Darunavir treatment was well tolerated. When compared with vehicle-dosed animals, a treatment-related increase in mortality was only seen in male mice dosed at 1000 mg/kg/day towards the end of the study. This led to termination of this group in Week 97 when survival reached 15 animals. In main animals, 38, 40, 41, 48, and 50 males and 37, 38, 34, 37, and 41 females, given vehicle 1, vehicle 2, 150, 450 and 1000 mg/kg/day respectively, were found dead or were sacrificed prematurely during the study. The principal cause of death/moribundity, common to all groups at similar incidences, including vehicle groups, were amyloidosis and hemolymphopoietic tumors. Treatment-related mortalities were reported in the high-dose group and were limited to 5 males, which died of atrial thrombosis and 10 males, which died of liver tumors. There were no relevant changes in clinical signs or in ophthalmic examination. No significant effects were seen in body weight and food consumption.

Changes in hematology consisted of minimal decreases in erythron parameters (red blood cells, hemoglobin and hematocrit) mainly in females and mean cell hemoglobin in males at 1000 mg/kg/day. Increases in thrombocyte (23%) and reticulocyte (29%) counts were seen in high dose females only. ALT (up to 2-fold in males and up to 5-fold in females), cholesterol (up to 2-fold in males and up to 3-fold in females), blood urea nitrogen (BUN, up to 2-fold in males), inorganic phosphorus (up to 27% in males) and bilirubin (up to 85% in females) increased at 450 and 1000 mg/kg/day. AST (3-fold) was also increased in high dose females.

Macroscopic examination and organ weights indicated an increase in liver weight (up to 73%) at all doses except in females given 150 mg/kg/day and

in kidney weight (7%) in females given 1000 mg/kg/day. The organ weight increases were accompanied by the presence of nodules, masses, swelling and/or discoloration.

Microscopically, liver changes consisted of an increase in the incidence of hepatocellular adenomas at 150 (8/65 males, 1/64 females), 450 (16/65 males, 8/65 females) and at 1000 mg/kg/day (38/62 males, 16/61 females) and carcinomas at 150 (3/65 males, 1/64 females), 450 (5/62 males, 1/63 females) and at 1000 mg/kg/day (7/62 males, 3/61 females). An increase in the incidence and severity of hepatocellular hypertrophy (all treated mice) and pigmented hepatocytes/macrophages (all treated mice except low dose female group) was also observed. Oval cell hyperplasia was also noted in males from 450 mg/kg/day. The liver findings correlated with the macroscopic findings, organ weight and clinical chemistry changes (ALT, AST, ALP, GGT, cholesterol, bilirubin and BUN) and are considered to be the result of liver enzyme induction and/or alterations in lipid metabolism.

The heart showed a moderate increase in atrial thrombosis from 450 mg/kg/day in males and at 1000 mg/kg/day in females. This has been reported to occur frequently in untreated mice of this strain. Vascular mineralization was observed in the heart, pancreas, tongue, seminal vesicles, epididymes and kidney; mineralization was also seen in the vibrissae and in the sclera of the eyes in high-dose males and/or females and correlated to the increases in inorganic phosphorus. Nephrosis with tubular pigmentation was seen from 450 mg/kg/day in a limited number of animals and was linked to the increase in kidney weight. In the spleen and the mesenteric lymph nodes, minor increases in hemopoiesis in high-dose males and/or females were correlated to changes in the erythron parameters. Given the hepatocellular changes seen at the lowest tested dose (150 mg/kg/day), a NOAEL was not established in this study. Systemic exposure expressed as C_{max} and AUC values are given in Table 13 below. For detailed results, see Tabulated Summary 2.6.7.10.A.

Table 13: Darunavir C_{max} and AUC Values After Repeated Oral Administration (24 Months) in Mice

TMC114 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M	F	M	F
150	Day 32	2.33	3.74	4.33	7.47
	Day 188	3.36	3.72	6.23	7.79
450	Day 32	4.94	7.89	22.0	41.8[a]
	Day 188	3.12	5.97	14.8	40.8
1000	Day 32	5.88	8.52	34.4	56.9
	Day 188	10.1	10.7	48.1	63.8

[a] AUC_{0-8h}

5.1.1.2. Rat Studies

For the rat carcinogenicity study, DRV formulated in PEG400 was administered once daily by oral gavage for 24 months[46].Two control (PEG400) and 3 treated groups were given 0 (vehicle 1), 0 (vehicle 2), 50, 150 or 500 mg/kg/day. Each group consisted of 70 male and 70 female Sprague-Dawley rats (main animals) along with 6 male and 6 female satellite animals (treated groups only) or 3 male and 3 female animals (vehicle group) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption. Ophthalmic examinations were performed on the first 20 surviving animals in the high-dose and the two vehicle groups at predose and 6, 12 and 24 months postdose. Clinical laboratory investigations (hematology, coagulation, blood chemistry and urine analysis) were carried out at 12 and 24 months postdose. A full necropsy was performed on all main animals, including those found dead or sacrificed moribund during the study, and all macroscopic changes were recorded. A number of organs were weighed and several tissues and organs were sampled and preserved for histological examination. Blood samples were collected on Days 31 and 184 after dosing for toxicokinetic analysis.

Darunavir treatment was well tolerated. When compared to vehicle-dosed rats, no treatment related increase in mortality was seen at any dose level; 44, 47, 39, 38, and 42 males and 49, 45, 49, 42, and 43 females, given vehicle 1, vehicle 2, 50, 150 or 500 mg/kg/day respectively were found dead or were sacrificed pre-terminally. Treatment-related mortality was limited to 1 male rat given 500 mg/kg/day which died of hepatocellular carcinoma, and 10 female rats given 150 (3) or 500 mg/kg/day (7), which died because of CPN. In addition, 427 animals were found dead or were sacrificed prematurely

during the study. The majority of these deaths were related to gavage accidents (37, 43, 35, 24 and 23 animals given 0, 0, 50, 150 or 500 mg/kg/day respectively), pituitary tumors (25, 25, 26, 25 and 16 animals given 0, 0, 50, 150 or 500 mg/kg/day respectively), or to coagulopathy (3, 0, 3, 12 and 15 animals given 0, 0, 50, 150, 500 mg/kg/day respectively). The cause of death was not evident in 14, 8, 14, 6 and 5 animals given vehicle 1, vehicle 2, 50, 150 or 500 mg/kg/day, respectively. Clinical signs included a dose-related increase in excessive salivation in all treated animals throughout the study. Decreases in soft feces from 50 mg/kg/day in males and from 150 mg/kg/day in females were also reported but the toxicological relevance of this finding is considered doubtful. Increases in the incidence of observations related to bleeding from 50 mg/kg/day were recorded in males (blood on the tray, the fur or the saliva) and in females (blood in saliva). These findings were mainly noted during the earlier phase of the study (up to the 5th month of dosing) and were related to an abnormally low vitamin K content in the diet, which was subsequently supplemented with vitamin K within a range required to maintain a normal health status in rats. Alopecia in females given 500 mg/kg/day and pale mucosa/skin in males dosed from 150 mg/kg/day were also noted. Body weight and weight gain were slightly to moderately reduced in high-dose males throughout the study and in females from Week 30 onwards. There were no relevant effects in food consumption or ophthalmic examination.

Changes in hematopoietic parameters at 12 and 24 months of dosing were noted and consisted of slight decreases in hemoglobin, hematocrit, mean cell volume and mean cell hemoglobin values in males and/or females at 150 and/or 500 mg/kg/day. Thrombocyte (up to 32%) and reticulocyte (up to 84%) counts were slightly increased at 150 (males) and 500 mg/kg/day (both sexes). Additionally, minimal increases in white blood cell (neutrophils and lymphocytes) and minimal decreases in eosinophil counts were seen in males given 500 mg/kg/day. There were slight increases in PT (up to7%, males) and fibrinogen values (up to 16%, females) at 500 mg/kg/day after 12 and 24 months of dosing. There were changes in clinical chemistry. Increases in GGT (up to 29-fold), potassium (up to 18%), bilirubin (up to 3-fold), calcium (up 6%), protein (8%) and BUN (up to 30%) values were recorded in males and/or females given 150 or 500 mg/kg/day. Triglyceride values were also decreased in rats dosed from 150 mg/kg/day. AST (46%),

cholesterol (up to 67%), albumin (up to 13%) and creatinine (30%) values were increased in females dosed at 150 or 500 mg/kg/day while ALT (76%) and ALP (49%) activities raised in males at 500 mg/kg/day. An increase in urinary volume at 150 or 500 mg/kg/day was noted in males and/or females throughout the study and accompanied by increases in protein (up to 65%) in females given 150 or 500 mg/kg/day.

Liver weight was moderately and markedly increased in both sexes at 150 and 500 mg/kg/day respectively. Macroscopic examination showed a dose related increase in dark discoloration, masses/nodules and swelling in the liver. The kidney presented dark discoloration in both sexes at 500 mg/kg/day. An irregular surface (from 150 mg/kg/day) and swelling (at 500 mg/kg/day) was also observed in females.

Microscopic liver changes consisted of hepatocellular tumors in animals dosed from 150 mg/kg/day. Increases in the incidence of adenomas were recorded at both 150 and 500 mg/kg/day. At 150 mg/kg/day this related to 16/70 males and 5/70 females, and at 500 mg/kg/day to 25/70 males and 15/70 females. There were also increases in the incidence of carcinomas at 150 mg/kg/day (4 males) and 500 mg/kg/day (19 males and 7 females). Hypertrophy, pigmentation (lipofuscin pigment in Kupffer cells, macrophages and hepatocytes; hemosiderin-containing Kupffer cells), vacuolation and foci of cellular alteration were observed in both sexes from 150 mg/kg/day. Oval cell hyperplasia in females given 500 mg/kg/day and cystic degeneration in all dosed males was also seen. The changes in liver were correlated to macroscopic findings, organ weight changes and clinical chemistry changes (ALT, AST, ALP, GGT, bilirubin, albumin, BUN, cholesterol and triglycerides) and are considered to be the result of liver enzyme induction and /or altered lipid metabolism. In the thyroid gland adenomas were present in males (4/70 at 150 mg/kg/day and 5/70 at 500 mg/kg/day). Focal hyperplasia of follicular cells in males at 500 mg/kg/day and hypertrophy in both sexes from 150 mg/kg/day were also reported. The changes in the thyroid were considered to be rat-specific associated with liver enzyme induction and increased elimination of thyroid hormones. In kidneys, a moderate increase in CPN (females) and tubular pigmentation (both sexes) was noted from 150 mg/kg/day. Bone marrow showed slight increases in cellularity in males from 150 mg/kg/day and in the spleen slight increases in extramedullary hematopoiesis were noted in

males at 500 mg/kg/day. These findings were linked to the red blood cell turnover and hematology changes. An increase in vacuolated cells was observed in the pituitary gland (males from 150 mg/kg/day) and was considered the result of liver enzyme induction (with increased thyroid stimulating hormone (TSH)-production in the pituitary gland). Vacuolation in the in adrenal glands (males at 500 mg/kg/day) and an increase in foamy macrophages in the lungs (females at 500 mg/kg/day) were noted and could be explained by lipid metabolism alteration (with changes in cholesterol and triglycerides). The results are displayed in Tabulated Summary 2.6.7.10.B.

The NOAEL in this study was considered to be 50 mg/kg/day based on the absence of any functional disturbances. Systemic exposure expressed as C_{max} and AUC values are given in Table 14 below.

Table 14: Darunavir C_{max} and AUC Values After Repeated Oral Administration (24 Months) in Rats

Darunavir Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M	F	M	F
50	Day 31	3.03	3.86	15.9	16.8
	Day 184	3.72	7.19	17.1	24.9
150	Day 31	7.53	8.1	50.1	39.4
	Day 184	7.02	10.1	50.4	51.7
500	Day 31	14.9	13	133	102
	Day 184	12.6	12.8	90.3	89.5

5.1.2. Short- or Medium-Term Studies

No short- or medium-term carcinogenicity studies have been conducted.

5.1.3. Other Studies

A **1-month** repeated dose study was conducted in **transgenic mice**.[47] Therefore DRV formulated in PEG400 was administered once daily at 0 (vehicle), 150, 450 and 1000 mg/kg/day in a dose volume of 10 mL/kg. . Each group consisted of 10 male and 10 female wild type TgRasH2 littermate, produced by crossing transgenic males C57BL/6J with non-transgenic BALB/cByJ females, (principal animals) together with 15 male and 15 female satellite animals (except for the vehicle group which comprised 12 males and 12 females). Details about the toxicologically relevant changes can be found in Tabulated Summary 2.6.7.10.

In Sprague-Dawley rats, DRV admixed with **die**t was fed ad libitum, for **3 months**.[48] One control group and three treated groups were given nominal doses of, 0 (vehicle), 260, 1040 and 2600 mg/kg/day as dietary concentrations of 5000, 20000 and 50000 ppm. The highest dose level corresponded to the maximum applicable diet substitution factor (5%). The mean achieved dosages ranged between 71% and 92% of the nominal doses. Each group consisted of 30 male and 30 female Sprague-Dawley rats (principal animals) together with 6 male and 6 female satellite animals (except in control group which had 3 males and 3 females) for toxicokinetic analysis. From the principal animals 10 males and 10 females were assigned to a 1-month interim kill. The remaining 20 males and 20 females were treated for 3-months. No NOAEL could be established due to clinical pathology changes seen at all doses (see Tabulated Summary 2.6.7.10.).

5.2. Cobicistat

5.2.1. Long Term Studies

5.2.1.1. Mouse Studies

Oral gavage studies in CD-1 mice with COBI were conducted in preparation for the 2-year carcinogenicity study (2-week study[24]; 13-week study)[27]; and to support a slight modification of the vehicle in the 2-year carcinogenicity study (2-week study)[25]. A 4-week study was conducted in wild type mice to assess the feasibility of the transgenic CB6F1-non TgrasH2 strain for a 6-month transgenic carcinogenicity study[26].

In a **dose range finding** study in mice (5/sex/group), COBI was administered at dose levels of 0 (vehicle; 95% PG, 5% EtOH, pH adjusted to 2.3 with HCl), 10, 30, 100, and 300 mg/kg/day in a dose volume of 10 mL/kg for 2 weeks[24]. Satellite groups of animals (18/sex/group/interval) were assigned for toxicokinetic assessment on Days 1 and 14. Due to deaths at 300 mg/kg/day on Day 1 or Day 2 (1 male and 5 females), the high dose was reduced to 200 mg/kg/day beginning on Day 3, and animals were replaced with an animal of similar body weight and of the same sex. Based on the results of the study (see Tabulated Summary 2.6.7.10), the NOAEL was set at 100 mg/kg/day in males and females.

In addition, a **2-week study** was conducted to compare the toxicity and determine the toxicokinetics of COBI when administered in two separate vehicles, pH-adjusted to 2.3 and pH-unadjusted, after daily oral gavage

administration to mice for 14 days (Tabulated Summary 2.6.7.10)[25]. This study was conducted to support a modification of the vehicle in the 2-year mouse carcinogenicity study. Male and female Crl:CD1(ICR) mice were assigned to 9 groups (24/sex/group). Animals were dosed daily via oral gavage at a dose volume of 5 mL/kg, and administered water, COBI in Vehicle 1 (95% PG, 5% EtOH, pH adjusted to 2.3) or COBI in Vehicle 2 (95% PG, and 5% EtOH) at 0, 5, 15, and 50 mg/kg/day (males) or at 0, 10, 30, and 100 mg/kg/day (females). The NOAEL is considered to be 50 mg/kg/day in males and 100 mg/kg/day in females.

The **4-week dose range finding** oral gavage toxicity and toxicokinetic study in **CByB6F1-Tg(HRAS)2Jic (wild type) mice** was conducted with COBI dose levels of 0 [vehicle (10% PG in 40 mM acetate buffer, pH 4.0)], 10, 30, and 100 mg/kg/day (dose volume = 5 mL/kg/day) (Tabulated Summary 2.6.7.10)[26]. The NOAEL was considered to be 100 mg/kg/day.

In the supportive **13-week** study, male and female Hsd:ICR(CD-1®) mice (15/sex/group) were dosed with COBI at 0 (vehicle; 95% PG, 5% EtOH, pH adjusted to 2.3 with HCl), 5, 15, and 50 mg/kg/day in a dose volume of 5 mL/kg (Tabulated Summary 2.6.7.10)[27]. The NOAEL of COBI is 50 mg/kg/day in females based on the absence of any toxicologically significant adverse effects, and 5 mg/kg/day in males. The results of the CYP activity measurements can also be found in Module 2.6.4/Section 5.5.2.1.

In the **carcinogenicity study in mice**, the carcinogenic potential of COBI was evaluated when administered daily via oral gavage to mice for at least 104 weeks (Tabulated Summary 2.6.7.10.C)[28]. Due to reduced survival, high-dose males were dosed for 95 weeks, and the remaining males were dosed for 96 weeks. High-dose females were dosed for 87 weeks, and the remaining females were dosed for 100 weeks. An early termination plan was agreed upon with the Division of Antiviral Products (DAVP) of the FDA (correspondence dated [redacted]).

Dose levels were selected based on results of a 2-week range-finding study and a 13-week toxicity study in mice, and after consultation with the DAVP and the Executive Carcinogenicity Assessment Committee (CAC) of the FDA (IND 101,283 Serial Number [redacted]; Serial Number[redacted]).

Male and female Crl:CD1(ICR) mice were assigned to 5 carcinogenicity groups (60 animals/sex/group) and administered water or COBI by daily oral gavage at 0 (vehicle), 5, 15, and 50 mg/kg/day (males), or 0 (vehicle), 10, 30, and 100 mg/kg/day (females) at a volume of 5 mL/kg. Blood samples were collected from satellite animals for toxicokinetic evaluation (on Day 1 and during Week 29) and from sentinel animals for viral screening.

From Day 1 through Week 13, the vehicle was 95% PG/5% EtOH (v/v) (pH 2.3 ± 0.1). Due to increased mortality in vehicle control animals compared to water control animals, the vehicle was modified slightly to 95% PG/5% EtOH (v/v) (not pH-adjusted) from Weeks 14 through 25. At Week 26, due to continued higher mortality in the vehicle control mice, the vehicle was changed to 10% PG in 90% 40 mM acetate buffer (v/v) (pH 4.0 ± 0.1) for the remainder of the study. Toxicokinetic data from a 14-day mouse study (TX-216-2032[25]) and this study (Table 17) indicate that there were no notable differences in COBI exposures associated with the change in vehicles.

Assessment of toxicity and carcinogenicity was based on mortality, clinical signs, body weight, body weight change, food consumption, ophthalmic examinations, and clinical and anatomic pathology.

No significant positive trend and/or COBI-related increase in neoplasms was observed compared to the vehicle control. No significant difference in neoplastic lesion incidence between the vehicle and water controls was observed. No COBI-related ophthalmic finding or development of hematologic neoplasia was observed.

An adequate number of mice survived an appropriate duration (ie, at least to Week 88 of the dosing phase) for a valid evaluation of the carcinogenic potential of COBI[29]. In COBI-treated males, there was no effect on mortality when compared with vehicle controls, although a significant increase in mortality was noted in vehicle controls compared with water controls (Table 15). In COBI-treated females, there was a significant positive trend in mortality resulting in a significant decrease in survival in the 100 mg/kg/day group compared with vehicle controls. Females in the vehicle and water control groups had comparable survival throughout the dosing period.

Table 15: Unadjusted Survival Rate (%) in the Mouse Carcinogenicity Study with COBI

Sex	Male					Female				
Dose Level (mg/kg/day)	**0 (W)**	**0 (V)**	**5**	**15**	**50**	**0 (W)**	**0 (V)**	**10**	**30**	**100**
Week 14	98	97	97	88	93	98	93	87	88	90
Week 26	92	88	90	85	78	97	88	83	83	80
Week 52	90	78	75	78	72	95	87	77	77	70
Week 78	83	55	58	67	45	67	72	57	62	40
Week 91	68	37	47	48	30	53	57	43	38	NA[d]
Termination	60[a]	33[a]	40[a]	45[a]	22[b]	38[c]	38[c]	32[c]	25[c]	23[d]

W = water control; V = vehicle control; NA = Not applicable.

Percent unadjusted survival at the end of each study week = [(survival/60) x 100].

a Terminal sacrifice on Day 673 (Week 97).

b Terminal sacrifice on Day 665 (Week 95).

c Terminal sacrifice on Day 699 (Week 100).

d Terminal sacrifice on Day 611 (Week 88).

Clinical signs with higher occurrences in both COBI- and vehicle control-dosed animals included few feces, hypoactivity, and audible/labored/irregular respiration. In COBI-dosed animals, a slightly higher occurrence of hunched posture and thin appearance was observed in 50 mg/kg/day males and 100 mg/kg/day females, respectively.

In general, there were no remarkable effects on body weight, body weight gain, or food consumption in COBI-treated animals compared to vehicle controls. However, decreases in mean body weight, body weight gain, and food consumption were noted in vehicle controls. When compared to water controls, decreases in body weights were minimal in vehicle control males, but were statistically significant throughout the study in vehicle control females. Decreases in mean body weight gain in vehicle control groups versus water controls reached statistical significance during Weeks 1#26 for both sexes, and throughout the dosing period (Weeks 1#86) for females. Reduced food consumption in most cases correlated with reduced body weight gain.

An increased incidence of direct upper respiratory tract irritation was noted on microscopic evaluation of animals administered the vehicle, with or without COBI, compared with water controls. Gavage errors and sequelae to

upper respiratory tract irritation were relatively common and disproportionably responsible for early deaths and unscheduled sacrifices among these animals (Table 16). The incidence of several parameters indicative of vehicle-induced upper respiratory tract irritation, including nasal turbinate neutrophilic inflammation, erosion/ulcer, and turbinate atrophy, as well as gavage-related deaths tended to be slightly higher in animals dosed with COBI compared to the vehicle control, primarily in the high-dose animals.

The incidences of hepatocellular hypertrophy and pigmented Kupffer cells were increased in the high-dose males and females compared with both the vehicle and water controls (Table 16). These liver findings are considered adaptive effects, secondary to microsomal enzyme induction, and are not considered adverse.

Exposure to COBI increased with the dose level from 5 to 50 mg/kg/day for males and from 10 to 100 mg/kg/day for females. Increases in C_{max} and AUC_{0-t} for males and females were not consistently dose-proportional. Potential accumulation of COBI was observed after multiple dosing.

In conclusion, daily oral gavage administration of COBI at dose levels up to 50 mg/kg/day (males) and 100 mg/kg/day (females) to CD1 mice for a minimum of 95 weeks (males) or 87 weeks (females) produced no carcinogenic effects. During Week 29, COBI exposures (AUC_{0-t}) at the high-dose levels (50 mg/kg/day for males and 100 mg/kg/day for females) were 75.0 μg.h/mL for males and 174 μg.h/mL for females.

Table 16: Notable Microscopic Observations in the Mouse Carcinogenicity Study with COBI

Sex	**Males**					**Females**				
Dose Level (mg/kg/day)	**0 (W)**	**0 (V)**	**5**	**15**	**50**	**0 (W)**	**0 (V)**	**10**	**30**	**100**
	Unscheduled Sacrifice and Early Death Animals									
Number of animals examined	24	40	36	33	46[a]	37	37	41	45	46
Deaths Attributed to Dosing and Direct Irritation[b]	1	8	9	4	21	3	4	12	15	19
Nasal Turbinates										
Infiltrate, Neutrophils, Airway	4	20	21	20	43	7	17	27	28	36
Liver										
Pigment, Kupffer cells	0	0	0	0	10	4	3	0	3	18
Hypertrophy, Hepatocellular	1	6	2	6	15	2	2	1	0	4
	Scheduled Sacrifice Animals									
Number of animals examined	36	20	24	27	13	23	23	19	15	14
Nasal Turbinates										
Infiltrate, Neutrophils, Airway	6	5	8	10	7	1	11	10	13	10
Liver										
Pigment, Kupffer cells	3	1	2	0	11	10	7	2	4	13
Hypertrophy, Hepatocellular	3	2	5	2	8	0	0	0	0	5

W = water control; V = vehicle control

a One male was found dead on Day 30, but was included with animals sacrificed or found dead between Days 1 and 28 of the dosing phase. Thus, only the esophagus, lung, and trachea were examined microscopically.

b Four death comment observations (airway occlusion; distention, abdominal; dosing-related trauma/irritation; and gavage-related death) from the microscopic data were summed.

Table 17: Mean Toxicokinetic Parameters of COBI in the Mouse Carcinogenicity Study with COBI

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
		Day 1	Week 29	Day 1	Week 29
5	Male	0.600	4.05	0.194	2.31
10	Female	4.26	13.9	2.00	7.88
15	Male	14.3	12.4	3.34	4.42
30	Female	36.0	72.1	7.07	13.3
50	Male	53.5	75.0	9.35	10.1
100	Female	100	174	16.8	21.6

5.2.1.2. Rat Studies

The objective of this study was to evaluate the carcinogenic potential of COBI when administered daily via oral gavage to rats for at least 104 weeks[30] (Tabulated Summary 2.6.7.10.D). Because the number of surviving animals in the vehicle control group had decreased to 20 animals earlier than expected, males were dosed for a minimum of 97 weeks and females were dosed for a minimum of 102 weeks. An early termination plan was agreed upon with the DAVP of the FDA (correspondence dated [redacted]).

Dose levels were selected based on results of a 26-week toxicity study in rats[14] and after consultation with the DAVP and the Executive CAC of the FDA (IND 101,283, Serial Number [redacted]; Serial Number [redacted]).

Male and female Crl:CD(SD) rats were assigned to 5 carcinogenicity groups (65 animals/sex/group) and administered water or COBI at 0 (vehicle; 95% PG [v/v] and 5% EtOH [v/v]), pH 2.3 [± 0.1] with HCl), 10, 25, or 50 mg/kg/day (males), or 0, 5, 15, or 30 mg/kg/day (females). Animals were dosed daily via oral gavage at a dose volume of 2 mL/kg.

Assessments of toxicity and carcinogenicity were based on mortality, clinical signs, body weight, body weight change, food consumption, ophthalmic examinations, and clinical and anatomic pathology. Blood samples were collected from satellite animals for toxicokinetic evaluation (on Day 1 and during Week 26) and from sentinel animals for viral screening.

Sufficient numbers of animals survived for an adequate duration to result in a valid evaluation of the carcinogenic potential of COBI[29]. Mortality was significantly increased for vehicle control males relative to the water control group, but was significantly decreased in males administered COBI at 25 or 50 mg/kg/day relative to the vehicle control. In females, no statistically significant effects on mortality were evident in any group.

Table 18: Adjusted Survival Rate (%) in the Rat Carcinogenicity Study with COBI

Sex	Male					Female				
Dose Level (mg/kg/day)	**0 (W)**	**0 (V)**	**10**	**25**	**50**	**0 (W)**	**0 (V)**	**5**	**15**	**30**
Week 13	100	100	100	100	98	100	100	98	98	97
Week 26	98	97	95	98	97	100	95	97	97	97
Week 52	92	92	92	95	94	95	92	92	94	95
Week 78	73	63	72	75	71	69	66	72	75	74
Week 91	55	37	49	58	62	51	50	55	57	65
Termination[a]	51	31	38	52	57	38	31	45	34	46

W = water control; V = vehicle control

Note: Data presented as percent adjusted survival at the end of Weeks 13, 26, 52, 78, and 91, and at termination.

a Termination during Week 98 for males and Week 103 for females.

No COBI-related clinical signs (including observations of masses), ophthalmic findings, evidence of hematopoietic neoplasia, or macroscopic observations were noted.

There were no notable differences in body weight or food consumption between vehicle and water controls of either sex, and there were no notable effects in females administered COBI. In males at 25 and 50 mg/kg/day, effects on body weight (significantly decreased vs. vehicle control by ≤ 12% at Week 98) and food consumption (decreased ≤ 12% at most assessment intervals through Week 77) were of minimal magnitude, did not demonstrate a clear dose response, and did not negatively impact survival.

Non-neoplastic and neoplastic microscopic findings attributed to COBI were limited to the thyroid and liver and considered secondary to hepatic microsomal enzyme induction. Neoplastic findings in the thyroid consisted of significant positive trends in thyroid follicular adenoma, carcinoma, and

combined adenoma and carcinoma in males and/or females, with significant increases in adenoma for males at 50 mg/kg/day, and in combined adenoma/carcinoma for males at ≥ 25 mg/kg/day and for females at 30 mg/kg/day (Table 19). The increase in thyroid follicular tumors was associated with follicular cell hypertrophy at ≥ 25 and ≥ 15 mg/kg/day in males and females, respectively (Table 20). Non-neoplastic changes in the liver included centrilobular hepatocyte hypertrophy in males administered ≥10 mg/kg/day and females administered 30 mg/kg/day, and hepatocyte karyocytomegaly in males administered ≥ 25 mg/kg/day.

Table 19: Neoplastic Findings in the Thyroid in the Rat Carcinogenicity Study with COBI

Sex	**Males**					**Females**				
Dose Level (mg/kg/day)	**0 (W)**	**0 (V)**	**10**	**25**	**50**	**0 (W)**	**0 (V)**	**5**	**15**	**30**
Number of animals examined	65	65	65	65	65	65	65	65	65	65
B-Adenoma, Follicular Cell	3	0	1	5	15[a]	0	0	0	2	6
M-Carcinoma, Follicular Cell	0	0	0	1	5	0	0	0	1	2
B-Adenoma and M-Carcinoma, Follicular Cell	3	0	1	6[a]	20[a]	0	0	0	3	8[a]

W = water control; V = vehicle control; B = benign; M = malignant

a Significant in pairwise comparison versus vehicle control

Table 20: Non-neoplastic Findings in the Thyroid and Liver in the Rat Carcinogenicity Study with COBI

Sex	Males					Females				
Dose Level (mg/kg/day)	**0 (W)**	**0 (V)**	**10**	**25**	**50**	**0 (W)**	**0 (V)**	**5**	**15**	**30**
Number of animals examined	65	65	65	65	65	65	65	65	65	65
Thyroid										
Hypertrophy, Follicular Cell	5	1	3	17	25	1	1	3	9	16
Hyperplasia, Follicular Cell	3	2	3	3	5	0	0	1	4	4
Liver										
Karyocytomegaly, Hepatocyte	1	0	1	8	13	4	3	3	7	8
Hypertrophy, Hepatocyte, Centrilobular	6	5	16	25	27	1	0	0	0	12

W = water control; V = vehicle control

Exposure to COBI increased with the increase in dose level for both males and females. Increases in C_{max} and AUC_{0-t} were generally greater than dose proportional. 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 (Table 21).

Table 21: Mean Toxicokinetic Parameters of COBI in the Rat Carcinogenicity Study with COBI

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
		Day 1	**Week 26**	**Day 1**	**Week 26**
10	Male	0.090	1.31	0.037	0.430
5	Female	0.038	0.807	0.017	0.725
25	Male	2.57	9.08	0.544	1.75
15	Female	3.88	6.38	1.05	1.75
50	Male	20.8	22.6	3.00	2.42
30	Female	12.0	19.9	2.18	3.23

In conclusion, daily oral administration of COBI to rats at doses of 10, 25, and 50 mg/kg/day (males) and 5, 15, and 30 mg/kg/day (females) for a

minimum of 97 weeks produced an increased incidence of combined thyroid follicular cell adenoma and carcinomas in males administered ≥ 25 mg/kg/day and in females administered 30 mg/kg/day. Non-neoplastic findings in the thyroid and liver included follicular cell hypertrophy in males and females at ≥25 and ≥15 mg/kg/day, respectively, and centrilobular hepatocyte hypertrophy in males at all dose levels and in females at 30 mg/kg/day. The thyroid and liver changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone changes, and consistent with findings in other studies in rats with COBI[14]. During Week 26, the mean COBI AUC_{0-t} was 22.6 and 19.9 μg.h/mL in males receiving 50 mg/kg/day and females receiving 30 mg/kg/day, respectively.

5.2.2. Short- or Medium-Term Studies

There were no short- or medium term carcinogeniticy studies conducted with COBI.

5.2.3. Other Studies

There were no other studies conducted with COBI.

6. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

6.1. Darunavir

Reproduction and development effects of DRV alone (in PEG400 vehicle) were evaluated by oral gavage in a fertility and early embryonic development study in rats, in embryo-fetal development studies in rat, rabbit and mouse, in a prenatal and postnatal development study in rats and in juvenile rat studies. Initially, DRV alone (in CMC/Tween 80) was evaluated in a rabbit embryo-fetal development study but to investigate the potential to increase exposure, a pilot study with RTV co-administered with DRV was conducted in rabbits. However, it was determined that higher exposure could be achieved in the mouse and, DRV alone (in PEG400) and in combination with RTV was evaluated in a mouse embryo-fetal development study. The combination was also evaluated in a rat and minipig embryo-fetal development study, a rat pre- and postnatal development study and two juvenile studies.

6.1.1. Fertility and Early Embryonic Development

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for 12 weeks (males) or 2 weeks (females) after which time the animals were mated[49]. One control group and three treated groups were given, 0 (vehicle), 40, 200 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 25 male and 25 female Sprague Dawley rats. Regular observations were made for clinical signs, body weight and food consumption. Vaginal smears were taken for vaginal cytology and to determine when mating occurred. The dams were sacrificed and necropsied on GD 14 to 16 (GD1 was the day mating was confirmed) and males were sacrificed and necropsied after all female necropsies were completed. Dosing in males continued until termination. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy and a limited number of organs were weighed from the animals sacrificed at the end of the study. Samples of a range of reproductive tissues and organs and gross lesions were preserved. Histological examination was conducted on control and high dose group animals only. Pregnancy rate, uterine data (e.g. number of corpora lutea, implantations and live fetuses) and sperm analysis data were collected and analyzed. No toxicokinetic evaluations were performed in this study. However the same strain of animals and dose levels were used in a dose range finding embryofetal development study[50,51] and these toxicokinetic (TK) data are considered representative of values expected in this present study (see Section 6.1.2.1.1).

There were no mortalities associated with DRV, but there were a number of accidental deaths associated with the dosing procedure, 1 at 40 and 1 at 1000 mg/kg/day. In addition, there were 5 deaths where the cause was not identified, 1 at 0, 1 at 40, 2 at 200 and 1 at 1000 mg/kg/day. There were no relevant clinical signs. Food consumption was slightly reduced for a short period following start of treatment in both sexes at 1000 mg/kg/day. Body weights were lower (7%) than controls in males given 1000 mg/kg/day but were greater (6%) than controls for males given 200 mg/kg/day. Body weight and body weight gain was reduced in females given 1000 mg/kg/day at the end of the post-mating period (6% and 16%, respectively).

There was no effect on vaginal cytology, mating, gross pathology, organ weights, histopathology, sperm number, sperm motility, and sperm

morphology or pregnancy rate. The number of corpora lutea, implantations and live fetuses in females given 1000 mg/kg/day were less than in control females; this effect on ovulation could possibly be a result of the lower body weight gain. It was concluded that there was no effect on male or female fertility at doses up to a dose producing effects on body weight gain (1000 mg/kg/day). The NOAEL was 200 mg/kg/day based on the absence of an adverse body weight effect in males and females (See Tabulated Summary 2.6.7.12.A).

6.1.2. Embryo-Fetal Development

6.1.2.1. Rats

6.1.2.1.1. Darunavir Alone

A **dose range finding study** of prenatal developmental toxicity was conducted in pregnant Sprague-Dawley rats[50,51]. Darunavir formulated in PEG400 was administered once daily, by oral gavage, during the period of GD7 to GD9 and GD13 to GD19 (GD1 is the day mating is confirmed) only, owing to the limited availability of test material. One control group and three treated groups were given, 0 (vehicle), 40, 200 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 6 time-mated, sexually mature female Sprague Dawley rats. Toxicokinetic analysis was also performed (see Table 22). The NOAEL was considered to be 1000 mg/kg/day based on the absence of any relevant findings (See Tabulated Summary 2.6.7.11).

Table 22: Darunavir C_{max} and AUC values after repeated p.o. administration in rats

TMC114 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
		F	F
40	Day 1 (GD7)	1.80	12.5
	Day 13 (GD19)	1.70	7.59
200	Day 1 (GD7)	11.1	73.8[b]
	Day 13 (GD19)	6.13	36.8
1000	Day 1 (GD7)	12.4	84.5
	Day 13 (GD19)	11.3	66.2

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{last} after repeated dose, [b] AUC_{last}

Based on this study, the doses for the **full size developmental study** were set at 0 (vehicle), 40, 200 and 1000 mg/kg/day (dose volume = 10 mL/kg).[52] Pregnant Sprague-Dawley rats (25/group) were administered DRV (in PEG400) once daily by oral gavage, during the period of GD7 to GD19. Regular observations were made for clinical signs, body weight and food

consumption. The dams were sacrificed and necropsied on GD22. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy. The uterus (with cervix and ovaries) was excised, weighed and examined. Uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution, were collected and analyzed. All live fetuses were examined externally and then approximately half of the fetuses were examined viscerally by fresh tissue examination. The heads of the animals selected for visceral examination were preserved in Bouin's fixative and examined by Wilson's sectioning. The remaining fetuses were eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities. No toxicokinetic evaluations were performed in this study, however, the same strain of animals and the dose levels were used in a dose range finding embryofetal development study[50,51] and these TK data are considered representative of values expected in this present study (see Table 22).

There were no mortalities associated with DRV, but there was one death at 1000 mg/kg/day where the cause was not identified. There were no relevant clinical signs. Body weights and food consumption were lower (5% and 10% respectively) than controls in animals given 1000 mg/kg/day. Body weight gain was also reduced (53% lower GD7-GD10 and 15% lower GD7-GD19). Both body weight and food consumption recovered in the days post-dose and there was no difference among groups on GD22.

There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal body weight, sex ratio or fetal abnormalities.

There was no evidence that DRV is teratogenic in rats. The maternal NOAEL was considered to be 200 mg/kg/day based on the body weight effect. There were no effects of DRV treatment on embryo-fetal development at any of the doses administered, including a maternally toxic dose. The embryo-fetal NOAEL was therefore considered to be 1000 mg/kg/day (See Tabulated Summary 2.6.7.13.A).

6.1.2.1.2. Darunavir Co-Administered with Ritonavir

In a **dose range finding study**, DRV (in PEG400) was administered alone or in combination with RTV (in PG) to Sprague-Dawley rats during the period

of organogenesis (from GD 6 to GD 17 inclusive)[53]. DRV/RTV at 500/0, 1000/0 or 500/50 mg/kg was administered b.i.d., 6 hours apart, by gavage at a volume of 5 mL/kg/dose to 3 groups of 12 animals each (6 females were dosed from GD 6 to GD 11 and 6 females from GD 12 to GD 17). A vehicle control group of 8 females received PEG400 alone from GD 6 to GD 17. The results of this study are shown in Tabulated Summary 2.6.7.11.

In the **full size embryo-fetal development study**, DRV formulated in PEG400 was administered with RTV formulated in PG orally by gavage to female Sprague-Dawley rats, b.i.d., 6 hours apart, at a volume of 5 mL/kg/dose during the period of organogenesis[54]. DRV/RTV 300/50 mg/kg or 500/50 mg/kg b.i.d were administered between GD 6 and GD 11 or GD 12 and GD 17. A similar group of females received PEG400 alone from GD 6 to GD 17 and served as a vehicle control group. Clinical signs, body weight and food consumption were recorded. All females were killed on Day 21 of pregnancy and a necropsy was performed during which they were examined for macroscopic abnormalities, pregnancy status, the numbers of corpora lutea of pregnancy, implantations, early and late resorptions and live and dead fetuses. The fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. The toxicokinetics of DRV were determined on GD 6, 11, 12 and 17.

Four females were sacrificed during the study due to gavage errors or gastro-intestinal disturbance, 1 in each of the groups receiving 300/50 mg/kg b.i.d (GD 12-17) and 500/50 mg/kg b.i.d (GD12-17) and 2 in the group receiving 500/50 mg/kg b.i.d (GD 6-11). Clinical signs included audible respiration, poor condition, decreased activity and hypothermia. There were 23, 23, 22, 21and 22 pregnant females at terminal sacrifice at 0/0, 300/50 (GD 6-11 and GD 12-17), 500/50 mg/kg b.i.d (GD 6-11 and GD 12-17) groups, respectively. Increases in the incidence of soft and /or pale feces were observed in all groups receiving DRV/RTV. In addition females receiving 500/50 mg/kg b.i.d showed an increased incidence of piloerection.

Between GD 6 and GD 11, transient non-dose related losses in body weight were observed at 300 and 500 mg/kg b.i.d. Food consumption was statistically significantly reduced throughout this dosing period. Body weight and food consumption showed recovery during the post-dosing period. Between GD 12 and GD 17, there was a non-dosage related loss in body

weight followed by a reduced body weight gain observed at 300/50 and 500/50 mg/kg b.i.d. At the end of study, the body weight gain remained low. Food consumption was statistically significantly reduced throughout the dosing period in DRV/RTV treated groups in comparison with the control animals and showed no recovery during the post-dosing period.

Pregnancy parameters were unaffected by treatment. No statistically significant increases in post-implantation loss were noted at 500/50 mg/kg b.i.d in (GD 6-11) groups. Decreases in fetal body weight were observed in all groups receiving DRV/RTV which was most pronounced in the groups dosed between GD 12 and GD 17. There were considered to be related to the observed reduced body weight gain in the females receiving DRV/RTV and not a direct effect on fetal growth. At fetal examination, sex ratio was normal. There were no relevant major changes at external or visceral examination in fetuses of dams dosed with DRV/RTV. There was an increased incidence of incompletely descended thymus gland in the groups dosed between GD 6 and GD 11, which is an indicator of a slight developmental delay and is commonly observed in fetuses of low body weight. Increases in a number of minor skeletal abnormalities and variants generally associated with a slight developmental delay as evidenced by the state of skeletal ossification, were observed in fetuses of all groups receiving DRV. These findings comprised absence or incomplete ossification of skull, vertebrae, sternum and metatarsal bones but were less marked in the groups dosed between GD 12 and GD 17. These findings generally showed a dosage relationship in the groups dosed between GD 12 and GD 17 and were considered to be related to the observed maternal toxicity with consequent reductions in fetal weight.

The NOAEL for maternal toxicity was considered to be less than 300/50 mg/kg b.i.d and the NOAEL for embryo-fetal development was considered to be 300/50 mg/kg b.i.d. Systemic exposure values, expressed as C_{max} and AUC, are given in Table 23 below (See Tabulated Summary 2.6.7.13.).

Table 23: Darunavir C_{max} and AUC $_{0\text{-}24h}$ values after oral administration in pregnant rats

	GD 6 to GD 11				GD 12 to GD 17			
DRV + RTV dose (mg/kg/day)	Sampling Day	C_{max1} (µg/mL)	C_{max2} (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)	Sampling Day	C_{max1} (µg/mL)	C_{max2} (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)

2 x 300 + 2 x 50	1 (GD 6) 6 (GD 11)	9.8 11	22 14	280 202	1 (GD 12) 6 (GD 17)	9.7 17	20 14	240 261
2 x 500 + 2 x 50	1 (GD 6) 6 (GD 11)	12 13	35 23	423 283	1 (GD 12) 6 (GD 17)	14 13	24 24	312 219

C_{max1} : after the first dosing; C_{max2} :after the second dosing; GD: gestation day; DRV:darunavir; RTV: ritonavir

6.1.2.2. Rabbits

6.1.2.2.1. Darunavir Alone

A **tolerability study** was performed in non-pregnant rabbits.[55,56] Darunavir formulated in 1% CMC/ 0.2% Tween 80 in deionised water was administered once, by oral gavage. One control group and three treated groups were given, 0 (vehicle), 250, 500 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 2 female New Zealand white rabbits. The two rabbits dosed at 1000 mg/kg/day were subsequently dosed at this same level on study days 3 to 7 to investigate tolerability after repeated dosing. The NOAEL was considered to be 1000 mg/kg/day based on the absence of any relevant findings (See Tabulated Summary 2.6.7.11).

In addition, a **dose range finding study** of prenatal developmental toxicity was performed in pregnant rabbits. Therefore DRV formulated in 1% CMC/ 0.2% Tween 80 in deionised water was administered once daily, by oral gavage, during the period of GD8 to GD20 (GD1 was the day mating confirmed)[57,58]. One control group and three treated groups were given, 0 (vehicle), 40, 200 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 6 time-mated, sexually mature female New Zealand white rabbits. The maternal and fetal NOAEL was considered to be 1000 mg/kg/day, based on the absence of any relevant findings (See Tabulated Summary 2.6.7.11). Toxicokinetic analysis was also performed (see Table 24).

Table 24: Darunavir C_{max} and AUC values after repeated p.o. administration in rabbits

TMC114 Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
		F	F
40	Day 1 (GD8) Day 13 (GD20)	0.03 0.15	0.13 0.47
200	Day 1 (GD8) Day 13 (GD20)	0.15 0.60	0.52 1.81
1000	Day 1 (GD8) Day 13 (GD20)	1.92 1.74	6.26[b] 6.00

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{last} after repeated dose, [b] AUC_{last}

Based upon these rabbit studies, a **full size embryo-fetal development study** was conducted in pregnant New Zealand white rabbits (20/group).[59,60] Darunavir formulated in 1% CMC/0.2% Tween 80 in deionised water was administered once daily, by oral gavage, during the period of GD8 to GD20 (GD1 was day mating confirmed). One control group and three treated groups were given, 0 (vehicle), 40, 200 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Regular observations were made for clinical signs, body weight and food consumption. The dams were sacrificed and necropsied on GD30. All animals found dead or sacrificed during or at the end of the study were subject to macroscopic examination at necropsy. The uterus (with cervix and ovaries) was excised, weighed and examined. Uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body weight and fetal sex distribution, were analyzed. All live fetuses were examined externally and viscerally (including the brain) by fresh tissue examination. The fetuses were then eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities. Limited toxicokinetic evaluations were performed in this study. Three satellite animals were given the highest dose and used for TK on GD20. Maternal and fetal plasma levels were determined. Maternal plasma concentrations ranged between 0.09 and 1.5 µg/mL and DRV was detected in the fetal plasma at up to 0.07 µg/mL. A complete toxicokinetic analysis, using the same strain of animals and dose levels, was done in a previous study[57,58] and these data are considered representative of values expected in this present study.

There were no mortalities associated with DRV, but there were two deaths where the cause was not identified, 1 at 40 and 1 at 1000 mg/kg/day. In addition, two animals given 1000 mg/kg/day aborted on GD20 and were sacrificed. There were no relevant clinical signs. There were no differences in mean body weight but examination of individual animal data reveals a higher number of animals in the high dose group, showing body weight gain decreases when compared to controls. Similarly, an effect on food consumption is present during the treatment period with individual high dose animals showing a decrease compared to controls. Whether this is due to the high dose treatment is not clear.

There was no effect on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-

implantation loss or live implantations, fetal body weight, sex ratio or fetal abnormalities.

The maternal and fetal NOAEL was considered to be 1000 mg/kg/day. There is no evidence that DRV is teratogenic in rabbits. However, these finding should be treated with caution given the apparent low exposure (See Tabulated Summary 2.6.7.13.C).

6.1.2.2.2. Darunavir Co-Administered with Ritonavir

Darunavir formulated in 0.5% hydroxypropyl methylcellulose/ 0.2% Tween 80 in deionised water was administered with and without RTV formulated in propylene glycol, once daily, by oral gavage, for 5 days[61]. The following combinations of DRV/RTV were given: 0/0 (vehicles), 0/40, 200/40, 1000/40 and 1000/0 mg/kg/day in a dose volume of 10 mL/kg for DRV and 0.5 mL/kg for RTV. Each group consisted of 4 female New Zealand white rabbits. The NOAEL was considered to be 1000 mg/kg/day based on the absence of any relevant findings (See Tabulated Summary 2.6.7.11).

6.1.2.3. Mice

Darunavir exposure in the rabbit was significantly low relative to other animal species (mouse and rat) and, as a consequence, the mouse was selected as a third species for embryo-fetal development studies.

6.1.2.3.1. Darunavir Alone

In a **dose range finding study**, DRV formulated in PEG400 was administered once daily, by oral gavage, during the period GD6 to GD15.[62] One control group and three treated groups were given, 0 (vehicle), 150, 450 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 10 time-mated, sexually mature female CD-1 mice. The NOAEL was considered to be 1000 mg/kg/day based on the absence of any relevant findings (See Tabulated Summary 2.6.7.11).

6.1.2.3.2. Darunavir Co-Administered with Ritonavir

In the **full size study**, DRV formulated in PEG400 was administered once daily, by oral gavage, during the period of GD6 to 15.[63] The following combinations of DRV/RTV were given: 0/0 (PEG400, vehicle), 150/0, 450/0, 1000/0, 1000/50 and 0/50 mg/kg/day in a dose volume of 10 mL/kg for DRV and 6.25 mL/kg for RTV. Each group consisted of 30 time-mated, sexually mature female CD-1 mice (principal animals), which were expected

to give 20 pregnant animals per group, together with 10 mated-female satellite animals (except in control group which had 8 mated-females) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption. The dams were sacrificed and necropsied on GD18. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy. Uterine data, the numbers of corpora lutea, implantations, live fetuses, fetal body and placental weight and fetal sex distribution, were analyzed. All live fetuses were sacrificed and examined externally. Approximately half of the fetuses were preserved in Bouin's fixative and were examined for visceral abnormalities. The remaining fetuses were eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities.

There were no mortalities associated with DRV, but 7 animals were found dead or were sacrificed prematurely during the study. In the principal animals these were 1, 1 and 2 given, 450/0, 1000/0 and 1000/50 mg/kg/day, respectively (in satellite animals, 1, and 1 given 1000/50 and 0/50 mg/kg/day, respectively). These deaths were associated with the dosing procedures but there was one death at 1000/0 mg/kg/day where the cause was not identified. There were no relevant clinical signs or effects on body weight and food consumption with DRV. With RTV alone, body weight gain and food consumption of the dams was reduced. In the group dosed with 1000 mg/kg/day DRV and 50 mg/kg RTV food consumption was also slightly reduced.

There were no relevant effects on gross pathology, gravid uterine weight, pregnancy rate, number of corpora lutea, number of pre-implantation loss, post-implantation loss or live implantations, fetal body and placental weight, sex ratio or fetal abnormalities. A statistically significant increase in the number of major abnormalities was observed in groups given 450/0, 1000/0 and 1000/50 mg/kg/day. This was primarily due to one litter in each group where an unusually high number of abnormalities were observed. Owing to the distribution of these findings it was considered that these were coincidental and unrelated to treatment with DRV or RTV.

The maternal NOAEL was considered to be 1000 mg/kg/day DRV alone based on the absence of any effects. Where DRV was given in combination with RTV only or where RTV was administered alone, a reduction in food

consumption was recorded. There were no effects of DRV treatment on embryo-fetal development at any of the doses administered. The embryo-fetal NOAEL was therefore considered to be 1000 mg/kg/day, and there was no evidence that DRV is teratogenic in mice (See Tabulated Summary 2.6.7.13.D).

Toxicokinetic analysis indicated non-linear pharmacokinetics of DRV and that combining DRV with RTV resulted in maintaining exposure to DRV. A decrease in exposure of RTV occurred when RTV was combined with DRV compared to administration of RTV alone. Systemic exposure, expressed as C_{max} and AUC values are given in Table 25 and Table 26 below:

Table 25: Darunavir C_{max} and AUC values after repeated oral administration in mice

TMC114/RTV Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
		F	F
150/0	GD6 GD15	8.38 5.99	24.2 12.5
450/0	GD6 GD15	15.7 8.00	69.8[b] 27.6[b]
1000/0	GD6 GD15	19.7 7.73	122 63.9
1000/50	GD6 GD15	25.3 16.1	158 81.6

[a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose, [b] AUC_{0-8h}

Table 26: RTV C_{max} and AUC values after repeated oral administration in mice

TMC114/RTV Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)	AUC[a] (µg.h/mL)
		F	F
0/50	GD6 GD15	13.7 9.36	37.0[b] 42.4[b]
1000/50	GD6 GD15	4.61 5.90	41.6 13.1

[.a] $AUC_{0-\infty}$ after single dose (day 1) or AUC_{0-24h} after repeated dose, [b] AUC_{0-8h}

6.1.2.4. Minipigs

A **dose range finding study** with DRV (in PEG400) alone or in combination with RTV (in PG) was conducted in non-pregnant female Göttingen SPF mini-pigs[64]. The study was conducted in 2 phases: in the first phase of the study, DRV alone was administered 6 hours apart at 500 or 1000 mg/kg b.i.d or in combination with RTV at 500/50 mg/kg b.i.d for one day. In the second phase of the study, repeated doses of DRV alone were administered b.i.d 9

hours apart for 6 days at 250 or 500 mg/kg or in combination with RTV at 250/50 mg/kg. Due to severe clinical signs (vomiting, passivity and soft/watery feces) with b.i.d administration, the treatment regimen was changed from b.i.d to once daily and the animals were dosed additionally for 7 days with DRV alone at 250 and 500 mg/kg/day or with DRV/RTV at 250/50 mg/kg/day. Mortality, clinical observations, body weight and food consumption were recorded for all dose levels. The toxicokinetics of DRV were determined on Days 1 (start of the first phase), 29 (start of the second phase) and 41 (end of study).

First phase

Most animals vomited on one or several occasions 1 or 2 hours after the second dosing on Day 1. Several animals were observed to be less active/passive or even subdued. These clinical signs were considered unacceptable and therefore doses were reduced in the subsequent repeated dose phase without change in frequency of administration.

Second phase (repeated doses)

In spite of reductions in daily oral doses, vomiting, passivity and soft/watery feces were seen in most animals. Some improvement in tolerability to the test item was observed over time. The clinical signs observed, however, were considered unacceptable for repeated administration. After lowering the frequency of administration and hence the total daily dose, the clinical condition of the animals improved, although passiveness and soft feces were still seen in all groups about 1 hr after dosing. As a result, no additional studies were performed in this species. Systemic exposure values, expressed as C_{max} and AUC, are given in Table 27 below (Tabulated Summary 2.6.7.11).

Table 27: Darunavir C_{max} and AUC $_{0\text{-}24h}$ values after single and repeated oral administration in non-pregnant female minipigs

DRV+ RTV dose (mg/kg/day)	First day of dosing				Last day of dosing		
	$C_{max\ 1}$ (µg/mL)	$C_{max\ 2}$ (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)	$AUC_{0\text{-}48h}$ (µg.h/mL)	$C_{max\ 1}$ (µg/mL)	$C_{max\ 2}$ (µg/mL)	$AUC_{0\text{-}24h}$ (µg.h/mL)
Day 1*							
2 x 500 + 0 (6h interval)	6.9	12	190	344			

2 x1000 + 0 (6h interval)	9.6	15	238	358			
2 x 500 + 2 x 50 (6h interval)	7.2	11	181	345			
Day 29*							
2 x 250 + 0 (9h interval)	3.7	8.0	109				
2 x 500 + 0 (9h interval)	3.9	14	159				
2 x 250 + 2 x 50 (9h interval)	4.4	8.7	115				
Day 41*							
250 + 0					6.0		59
500 + 0					8.1		82
250 + 50					5.6		71

* toxicokinetic sampling day

6.1.3. Prenatal and Postnatal Development, Including Maternal Function

6.1.3.1. Darunavir Alone

In a **dose range finding study**, DRV formulated in PEG400 was administered once daily, by oral gavage, to mated females from day 6 of gestation to day 7 of lactation.[65] Two control groups and three treated groups were given, 0 (vehicle), 0 (vehicle), 40, 200 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Each group consisted of 6 time-mated female Sprague Dawley rats. Regular observations were made for maternal clinical signs, body weight and food consumption. The females were allowed to litter, and nesting and nursing behavior were observed. Parturition, litter size and numbers of each sex were recorded, as were clinical observations and pup body weight. On day 6 of lactation, 8 male and 8 female pups per group were selected for direct dosing from day 12 until day 25 of age. One control group and four treated groups were given, 0 (vehicle), 1000, 40, 200 and 1000 mg/kg/day in a dose volume of 10 mL/kg. Group 2 pups (1000 mg/kg/day) were selected from litters where dams had previously been given vehicle only. Owing to unexpected deaths and adverse findings after direct dosing of pups on day 12 of age, the dose level of 1000 mg/kg/day (groups 2 and 5) was reduced to 500 mg/kg/day. This dose level also resulted in unexpected deaths and adverse findings after direct dosing of pups on day 13 and, therefore, these groups were terminated. On day 7 of lactation, 12 male and 12 female pups from each group, which had not been selected for direct dosing, were used for blood sampling during the first 6 hours after dosing of the dams, for toxicokinetic analysis. On day 12 at 1, 3 and 5 hours

after dosing and on day 25 up to 8 hours after dosing, terminal blood samples were taken from pups directly dosed with 40 and 200 mg/kg/day. In addition, on day 26 blood samples were taken up to 24 hours after dosing from 10 male and 10 female pups, which had not been previously selected for direct dosing, after a single direct dose of 1000 mg/kg/day. All pups directly dosed (following the collection of blood) and all pups found dead or sacrificed during lactation were subject to macroscopic examination at necropsy. The dams and pups not directly dosed and not used for blood samples were sacrificed (on day 21 of lactation) and not examined. Brain and liver were collected from directly dosed pups, after single or repeated dosing to assess the effect of age on DRV exposure.

In the dams there were no mortalities associated with DRV, but two dams were found dead (one given vehicle and one given 200 mg/kg/day). The cause of death was not stated but for the animal given 200 mg/kg/day it may have been associated with the dosing procedure based on clinical signs. During lactation, 3 animals were sacrificed owing to total litter death (2 dams, one given vehicle and one given 1000 mg/kg/day) and failure to produce milk (1 dam, 40 mg/kg/day). In dams given 1000 mg/kg/day body weight gain was lower than controls after the start of dosing and during days 1 to 7 of lactation. Food consumption was slightly reduced between days 6 and 9 of gestation (18%).

There was no effect of maternal treatment on parturition or the numbers of pups born. Pup bodyweight gain to day 6 of lactation (pups not directly dosed) was lower in groups given 200 and 1000 mg/kg/day (up to 29%). Pup survival up to start of pup direct dosing on day 12 post-partum was unaffected by maternal treatment.

At the start of direct dosing of the pups, on day 12 post-partum, all pups given 500 and 1000 mg/kg/day, died or were sacrificed prematurely owing to adverse clinical signs on day 12 or 13. In pups directly dosed with 40 and 200 mg/kg/day, 5/16 and 6/16 pups died or were sacrificed prematurely owing to adverse clinical signs. Of the pups selected for direct dosing at 40 and 200 mg/kg/day there was no difference in the group mean body weight at the start of dosing or at the end of the repeat direct dosing period. The deaths were considered to be associated with DRV and may reflect increased exposure in juvenile animals. At necropsy there were no findings in the pups

that could be unequivocally attributed to direct dosing of pups or maternal treatment.

The maternal NOAEL was considered to be 200 mg/kg/day based on the body weight effect of dams. For pups, not directly dosed, the NOAEL was 40 mg/kg/day based on the body weight effect to day 6 of lactation. For pups directly dosed a NOAEL could not be established owing to the deaths and adverse clinical signs (See Tabulated Summary 2.6.7.14.A).

From dams dosed at 40, 200 and 1000 mg/kg/day from GD6 to day 7 of lactation, data indicated that the pups were indirectly exposed to DRV via the dam's milk and the exposure seemed to increase with increasing dose levels. A mean DRV concentration of up to 0.61 µg/mL was detected in pups whose mothers received DRV at 1000mg/kg/day. At 1000 mg/kg/day, exposure was higher after a single direct dose to juvenile rats on day 12 than on day 25 of age. Exposure on day 12 of age, expressed as $AUC_{0\text{-}5h}$, 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, $AUC_{0\text{-}5h}$ 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. After repeated administration in juvenile rats, directly dosed, plasma exposure on day 25, expressed as C_{max} was 1.53 (females only) and 5.63 to 7.21 µg/mL, and AUC_{last} was 6.31 (females only) and 28.6 to 29.8 µg.h/mL, in pups given 40 and 200 mg/kg/day, respectively. Above data indicate that exposure to DRV in juvenile animals, directly dosed, was dose and age dependent and very likely influenced by the maturation of the liver enzymes involved in the elimination of DRV. Distribution of DRV to the brain seems was probably influenced by the maturation of the blood-brain barrier.

6.1.3.2. Darunavir Co-Administered with Ritonavir

In a **full size pre- and postnatal developmental study**, DRV formulated in PEG400 was administered once daily, by oral gavage, to mated females from day 6 of gestation to day 20 of lactation.[66] The F1 generation was allowed to mature, untreated. The following combinations of DRV/RTV were given: 0/0 (PEG400, vehicle), 40/0, 200/0, 1000/0, 1000/75(50), 1000/50 and 0/75(50) mg/kg/day in a dose volume of 10 mL/kg for DRV and 1.25 mL/kg for RTV. Where appropriate RTV was administered first immediately followed by DRV. Each group consisted of 25 time-mated female Sprague Dawley rats. Regular observations were made for maternal clinical signs,

body weight and food consumption. The females were allowed to litter and nesting and nursing behavior were observed. Parturition, litter size and numbers of each sex were recorded, as were clinical observations in pups and pup body weight. The number with ears open, the static righting reflex, eyes open, startle response and papillary light reflex were recorded. The F0 females were sacrificed at weaning of their litters. All females were examined microscopically and the number of implantation scars in each uterine horn was counted. Approximately one week after the start of weaning of the F1 generation, 20 male and 20 females offspring were randomly selected (except from the group dosed at 1000/75[50]) and effects on growth, development, behavior and reproductive performance assessed. No toxicokinetic determinations were made throughout the study but data generated from the previous dose range finding study and other toxicity studies considered representative of the values expected in this study (See Table 11 and Table 30).

In the dams given 1000/75 mg/kg/day, the RTV dose was reduced to 50 mg/kg/day at the end of gestation because of poor clinical condition and body weight gain. The RTV dose in the RTV alone group was also reduced. During lactation poor body weight gain of the F1 pups in this group was observed and dosing was stopped from approximately day 14 of lactation. A limited number of selected F1 pups (13 males and 13 females) from this group underwent mating assessment and E-maze development test only. An additional group of time-mated females was given 1000/50 mg/kg/day from day 6 to day 20 of gestation only and not treated during lactation owing to the effects seen.

Maternal treatment with DRV at 1000 mg/kg/day throughout the gestation and lactation periods was associated with clinical signs and reduced bodyweight gain and food consumption. At 200 mg/kg/day there was a single interval of lower bodyweight gain and reduced mean food intake. The NOAEL for maternal treatment was therefore considered to be 40 mg/kg/day. When DRV was administered together with RTV at 1000/75(50) mg/kg/day similar observations were recorded but the effect was greater than with DRV alone. Maternal treatment with RTV only at a dose level of 75(50) mg/kg/day was associated with maternal clinical signs and lower mean food consumption and bodyweight gain in comparison with

the controls, but values were less affected than those in the combination groups.

Maternal administration of DRV alone had no effect upon pup survival during lactation at dose levels up to and including 1000 mg/kg/day. An overall lower mean pup bodyweight throughout lactation and F1 maturation was associated with treatment at 1000 mg/kg/day. There was a slight delay in the acquisition of the developmental milestones pinna detachment and eyes open at 200 and 1000 mg/kg/day related to the lower bodyweight of the pups in these groups during lactation. There was no effect of maternal treatment on F1 developmental tests or mating and fertility at any dose level. The NOAEL for pup development after maternal treatment with the test article was therefore considered to be 40 mg/kg/day.

When DRV was administered together with RTV at 1000/75(50) mg/kg/day, similar observations were recorded for bodyweight and developmental milestones but the effects were more pronounced than with DRV alone. In addition pup survival during lactation was reduced. The reduced pup bodyweight gain during lactation was observed only in the group where dosing of mothers was continued until day 14 of lactation. In the group where dosing stopped at the end of gestation, bodyweight performance of F1 pups was comparable to that of the control animals. This confirms that this finding was directly related to DRV/RTV administration to the mothers during lactation and not resulting from in utero exposure. Lower mean bodyweight was also evident for F1 post-weaning but there was considered to be no effect on F1 developmental tests or the fertility and mating performance of the F1 animals.

Maternal administration of RTV at 75(50) mg/kg/day was associated with decreased pup survival during the second half of lactation and poor bodyweight performance of surviving pups and a delay in the acquisition of the developmental milestones. Lower mean bodyweight was evident for F1 selected animals post-weaning but there was no effect on F1 development or the fertility and mating performance of the F1 animals (See Tabulated Summary 2.6.7.14.B).

Darunavir exposure, expressed as C_{max} and AUC at 1000 mg/kg in pregnant rats was 11.3 µg/mL and 66.2 µg.h/mL respectively (see Tabulated Summary 2.6.7.11). These values were 13.6 µg/mL and 126 µg.h/mL when

DRV (1000 mg/kg/day) was combined with RTV (50 mg/kg/day) in non-pregnant rats (see TMC114-NC141[20], Tabulated Summary 2.6.7.6).

6.1.4. Studies in which the Offspring are Dosed

6.1.4.1. Darunavir Alone

A **dose range finding study** for a main study of prenatal and postnatal developmental toxicity was conducted and this included assessment of juvenile toxicity[65]. This study is described in section 6.1.3 above. For pups directly dosed at 40 and 200 mg/kg/day from **day 12 until day 25 of age**, a NOAEL could not be established owing to the deaths and adverse clinical signs. Doses of 500 and 1000 mg/kg/day were not tolerated. The exposure in juvenile animals directly dosed was age dependent and influenced by the maturation of the liver enzymes involved in the elimination of DRV.

In **a juvenile rat tolerance study**, DRV formulated in PEG400 was administered as a single oral gavage dose on one of three occasions to the pups of an individual litter[67]. On postnatal Day 5 (**PND 5**) (Day 1 was the day of birth), the doses were 20, 40 or 80 mg/kg, on **PND 8**, the doses were 40, 80 or 120 mg/kg, and on **PND 11**, the doses were 40, 80 or 160 mg/kg. Each main group consisted of 1 litter (minimum litter size 10 pups) from a single Sprague-Dawley dam. In addition, a further litter (minimum litter size 12 pups) from a single Sprague-Dawley dam was also dosed and used for plasma, brain and liver toxicokinetic analysis. The dams were not dosed. Details concerning this study can be found in Tabulated Summary 2.6.7.15.A.

6.1.4.2. Darunavir Co-Administered with Ritonavir

A **second dose range finding study** was conducted in which DRV formulated in PEG400 was administered alone or in combination with RTV formulated in propylene glycol (PG), once daily, by oral gavage to juvenile Sprague-Dawley rats, for 2 weeks (**from Day 23 to Day 36 of age**).[68] Six groups each consisting of 6 male and 6 female pups were given the following combinations of darunavir/RTV: 0/0 (PEG400 vehicle), 20/0, 100/0, 500/0, 500/50 or 0/50 mg darunavir/RTV/kg/day, in a dose volume of 10 mL/kg for darunavir or 1.25 mL/kg for RTV. A further 12 male and 12 female pups were allocated to each group for toxicokinetic analysis (satellite animals) and were given a single dose on Day 23 only. RTV was always given first, immediately followed by darunavir (see Tabulated Summary 2.6.7.15.A).

Then DRV formulated in PEG400 was administered alone or in combination with RTV formulated in PG, once daily, by oral gavage to juvenile rats, for 4 weeks (**from Day 23 to Day 50 of age**)[69]. This was followed by a subsequent 25-day period without dosing to assess reversibility of any changes. Six groups each consisting of 30 male and 30 female juvenile Sprague-Dawley rats (15 male and 15 female rats in each group allocated either as main animals or recovery animals) were given the following combinations of darunavir/RTV: 0/0 (PEG400 vehicle), 40/0, 200/0, 500/0, 500/75 or 0/75 mg darunavir/RTV/kg/day, in a dose volume of 10 mL/kg for darunavir or 1.25 mL/kg for RTV. A further 12 male and 12 female juvenile rats were allocated to each group for toxicokinetic analysis (satellite animals). RTV was always given first, immediately followed by darunavir. The results of this study can be found in Tabulated Summary 2.6.7.15.B.

6.2. Cobicistat

6.2.1. Fertility and Early Embryonic Development

The potential adverse effects of COBI were assessed when administered daily via oral gavage to male and female rats prior to mating and until termination (males) or through early gestation (females) [31] (Tabulated Summary 2.6.7.12.B).

Male and female Crl:CD(SD) rats were assigned to 4 groups (22/sex/group) and administered 0 [vehicle: 95% PG, 5% EtOH, pH 2.3], 10, 30, and 100 mg/kg/day COBI in a dose volume of 2.5 mL/kg. All males were dosed for at least 28 days prior to mating and throughout the mating phase (minimum of 10 weeks). Females were dosed for at least 14 days prior to mating (premating phase), throughout the mating phase and through GD7. Treated males were paired with treated females during the mating phase. On GD 13, females were necropsied and the uterus of each was examined for the number of live and dead fetuses and resorptions. Ovaries were examined for the number of corpora lutea. All males were necropsied and evaluated for reproductive capacity.

Assessment of toxicity was based on mortality, clinical signs, body weight, body weight gain, food consumption, organ weights, macroscopic findings, sperm analysis (sperm motility, morphology and counts), pregnancy status, and cesarean section evaluations.

All animals survived until the scheduled termination. Treatment-related clinical observations for the males and females during the premating phase included clear, oral discharge and alopecia in the 30 and 100 mg/kg/day groups. Clear oral discharge was also observed immediately after dosing in the 100 mg/kg/day females during the gestation phase.

Treatment with COBI produced significantly decreased mean body weight gain in the 100 mg/kg/day males at the initiation of dosing (Days 0#3) and over the entire dosing phase (Days 0#71). Significantly decreased mean body weight gain was observed in the 100 mg/kg/day males beginning on Day 49 and continued through the remainder of the dosing phase. At the initiation of dosing (Days 0#3), over the entire premating phase (Days 0#14), and from GD 0#3, a slight decrease in mean body weight gain was observed in the 100 mg/kg/day females. Significantly decreased mean food consumption was observed in the 100 mg/kg/day males and females and correlated with the observed effects on body weight.

Although there were no parental necropsy findings attributed to treatment, liver and thyroid weights were significantly increased in the 100 mg/kg/day males and females.

No biologically relevant effects were noted on the estrous cycle, and the majority of females were confirmed to have mated within the first 4 days of mating. Male/female copulation and fertility indices were unaffected by treatment. Cesarean section data were unremarkable. Mean percent preimplantation losses were similar across groups, indicating no treatment-related effects on the females. Mean postimplantation loss and mean percent live fetuses were similar across groups, indicating that COBI had no effect on embryo/fetal viability.

Mean sperm assessment parameter values were generally similar across groups, although a slight decrease in total sperm count was observed for the 100 mg/kg/day males. The apparent decrease was due to a single male, not the result of a general trend across multiple animals in the group, and therefore was not attributed to treatment.

In conclusion, the NOAEL for parental toxicity for COBI when administered daily via oral gavage to male and female rats prior to mating and until termination (males) or through early gestation (females) is 30 mg/kg/day for

both sexes based on significant decreases in body weight gain and food consumption at 100 mg/kg/day. The no observed effect level (NOEL) for male and female reproductive performance and competency (fertility), and embryo/fetal viability is 100 mg/kg/day.

6.2.2. Embryo-Fetal Development

6.2.2.1. Rats

A **dose range finding embryo-fetal development study** was conducted in pregnant rats[32]. Therefore time-mated female Crl:CD(SD) rats were assigned to 4 main groups (8/group) and administered 0 [vehicle: 95% PG, 5% EtOH, pH 2.3], 25, 75, and 200 mg/kg/day COBI. Doses were administered by oral gavage on GD 6 through 17 in a dose volume of 5 mL/kg. Additional time-mated rats (3#9/group) were used for toxicokinetic analysis on GD 6 and 17. Based upon the results of this study, doses for the full size development study were selected (Tabulated Summary 2.6.7.11).

In the **full size study**, the maternal and embryo/fetal toxicity, teratogenic potential and toxicokinetics of COBI was determined when administered daily via oral gavage to pregnant rats during the period of organogenesis (Tabulated Summary 2.6.7.13.E)[33]**.**

Groups of 25 time-mated female Crl:CD(SD) rats were administered 0 [vehicle: 95% PG, 5% EtOH, pH 2.3], 25, 50, or 125 mg/kg/day COBI. Doses were administered by oral gavage on GD 6 through 17 in a dose volume of 5 mL/kg. Additional time-mated rats (3#9/group) were used for toxicokinetic analysis to determine maternal COBI plasma concentrations at selected time points on GD 6 and 17.

Assessment of toxicity was based on mortality, clinical signs, body weights, food consumption, maternal necropsy, cesarean section, fetal external, and visceral and skeletal evaluations.

All animals survived to their scheduled termination. A dose-related increase in the incidence of clear oral discharge was observed in all treated groups during the dosing phase. Treatment-related clinical signs of thin appearance, hypoactivity, audible and/or irregular respiration, alopecia, rough haircoat, and few or no feces were limited to the 125 mg/kg/day group.

A significant treatment-related decrease in mean maternal body weight, body weight gain, and food consumption was noted at 125 mg/kg/day compared to control means. During the dosing phase, mean maternal body weight (85% of control), total mean weight gain (47% of control), and mean food consumption (66% of control) were significantly decreased compared to control group means. The effects on mean body weight gain and food consumption were partially reversed during the postdose phase (GD 18#20), in which mean weight gain and food consumption were significantly increased versus the control group. However, mean body weight remained significantly decreased (11% of controls) on GD 20 at 125 mg/kg/day.

A transient, but statistically significant, decrease in mean body weight gain was noted for the 50 mg/kg/day group during the initial dosing interval correlated with significantly decreased mean food consumption. These effects were transient and not considered adverse.

There were no treatment-related necropsy findings.

The mean gravid uterine weight and corrected maternal body weights were significantly decreased in the 125 mg/kg/day group.

The cesarean section data were unremarkable with the exception of a slight increase in the mean percent of postimplantation loss at 125 mg/kg/day that corresponded to a slight decrease in mean percent of live fetuses. The covariate-adjusted mean fetal weight was significantly decreased for the 125 mg/kg/day fetuses, corresponding to the decrease in mean gravid uterine weight and was likely an indirect effect from the treatment-related decrease in mean maternal body weights.

There were no test article-related malformations. Treatment-related fetal anomalies were limited to skeletal variations related to ossification issues in the spinal column and sternebra in the 125 mg/kg/day fetuses. The increase in these skeletal variations correlated with significantly decreased mean fetal weights for this high-dose group. However, these variations had no effect on body conformity or the well-being of the animal and are not considered adverse.

Exposure to COBI increased with the increase in COBI dose level from 25 to 125 mg/kg/day (Table 28). The increases in C_{max} and AUC_{0-t} were generally

dose proportional. No accumulation of COBI was observed after multiple dosing of COBI in pregnant rats.

Table 28: Mean Toxicokinetic Parameters of COBI in Pregnant Rats Following Daily Oral Doses of COBI

Dose Level (mg/kg/day)	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
	GD 6	GD 17	GD 6	GD 17
25	10.0	6.7	1.4	0.9
50	27.1	14.8	2.7	1.7
125	57.9	61.4	5.1	4.2

GD = Gestation Day

In conclusion, the NOAEL for maternal toxicity for COBI when administered by oral gavage to pregnant rats during the period of organogenesis is 50 mg/kg/day. The NOEL for embryo/fetal viability and growth and developmental toxicity is 50 mg/kg/day (GD17 AUC_{0-t}: 14.8 µg.h/mL), based on increased postimplantation loss and decreased fetal weights associated with significant decreases in maternal body weights at 125 mg/kg/day.

6.2.2.2. Rabbits

This **exploratory study** determined the maternal and in-utero toxicity and toxicokinetics of COBI when administered daily via oral gavage to pregnant rabbits during the period of organogenesis for the purpose of setting dosage levels for a definitive developmental study[34]. Time-mated female Hra:(NZW)SPF rabbits were randomly assigned to 5 main groups (5 animals/group) and administered 0 [vehicle: 95% PG, 5% EtOH, pH 2.3], 50, 100, 175, and 300 mg/kg/day COBI. Doses were administered on GD 7 through 20 in a dose volume of 1 mL/kg. All surviving rabbits were sacrificed on GD 29, cesarean-sectioned, and fetuses were weighed and examined for gross external alterations and sexed. Additional time-mated rabbits (3/group) were used for toxicokinetic analysis at selected time points on GD 7 and 20. Based on the results of this study (Tabulated Summary 2.6.7.11), the doses for the full size embryo-fetal development study were selected.

Doses of 0 [vehicle: 95% PG, 5% EtOH, pH 2.3], 25, 50, or 100 mg/kg/day COBI were given to 20 time-mated female Hra:(NZW)SPF rabbits per group, in order to study **maternal and embryo/fetal toxicity and teratogenic potential**[35]. Doses were administered by oral gavage on GD 7 through 20 in a dose volume of 1 mL/kg. Additional time-mated rabbits (3/group) were used for toxicokinetic analysis to determine maternal COBI plasma concentrations at selected time points on GD 7 and 20.

Assessment of toxicity was based on mortality, clinical observations, body weight, food consumption, necropsy, cesarean section, and fetal external, soft tissue and skeletal evaluations.

There were no unscheduled deaths or clinical signs related to COBI-treatment.

During the dosing phase, total mean body weight gain (GD 7#21) was decreased for the 100 mg/kg/day group and corresponded to decreased mean food consumption. However, these effects were not considered adverse since overall mean body weight was similar to control throughout the dosing phase, and the effects on mean body weight gain and food consumption were reversed during the postdose phase (GD 21#29).

There were no necropsy findings in any animals. The mean gravid uterine weight and corrected maternal body weights were unremarkable for all groups.

The cesarean section data were unremarkable and indicated COBI had no effect on embryo/fetal viability.

There were no effects on the covariate-adjusted mean fetal weights in any group or any treatment-related fetal anomalies.

Exposure to COBI generally increased with the increase in dose level from 25 to 100 mg/kg/day (Table 29). The increases in mean C_{max} and AUC_{0-t} were greater than dose proportional. Accumulation of COBI was observed after multiple dosing in pregnant rabbits.

Table 29: Mean Toxicokinetic Parameters of COBI in Pregnant Rabbits Following Daily Oral Doses of COBI

Dose Level (mg/kg/day)	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
	GD 7	GD 20	GD 7	GD 20
25	0.04	1.6	0.04	0.7
50	0.24	19.2	0.19	4.6
100	10.6	35.7	2.8	3.0

GD = Gestation Day

The NOAEL for maternal effects of COBI when administered by oral gavage to pregnant rabbits during the period of organogenesis is 100 mg/kg/day. There were no treatment-related effects on embryo/fetal viability and growth and no fetal anomalies; therefore, the NOEL for developmental toxicity is 100 mg/kg/day (GD 20 AUC_{0-t}: 35.7 µg.h/mL) (Tabulated Summary 2.6.7.13.F).

6.2.3. Prenatal and Postnatal Development, Including Maternal Function

An oral pre-and postnatal development study, including a 28-day juvenile toxicity study, was performed to investigate the effects of COBI upon gestation, parturition and lactation (treated from GD 6 to PND 20, 21, or 22) in the dam, the development of the pups and their survival, physical development, behavior and reproductive performance (Phase I) and to determine the effects of COBI on development when given by oral gavage daily for 4 weeks (PND 22 to 49) to juvenile rats (Phase II)[36].

During Phase 1, COBI was administered by oral gavage to time mated F_0 female rats (24 per group) at 0 [Vehicle: 10% (v/v) PG in 90% (v/v) 40 mM acetate buffer (pH 4)], 10, 30, and 75 mg/kg/day in a dose volume of 5 mL/kg. One or 2 weanlings/sex/litter were randomly selected to form the first (F_1) generation (Behavioral/Reproductive Phase, 24 animals/sex/group) and were not directly treated.

Toxicity assessment for the F_0 generation was based on mortality, clinical signs, body weight, food consumption, premature deliveries, maternal necropsy, and cesarean evaluations. Rats were also evaluated for adverse clinical signs during parturition, the duration of gestation, litter size, and maternal behavior. Pup viability, pup body weights, and clinical signs were

recorded for each litter. Blood and milk samples for TK analysis were collected from satellite groups of dams on PND 10.

F_0 Generation (Phase I)

There was no COBI-related mortality in F_0 generation female rats. Cobicistat-related clinical observations included a higher incidence of salivation, and wet fur of the muzzle and/or lower jaw during the gestation and lactation (PND 0 to 20) periods in dams administered 30 and 75 mg/kg/day. Decreases in body weight gains and food consumption were noted from GD 6 through GD 11 at 75 mg/kg/day. These differences resulted in significantly decreased body gain during the gestation period (decreased 15% versus control) and significantly lower mean body weight (from PND 0 to 12) during the lactation period at 75 mg/kg/day. At 10 and 30 mg/kg/day, transient decreases in body weight gain were noted during GD 6 through 8, only. Body weight gains over the entire postnatal period (PND 0 to 21) were unaffected by treatment at any dose level.

Cobicistat had no effect on pregnancy rate, gestation index, length of gestation, duration of parturition, number of live, dead and malformed pups, sex ratio, number of implantation scars, and the live birth index.

There were no gross pathologic findings in the dams attributed to COBI at any dose level.

There were no COBI-related effects on pup survival (viability, survival, and lactation indices), litter size, body weights, clinical signs, reflex development (righting reflex, negative geotaxis and auricular startle response), and gross pathology findings.

Exposure to COBI increased with the increase in dose level from 10 to 75 mg/kg/day (Table 30). Increases in C_{max} and AUC_{0-t} were approximately proportional between the 10 to 30 mg/kg/day dose levels and less than proportional between 30 and 75 mg/kg/day. Cobicistat was present in milk samples 2 hours postdose on PND 10 with mean milk to plasma ratios ranging from 1.3 to 1.9 (Table 31).

Table 30: Mean Toxicokinetic Parameters of COBI in Female Lactating Rats on Postnatal Day 10

Dose Level (mg/kg/day)	AUC_{0-t} (µg.h/mL)	C_{max} (µg/mL)
10	1.9	0.4
30	6.4	1.1
75	9.9	1.9

Table 31: Mean Concentrations of COBI in Rat Plasma and Milk 2 hours Postdose on Postnatal Day 10

Dose Level (mg/kg/day)	COBI (Plasma) (µg/mL)	COBI (Milk) (µg/mL)	Milk:Plasma Ratio
10	0.363	0.479	1.265
30	0.883	1.487	1.904
75	1.693	3.240	1.744

Behavioral/Reproductive Assessment (F_1 Generation)

For the F_1 generation animals, mortality, clinical signs, body weight, food consumption, physical signs of sexual maturation (vaginal opening from PND 26; preputial separation from PND 35), visual function (on PND 21), behavioral performance (motor activity at approximately PNDs 35 and 60; auditory startle habituation at approximately PND 55; 'E' water maze between PND 60 and 70), and estrous cycles for 10 days before placement for mating, were assessed. At approximately 79 to 83 days of age, the F_1 generation rats were assigned to a 14-day cohabitation period. All male rats were euthanized 2#3 weeks after completion of the cohabitation period, a gross necropsy was performed and testes, epididymides, seminal vesicles, and prostates were weighed. All mated female rats were sacrificed on GD 13, caesarean-sectioned, and a gross necropsy was performed.

There were no COBI-related effects on mortality and clinical signs, and no adverse effects on body weight, body weight gain, or food consumption.

There were no effects on visual function and no effects on physical and behavioral development.

There were no effects on estrous cycles, mating and fertility indices, conception rate, and mean day to mating. There were no COBI-related effects on caesarian section parameters, and no gross pathologic findings.

Juvenile Toxicity

For the juvenile toxicity phase (Phase II), F_1 generation rats (10/sex/group) were administered COBI at 0 (vehicle), 10, 30, and 75 mg/kg/day from PND 22 to PND 49 in a dose volume of 5 mL/kg. Assessment of toxicity was based on mortality, clinical signs, body weight, food consumption, clinical and anatomic pathology. Blood samples for TK analysis were collected from satellite animals on PND 22 and PND 49.

There was no COBI-related mortality. At 30 and 75 mg/kg/day, males and females exhibited a dose-related, but nonadverse, increase in postdose salivation, and red staining of the fur of the lower jaw.

Slight, but not statistically significant, decreases in mean body weight, mean body weight gain and mean food consumption were noted for males at 75 mg/kg/day during the dosing period. There were no notable effects on body weight or food consumption in males at 10 and 30 mg/kg/day, or in females at any dose level.

There were no treatment-related effects on hematology, coagulation, or urinalysis parameters. Clinical chemistry changes noted at 30 and 75 mg/kg/day were not considered adverse. These changes included minimal, but statistically significant, decreases in ALP levels in males at ≥ 30 mg/kg/day and in females at 75 mg/kg/day, and significant increases in globulin, with decreases in the albumin to globulin (A:G) ratio, in both sexes at ≥ 30 mg/kg/day.

Cobicistat-related increases in TSH, decreases in T4 and/or triiodothyronine (T3) were noted in both sexes at all dose levels.

There were no COBI-related macroscopic findings. Significantly increased liver weights (males at 75 mg/kg/day and females at ≥ 30 mg/kg/day) and increased thyroid weights (females at all dose levels) were associated with hepatocellular hypertrophy (females at 75 mg/kg/day) and thyroid follicular cell hypertrophy (in males at 75 mg/kg/day and in females at ≥ 30 mg/kg/day), respectively. In addition, there was an increased incidence

of hepatocellular vacuolation in females at ≥ 30 mg/kg/day. These liver and thyroid effects are considered to be adaptive changes, commonly seen in rodents with microsomal enzyme inducers, and previously observed with COBI in repeat-dose studies with adult rats (Section 3.2.1.1) and not considered adverse under the conditions of this study.

Exposure to COBI (Table 32) increased with the increase in dose level from 10 to 75 mg/kg/day. Increases in C_{max} and AUC_{0-t} were greater than proportional between the 10 and 75 mg/kg/day. C_{max} and AUC_{0-t} values were generally similar on PNDs 22 and 49, except for higher values in females at the 10 mg/kg/day dose level, indicating no accumulation of COBI after multiple dosing to juvenile rats. Sex differences were generally less than 2-fold in C_{max} and AUC_{0-t} values, except at 10 mg/kg/day on PND 49, where females had 3- to 6-fold higher C_{max} and AUC_{0-t} values, respectively, than males.

Table 32: Mean Toxicokinetic Parameters of COBI in Juvenile Rats Following Daily Oral Doses of COBI

Dose Level (mg/kg/day)	Sex	AUC_{0-t} (µg.h/mL)		C_{max} (µg/mL)	
		PND 22	PND 49	PND 22	PND 49
10	Male	0.3	0.3	0.1	0.1
	Female	0.5	1.8	0.2	0.3
30	Male	9.1	5.0	1.7	1.1
	Female	6.5	8.5	1.7	1.9
75	Male	32.1	20.6	3.7	3.2
	Female	18.6	21.2	3.1	3.1

PND = postnatal day

In conclusion, in the development and peri-natal/postnatal reproduction phase of the study, including a postnatal behavioral/functional evaluation, daily oral gavage administration of COBI resulted in lower body weights and food intake at 75 mg/kg/day during gestation and lactation but with no effects on maternal performance. In the untreated offspring (F_1 generation), there were no effects on survival, growth, physical and behavioral development, reflex responses, visual function, gross pathology or reproductive parameters. Based on these results, the NOAEL for maternal toxicity for the F_0 generation was considered to be 30 mg/kg/day, and the NOAEL for the reproduction in the dams and viability, growth, and

development of the offspring was considered to be 75 mg/kg/day (Maternal PND 10: C_{max} and AUC_{0-t}: 1.9 µg/mL and 9.9 µg.h/mL, respectively).

In the juvenile toxicity phase of the study, daily oral gavage administration of COBI to F_1 generation pups from PND 22 to 49 resulted in slight decreases in body weight and food consumption, nonadverse clinical chemistry changes, and adaptive changes in liver and thyroid. Based on these results, the NOAEL for the juvenile males and females was considered to be 75 mg/kg/day (PND 49 C_{max} and AUC_{0-t}: 3.2 and 3.1 µg/mL, and 20.6 and 21.2 µg.h/mL, in males and females, respectively) (Tabulated Summary 2.6.7.14.C).

6.2.4. Studies in which the Offspring are Dosed

The potential effects of COBI in juvenile animals were evaluated in the pre- and postnatal development toxicity study in rats (Section 6.2.3).

7. LOCAL TOLERANCE

7.1. Darunavir

7.1.1. Skin Sensitization: Local Lymph Node Assay

In a mouse local lymph node assay study, DRV was found to be unlikely to cause skin sensitization[70]. The assay determines the level of T lymphocyte proliferation in lymph nodes draining the site of chemical application by measuring the amount of radiolabelled thymidine incorporated into dividing cells. Darunavir was applied at 10, 25 and 50% w/v preparations in dimethyl formamide (DMF).

A second study[71] was conducted in which DRV was applied at 2.5, 5, 10, 25 or 50% w/v (in DMF). Darunavir did not induce delayed contact hypersensitivity in the mouse local lymph node assay and is considered unlikely to have the potential to cause skin sensitization. No cutaneous reactions and no increase in ear thickness occurred in DRV treated animals and, therefore, DRV is considered unlikely to have the potential to cause skin irritation.

For both studies, please refer to Tabulated Summary 2.6.7.16.

7.1.2. In Vivo Skin Irritation

Darunavir was evaluated for possible skin irritation potential according to the OECD test guideline no. 404, “Acute Dermal Irritation / Corrosion”[72].

Darunavir was applied by topical semi-occlusive application of 0.5 g (as 1% w/w solution) to the intact clipped skin of 3 New Zealand white rabbits for 4 hours. The scoring of skin reactions was performed at 1, 24, 48 and 72 hours after removal of the dressing.

All animals survived and there were no noteworthy clinical observations or effects on body weight. No skin reactions, staining of skin or corrosive effects were observed at any time point in any animal. Darunavir was classified as "non-irritant" to rabbit skin. Refer to Tabulated Summary 2.6.7.16.

7.1.3. In Vitro Ocular Irritation

Darunavir was evaluated for possible ocular irritation potential using the bovine corneal opacity-permeability (BCOP) assay, by measuring the opacity and permeability after exposure to the corneal epithelium[73]. Darunavir was applied for 4 hours as 20% w/w suspension in saline and induced no increase in corneal opacity and no increase in permeability. Darunavir was classified as a "non-irritant" to the bovine eye, in vitro (See Tabulated Summary 2.6.7.16).

7.2. Cobicistat

7.2.1. Skin Sensitization: Local Lymph Node Assay

The purpose of this study was to assess the skin sensitization potential of COBI in the murine local lymph node assay (LLNA) according to OECD Guideline 429[37]. Cobicistat was formulated in an acetone:olive oil (4:1) vehicle at 2.5%, 5%, and 10% (w/v). Twenty-five microliters of vehicle, positive control (25% hexyl cinnamic aldehyde) and each concentration of COBI were applied for 3 consecutive days on the dorsal surface of both ears of 5 female CBA/Ca mice per group. Three days later, all animals received an intravenous injection of [^{3}H]methyl thymidine. Five hours later they were euthanized and the radioactivity in the auricular lymph nodes was measured.

There were no deaths and no signs of ill health or toxicity were observed during the study. No signs of erythema were seen on the ears during the study. The test to control ratio (SI) of radioactivity from thymidine incorporated in the draining lymph node for each concentration ranged from 2.2 to 2.9, indicating that COBI did not show the potential to induce skin sensitization. The SI for the positive control substance was 14.8, demonstrating the validity of the test system (Tabulated Summary 2.6.7.16).

7.2.2. In Vivo Skin Irritation

The purpose of this study was to evaluate the skin irritation potential of COBI in New Zealand white rabbits according to OECD Guideline 404[38]. Three female rabbits received a single 4-hour, semi-occlusive administration (intact clipped skin moistened with 0.5 mL of reverse osmosis water) of approximately 0.5 g COBI. An additional control site was similarly treated without COBI. Skin reactions were scored 1, 24, 48, and 72 hours after removal of the semi-occlusive dressing and observations continued for up to 13 days. All animals survived and there were no noteworthy clinical observations. Very-slight erythema was evident at the test-site of each animal during the first seven days after bandage removal, persisting in one animal for a further day, and in the final animal for an additional six days. The Primary Irritation Index (PII) was calculated to be 1.0 and COBI was classified as "mildly-irritating" (PII > 0 – 2.0) to rabbit skin (Tabulated Summary 2.6.7.16).

7.2.3. In Vitro Ocular Irritation

The purpose of this study was to evaluate the ocular irritancy potential of COBI in the BCOP assay according to OECD Guideline 437[39]. Cobicistat was applied for 4 hours as a 20% w/w suspension in 0.9% saline to the corneal epithelium. Cobicistat elicited an In Vitro Irritancy Score of -1.6 ± 0.0 and was predicted to be a noncorrosive/nonsevere eye irritant. The In Vitro Irritancy Score of imidazole (positive control) was 161.8 ± 25.5, thereby confirming the ability of the assay to detect corrosive/severe irritants (Tabulated Summary 2.6.7.16).

7.2.4. Phototoxicity

Phototoxicity studies are not considered necessary for COBI. Cobicistat does not absorb light within the range 290-700 nm, and is photostable. There are no nonclinical or clinical findings indicative of phototoxicity with COBI, and no indications from structurally-related HIV protease inhibitors of a potential for phototoxicity.

8. OTHER TOXICITY STUDIES

8.1. Darunavir

8.1.1. Immunotoxicity

Darunavir formulated in PEG400 was co-administered without and with RTV formulated in PG, once daily, by oral gavage, for at least 4-

weeks[74].The following controls and combinations of DRV/RTV were used: 00 (purified water) 0 (PEG400), 20/0, 100/0, 500/0, 0/50 and 100/50 mg/kg/day in a dose volume of 5 mL/kg for DRV and 2.5 mL/kg for RTV. The RTV was given first immediately followed by the DRV. Each group consisted of 8 male and 8 female Sprague-Dawley rats (principal animals) together with 6 male and 6 female satellite animals (except for the purified water and PEG400 vehicle groups) for toxicokinetic analysis. Regular observations were made for clinical signs, body weight and food consumption, together with hematology (blood and bone marrow, at termination). The antibody response to the T-cell dependent antigen Keyhole Limpet Hemocyanin (KLH) was evaluated by subcutaneous administration of KLH on day 22, followed by ELISA determination of IgM levels in serum samples taken at necropsy. Blood samples were collected on day 25/26 during the 24 hours after dosing for TK analysis. All principal animals sacrificed at the end of the study were subject to macroscopic examination at necropsy, and a limited number of organs were weighed. Samples of a range of lymphoid tissues and organs were preserved and were examined microscopically.

There were no mortalities associated with DRV and there were no relevant clinical signs, or effects on body weight or food consumption.

A decrease (7%) in hemoglobin in females, with changes in RBC derived parameters in males and females, and an increase in reticulocyte counts (up to2-fold) in males and females occurred in animals given 500 mg/kg/day. When the myelograms of treated animals were compared to those given PEG400, a lower myeloid/erythroid ratio (up to 35%) was noted in animals given 500 mg/kg/day. In females this was due to higher total number erythroid elements as well as the number of different erythroblasts and normoblasts.

The immune response of treated animals, as measured by IgM production, was not affected by treatment.

Macroscopic examination and organs weights at necropsy indicated an increase in liver weight in animals given 500 mg/kg/day and with DRV alone or in combination with RTV or with 50 mg/kg/day RTV alone. The relative liver weights increased up to 72%. The increase in liver weight of animals dosed with 100/50 mg/kg/day DRV/RTV was greater than RTV

alone and comparable to that of animals dosed with 500 mg/kg/day DRV. Only livers that appeared abnormal at necropsy were examined microscopically and in these animals the liver weight changes were confirmed as hepatocellular hypertrophy. There were no other relevant histopathological findings in lymphoid tissues.

Darunavir was found not to result in any immunotoxicological response at doses up to 500 mg/kg/day. Similarly, for RTV alone or DRV in combination with RTV (0/50 and 100/50 mg/kg/day, respectively), no immunotoxicological response was observed. The NOAEL was considered to be 20 mg/kg/day based on the absence of any hematological or histopathological changes (See Tabulated Summary 2.6.7.17.A).

Systemic exposure, expressed as C_{max} and AUC values are given in Table 33 and Table 34 below:

Table 33: Darunavir C_{max} and AUC values after repeated oral administration (4 weeks) in rats

TMC114/RTV Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M	F	M	F
20/0	Week 4	0.75	1.71	4.20	4.68
100/0	Week 4	3.87	6.54	25.2	30.0
500/0	Week 4	13.6	9.96	102	84.4
100/50	Week 4	6.62	6.63	59.0	57.7

Table 34: RTV C_{max} and AUC values after repeated oral administration (4 weeks) in rats

TMC114/RTV Dose (mg/kg/day)	Sampling Period	C_{max} (µg/mL)		AUC_{last} (µg.h/mL)	
		M	F	M	F
0/50	Week 4	3.43	5.45	21.8	37.3
100/50	Week 4	1.92	2.70	14.4	20.1

8.1.2. Mechanistic Studies

A 1-month mechanistic study was conducted in Sprague-Dawley rats who were dosed once daily at 0 (vehicle), 50 and 500 mg/kg/day in a dose volume of 5 mL/kg, in order to investigate the effects of TMC114 on the pituitary-thyroid-axis[75]. Each group consisted of 20 male and 20 female rats (main animals) together with 6 (treated groups) or 3 (vehicle group) male and female satellite animals for toxicokinetic analysis. The results are tabulated in Tabulated Summary 2.6.7.17.B.

8.1.3. Studies on Impurities

8.1.3.1. Batch Qualification Studies

Batch qualification studies were conducted on batch, [redacted] (limited Ames test) and batches, [redacted], [redacted] and [redacted] (limited Ames test and 14-day dog study).

Bacterial Reverse Mutation Assay

Darunavir, [redacted] formulated in DMSO, was tested in a limited bacterial reverse mutation assay (Ames test), with 2 histidine-requiring strains of *Salmonella typhimurium* (TA98 and TA100)[76]. Darunavir was tested in the 2 tester strains of *Salmonella typhimurium*, in the absence or presence of S9-metabolic activation system (Aroclor-induced rat liver S9-mix) up to 5000 µg/plate. As previously observed, DRV precipitated on the plates at the higher dose level tested but the bacterial background lawn was not reduced at any of the concentrations tested and no decrease in the number of revertants was observed. Darunavir did not induce a dose-related, more than two-fold increase in the number of revertants in any of the tester strains in the absence or presence of S9-metabolic activation system and these observations were confirmed in 3 independently repeated experiments. Vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. Darunavir, batch [redacted] was not mutagenic under the conditions of this reverse gene mutation assay (See Tabulated Summary 2.6.7.17.C).

Darunavir, batches [redacted], [redacted] and [redacted] formulated in DMSO, were tested in a limited bacterial reverse mutation assay (Ames test), with 2 histidine-requiring strains of *Salmonella typhimurium* (TA98 and TA100)[77]. Darunavir was tested in the 2 tester strains of *Salmonella typhimurium*, in the absence or presence of S9-metabolic activation system (Aroclor-induced rat liver S9-mix) up to 5000 µg/plate. As previously observed, DRV precipitated on the plates at the higher dose level tested but the bacterial background lawn was not reduced at any of the concentrations tested and no decrease in the number of revertants was observed. Darunavir did not induce a dose-related, more than two-fold increase in the number of revertants in any of the tester strains in the absence or presence of S9-metabolic activation system and these observations were confirmed in 3 independently repeated experiments.

Vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. Darunavir, batches [redacted], [redacted] and [redacted] were not mutagenic under the conditions of this reverse gene mutation assay (See Tabulated Summary 2.6.7.17.C).

14-Day Repeated Dosing

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for at least 14 days[78,79]. One control group and three treated groups were given, 0 (vehicle), 120 [redacted]), 120 ([redacted]) and 120 ([redacted]) mg /kg/day in a dose volume of 1 mL/kg. The vehicle control group consisted of 1 male and 1 female and each treated group consisted of 2 male and 2 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption together with ophthalmoscopic examination, electrocardiography and clinical laboratory investigations (hematology, blood chemistry and urine analysis, at termination). Blood samples were collected on days 1 and 14 during the first 24 hours after dosing for toxicokinetic analysis. All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all groups and all gross lesions were examined microscopically.

There were no mortalities in the study and there were no relevant clinical signs, or effects on body weight, food consumption, ophthalmoscopy or electrocardiology. Hematology, serum chemistry and urinalysis parameters were unchanged with treatment of above batches of DRV.

Macroscopic examination at necropsy, organ weights and histopathological examination indicated no relevant changes that could be attributable to treatment. No differences in effects were noted with the three different batches (See Tabulated Summary 2.6.7.17.C).

Toxicokinetic analysis indicated that the three different batches gave comparable systemic exposure. The exposure of DRV, expressed as AUC_{last}, after repeated dosing of DRV on day 14 ranged from 22.5 to 95.4 µg.h/mL.

8.1.3.2. Impurities 不純物A* ***and*** 不純物B*

Darunavir drug substance impurities, 不純物A* and 不純物B*, were investigated in an Ames test, a 14-day oral rat study and a

chromosomal aberration test (CAT), where spiked DRV material was evaluated and compared to a reference batch.

Bacterial Reverse Mutation Assay

Darunavir spiked with 1% of each of 不純物A* and 不純物B* (DRV drug substance impurities), formulated in DMSO, was tested in a classical bacterial reverse mutation assay (Ames test), with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[80]. Following a dose-range finding test, DRV was tested in 2 independent assays, in the 5 tester strains of *Salmonella typhimurium*, in the absence or presence of S9-metabolic activation (Aroclor-induced rat liver S9-mix) up to 5000 µg/plate, and in a third confirmatory experiment without S9-mix. As previously observed, DRV precipitated on the plates at the higher dose level tested but the bacterial background lawn was not reduced at any of the concentrations tested and no decrease in the number of revertants was observed. Darunavir spiked with 1% of each of 不純物A* and 不純物B* did not induce a dose-related, more than two-fold increase in the number of revertants in any of the tester strains in the absence or presence of S9-metabolic activation system and these observations were confirmed in 3 independently repeated experiments. Vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. Darunavir spiked with 1% of each of 不純物A* and 不純物B* was not mutagenic under the conditions of this reverse gene mutation assay (See Tabulated Summary 2.6.7.17.C).

14-Day Repeated Dosing

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for 14 days[81]. One control group and four treated groups were given, 0 (vehicle), 20 (ref. DRV), 500 (ref. DRV), 20 (spiked DRV) and 500 mg/kg/day (spiked DRV) in a dose volume of 5 mL/kg. The DRV reference batch was spiked with 1% of each of 不純物A* and 不純物B*. Each group consisted of 10 male and 10 female Sprague-Dawley rats. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (hematology, blood chemistry and urine analysis, at termination). All animals sacrificed at the end of the study were subject to macroscopic

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examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. A limited list of relevant tissues and organs from all animals and all gross lesions, were examined microscopically. A limited list of tissues and organs from all animals in all groups and a full list of tissues and organs from high dose and control animals sacrificed at the end of the study and all gross lesions, were examined microscopically.

There were no mortalities associated with DRV and there were no relevant changes on clinical signs, body weight or food consumption.

The previously recorded effects on the hematopoietic system, including increased hematopoietic activity in the spleen, changes in serum analysis, liver, thyroid and adrenocortical zona fasciculata were again observed in this study at 500 mg/kg/day.

The overall toxicity profile of DRV spiked with 1% of each of 不純物A* and 不純物B* was identical to that of the reference, non-spiked drug substance (See Tabulated Summary 2.6.7.17.C).

Chromosomal Aberration Test

Darunavir spiked with 1% of each of 不純物A* and 不純物B* (darunavir drug substance impurities) formulated in DMSO was tested for the induction of chromosomal aberrations in cultured peripheral human lymphocytes, in the absence and presence of S9 metabolic activation system (Aroclor-induced rat liver S9 mix)[82]. In an initial assay, in the absence and presence of S9 mix darunavir was tested at concentrations up to 1000 µg/mL, for a 4-hour treatment time. In the confirmatory assay, darunavir was tested at concentrations up to 500 µg/mL in the absence of S9 mix for a 20-hour treatment time and in the presence of S9 mix for a 4-hour treatment time. In both assays, cells were harvested at 20 hours and only concentrations up to 400 µg/mL were selected for microscopic assay owing to precipitation of the test solution above this concentration.

Darunavir spiked with 1% of each of 不純物A* and 不純物B* did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations in the absence or presence of S9 mix, in 2 independently repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was

concluded that darunavir spiked with above impurities was not clastogenic in human lymphocytes under the conditions of this assay (Tabulated Summary 2.6.7.17.C).

8.1.3.3. Impurities 不純物C and 不純物D**

Darunavir drug substance impurities, the two isomers of a racemate, 不純物C* and 不純物D* were investigated in an Ames test, a 14-day oral rat study and a CAT, where spiked DRV material was evaluated and compared to a reference batch.

Bacterial Reverse Mutation Assay

Darunavir spiked with 1% of each of 不純物C* and 不純物D* (DRV drug substance impurities), formulated in DMSO, was tested in a classical bacterial reverse mutation assay (Ames test), with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[83]. Following a dose-range finding test, DRV was tested in 3 independent assays, in the 5 tester strains of *Salmonella typhimurium*, in the absence or presence of S9-metabolic activation system (Aroclor-induced rat liver S9-mix) up to 5000 µg/plate. As previously observed, DRV precipitated on the plates at the higher dose level tested but the bacterial background lawn was not reduced at any of the concentrations tested and no decrease in the number of revertants was observed. Darunavir spiked with 1% of each of 不純物C* and 不純物D* did not induce a dose-related, more than two-fold increase in the number of revertants in any of the tester strains in the absence or presence of S9-metabolic activation and these observations were confirmed in 3 independently repeated experiments. Vehicle control and appropriate positive control reference articles confirmed the adequacy of the test system. Darunavir spiked with 1% of each of 不純物C* and 不純物D* was not mutagenic under the conditions of this reverse gene mutation assay (See Tabulated Summary 2.6.7.17.C).

14-Day Repeated Dosing

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for **14 days**[84]. One control group and four treated groups were given, 0 (vehicle), 20 (ref. DRV), 500 (ref. DRV), 20 (spiked DRV) and 500 mg/kg/day (spiked DRV) in a dose volume of 5 mL/kg. The DRV

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reference batch was spiked with 1% of each of 不純物C* and 不純物D* . Each group consisted of 10 male and 10 female Sprague-Dawley rats. Regular observations were made for clinical signs, body weight and food consumption, together with clinical laboratory investigations (hematology, blood chemistry and urine analysis, at termination). All animals sacrificed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. A limited list of tissues and organs from all animals sacrificed at the end of the study and all gross lesions were examined microscopically.

There were no mortalities associated with DRV and there were no relevant changes on clinical signs, body weight or food consumption.

The previously recorded effects on the hematopoietic system including increased hematopoietic activity in the spleen, changes in serum analysis, liver and thyroid (weight and histology) were again observed in this study at 500 mg/kg/day.

The overall toxicity profile of DRV spiked with 1% of each of 不純物C* and 不純物D* was identical to that of the reference, non-spiked drug substance (See Tabulated Summary 2.6.7.17.C).

Chromosomal Aberration Test

Darunavir spiked with 1% of each of 不純物C* and 不純物D* (darunavir drug substance impurities) formulated in DMSO was tested for the induction of chromosomal aberrations in cultured peripheral human lymphocytes, in the absence and presence of S9 metabolic activation system (Aroclor-induced rat liver S9 mix)[85]. In an initial assay, in the absence and presence of S9 mix darunavir was tested at concentrations up to 1000 µg/mL, for a 5-hour treatment time. In the confirmatory assay, darunavir was tested at concentrations up to 500 µg/mL in the absence of S9 mix for a 20-hour treatment time and in the presence of S9 mix for a 4-hour treatment time. In both assays, cells were harvested at 20 hours and only concentrations up to 300 µg/mL were selected for microscopic assay owing to precipitation of the test solution above this concentration.

Darunavir spiked with 1% of each of 不純物C* and 不純物D* did not induce a statistically or biologically significant increase in the number of

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cells with chromosome aberrations in the absence or presence of S9 mix, in 2 independently repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was concluded that darunavir spiked with above impurities was not clastogenic in human lymphocytes under the conditions of this assay (Tabulated Summary 2.6.7.17.C).

8.1.3.4. Impurities 不純物E , 不純物F* and 不純物G**

Darunavir drug substance impurities 不純物E* , 不純物F* and 不純物G* were investigated in 2 Ames tests and a CAT, where spiked DRV material was evaluated and compared to a reference batch.

Bacterial Reverse Mutation Assay

Darunavir spiked with 1% of each of 不純物E* , 不純物F* and 不純物G* (darunavir drug substance impurities), formulated in DMSO, was tested in a classical Ames test, with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[86]. No dose range finding test was performed; concentrations levels were selected from a screening study[87]. Darunavir was tested in 2 independent assays; using the 5 tester strains of *Salmonella typhimurium*, in the absence and presence of S9 metabolic activation (Aroclor induced rat liver S9- mix) up to 5000 µg/plate.

In both experiments darunavir spiked with 1% of each of 不純物E* , 不純物F* and 不純物G* did not induce a dose-related, more than 2-fold increase in the number of revertants in any of the tester strains in the absence or presence of S9 mix. Above 1250 µg/plate, a concentration-related increase in precipitation was observed with all strains in the absence and presence of S9 mix. Vehicle control and appropriate positive controls confirmed the adequacy of the test system. Darunavir spiked with 1% of each of 不純物E* , 不純物F* and 不純物G* was not mutagenic under the conditions of this reverse gene mutation assay (Tabulated Summary 2.6.7.17.C).

Chromosomal Aberration Test

Darunavir spiked with 1% of each of 不純物E* , 不純物F* and 不純物G* (darunavir drug substance impurities) formulated in DMSO

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was tested for the induction of chromosomal aberrations in cultured peripheral human lymphocytes, in the absence and presence of S9 metabolic activation system (Aroclor-induced rat liver S9 mix)[88]. In an initial assay, in the absence and presence of S9 mix darunavir was tested at concentrations up to 1000 µg/mL, for a 4-hour treatment time. In the confirmatory assay, darunavir was tested at concentrations up to 500 µg/mL in the absence of S9 mix for a 20-hour treatment time and in the presence of S9 mix for a 4-hour treatment time. In both assays, cells were harvested at 20 hours and only concentrations up to 350 µg/mL were selected for microscopic assay owing to precipitation of the test solution above this concentration.

Darunavir spiked with 1% of each of 不純物E* , 不純物F* and 不純物G* did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations in the absence or presence of S9 mix, in 2 independently repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was concluded that darunavir spiked with above impurities was not clastogenic in human lymphocytes under the conditions of this assay (Tabulated Summary 2.6.7.17.C).

8.1.3.5. Impurities 不純物H and 不純物I**

Darunavir drug substance impurities 不純物H* and 不純物I* were investigated in an Ames test and a CAT, where spiked DRV material was evaluated and compared to a reference batch. In addition a 14-day study with 1% each of those impurities was tested.

Bacterial Reverse Mutation Assay

Darunavir spiked with 1% of each of 不純物H* and 不純物I* (darunavir drug substance impurities), formulated in DMSO, was tested in a classical bacterial reverse mutation assay (Ames test), with 5 histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA102, TA1535 and TA1537)[89]. Following a dose range finding test, darunavir was tested in 2 independent assays; using the 5 tester strains of *Salmonella typhimurium*, in the absence and presence of S9 metabolic activation (Aroclor induced rat liver S9 mix) up to 5000 µg/plate.

In both experiments darunavir spiked with 1% of each of 不純物H* and 不純物I* did not induce a dose-related, more than 2-fold increase in the

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number of revertants in any of the tester strains in the absence or presence of S9 mix. Weak bacteriotoxic effects, visualized by a reduction in the number of revertant colonies were observed, with some strains in the presence of S9 mix. In addition, a concentration-related increase in precipitation was observed with all strains in the absence (from 1250 µg/plate) and presence (from 2500 µg/plate) of S9 mix. Vehicle control and appropriate positive controls confirmed the adequacy of the test system. Darunavir spiked with 1% of each of 不純物H* and 不純物I* was not mutagenic under the conditions of this reverse gene mutation assay (Tabulated Summary 2.6.7.17.C).

Chromosomal Aberration Test

Darunavir spiked with 1% of each of 不純物H* and 不純物I* (darunavir drug substance impurities) formulated in DMSO was tested for the induction of chromosomal aberrations in cultured peripheral human lymphocytes, in the absence or presence of S9 metabolic activation system (Aroclor-induced rat liver S9 mix)[90]. In an initial assay, in the absence and presence of S9 mix darunavir was tested at concentrations up to 500 µg/mL, for a 3-hour treatment time and the cells were harvested at 20 hours. In the confirmatory assay, darunavir was tested at concentrations up to 500 µg/mL in the absence of S9 mix for a 20-hour treatment time and in the presence of S9 mix for a 3-hour treatment time and the cells were harvested at 20 hours and 44 hours. Precipitation was noted at concentrations of 500 µg/mL.

Darunavir spiked with 1% of each of 不純物H* and 不純物I* did not induce a statistically or biologically significant increase in the number of cells with chromosome aberrations in the absence or presence of S9 mix, in 2 independently repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was concluded that darunavir spiked with above impurities was not clastogenic in human lymphocytes under the conditions of this assay (Tabulated Summary 2.6.7.17.C).

14-Days Repeated Dosing

Darunavir formulated in PEG400 was administered once daily, by oral gavage, for at least 14 days[91]. One control and two treated groups were given 0 (vehicle), 120 (reference darunavir) and 120 (spiked darunavir) mg/kg/day

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in a dose volume of 2 mL/kg. The darunavir reference batch was spiked with 1% each of 不純物H* and 不純物I* (darunavir drug substance impurities). Each group consisted of 3 male and 3 female Beagle dogs. Regular observations were made for clinical signs, body weight and food consumption, together with ophthalmic examination, electrocardiography and clinical laboratory investigations (hematology, blood chemistry and urine analysis, before dosing and at termination). All animals killed at the end of the study were subject to macroscopic examination at necropsy and a number of organs were weighed. Samples of a range of tissues and organs were preserved for histological examination. All tissues and organs from animals in all groups and all gross lesions were examined microscopically.

There were no mortalities in the study but clinical signs, such as increased frequency of soft and mucoid feces and vomiting were observed in darunavir treated animals, however these were comparable between the two darunavir treated groups. There were no relevant effects on body weight, food consumption, ophthalmoscopy, electrocardiography and the clinical laboratory investigations (hematology, blood chemistry and urine analysis). Macroscopic examination at necropsy, organs weights and histological examination revealed no relevant changes that could be attributed to darunavir treatment.

The overall toxicity profile of darunavir spiked with 1% each of 不純物H* and 不純物I* was identical to that of the reference, non-spiked drug substance (Tabulated Summary 2.6.7.17.C).

8.1.4. Other Studies

8.1.4.1. Platelet Aggregation

The direct effect of DRV, lopinavir and tipranavir on human platelets was measured in an in vitro platelet aggregation study[92]. The results can be found in Tabulated Summary 2.6.7.17.D.

8.2. Cobicistat

8.2.1. Immunotoxicity

A 4-week oral gavage T-cell dependent antibody assay was conducted with COBI I rats[40]. Therefore male and female Crl:CD(SD) rats were assigned to 5 toxicity groups. Each group received COBI at dose levels of 0 (vehicle [95% PG, 5%EtOH]), 20, 50 or 150 mg/kg/day, or cyclosporine A (positive control) at 30 mg/kg/day via oral gavage. All toxicity animals received one

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dose of KLH by intravenous injection into a tail vein on Day 5 of the dosing phase. On Days 5 (pre-KLH injection), 15, 22, and 29, blood samples were collected for analysis of serum anti-KLH immunoglobulin M (IgM; Days 5, 15, and 22) and anti-KLH IgG (Days 5, 22 and 29) using ELISA methods. Blood samples, analyzed to confirm COBI exposure, were collected from toxicokinetic animals on Day 1 and during Week 4.

Assessments of general toxicity and immunotoxicity (T-cell dependent antibody assay alterations) were based on mortality, clinical signs, food consumption, body weights, anti-KLH antibody analysis, toxicokinetic evaluation, immunohistochemistry, and anatomic pathology.

No test article-related deaths occurred in the study. Test article-related clinical signs were limited to clear oral discharge and skin/pelage findings (red skin and red haircoat) and were noted more frequently in animals given 50 or 150 mg/kg/day COBI.

Decreased mean body weights and body weight gain and/or lower food consumption were noted in males given 10 mg/kg/day COBI or cyclosporin A when compared to control.

No COBI-related changes in the anti-KLH IgM antibody response were noted at any dose. Statistically significant decreases in the anti-KLH IgG antibody responses were noted in females at 50 and 150 mg/kg/day COBI. In males, decreases in the anti-KLH IgG response at 150 mg/kg/day did not reach statistical significance. Animals given cyclosporin A produced almost no anti-KLH IgM or IgG antibodies, consistent with the immunosuppressive properties of cyclosporin A.

No test article-related macroscopic findings were noted. Dose-related increases in liver weights were observed at 50 or 150 mg/kg/day COBI in both sexes and increases in thyroid/parathyroid weights were noted at 150 mg/kg/day in males. These organ weight changes are consistent with previous data with COBI in rats, and are considered adaptive changes commonly noted with hepatic microsomal enzyme inducers. Microscopic findings of lymphoid depletion of germinal centers in the spleen were noted in control, COBI, and cyclosporin A-treated animals. Although the incidence and/or severity of lymphoid depletion appeared to be higher in males and

females administered COBI at 50 and 150 mg/kg/day, the relationship to T-cell dependent antibody response findings is unclear.

Immunohistochemical (IHC) analysis of the spleen revealed trends toward lymphoid depletion of germinal centers and peanut agglutinin (PNA)-positive B-cells at 150 mg/kg/day in males, trends towards decreases in KiB1R-positive B cells at 50 and 150 mg/kg/day in males, and a slight decrease PNA-positive B cells in females given 50 mg/kg/day. No changes in CD3-positive T cells or number of germinal centers/follicular centers in COBI-treated males or females were noted. Animals given cyclosporin A had significant decreases in periarteriolar lymphoid sheath cellularity, lymphoid depletion in germinal centers, decreases in CD3-positive T cells, decreases in KiB1R-positive/PNA-positive B cells, and the almost complete absence of KiB1R-/PNA-positive germinal centers/follicular centers. The IHC changes were judged not to be reflective of the decreases noted in anti-KLH IgG levels, as greater decreases in anti-KLH IgG levels were observed in females versus males, but greater IHC changes were noted in males. For females, there was a notable lack of any dose-response with respect to the IHC changes.

A limited number of blood samples were taken for toxicokinetic analysis (predose, 2, 8, and 24 hours postdose, only). Cobicistat values were measurable only at 2 and 8 hours postdose during Week 4 at dose levels of 20 and 50 mg/kg/day, and at 2, 8, and 24 hours postdose at the 150 mg/kg/day dose. Exposure to COBI increased with the increase in dose level from 20 to 150 mg/kg/day. The increases in C_{max} and AUC_{0-t} were not consistently dose proportional. Females generally had higher COBI C_{max} and AUC_{0-t} values than males. No accumulation of COBI in rat plasma was observed after multiple dosing.

In conclusion, administration of COBI to rats by oral gavage for at least 4 weeks produced immunosuppressive effects in females at 50 and 150 mg/kg/day, based on decreased response to KLH immunization (lower anti-KLH IgG antibody titers). Decreased anti-KLH IgG responses in males did not reach statistical significance at 150 mg/kg/day. No COBI-related changes in the anti-KLH IgM response in males and females were noted. In addition, clinical signs, decreases in body weight gain and/or food consumption, increases in liver and thyroids weights, and lymphoid depletion of germinal

centers in the spleen were observed at 50 and/or 150 mg/kg/day. The NOEL for the TDAR is considered to be 20 mg/kg/day in females (Week 4 AUC_{0-t} 5.7 μg.h/mL), and 50 mg/kg/day in males (Week 4 AUC_{0-t} 11.2 μg.h/mL). Based on the observed changes in the spleen, the NOAEL for the study is considered to be 20 mg/kg/day in males and females (Week 4 AUC_{0-t} of 2.3 and 5.7 μg.h/mL, respectively) (Tabulated Summary 2.6.7.17.E).

8.2.2. Mechanistic Studies

No specific mechanistic studies were conducted for COBI. In the repeat-dose toxicity studies (Section 3.2), specific endpoints were incorporated into study designs to help interpret the effects of COBI on liver, thyroid, heart, the immune system and on urine volume. Hepatic microsomal CYP450 enzymes (mice, rats and dogs), thyroid hormones (mice and rats), ECGs (dogs), immunophenotyping (rats and dogs), and vasopressin and aldosterone levels (rats) were measured. Further, the potential for COBI to activate human and rat PXR was evaluated in vitro (Module2.6.4/Section5.5.2.2.1).

8.2.3. Studies on Impurities

8.2.3.1. 4-Week Oral Gavage Qualification Toxicity and Toxicokinetic Study with COBI in Rats

The potential toxicity and qualified potential impurities of COBI were evaluated by comparing a pure lot (COBI-A) to a lot spiked with up to 4.9% impurities (COBI-B) administered daily via oral gavage to rats for at least 28 days (Tabulated Summary 2.6.7.17.F)[41].

Female Crl:CD(SD) rats were randomized to 4 toxicity groups (10/group) and were dosed daily via oral gavage administration with the vehicle control article [95% PG and 5% EtOH (v/v)] and 100 mg/kg/day COBI-A, or 30 and 100 mg/kg/day COBI-B in a dose volume of 2.5 mL/kg. Blood was collected for toxicokinetic analysis from satellite groups of animals on Days 1 and 28. Female rats only were evaluated in this study due to the slightly higher exposures achievable in this sex.

Assessment of toxicity was based on mortality, clinical signs, body weights, food consumption, and clinical and anatomic pathology.

All animals survived to their scheduled sacrifice. Transient COBI-related clinical signs were limited to clear oral discharge, with a higher incidence in

animals given COBI-B. There were no COBI-related effects on mean body weight or food consumption for either lot of COBI.

Clinical pathology changes were minor, similar with both lots of the test article, and not considered adverse. Several minor findings were observed at 30 and 100 mg/kg/day and included minimally lower red blood cell counts, hemoglobin and hematocrit, minimally to mildly higher white blood cell, absolute neutrophil, lymphocyte, monocyte, eosinophil, and platelet counts and minimally to mildly higher glucose, cholesterol, calcium, total protein, globulin, ALT activity, urine volume, and urine pH and lower A:G ratio, BUN, chloride, and urine specific gravity.

No test article-related macroscopic observations were noted. Significantly increased mean liver weights were noted in all COBI-treated animals compared to controls, and the increases were similar with both lots at the 100 mg/kg/day dose level. With both lots, increased liver weights correlated microscopically with hepatocellular hypertrophy. Slight increases in mean thyroid/parathyroid weights were observed in all COBI-treated groups, and correlated with follicular cell hyperplasia. These liver and thyroid/parathyroid findings were considered adaptive changes and not considered adverse.

Exposure to COBI-B increased with the increase in dose level from 30 to 100 mg/kg/day, and exposures were generally less than dose proportional. Values for C_{max} and AUC_{0-t} at 100 mg/kg/day COBI-A and COBI-B were similar on Days 1 and 28.

In conclusion, both the pure lot and the spiked impurities lot of COBI (COBI-A and COBI-B, respectively) given once daily by oral gavage to female rats for 29 days were well-tolerated up to 100 mg/kg/day, the highest dose administered. The NOAEL was considered to be 100 mg/kg/day for both COBI-A and COBI-B (Day 28 AUC_{0-t} of 40.3 and 36.0 μg.h/mL, respectively).

8.2.3.2. Qualification of Impurities

Over 15 impurities and degradation products related to COBI have been identified in batches of the active pharmaceutical ingredient (API) or drug product. The impurity profiles for batches of API or drug product used in nonclinical toxicology studies are provided in Tabulated Summary 2.6.7.4.

A 4-week study was conducted in rats to determine if there were unexpected toxicologic effects of COBI which had been spiked with impurities (Tabulated Summary 2.6.7.17.F)[41]. No adverse treatment-related findings were observed following at least 28 days of oral gavage dosing of either COBI or COBI lots spiked with impurities at doses up to 100 mg/kg/day to rats. No differences between treatment with either COBI or COBI spiked with impurities were noted.

In silico evaluation of several process intermediates and potential impurities in COBI for potential mutagenicity, chromosome damage, genotoxicity and carcinogenicity revealed no structural alerts (Tabulated Summary 2.6.7.17.F)[42,43]. Representative process impurities (不純物CA* , 不純物CB* , 不純物CC* and 不純物CD*) were also evaluated in in vitro reverse mutation (Ames) studies (Tabulated Summary 2.6.7.17.F)[44] and in mouse lymphoma forward mutation assays (Tabulated Summary 2.6.7.17.F)[45], and were considered non-genotoxic.

Based on their impurity profiles, the multiple GLP batches of COBI tested in the toxicology program are considered, in composite, to be representative of the GMP material and support the specified limits of impurities proposed for commercial production.

8.2.4. Other Studies

No other relevant studies with COBI were conducted.

9. DISCUSSION AND CONCLUSIONS

This section discusses the significance of the results obtained in the toxicology studies with DRV and COBI. The relevance of the effects observed for human health and the margin of safety will be discussed in Module 2.4 Nonclinical Overview. The toxicology program comprised oral single-dose toxicity studies in mice, rats and dogs and single dose studies with DRV in rats and dogs. Repeat-dose with DRV alone, DRV co-administered with RTV, COBI alone and COBI co-administered with ATV were performed in mice, rats and/or dogs. A standard package of in vitro genotoxicity studies was conducted, as well as an in vivo micronucleus test in mice (DRV) or rats (COBI). For DRV, the reproductive and developmental toxicity studies with or withoutRTV comprised a male and female fertility study in rats, embryo-fetal developmental studies in rats, rabbits, mice or minipigs, pre- and postnatal developmental studies in rats

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and juvenile studies in rats. For COBI a fertility study was performed in rats, embryo-fetal developmental studies in rats and rabbits and a pre- and postnatal developmental study including a juvenile toxicity study in rats. The comprehensive package of safety studies was completed with local tolerance, immunotoxicity, mechanistic studies and safety studies with impurities encountered in the drug substance.

9.1. Darunavir

9.1.1. Single and Repeated Dosing-Target Organ Toxicity

In single dose and repeated dose toxicity studies, up to 6 months in rats and 12 months in dogs, there were only limited effects of treatment with DRV. In the rat the key target organs/systems identified were the hematopoietic system, the blood coagulation system, liver and thyroid. No major toxicity findings or key target organs/systems were identified in the dog but after 12 months of treatment limited changes in the liver did occur. No new target organs were identified in mouse studies up to 3 months duration.

To increase systemic exposure to DRV in animals and to assess the toxicity effect of the combination, several nonclinical studies were done with DRV and RTV. The two compounds were given in separate formulations orally; RTV first followed by DRV. This approach was successful in rodents and subsequently a 6-month combination study was conducted. Various combinations and dose regimens of DRV with RTV were evaluated in pilot studies in dogs but as this was unsuccessful due to limited tolerability and no increase in DRV exposure, no further combination studies were conducted.

Throughout all of the rodent toxicology studies there were no mortalities associated with DRV. The majority of deaths were gavage accidents, related to the viscous and irritant nature of the vehicle used, namely PEG400. In combination studies with RTV, double gavaging was performed, which further contributed to gavage errors. In the 6-month rat study with DRV administered in combination with RTV, the cause of death was not evident in some animals and a relationship between mortality and RTV treatment at 75 mg/kg/day could not be excluded.

In rats, no relevant changes were seen at the NOAEL of 20 mg/kg/day, DRV alone, where exposures ($AUC_{0\text{-}24h}$) were in the range of 2 to 4 µg.h/mL.

Effects on the hematopoietic system were observed with DRV alone and in combination with RTV (or with RTV alone). These changes included a variable, but limited, decrease in the RBC count, hemoglobin and hematocrit and an increase in reticulocytes. These changes, with an increase in bilirubin levels, are all indicative of an increased RBC turnover, which was generally confirmed microscopically by the presence of extramedullary hematopoiesis in the spleen and was sometimes accompanied by an increase in spleen weight. Generally, an increase in the APTT was observed but this was not associated with any gross or microscopic evidence of bleeding. However, in the 6-month study with DRV co-administered with RTV, after an initial increase, APTT was decreased at the end of the study and also PT was lower in treated groups, indicating adaptation of the animals to these changes. The changes in coagulation times occurred, in general, in the presence of increased platelet counts.

Hepatocellular hypertrophy, associated with liver weight increases were seen at doses from 100 mg/kg/day onwards in rats given DRV alone. Corresponding exposure levels, expressed as mean C_{max} and $AUC_{0\text{-}24h}$, were in the range of 7 to 25 µg/mL and 32 to 121 µg.h/mL, respectively. The hepatocellular hypertrophy is considered to reflect an adaptive response to enzyme induction after administration of a xenobiotic, rather than a direct adverse effect of DRV. The lipid and protein changes were probably secondary to the effects on the liver. Increases in lipofuscin in the liver are likely linked to this.

In rat oral studies of DRV and RTV in combination, effects on the liver were similar to those seen in the 6-month study with DRV alone but were exacerbated owing to increased DRV exposure and also to the effect of RTV on the liver. Exposure in the combination study, expressed as mean C_{max} and $AUC_{0\text{-}24h}$ was in the range of 2 to 27 µg/mL and 11 to 318 µg.h/mL, respectively, at the end of the 6-month study. In this study, increases in liver weight and centrilobular hypertrophy were accompanied by multinucleated hepatocytes and some single cell necrosis. The latter findings were also noted in groups treated only with RTV. Increases in liver transaminase enzyme activities were noted in males after treatment with RTV only and to a lesser extent in some groups treated with DRV and RTV. No NOAEL was established in the rat studies with the combination.

Diffuse follicular hypertrophy and hyperplasia in the thyroid was also observed to varying extents in the different rat studies, but not in the 6-month rat study with DRV alone. It is possible that this is a rodent-specific adaptive response to liver enzyme induction and an enhanced metabolism of thyroid hormones. Both cytochrome P450 and uridine 5'-diphosphate glucuronosyltransferase (UDPGT) are responsible for eliminating numerous substrates, both endogenous and exogenous, rendering them water-soluble and excretable in urine and bile. Increased elimination of thyroid hormones, especially thyroxine (T4), via UDPGT induction may stimulate the thyroid and result in the changes seen. Both of these enzymes were induced in rats following repeated oral administration with DRV alone and in combination with RTV (Module 2.6.4/Section 5.5.1).

Additional findings were seen in rats and included an increased incidence in males of focal hyperkeratosis at the limiting ridge of the non-glandular stomach at 500 mg/kg/day only, in the 3-month study with DRV alone. As there is no equivalent for this structure in the human stomach, the clinical relevance of this finding is limited. Moreover this finding was not observed in the 6-month studies. In various studies, changes in creatinine, minor changes in urine (volume and electrolytes) and increases in weight of the kidneys, were not associated with any relevant histopathological changes in the kidney. The hyaline droplets that were present in the kidneys of male rats in the 3-month study with DRV alone, were associated with lipofuscin disposition and were not seen in the subsequent 6-month study with DRV alone. Nephropathy, observed in the 6-month combination study with RTV, was not seen in studies with DRV alone and was also observed in the RTV-only group. Hence, the nephropathy was at least partially related to RTV. In the 6-month combination study there was a change in the pancreas (minimally increased incidence/severity of islet fibrosis/siderocytes in males) that was observed in animals treated with DRV in combination with RTV, mainly at doses of 500/75 and 1000/75 mg/kg/day. A slightly increased severity of adrenal cortical vacuolation was observed at the same doses in that study. The exposure to DRV at these doses, expressed as mean C_{max} and $AUC_{0\text{-}24h}$ was similar and in the range of 11 to 27 µg/mL and 183 to 318 µg.h/mL, respectively, at the end of the study.

In rat oral studies of DRV and RTV in combination, it was not possible to establish a NOAEL and given the complex nature of these studies and the potential effects of both compounds these results should be considered in comparison with other studies. If this is done then it is considered that there is evidence of a small additive effect of the combination of DRV and RTV in the 6-month rat study on RBC parameters, liver and thyroid. These changes appear to reflect the sustained systemic exposure to DRV in the presence of RTV but were not of a different order of magnitude observed in animals with each compound alone. It needs to be taken into account that these changes were also observed with RTV alone.

In dogs, the NOAEL dose in the 12-month study was 30 mg/kg/day. Exposure at this dose, expressed as mean C_{max} and AUC_{last} for males and females, was 12 and 9 µg/mL and 32 and 21 µg.h/mL, respectively, at the end of the 12-month study. However, in dogs, 6 months treatment at 120 mg/kg/day was without any significant toxicological effect and only after 12 months of treatment was there a limited response in the liver, consisting of an increase in liver weight and some hepatocellular vacuolation and pigmentation. After 12 months treatment at 120 mg/kg/day, exposure (C_{max} and AUC_{last}) was 27.8 and 23.9 µg/mL and 130 and 100 µg.h/mL, for males and females, respectively. These values are higher (for C_{max}) or broadly equivalent (AUC) to the anticipated exposure in the clinic at the recommended dose.

9.1.2. Genotoxicity

Darunavir was not genotoxic in an Ames assay, an in vitro chromosome aberration assay and an in vivo micronucleus assay.

9.1.3. Carcinogenicity

In the mouse and rat carcinogenicity study, DRV was well tolerated. Mortalities related to DRV exposure occurred in high-dose male mice only and were due to atrial thrombosis (5/65) and liver tumors (10/65). In rats, test-article related mortality was seen in 1 high-dose male (hepatocellular carcinoma), and 10 females given 150 (3) or 500 mg/kg/day (7) (CPN). In mice and rats, dose-related increases in the incidence of hepatocellular adenomas and carcinomas, hypertrophy, foci of cellular alteration, pigmentation and oval cell hyperplasia in the liver were observed and correlated with presence of masses/nodules/discoloration, organ weight

changes and clinical chemistry changes. These findings are considered to be related to liver enzyme induction and/or alterations in lipid metabolism. Other treatment-related changes in mice were nephrosis and/or tubular pigmentation that linked to the increase in kidney weight. The atrial thrombosis seen in the heart is considered mouse-specific age-related change. Vascular mineralization was observed in the heart, pancreas, tongue, seminal vesicles, epididymes and kidney; mineralization was also present in the vibrissae and in the sclera of the eyes in high-dose male and/or female mice and correlated to the increases in inorganic phosphorus. The spleen and the mesenteric lymph nodes showed minimal increases in hemopoiesis that correlated with the erythron parameter changes. Given the hepatocellular changes seen at the lowest tested dose (150 mg/kg/day), an NOAEL was not established in the mouse study. At 150 mg/kg/day $AUC_{0\text{-}24h}$ was 6.23 and 7.79 µg.h/mL in male and female mice, respectively. In the rat the slight increase in bone marrow cellularity in males dosed from 150 mg/kg/day and the slight increase in splenic extramedullary hematopoiesis in males at 500 mg/kg/day correlated with hematology changes. The NOAEL in rats was 50 mg/kg/day corresponding to an $AUC_{0\text{-}24h}$ of 17.1 and 24.9 µg.h/mL in males and females, respectively.

9.1.4. Reproductive Toxicity

Darunavir did not affect male or female fertility at doses up to a dose producing effects on body weight gain (1000 mg/kg/day). The NOAEL was 200 mg/kg/day based on the absence of an adverse body weight effect in males and females, where exposure (AUC_{last}) would be about 37 µg.h/mL, as determined in a supporting dose range finding study.

Darunavir has shown no teratogenic potential in rats, rabbits and mice. Initially, DRV alone was evaluated in rat and rabbit embryo-fetal development studies. The maternal NOAEL in the rat study was 200 mg/kg, based on the body weight effect, whereas the fetal NOAEL was 1000 mg/kg, based on the absence of any effects. In rabbits, the maternal and fetal NOAEL was considered to be 1000 mg/kg/day but exposure in rabbits was significantly lower and substantially below human exposure. Higher exposure was achieved in the mouse and, therefore, DRV alone as well as in combination with RTV was evaluated in a mouse embryo-fetal development study. There were no effects of DRV treatment on embryo-fetal development

at any of the doses administered. The embryo-fetal NOAEL was, therefore, considered to be 1000 mg/kg/day.

Also a combination study in the rat and minipig was performed. In rats, the NOAEL of DRV when co-administered with RTV, for maternal toxicity was considered to be less than 300/50 mg/kg b.i.d and the NOAEL for embryo-fetal development was considered to be 300/50 mg/kg b.i.d. Due to the poor tolerability of the formulation, dosing could not be maintained for the repeated administration phase in minipigs.

A dose range finding study and a main study of prenatal and postnatal developmental toxicity were conducted in the rat and the dose range finding study included assessment of juvenile toxicity. For pups directly dosed at 40 and 200 mg/kg/day from day 12 until day 25 of age, a NOAEL could not be established owing to the deaths and adverse clinical signs. A dose of 500 or 1000 mg/kg/day was not tolerated in juvenile rats. The exposure in juvenile animals directly dosed was dose and age dependent and influenced by the maturation of the liver enzymes involved in the elimination of darunavir. Comparing with adult rats, several oxidative metabolites were not formed in the liver of juvenile rats.

In the rat pre- and postnatal development study , the NOAEL for maternal treatment was considered to be 40 mg/kg/day, based on effects on body weight and food consumption at higher dose levels of DRV alone. When DRV was administered with RTV at 1000/75(50) mg/kg/day, maternal effects were greater than with DRV alone. Maternal treatment with RTV alone at a dose level of 75(50) mg/kg/day was associated with maternal clinical signs and lower mean food consumption and bodyweight gain. Based on pup body weight gain reduction at 1000 mg/kg DRV alone and a slight delay in developmental milestones at 200 mg/kg/day, the NOAEL for pup development after maternal treatment was considered to be 40 mg/kg/day. When DRV was administered with RTV at 1000/75(50) mg/kg/day the effects on pup development were more pronounced than with DRV alone. In addition pup survival during lactation was reduced. The reduced pup bodyweight gain in the combination group during lactation was directly related to administration of DRV/RTV to the mothers and not resulting from *in utero* exposure. Maternal administration of RTV at 75(50) mg/kg/day was associated with decreased pup survival during the second half of lactation

and poor bodyweight performance of surviving pups and a delay in the acquisition of the developmental milestones. Lower mean bodyweight was evident for F1 selected animals postweaning. There was no effect on F1 developmental tests or the fertility and mating performance of the F1 animals in any treated group during the study.

9.1.5. Local Tolerance

Local tolerance studies, including skin irritation and sensitization and eye irritation, indicated that there was no irritation or sensitization potential.

9.1.6. Immunotoxicity

To evaluate the potential for immunotoxicity in rats, DRV was administered daily, alone or in combination with RTV in a 4-week study. There were no changes in the immune response of treated animals, as measured by IgM production, at mean exposures ($AUC_{0\text{-}24h}$) of up to 102 µg.h/mL, in the presence of changes in the hematopoietic system and liver as previously described.

9.1.7. Batch Qualification/Impurities

Drug substance impurities of DRV were investigated and Batch qualification studies as necessary were conducted and no differences in toxicity were found.

9.2. Cobicistat

9.2.1. Target Organ Toxicity

In repeat-dose studies (up to 13 weeks in mice, up to 26 weeks in rats; up to 39 weeks in dogs) and the 2-year carcinogenicity studies, target organs identified were liver (mouse, rat, and dog) and thyroid (rat). Slight hematological changes were noted in rats; clinical chemistry changes were observed in mice, rats, and dogs; and urinalysis changes were noted in rats and dogs. Decreased IgG levels were noted in one study, and only in female rats. Combination toxicity study indicate that administration of COBI with ATV is unlikely to exacerbate the known toxicities of the individual agents, or lead to unexpected toxicities. Cardiovascular evaluations conducted in the repeat-dose toxicity studies in dogs suggest that COBI has a low potential for QT prolongation, but may have a tendency to slightly prolong the PR interval. The potential for cardiovascular changes is discussed in more detail in Module 2.6.2/Section 4.2.

Liver

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

In the 13-week mouse study, mild to marked elevations in ALT and AST were noted in males at 15 and 50 mg/kg/day, respectively. These changes were associated with increases in liver weight, microsomal enzyme induction, and minimal hepatocellular hypertrophy at 50 mg/kg/day. Female mice were notably less sensitive; a marked elevation in ALT and AST was noted in only one high-dose female (50 mg/kg/day). In the 2-year mouse carcinogenicity study, COBI-related effects in the liver, considered secondary to microsomal enzyme induction, included hepatocellular hypertrophy and pigment containing Kupffer cells in high-dose males and females administered 50 and 100 mg/kg/day, respectively. Similar liver findings were noted in the 4-week toxicity in HRAS wild type mice. In rats, increases in liver weights were associated with microsomal enzyme induction and hepatocellular hypertrophy. These findings were shown to be reversible and were not considered adverse. In the 2-year rat carcinogenicity study (TX-216-2031), COBI-related effects in the liver included centrilobular hypertrophy and hepatocyte karyocytomegaly. The liver effects are considered adaptive changes, are commonly seen in rodents with microsomal enzyme inducers, and are considered secondary to microsomal enzyme induction[46,47]. Unlike in humans, COBI induces hepatic CYP3A activity in rats and in mice likely due to a species-specific activation of rodent PXR (Module2.6.4/Section5.5.2).

In the 2-year carcinogenicity studies with COBI, there were no increases in the incidence of liver tumors in COBI-treated mice or rats.

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

hypertrophy) were observed in males at 10 mg/kg/day, and in both sexes at 20 mg/kg/day. These hepatic changes observed in the 39-week study are considered an adaptive response, and not adverse based on their minimal severity, the absence of degeneration, and their reversibility after cessation of dosing[46,47].

The nature and degree of the observed effects in serum chemistry, liver histopathology, and liver enzyme induction parameters, as well as the absence of significant bioaccumulation, generation of reactive metabolites, and immune-related hepatic effects support the conclusion that COBI has a low potential for inducing hepatotoxicity[48]. Phase 2 and 3 safety data with COBI do not indicate a clinically relevant adverse effect on the liver.

Thyroid

Effects on the thyroid glands in rats in the 26-week study were characterized by decreases in T4 in males at 100 mg/kg/day, increases in TSH in 10 mg/kg/day females, and in both sexes at 30 and 100 mg/kg/day, increased thyroid weights at 30 and 100 mg/kg/day, and thyroid follicular cell hypertrophy (in one female at 10 and 30 mg/kg/day, and in most male and female animals at 100 mg/kg/day). These findings were reversible and were not considered adverse. In the 2-year rat carcinogenicity study, neoplastic findings in the thyroid consisted of significant positive trends in thyroid follicular adenoma, carcinoma, and combined adenoma and carcinoma in males and/or females, with significant increases in adenoma for males at 50 mg/kg/day, and in combined adenoma/carcinoma for males at $\geq$ 25 mg/kg/day and for females at 30 mg/kg/day. The increase in thyroid follicular tumors was associated with follicular cell hypertrophy at $\geq$ 25 and (15 mg/kg/day in males and females, respectively.

There are no indications that COBI has a direct effect on the thyroid gland, or a particular affinity for thyroid tissue (Module2.6.4/Section 4.1.2). These clinical and anatomic pathology changes are considered adaptive changes, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance. The thyroid effects are considered rodent specific, and predispose rats, but not humans, to thyroid neoplasms. It is unlikely that COBI presents a risk to the human thyroid[1,2,3,4,5,6]. No clinically relevant adverse effects on thyroid function have been observed in clinical studies conducted to date with COBI.

Urinalysis

Urinalysis changes, noted primarily in high-dose rats (at 100 mg/kg/day) and in dogs (at ≥ 30 mg/kg/day), included higher urine volume, lower urine specific gravity, and increases in electrolyte excretion. These changes showed no progression after long term dosing, were not associated with remarkable serum clinical chemistry changes, including serum electrolytes, serum creatinine and BUN, were without histopathological correlates, and were reversible. Although the mechanism associated with these urinalysis changes is not understood (there were no treatment-related changes in serum vasopressin or aldosterone levels in the 26-week rat study), similar findings have been reported with other structurally-related agents, including ATV[49], DRV and RTV[50]. In the 13-week combination toxicity study in rats with COBI and ATV, increases in urine volume were noted in groups given COBI and ATV alone, and in combination. However, these effects were slight, not additive when COBI and ATV were administered in combination, nor associated with microscopic correlates and were reversible; therefore these changes were not considered adverse.

Hematology, Coagulation and Clinical Chemistry

Red blood cells

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

Coagulation

In rats, increases (up to 38%) in mean platelet counts were noted in the 4-week study at doses (50 mg/kg/day, and in the 26-week study in males at (30 mg/kg/day and in females at 100 mg/kg/day. In dogs, increases in platelet counts (up to 43%) were noted after 13-, 26- and 39-weeks dosing in high dose (20 mg/kg/day) females, and in 10 mg/kg/day females at Week 26, only. Further, in dogs minimal decreases (up to 15%) in activated partial thromboplastin time (APTT) were noted in 10 mg/kg/day females and in both sexes at 20 mg/kg/day. Similar changes were not observed in the 4-week dog study at doses up to 45/30 mg/kg/day. In all cases, there were no

associated effects on bleeding, the changes were reversible, and they were not considered adverse. Since these changes only occurred at high doses, the relevance of these limited changes is questionable.

Clinical Chemistry

Slight but statistically significant increases in cholesterol were observed in the 13-week mouse study (up to 46% increase at dose levels ≥ 15 mg/kg/day; and in female rats in the 26-week study (up to 35% increase at dose levels ≥ 30 mg/kg/day). Cobicistat is a rat PXR (rPXR) inducer (Module2.6.4/Section 5.5.2.2), with activation similar to known PXR inducers (RTV and miconazole). Induction of PXR can reduce Cyp7a1 transcription and cholesterol 7a-hydroxylase activity, which are involved in the conversion of cholesterol to bile acids[51]. Down-regulation of Cyp7a1 results in less cholesterol being converted to bile acids and consequently more free cholesterol. An approximately 6-fold induction of rPXR was observed with COBI at 10 μM. In the 13-week mouse and 26-week rat toxicity studies, COBI C_{max} values associated with cholesterol changes were 2.7 to 11.4 μg/mL (3.5 to 14.7 μM), suggesting adequate PXR activation to explain the slight increase in cholesterol levels observed with COBI. At clinically-relevant concentrations, COBI would not activate human PXR (Module2.6.4, Section5.5.2.2).

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

Immune System

Results from a rat immunotoxicity study showed lower anti-KLH IgG antibody titers in females at ≥ 50 mg/kg/day. There were no COBI-related

changes in the anti-KLH IgM antibody response at any dose in either sex. The NOEL for the T-cell dependent antibody response is considered to be 20 mg/kg/day in females and 50 mg/kg/day in males. The NOAEL for this study was 20 mg/kg/day, based on clinical signs, decreases in body weight gain and/or food consumption, increases in liver and thyroids weights, and lymphoid depletion of germinal centers in the spleen at (50 mg/kg/day. Additional IHC analysis of spleens from all animals was conducted, as described in the International Conference on Harmonization S8 Guideline, Immunotoxicity Studies for Human Pharmaceuticals[52]. Formalin fixed, paraffin embedded tissues (spleen) were evaluated for detection of B cells (KiB1R-positive), T cells (CD3-positive), and germinal centers (PNA-positive). Immunohistochemical findings observed in COBI-treated animals did not correlate with the decreases noted in anti-KLH IgG levels, as greater decreases in anti-KLH IgG levels were observed in females versus males, but IHC trends were noted only in males. In females, there was a notable lack of any dose-response with respect to the IHC changes.

To further assess the potential for immunotoxicity, immune system tissues (spleen, thymus, lymph nodes, and Peyers patches) from rats administered COBI in the 4-week and 26-week toxicity studies were subjected to a pathology peer-review. Evaluation was conducted according to recommendations for rat lymphoid tissue evaluation and included an estimate of the numbers of germinal centers in the spleen for each animal[53,54,55]. The peer reviewing pathologist was in agreement with the overall interpretations and conclusion of the histopathology diagnoses and conclusions of the studies regarding the lack of treatment-related effects on rat lymphoid tissues. The NOAELs for COBI in the 4-week and 26-week studies were 50 and 30 mg/kg/day, respectively.

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

In the 39-week dog study, peripheral blood immunophenotyping (total T cells, helper T cells, cytotoxic T cells, and B cells) conducted during Week 26 revealed no treatment-related changes. Histopathological changes in

immune system tissues were limited to minimal to moderate thymic involution in high dose males at the 13-week interim sacrifice. The thymic changes after 13 weeks of dosing were attributed to stress (thin appearance, decreased body weight and food consumption, excessive salivation, emesis and abnormal feces) and not considered direct COBI-related effects. Further, these changes were no longer apparent in terminal sacrifice or recovery sacrifice animals suggesting tolerance to these stress-related changes. The NOAEL after 39 weeks daily oral gavage dosing to dogs is considered to be 10 mg/kg/day.

In the 2-year carcinogenicity studies in mice and rats, there were no notable COBI-related changes in immune system tissues, no increase in opportunistic infections in COBI-treated animals, and no COBI-related increase in tumors, beyond thyroid follicular cell changes in rats. As noted, the follicular cell findings are considered to be rat specific, secondary to species-specific activation of PXR, hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans.

In conclusion, in standard 13-week mouse, 26-week rat and 39-week dog toxicity studies, at doses up to 50 mg/kg/day, 100 mg/kg/day and 20 mg/kg/day in mouse, rat and dog, respectively, microscopic changes suggestive of immunotoxicity were not observed in lymphoid organs. Immunophenotyping of peripheral blood cells evaluated in the chronic rat and dog toxicity studies did not reveal any adverse effects, and there were no signs of potential immunosuppression as assessed by animal health status (ie, no signs of opportunistic infections) and clinical chemistry and hematological analyses. Further, there was no evidence in the 2-year carcinogencity studies in mice and rats suggestive of immunosuppression. The clinical significance of the decrease in anti-KLH IgG levels in a single study in female rats is unclear considering that no adverse effects on hematological parameters, IgG levels, or rate of infections considered related to study drug that could be suggestive of immunosuppression have been observed in clinical studies conducted with COBI.

9.2.2. Genotoxicity

The potential of COBI to induce mutagenesis has been investigated in vitro in the Ames test and the mouse lymphoma assay, and in vivo in the rat bone

marrow micronucleus test. No evidence for mutagenic or genotoxic activity was identified in these studies.

9.2.3. Carcinogenicity

In the 2-year carcinogenicity study in mice, no drug-related increase in tumor incidence was observed at exposures 9 to 21 times (males and females, respectively) the human systemic exposure at the therapeutic daily dose. In the 2-year carcinogenicity study in rats, increases in follicular cell adenomas and/or carcinomas in the thyroid gland were observed at doses of 25 and 50 mg/kg/day in males, and at 30 mg/kg/day in females. The follicular cell findings are considered to be rat-specific, secondary to hepatic microsomal enzyme induction and thyroid hormone imbalance, and are not relevant for humans[3,5,6]. At the highest doses tested in the rat carcinogenicity study, systemic exposures were approximately 2.6 times the human systemic exposure at the therapeutic daily dose.

In rats, COBI induces hepatic CYP3A activity due to a species-specific activation of PXR, which does not occur in humans (Module2.6.4/Section5.5.2.2). The observed toxicity profile on the thyroid is rodent-specific and it is unlikely that COBI presents a risk to the human thyroid. These epigenetic effects, associated with liver enzyme induction, bear no relevance for man as a similar association between liver enzyme induction and carcinogenesis does not exist in man.

9.2.4. Reproductive Toxicity

No adverse effects on embryo-fetal viability or on male or female fertility were observed in a rat fertility study; the NOEL for reproductive parameters was 100 mg/kg/day. No teratogenic effects were observed in rat and rabbit developmental toxicity studies. In rats at 125 mg/kg/day, increases in postimplantation loss and decreased fetal weights were associated with significant maternal toxicity. NOAELs for developmental toxicity in the rat and rabbit studies were 50 and 100 mg/kg/day, respectively. In the pre- and postnatal developmental toxicity study, there were no adverse effects on reproduction and postnatal development at doses up to 75 mg/kg/day. Cobicistat was also well tolerated in juvenile rats at doses up to 75 mg/kg/day, with adaptive liver and thyroid changes observed at similar dose levels and exposures to adult animals.

9.2.5. Local Tolerance

Cobicistat-related emesis and salivation was observed more frequently at doses of (15 mg/kg/day in the 7-day, 4-week, and 39-week dog studies. There were no associated pathological changes in the 4- and 39-week studies. In a 14-day range-finding rat study with COBI, erosion/ulceration of the nonglandular stomach was noted in 1 of 5 animals at 100 mg/kg/day, and in animals dosed at 300 mg/kg/day for 5 days. There were no changes in the GI tract of rats dosed at 30 mg/kg/day for 14 days, 175 mg/kg/day for 8 days, or in longer term studies at doses up to 100 mg/kg/day for up to 26-weeks or in the 2-year study at doses up to 50 mg/kg/day.

Cobicistat was mildly irritating to skin, not a severe irritant to eyes, and showed no potential for phototoxicity or sensitization.

9.2.6. Metabolites/Impurities

There are no major human metabolites, therefore, no specific toxicology studies with COBI metabolites were conducted. The identified metabolites and impurities have been assessed as part of the general toxicology and qualification (of impurities) studies. Exposures to the most prominent COBI metabolite, GS-9612, were evaluated in the 13-week mouse study, and in the chronic rat and dog toxicity studies. Exposures achieved in these studies were above clinical exposures levels.

9.2.7. Conclusions

A complete nonclinical toxicology program was conducted for COBI, including single-dose oral toxicity studies in mice and rats; repeat-dose oral toxicity studies in mice (up to 13 weeks), rats (up to 26 weeks), and dogs (up to 39 weeks); combination toxicity studies in rats with ATV (13 weeks); in vitro and in vivo genotoxicity tests; 2-year oral carcinogenicity studies in mice and rats; developmental toxicity studies in rats and rabbits; fertility, peri/postnatal, and juvenile toxicity studies in rats; and local tolerance studies.

The toxicity profile of COBI has been well characterized. Target organs were the liver (mouse, rat, and dog) and thyroid (rat). Liver changes included microsomal enzyme induction, increased weights, and hepatocellular hypertrophy and/or vacuolation. The thyroid changes are considered rodent-specific, secondary to microsomal enzyme induction and thyroid hormone imbalance, and it is unlikely that COBI presents a risk to the human

thyroid[1,2,3,4,5,6]. Apart from the thyroid neoplasia, the liver and thyroid changes appeared to be completely reversible and are considered to be nonadverse, adaptive responses. Hematological and clinical chemistry changes occurred at high doses, were minimal to mild in severity, reversible, and not considered adverse. Urinalysis changes (higher urine volume, lower urine specific gravity, increases in electrolyte excretion) showed no progression after long term dosing, were reversible, were not associated with remarkable clinical chemistry changes, including serum creatinine and BUN, and were without morphological evidence of kidney damage. The clinical significance of the decrease in anti-KLH IgG levels in female rats is questionable, considering the lack of effects on the immune system in long-term studies in mice, rats, and dogs, and the lack of adverse, immune system-related changes in clinical studies with COBI. Other potential toxicities related to COBI that were observed in nonclinical studies include PR interval prolongation in the 4-week dog toxicity study and decreases in LV function in isolated rabbit hearts (Module2.6.2/Section 4.2).

Combination toxicity studies of COBI with ATV did not result in unexpected or additive toxicity.

Cobicistat was negative for mutagenic potential in a bacterial reverse mutation test, negative in a forward mutation test in mouse lymphoma cells, and negative in a rat micronucleus assay. The overall potential for genetic toxicity is considered to be low.

Long-term carcinogenicity studies with COBI in mice and rats did not reveal effects relevant for humans.

No adverse effects on fertility or embryo-fetal viability were observed in a fertility study in male and female rats, and no teratogenic effects were observed in rat and rabbit developmental toxicity studies. In rats at 125 mg/kg/day, increases in postimplantation loss and decreased fetal weights were associated with significant maternal toxicity. There were no significant effects on peri- or postnatal development in rats. Cobicistat was also well tolerated in juvenile rats at dose levels and exposures similar to those used in the repeat-dose studies with older rats.

Cobicistat was a mild irritant to rabbit skin, was not a strong eye irritant, and was negative for delayed-type hypersensitivity.

Identified impurities and degradants have been qualified as part of the routine toxicology or qualification studies with the impurities themselves. Several process intermediates and potential impurities were also evaluated in silico or qualified in genotoxicity studies.

Overall, the toxicology profile of COBI as described in this document, whether dosed alone or in combination with ATV, support the safe use of this agent as a pharmacoenhancer in combination therapy for the treatment of HIV-1 infection.

10. TABLES AND FIGURES

Whenever needed, tables were included throughout this summary. Tabulated Summaries are located in Module 2.6.7.

11. REFERENCES

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

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MODULE 2.6.7

TOXICOLOGY TABULATED SUMMARY

2.6.7.1 Toxicology: Overview- Darunavir

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Single-Dose Toxicity								
	Mouse/ CD-1	Oral/gavage	Single dose	0 (vehicle), 100, 300, 1000	Yes	[redacted]	TMC114-NC111	4.2.3.1
	Rat/ Wistar	Oral/gavage	Single dose	0 (vehicle), 20, 100, 500	Yes	[redacted]	TMC114-NC101	4.2.3.1
	Rat/ Wistar	Oral/gavage	Single dose	0 (vehicle), 500, 1000, 1500, 2000	Yes	[redacted]	TMC114-NC104	4.2.3.1
	Rat/ Wistar	Intravenous/infusion	Single dose	0 (vehicle), 10, 20, 40	Yes	[redacted]	TMC114-NC110	4.2.3.1
	Dog/Beagle	Oral/gavage	Single dose escalation followed by 3 days repeated dose at MTD	0 (vehicle), 20, 80, 320 (ESC) and 80 (MTD)	Yes	[redacted]	TMC114-NC102	4.2.3.1
	Dog/Beagle	Intravenous/injection	Single dose escalation followed by 3 days repeated dose at MTD	0 (vehicle), 10, 20, 40 (ESC) and 10 (MTD)	Yes	[redacted]	TMC114-NC109	4.2.3.1
Repeat-Dose Toxicity								
	Rat/Wistar	Oral/gavage	14 days	0 (vehicle), 40, 200, 1000	Yes	[redacted]	TMC114-NC107	4.2.3.2
	Rat/Sprague-Dawley	Oral/gavage	3 months	0 (vehicle), 20, 100, 500	Yes	[redacted]	TMC114-NC130	4.2.3.2
	Rat/Sprague-Dawley	Oral/gavage	6 months	0 (vehicle), 20, 100, 500	Yes	[redacted]	TMC114-NC132	4.2.3.2

(Continued)

2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Repeat-Dose Toxicity								
	Rat/Sprague-Dawley	Intravenous/bolus	2 weeks	0 (vehicle), 2.5, 10, 25	Yes	[redacted]	TMC114-NC160	4.2.3.2
	Rat/Wistar	Oral/gavage	2 weeks	0/0 (vehicles), 0/50, 150/25, 500/25, 1000/50	Yes	[redacted]	TMC114-NC141	4.2.3.2
	Rat/Wistar	Oral/gavage	2 weeks	0/0 (vehicles), 0/75, 2000/0, 2000/75	Yes	[redacted]	TMC114-NC143	4.2.3.2
	Rat/Wistar	Oral/gavage	6 months	0, 0/75/ 20/50, 100/50, 500/75, 1000/75	Yes	[redacted]	TMC114-NC146	4.2.3.2
	Dog/Beagle	Oral/gavage	2 weeks	0 (vehicle), 30, 60, 120	Yes	[redacted]	TMC114-NC106	4.2.3.2
	Dog/Beagle	Oral/gavage	2 weeks	360 single dose at 120	No	[redacted]	TMC114-NC173	4.2.3.2
	Dog/Beagle	Oral/gavage	3 months	0 (vehicle), 30, 60, 120	Yes	[redacted]	TMC114-NC131	4.2.3.2
	Dog/Beagle	Oral/gavage	6 months	0 (vehicle), 30, 60, 120	Yes	[redacted]	TMC114-NC133	4.2.3.2
	Dog/Beagle	Oral/gavage	12 months	0 (vehicle), 30, 60, 120	Yes	[redacted]	TMC114-NC145	4.2.3.2
	Dog/Beagle	Intravenous/bolus	2 weeks	0 (vehicle), 3.12, 6.25, 12.5	Yes	[redacted]	TMC114-NC199	4.2.3.2
	Dog/Beagle	Oral/gavage	2 weeks	0/10, 40/10, 120/10, 360/10	Yes	[redacted]	TMC114-NC140	4.2.3.2

(Continued)

2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Repeat-Dose Toxicity	Dog/Beagle	Oral/capsule or gavage	2 weeks	0/200, 240/200 (capsule) 120/10, 120/20 (gavage)	Yes	[redacted]	TMC114-NC144	4.2.3.2
Genotoxicity	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP2uvrA	In vitro	48 hours	33, 100, 333, 1000, 3330 µg/plate	Yes	[redacted]	293063	4.2.3.3.1
	Human peripheral lymphocytes	In vitro	up to 48 hours	33, 100, 130, 180, 240 and 333 µg/mL	Yes	[redacted]	294288	4.2.3.3.1
	Mouse / NMRI BR	Oral/gavage	Single dose	2000	Yes	[redacted]	TMC114-NC114	4.2.3.3.2
Carcinogenicity	Mouse/ CD-1	Oral/gavage	2 weeks	0 (vehicle), 50, 150, 450, 1000	No	J&JPRD	TMC114-NC190	4.2.3.4.1
	Mouse/ CD-1	Oral/gavage	3 months	0 (vehicle), 150, 450, 1000	Yes	J&JPRD	TMC114-NC157	4.2.3.4.1
	Mouse/ CD-1	Oral/gavage	24 months	0, 0, 150, 450 or 1000	Yes	J&JPRD	TMC114-NC159	4.2.3.4.1
	Rat/ Sprague-Dawley	Oral/gavage	24 months	0, 0, 50, 150 or 500 0, 0, 150, 450 or 1000	Yes	J&JPRD	TMC114-NC158	4.2.3.4.1

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2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Carcinogenicity								
	Mouse/ CB6F1-nonTgrasH2	Oral/gavage	4 weeks	0 (vehicle), 150, 450, 1000	Yes	[redacted]	TMC114-NC194	4.2.3.4.3
	Rat/ Sprague-Dawley	Oral/dietary admixture	13 weeks	0 (vehicle), 260, 1040, 2600	Yes	[redacted]	TMC114-NC196	4.2.3.4.3
Reproductive and Developmental Toxicity								
	Rat/ Sprague-Dawley	Oral/gavage	M: 12 weeks before mating until necropsy; F: 2 weeks prior to mating up to GD14-16	0 (vehicle), 40, 200, 1000	Yes	[redacted]	TMC114-NC129	4.2.3.5.1
	Rat/ Sprague-Dawley	Oral/gavage	GD7-9 and GD13-19	0 (vehicle), 40, 200, 1000	Yes	[redacted]	TMC114-NC127	4.2.3.5.2
	Rat/ Sprague-Dawley	Oral/gavage	GD7-19	0 (vehicle), 40, 200, 1000	Yes	[redacted]	TMC114-NC128	4.2.3.5.2
	Rat/ Sprague-Dawley	Oral/gavage	GD6-17	Vehicle, 1000/0, 2000/0 or 1000/100 DRV/RTV	No	J&J PRD	TMC114-NC397	4.2.3.5.2
	Rat/ Sprague-Dawley	Oral/gavage	GD6-17	Vehicle, 600/100 or 1000/100 DRV/RTV	Yes	J&J PRD	TMC114-NC398	4.2.3.5.2
	Rabbit/New Zealand white	Oral/gavage	Single dose followed by 5 days at 1000 mg/kg body weight	250, 500, 1000	No	[redacted]	TMC114-NC124	4.2.3.5.2

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2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Reproductive and Developmental Toxicity								
	Rabbit/New Zealand white	Oral/gavage	GD8-20	0 (vehicle), 40, 200, 1000	Yes	[redacted]	TMC114-NC125	4.2.3.5.2
	Rabbit/New Zealand white	Oral/gavage	GD8-20	0 (vehicle), 40, 200, 1000	Yes	[redacted]	TMC114-NC126	4.2.3.5.2
	Rabbit/New Zealand white	Oral/gavage	5 days	0/0 (vehicle), 0/40, 200/40, 1000/40, 1000/0	No	J&JPRD	TMC114-NC189	4.2.3.5.2
	Mouse/CD-1	Oral/gavage	GD6-15	0 (vehicle), 150, 450, 1000	Yes	[redacted]	TMC114-NC175	4.2.3.5.2
	Mouse/CD-1	Oral/gavage	GD6-15	0 (vehicle), 150/0, 450/0, 1000/0, 1000/50, 0/50	Yes	[redacted]	TMC114-NC172	4.2.3.5.2
	Minipig, Göttingen SPF	Oral/gavage	SD	Vehicle, 1000/0, 2000/0 or 1000/100 DRV/RTV	No	[redacted]	TMC114-NC399	4.2.3.5.2
			6 days	Vehicle, 500/0, 1000/0 or 500/100 DRV/RTV				
			7 days	Vehicle, 250/0, 500/0 or 250/50 DRV/RTV				
	Rat/ Sprague-Dawley	Oral/gavage	F: GD6-LD7 F1: day 12 to 25 of age	0 (vehicle), 40, 200, 1000	No	[redacted]	TMC114-NC178	4.2.3.5.3

(Continued)

2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Reproductive and Developmental Toxicity								
	Rat/ Sprague-Dawley	Oral/gavage	F: GD6-LD20 F: GD6-GD20	20/0, 200/0, 1000/0, 1000/75 (50) b, 0/75 (50) b, 1000/50	Yes	[redacted]	TMC114-NC156	4.2.3.5.3
	Rat pups/ Sprague-Dawley	Oral/Gavage	Postnatal Days 5, 8 and 11	20, 40, 80 (Day 5) 40, 80, 120 (Day 8) 40, 80, 160 (Day 11)	Yes	[redacted]	TMC114-NC240	4.2.3.5.4
	Rat pups/ Sprague-Dawley	Oral/Gavage	Postnatal Day 23 to Day 36	20/0, 100/0, 500/0, 500/50, 0/50 darunavir/RTV	No	[redacted]	TMC114-NC248	4.2.3.5.4
	Rat pups/ Sprague-Dawley	Oral/Gavage	Postnatal Day 23 to Day 51	40/0, 200/0, 500/0, 500/75, 0/75 darunavir/RTV	Yes	[redacted]	TMC114-NC241	4.2.3.5.4
Local Tolerance								
	Mouse	Dermal application	single	10%, 25% and 50% w/v	No	[redacted]	TMC114-NC245	4.2.3.6
	Mouse/CBA/J	Topical skin application	Day 1, 2 and 3	2.5, 5, 10, 25 or 50% w/v	Yes	[redacted]	TMC114-NC330	4.2.3.6
	Rabbit/New Zealand white	Topical skin application	Single	0.5 g as 1% w/w	Yes	[redacted]	TMC114-NC316	4.2.3.6
	Bovine eye	In vitro	---	20% w/v	No	J&J PRD	TMC114-NC166	4.2.3.6

(Continued)

2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Other Toxicity Studies								
Immunotoxicity	Rat/Sprague-Dawley	Oral/gavage	4 weeks	0 (vehicle), 00(water), 20/0, 100/0, 500/0, 0/50, 100/50	Yes	[redacted]	TMC114-NC187	4.2.3.7.2
Mechanistic	Rat/ Sprague-Dawley	Oral/Gavage	28/29 days	0 (vehicle), 50, 500	Yes	J&J PRD	TMC114-NC162	4.2.3.7.3
Impurities	*Salmonella typhimurium* TA98, TA100	In vitro	48 hours	3, 10, 33, 100, 333, 1000, 3330, 5000 µg/plate	Yes	[redacted]	TMC114-NC150	4.2.3.7.6
	Salmonella typhimurium TA98, TA100	In vitro	48 hours	3, 10, 33, 100, 333, 1000, 3330, 5000 µg/plate	Yes	[redacted]	TMC114-NC170	4.2.3.7.6
	Dog/Beagle	Oral/gavage	2 weeks	0 (vehicle), 120	Yes	[redacted]	TMC114-NC176	4.2.3.7.6
	Salmonella typhimurium TA98, TA1537, TA100, TA1535, TA102	In vitro	---	0 (vehicle), 312.5, 625, 1250, 2500, 5000 !g/plate	Yes	[redacted]	TMC114-NC228	4.2.3.7.6
	Rat/ Sprague-Dawley	Oral/Gavage	2 weeks	0 (vehicle), 20, 500	Yes	J&JPRD	TMC114-NC227	4.2.3.7.6

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2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Impurities								
	Chromosomal aberration – peripheral human lymphocytes	In vitro	+/-S9 4h –S9 20h	TMC114 spiked with 1% of each impurity (不純物A* and 不純物B*) 0 (vehicle), 25 to 1000 µg/mL	Yes	[redacted]	TMC114-NC355	4.2.3.7.6
	Salmonella typhimurium TA98, TA1537, TA100, TA1535, TA102	In vitro	---	0 (vehicle), 78.13, 156.25, 312.5, 625, 1250, 2500, 5000 !g/plate	Yes	J&JPRD	TMC114-NC244	4.2.3.7.6
	Rat/ Sprague-Dawley	Oral/Gavage	2 weeks	0 (vehicle), 20, 500	Yes	J&JPRD	TMC114-NC242	4.2.3.7.6
	Chromosomal aberration – peripheral human lymphocytes	In vitro	+/-S9 5h +S9 4h –S9 20h	TMC114 spiked with 1% of each impurity (不純物C* and 不純物D*) 0 (vehicle), 25 to 1000 µg/mL	Yes	[redacted]	TMC114-NC354	4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537	In vitro	---	TMC114 spiked with 1% of each impurity (不純物E* , 不純物F* and 不純物G*) 0 (vehicle), 78.13 to 5000 µg/plate	Yes	J&J PRD	TMC114-NC359	4.2.3.7.6

(Continued)

*新薬承認情報提供時に置き換え

2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Impurities								
	Salmonella typhimurium (TA98, TA100, TA102, TA1535 and TA1537)	In vitro	---	TMC114 spiked with 1% of each impurity (不純物E*, 不純物F* and 不純物G*)	Yes	J&J PRD	TMC114-NC358	4.2.3.7.6
	Chromosomal aberration – peripheral human lymphocytes	In vitro (DMSO)	+/-S9 4h –S9 20h	0 (vehicle), 50 to 1000 µg/mL TMC114 spiked with 1% of each impurity (不純物E*, 不純物F* and 不純物G*) 0 (vehicle), 50 to 1000 µg/mL	Yes	[redacted]	TMC114-NC357	4.2.3.7.6
	Salmonella typhimurium TA98, TA100, TA102, TA1535, TA1537	In vitro	---	TMC114 spiked with 1% of each impurity (不純物H* and 不純物I*) 0 (vehicle), 78.13 to 5000 µg/plate	Yes	J&J PRD	TMC114-NC336	4.2.3.7.6
	Chromosomal aberration – peripheral human lymphocytes	In vitro (DMSO)	+/-S9 3h –S9 20h	TMC114 spiked with 1% of each impurity (不純物H* and 不純物I*) 0 (vehicle), 2.5 to 500 µg/mL	Yes	[redacted]	TMC114-NC337	4.2.3.7.6

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2.6.7.1 Toxicology: Overview- Darunavir (Continued)

Test Article: Darunavir

Type of Study	Species and Strain	Route (Vehicle/Formulation)	Duration of Dosing	Doses (TMC114[/RTV]) (mg/kg)[a]	GLP Compliance	Testing Facility	Study No.	Location in CTD
Impurities								
	Dog/ Beagle	Oral/gavage	2 Weeks	TMC114 spiked with 1% of each impurity (不純物H* and 不純物I*) 0 (vehicle), 120 (ref TMC114), 120 (spiked TMC114)	Yes	J&J PRD	TMC114-NC338	4.2.3.7.6
Other								
	Human platelets	In vitro	---	TMC114: 6, 50 µM Lopinavir: 10, 50 µM Tipranavir: 30, 50 µM.	No	J&J PRD	TMC114-NC394	4.2.3.7.7

Esc = escalating, MTD = maximum tolerated dose; F = female, F1 = pups, GD = gestation day, LD = lactation day; DRV: darunavir, RTV: ritonavir, SD: single dose.
For repeat-dose toxicity, the highest NOAEL (No Observed Adverse Effect Level) is underlined
[a] unless otherwise stated
[b] Due to the poor bodyweight performance and clinical condition of the first group of dams the RTV dose was lowered from 75 to 50 mg/kg/day at end of the gestation period ± 1 day; due to poor
body weight gain of the pups during lactation dosing was discontinued from approximately day 14 ± 1 of lactation.

*新薬承認情報提供時に置き換え

2.6.7.1 Toxicology: Overview- Cobicistat

Test Article: Cobicistat

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. Location in CTD
Single-Dose Toxicity of COBI						
Mouse/CByB6F1-Tg (HRAS)2Jic (wild type)	Oral Gavage	1 Day	30, 100, 300	No	[redacted], [redacted], [redacted] USA	PC-216-2013 4.2.2.2
Rat/Sprague-Dawley	Oral Gavage	1 Day	0, 125, 250, 500	Yes	[redacted], [redacted], [redacted] USA	TX-216-2003 4.2.3.1
In Vivo/Micronucleus /Rat/Sprague-Dawley – Single dose data	Oral Gavage	1 Day	M: 0, 212.5, 425, 850; F: 0, 125, 250, 500	Yes	[redacted], [redacted], Canada	TX-216-2012 4.2.3.3.2
Repeat-Dose Toxicity of COBI						
Rat/Sprague-Dawley	Oral Gavage	2 Weeks	COBI: 0, 30, 100, 175, 300 RTV: 0, 100	No	[redacted], [redacted], [redacted] USA	TX-216-2001 4.2.3.2
Rat/Sprague-Dawley	Oral Gavage	4 Weeks	0, 10, 20, 50, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2004 4.2.3.2
Rat/Sprague-Dawley	Oral Gavage	26 Weeks	0, 10, 30, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2017 4.2.3.2
Rat/ Sprague-Dawley	Oral Gavage	5 Days	COBI/ATV: 50/0, 0/150, 30/100, 50/150	No	[redacted], [redacted], [redacted] USA	TX-216-2027 4.2.3.2
Rat/ Sprague-Dawley	Oral Gavage	13 Weeks	COBI/ATV: 0, 30/0, 0/20, 0/50, 30/20, 30/50	Yes	[redacted], [redacted], [redacted] USA	TX-216-2024 4.2.3.2
Dog/Beagle	Oral Gavage	1 Week	COBI: 0, 50[d], 125, 250 RTV: 0, 125	No	[redacted], [redacted], [redacted] USA	TX-216-2002 4.2.3.2
Dog/Beagle	Oral Gavage	4 Weeks	0, 5, 15, 45/30	Yes	[redacted], [redacted], [redacted] USA	TX-216-2005 4.2.3.2
Dog/Beagle	Oral Gavage	39 Weeks	0, 5, 10, 20	Yes	[redacted], [redacted], [redacted] USA	TX-216-2016 4.2.3.2
Genotoxicity of COBI						
In Vitro/*S. typhimurium* and *E. coli*	In Vitro	48 to 72 Hours	15.8 to 5000 µg/plate	Yes	[redacted], [redacted], Canada	TX-216-2010 4.2.3.3.1
In Vitro/Mouse Lymphoma/L5178Y $TK^{+/-}$ Cells	In Vitro	3 to 24 Hours	13.4 to 80 µg/mL	Yes	[redacted], [redacted], Canada	TX-216-2011 4.2.3.3.1
In Vivo/Micronucleus /Rat/Sprague-	Oral Gavage	1 Day	M: 0, 212.5, 425,	Yes	[redacted], [redacted], Canada	TX-216-2012

Test Article: Cobicistat

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. Location in CTD
Dawley			850; F: 0, 125, 250, 500			4.2.3.3.2
Carcinogenicity of COBI						
Mouse/CD-1	Oral Gavage	2 Weeks	0, 10, 30, 100, 300/200[c]	No	[redacted], [redacted], [redacted] USA	TX-216-2025 4.2.3.4.1
Mouse/CD-1	Oral Gavage	2 Weeks	M: 0, 5, 15, 50 F: 0, 10, 30, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2032 4.2.3.4.1
Mouse/CByB6F1-Tg (HRAS)2Jic (wild type)	Oral Gavage	4 Weeks	0, 10, 30, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2041 4.2.3.4.1
Mouse/CD-1	Oral Gavage	13 Weeks	M: 0, 5, 15, 50 F: 0, 5, 15, 50	Yes	[redacted], [redacted], [redacted] USA	TX-216-2026 4.2.3.4.1
Mice/CD-1	Oral Gavage	104 Weeks	M: 0, 5, 15, 50; F: 0, 10, 30, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2030 4.2.3.4.1
Rat/ Sprague-Dawley	Oral Gavage	104 Weeks	M: 0, 10, 25, 50; F: 0, 5, 15, 30	Yes	[redacted], [redacted], [redacted] USA	TX-216-2031 4.2.3.4.1
Reproductive and Developmental Toxicity of COBI						
Fertility/Rat/ Sprague-Dawley	Oral Gavage	F:14 Days before to mating to GD 7; M:28 days before mating through mating phase	0, 10, 30, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2023 4.2.3.5.1
Developmental DRF/Rat/ Sprague-Dawley	Oral Gavage	GD 6-17	0, 25, 75, 200	Yes	[redacted], [redacted], [redacted] USA	TX-216-2018 4.2.3.5.2
Developmental/Rat/Sprague-Dawley	Oral Gavage	GD 6-17	0, 25, 50, 125	Yes	[redacted], [redacted], [redacted] USA	TX-216-2020 4.2.3.5.2
Developmental DRF/Rabbit/NZW	Oral Gavage	GD 7-20	0, 50, 100, 175, 300	Yes	[redacted], [redacted], [redacted] USA	TX-216-2019 4.2.3.5.2
Developmental/Rabbit/NZW	Oral Gavage	GD 7-20	0, 25, 50, 100	Yes	[redacted], [redacted], [redacted] USA	TX-216-2021 4.2.3.5.2
Peri-postnatal Development/Rat/ Sprague-Dawley	Oral Gavage	F_0 GD 6– PND 22; F_1 PND 22-49	0, 10, 30, 75	Yes	[redacted], [redacted], Canada	TX-216-2033 4.2.3.5.3
Local Tolerance of COBI						
In Vitro Tests/Bovine Corneal Opacity and Permeability Assay (BCOP)	In Vitro	Opacity = 4 hrs; Permeability = 90 min	20% w/w	Yes	[redacted], [redacted], UK	TX-216-2043 4.2.3.6
In Vivo Tests/Dermal Irritation	Dermal	4 Hours	0.5 g	Yes	[redacted], [redacted], UK	TX-216-2044 4.2.3.6

Test Article: Cobicistat

Type of Study/Species/Strain	Method of Administration	Duration of Dosing	Dose (mg/kg)[a]	GLP[b]	Testing Facility	Study No. Location in CTD
Other Toxicity Studies with COBI						
Antigenicity/Local Lymph Node Assay (LLNA) /Mouse/CBA, Ca	Topical	3 Days	2.5, 5 and 10% w/v	Yes	[redacted], UK	TX-216-2042 4.2.3.6
Immunotoxicity/ Rat/Sprague-Dawley	Oral Gavage	4 Weeks	0, <u>20</u>, 50, 150	Yes	[redacted], USA	TX-216-2022 4.2.3.7.2
Impurities/In Silico	NA	NA	NA	No	[redacted], USA	TX-216-2046 4.2.3.7.6
Impurities/In Silico	NA	NA	NA	No	[redacted], USA	TX-216-2054 4.2.3.7.6
Impurities/Genotoxicity/In Vitro/ *S. typhimurium* and *E. coli*	In Vitro	Pre-incubation and Plate incorporation	1.58 to 5000 µg/plate[e]	Yes	[redacted], Canada	TX-216-2052 4.2.3.7.6
Impurities/Genotoxicity/In Vitro/Mouse Lymphoma/L5178Y $TK^{+/-}$ Cells	In Vitro	3 to 21 Hours	2.5 to 2510 µg/mL[e]	Yes	[redacted], Canada	TX-216-2053 4.2.3.7.6
Impurities/ Rat/Sprague-Dawley	Oral Gavage	4 Weeks	0, <u>100</u>[f], 30[g], <u>100</u>[g]	Yes	[redacted], USA	TX-216-2045 4.2.3.7.6

a Unless otherwise specified, the highest No-Observed-Adverse-Effect Level (NOAEL) is underlined.
b 'Yes', indicates study includes a GLP Compliance statement.
c Due to animal deaths in the high dose group at 300 mg/kg/day, the high dose animals were not dosed on Dosing Phase Day 2. Beginning on Dosing Phase Day 3, the high dose level was reduced to 200 mg/kg/day.
d NOAEL: < 50 mg/kg/day COBI.
e 不純物CA* (Lot # [redacted]), 不純物CB* (Lot # [redacted]), 不純物CC* (Lot # [redacted]) and 不純物CD* (Lot # [redacted]).
f GS-9350, Lot # 4646-065-18.
g GS-9350, Lot # 4646-067-15 (spiked with impurities).
h GS-9350/GS-9137 non-degraded crushed tablets, Lot # RJ-4610-25B.
i GS-9350/GS-9137 degraded crushed tablets, Lot # RJ-4610-25A.

JTK-303 = GS-9137, elvitegravir (EVG); GS-9350 = cobicistat (COBI); NA = not applicable; RTV = ritonavir; ATV = atazanavir; DRF = dose range-finding; GD = gestation day; LD = lactation day; PPD = post-partum day; PND = post natal day; w/w = weight/weight; w/v = weight/volume; CA = citric acid; SC = subcutaneous; IV = intravenous; F = female; M = male; bid =twice daily; qd = every day; q2d = every two days; q7d = every 7 days; * = studies ongoing

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies-Darunavir

Test Article: darunavir

Type of Study	Test System	Route/Method of Administration	TMC114 (/RTV) Nominal Doses (mg/kg)	GLP Compliance	Study/report Number	Location in CTD
Single-Dose Toxicity	Mouse/ CD-1	Oral/gavage	0 (vehicle), 100, 300, 1000	No	TMC114-NC111	4.2.3.1
	Rat/ Wistar	Oral/gavage	0 (vehicle), 20, 100, 500	No	TMC114-NC101	4.2.3.1
	Rat/ Wistar	Oral/gavage	0 (vehicle), 500, 1000, 1500, 2000	No	TMC114-NC104	4.2.3.1
	Rat/ Wistar	Intravenous /infusion	0 (vehicle), 10, 20, 40	No	TMC114-NC110	4.2.3.1
	Dog/ Beagle	Oral/gavage	0 (vehicle), 20, 80, 320 (ESC) and 80 (MTD)	No	TMC114-NC102	4.2.3.1
	Dog/ Beagle	Intravenous/ injection	0 (vehicle), 10, 20, 40 (ESC) and 10 (MTD)	No	TMC114-NC109	4.2.3.1
Repeat-Dose Toxicity	Rat/ Wistar	Oral/gavage	0 (vehicle), 40, 200, 1000	No	TMC114-NC107	4.2.3.2
	Rat/ Sprague-Dawley	Oral/gavage	0 (vehicle), 20, 100, 500	No	TMC114-NC130	4.2.3.2
	Rat/ Sprague-Dawley	Oral/gavage	0 (vehicle), 20, 100, 500	Yes	TMC114-NC132	4.2.3.2
	Rat/ Sprague-Dawley	Intravenous/ bolus	0 (vehicle), 2.5, 10, 25	Yes	TMC114-NC160	4.2.3.2
	Rat/ Wistar	Oral/gavage	0/0 (vehicles), 0/50, 150/25, 500/25, 1000/50	No	TMC114-NC141	4.2.3.2
	Rat/ Wistar	Oral/gavage	0/0 (vehicles), 0/75, 2000/0, 2000/75	No	TMC114-NC143	4.2.3.2
	Rat/ Wistar	Oral/gavage	0, 0/75/ 20/50, 100/50, 500/75, 1000/75	Yes	TMC114-NC146	4.2.3.2

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies-Darunavir (Continued)

Test Article: darunavir

Type of Study	Test System	Route/Method of Administration	TMC114 (/RTV) Nominal Doses (mg/kg)	GLP Compliance	Study/report Number	Location in CTD
Repeat-Dose Toxicity (continued)	Dog/ Beagle	Oral/gavage	0 (vehicle), 30, 60, 120	No	TMC114-NC106	4.2.3.2
	Dog/ Beagle	Oral/gavage	360 single dose at 120	No	TMC114-NC173	4.2.3.2
	Dog/ Beagle	Oral/gavage	0 (vehicle), 30, 60, 120	No	TMC114-NC131	4.2.3.2
	Dog/ Beagle	Oral/gavage	0 (vehicle), 30, 60, 120	No	TMC114-NC133	4.2.3.2
	Dog/ Beagle	Oral/gavage	0 (vehicle), 30, 60, 120	Yes	TMC114-NC145	4.2.3.2
	Dog/ Beagle	Intravenous/ bolus	0 (vehicle), 3.12, 6.25, 12.5	Yes	TMC114-NC199	4.2.3.2
	Dog/ Beagle	Oral/gavage	0/10, 40/10, 120/10, 360/10	Yes	TMC114-NC140	4.2.3.2
	Dog/ Beagle	Oral/capsule and gavage	0/200, 240/200 (capsule) 120/10, 120/20 (gavage)	No	TMC114-NC144	4.2.2.2
Carcinogenicity	Mouse/ CD-1	Oral/gavage	0 (vehicle), 150, 450, 1000	Yes	TMC114-NC157	4.2.3.4.1
	Rat/ Sprague-Dawley	Oral/dietary admixture	0 (vehicle), 260, 1040, 2600	Yes	TMC114-NC196	4.2.3.4.3
	Mouse/ CB6F1-nonTgrasH2	Oral/gavage	0 (vehicle), 150, 450, 1000	Yes	TMC114-NC194	4.2.3.4.3
	Mouse	Oral/Gavage	0, 0, 150, 450 or 1000	Yes	TMC114-NC159	4.2.3.4.1
	Rat	Oral/Gavage	0, 0, 50, 150 or 500	Yes	TMC114-NC158	4.2.3.4.1

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies-Darunavir (Continued)

Test Article: darunavir

Type of Study	Test System	Route/Method of Administration	TMC114 (/RTV) Nominal Doses (mg/kg)	GLP Compliance	Study/report Number	Location in CTD
Reproductive and Developmental	Rat/ Sprague-Dawley	Oral/gavage	0 (vehicle), 40, 200, 1000	No	TMC114-NC127	4.2.3.5.2
	Rabbit/ New Zealand White	Oral/gavage	250, 500, 1000	No	TMC114-NC124	4.2.3.5.2
	Rabbit/ New Zealand White	Oral/gavage	0 (vehicle), 40, 200, 1000	No	TMC114-NC125	4.2.3.5.2
	Rabbit/ New Zealand White	Oral/gavage	0 (vehicle), 40, 200, 1000	No	TMC114-NC126	4.2.3.5.2
	Rabbit/ New Zealand White	Oral/gavage	0/0 (vehicle), 0/40, 200/40, 1000/40, 1000/0	No	TMC114-NC189	4.2.3.5.2
	Mouse/ CD-1	Oral/gavage	0 (vehicle), 150/0, 450/0, 1000/0, 1000/50, 0/50	Yes	TMC114-NC172	4.2.3.5.2
	Rat/ Sprague-Dawley	Oral/gavage	0 (vehicle), 40, 200, 1000	No	TMC114-NC178	4.2.3.5.3
	Rat pups/ Sprague-Dawley	Oral/Gavage	20, 40, 80 (Day 5) 40, 80, 120 (Day 8) 40, 80, 160 (Day 11)	No	TMC114-NC240	4.2.3.5.4

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies-Darunavir (Continued)

Test Article: darunavir

Type of Study	Test System	Route/Method of Administration	TMC114 (/RTV) Nominal Doses (mg/kg)	GLP Compliance	Study/report Number	Location in CTD
Reproductive and Developmental (continued)	Rat pups/ Sprague-Dawley	Oral/Gavage	20/0, 100/0, 500/0, 500/50, 0/50 darunavir/RTV	No	TMC114-NC248	4.2.3.5.4
	Rat pups/ Sprague-Dawley	Oral/Gavage	40/0, 200/0, 500/0, 500/75, 0/75 darunavir/RTV	Yes	TMC114-NC241	4.2.3.5.4
OtherToxicity Studies	Rat/ Sprague-Dawley	Oral/gavage	0 (vehicle), 00 (water), 20/0, 100/0, 500/0, 0/50, 100/50	Yes	TMC114-NC187	4.2.3.7.2
	Rat/ Sprague-Dawley	Oral/Gavage	0 (vehicle), 50, 500	Yes	TMC114-NC162	4.2.3.7.3
	Dog/ Beagle	Oral/gavage	0 (vehicle), 120	Yes	TMC114-NC176	4.2.3.7.6

ESC = escalating; MTD = maximum tolerated dose; RTV = ritonavir

2.6.7.2 Toxicokinetics: Overview of Toxicokinetics Studies-Cobicistat

Test Article: Cobicistat

Type of Study	Test System	Method of Administration	Dose (mg/kg/day)	GLP[a]	Gilead Study No. Location In CTD
4-Week Repeat Dose	Rat	Oral Gavage	0, 10, 20, 50, 100	Yes	TX-216-2004 4.2.3.2
13-Week Repeat Dose	Rat	Oral Gavage	0, 10, 30, 100	Yes	TX-216-2017 4.2.3.2
13-Week Repeat Dose with COBI/atazanavir	Rat	Oral Gavage	0, 30/0, 0/20, 0/50, 30/20, 30/50	Yes	TX-216-2024 4.2.3.2
13-Week Repeat Dose with COBI/elvitegravir	Rat	Oral Gavage	0, 30/0, 0/1000, 30/100, 30/1000	Yes	TX-216-2001 4.2.3.2
4-Week Repeat Dose	Dog	Oral Gavage	0, 5, 15, 45/30	Yes	TX-216-2005 4.2.3.2
39-Week Repeat Dose	Dog	Oral Gavage	0, 5, 10, 20	Yes	TX-216-2016 4.2.3.2
4-Week Repeat Dose	Mouse	Oral Gavage	0, 10, 30, 100	Yes	TX-216-2041 4.2.3.4.1
13-Week Repeat Dose	Mouse	Oral Gavage	0, 5, 15, 50	Yes	TX-216-2026 4.2.3.4.1
2-year Carcinogenicity	Mouse	Oral Gavage	0 (water), 0 (vehicle), 5, 15, 50 (males); 0 (water), 0 (vehicle), 10, 30, 100 (females)	Yes	TX-216-2030 4.2.3.4.1
2-year Carcinogenicity	Rat	Oral Gavage	0 (water), 0 (vehicle), 10, 25, 50 (males); 0 (water), 0 (vehicle), 5, 15, 30 (females)	Yes	TX-216-2031 4.2.3.4.1
Embryo/Fetal Development	Rat	Oral Gavage	0, 25, 50, 125	Yes	TX-216-2020 4.2.3.5.2
Embryo/Fetal Development	Rabbit	Oral Gavage	0, 25, 50, 100	Yes	TX-216-2021 4.2.3.5.2
Perinatal/Postnatal/Juvenile Toxicity	Rat	Oral Gavage	0, 10, 30, 75	Yes	TX-216-2033 4.2.3.5.3

Test Article: Cobicistat

Type of Study	Test System	Method of Administration	Dose (mg/kg/day)	GLP[a]	Gilead Study No. Location In CTD
Immunotoxicity	Rat	Oral Gavage	0, 20, 50, 150	Yes	TX-216-2022 4.2.3.7.2

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data-Darunavir

Test article: darunavir

C_{max} at steady state (µg/mL)									
Daily Dose (mg/kg/day)		Study Duration (days)	Mouse		Rat		Rabbit	Dog	
TMC114	RTV		M	F	M	F	F	M	F
20	-	177[d]			1.03	1.77			
20	50	176[e]			1.66	1.95			
30	-	358[h]						11.5	9.11
40 (pregnant)	-	12[f]				1.70			
40 (pregnant)	-	13[g]					0.151		
50	-	184[i]			3.72	7.19			
60	-	358[h]						15.4	13.5
100	-	177[d]			7.11	12.8			
100	50	176[e]			5.30	6.42			
120	-	358[h]						27.8	23.9
150	-	87[a]	1.78	2.89					
150	-	184[i]			7.02	10.1			
150	-	188[g]	3.36	3.72					
150 (pregnant)	-	10[b]		5.99					
200 (pregnant)	-	12[f]				6.13			
200 (pregnant)	-	13[g]					0.601		
260 (diet)	-	91[c]			1.42	1.89			
450	-	87[a]	4.79	8.45					
450	-	188[g]	3.12	5.97					
450 (pregnant)	-	10[b]		8.00					
500	-	177[d]			10.3	24.5			
500	-	184[i]			12.6	12.8			
500	75	176[e]			11.5	26.6			

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data-Darunavir (Continued)

Test article: darunavir

C_{max} at steady state (µg/mL)									
Daily Dose (mg/kg/day)		Study Duration (days)	Mouse		Rat		Rabbit	Dog	
TMC114	RTV		M	F	M	F	F	M	F
1000	-	87[a]	4.18	6.80					
1000	-	188[g]	10.1	10.7					
1000	75	176[e]			14.4	24.8			
1000 (pregnant)	-	10[b]		7.73					
1000 (pregnant)	-	12[f]				11.3			
1000 (pregnant)	-	13[g]					1.74		
1040 (diet)	-	91[c]			3.03	3.47			
2600 (diet)	-	91[c]			4.56	6.30			

Only the long-term studies in mice, rats, rabbits and dogs after oral administration of darunavir alone and the long-term study in rats with RTV were presented in this table.

[a] : NC157; [b] : NC172; [c] : NC196; [d] : NC132; [e] : NC146; [f] : NC127; [g] : NC125; [h] : NC145; [i] : NC158; [g] : NC159

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data-Darunavir (Continued)

Test article: darunavir

$AUC_{0\text{-}24h}$ at steady state (µg.h/ml)									
Daily Dose (mg/kg/day)		Study Duration (days)	Mouse		Rat		Rabbit	Dog	
			M	F	M	F	F	M	F
TMC114	RTV								
20	-	177[d]			2.92	4.38			
20	50	176[e]			11.2[k]	13.4			
30	-	358[h]						31.6[l]	21.2[l]
40 (pregnant)	-	12[f]				7.6[k]			
40 (pregnant)	-	13[g]					0.465[k]		
50	-	184[i]			17.1	24.9			
60	-	358[h]						71.4[l]	50.3[l]
100	-	177[d]			31.6	35.8			
100	50	176[e]			69.0	70.4			
120	-	358[h]						130[l]	100[l]
150	-	87[a]	6.03	8.48					
150	-	184[i]			50.4	51.7			
150	-	188[g]	6.23	7.79					
150 (pregnant)	-	10[b]		12.5					
200 (pregnant)	-	12[f]				36.8[k]			
200 (pregnant)	-	13[g]					1.81[k]		
260 (diet)	-	91[c]			23.9	30.8			
450	-	87[a]	22.4	45.8					
450	-	188[g]	14.8	40.8					
450 (pregnant)	-	10[b]		27.6[k]					
500	-	177[d]			63.8	121			
500	-	184[i]			90.3	89.5			
500	75	176[e]			183	318			

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data-Darunavir (Continued)

Test article: darunavir

$AUC_{0\text{-}24h}$ at steady state (µg.h/ml)									
Daily Dose (mg/kg/day)		Study Duration (days)	Mouse		Rat		Rabbit	Dog	
TMC114	RTV		M	F	M	F	F	M	F
1000	-	87[a]	33.0	50.3					
1000	-	188[g]	48.1	63.8					
1000	75	176[e]			198	297			
1000 (pregnant)	-	10[b]		63.9					
1000 (pregnant)	-	12[f]				66.2[k]			
1000 (pregnant)	-	13[g]					5.99[k]		
1040 (diet)	-	91[c]			47.4	66.4			
2600 (diet)	-	91[c]			72.2	123			

Only the long-term studies in mice, rats, rabbits and dogs after oral administration of darunavir alone and the long-term study in rats with RTV were presented in this table.

[a] : NC157; [b] : NC172; [c] : NC196; [d] : NC132; [e] : NC146; [f] : NC127; [g] : NC125; [h] : NC145; [i] : NC158; [g] : NC159

[k] : $AUC_{0\text{-}8h}$; [l] : $AUC_{0\text{-}last}$

2.6.7.3 Toxicokinetics: Overview of Toxicokinetics Data-Cobicistat

Test Article: Cobicistat

	Steady State AUC_{0-t} (µg•h/mL)						
Species	**Mouse**		**Rat**		**Dog**		**Rabbit**
Method of Administration	**Oral Gavage**		**Oral Gavage**		**Oral Gavage**		**Oral Gavage**
Gender (M/F)	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Female**
Dose (mg/kg/day)							
5	0.93[b], 4.05[l]	1.19[b]		0.81[m]	4.39[g], 5.27[h]	2.18[g], 9.12[h]	
10	6.58[a]	3.85[a], 13.9[l]	1.23[c], 2.09[d], 1.31[m]	3.05[c], 3.52[d]	19.6[h]	16.8[h]	
15	16.3[b], 12.4[l]	11.3[b]		6.38[m]	22.9[g]	34.7[g]	
20			5.76[c], 2.33[k]	7.48[c], 5.69, 5.69[k]	71.3[h]	99.7[h]	
25			9.08[m]	6.66[i]			1.59[j]
30	33.2[a]	30.8[a], 72.1[l]	9.92[d], 7.41[e], 10.8[f]	13.3[d], 15.4[e], 12.8[f], 19.9[m]	NA	53.6[g]	
30 (+ EVG 100 mg/kg/day)			8.95[e]	16.4[e]			
30 (+ EVG 1000 mg/kg/day)			5.18[e]	7.55[e]			
30 (+ ATV 20 mg/kg/day)			4.58[f]	11.2[f]			
30 (+ ATV 50 mg/kg/day)			6.10[f]	6.34[f]			
45					89.0[g]	NA	
50	46.2[b], 75.0[l]	60.1[b]	21.2[c], NA, 11.2[k], 22.6[m]	24.8[c], 14.8[i], 10.9[k]			19.2[j]
100	65.3[a]	90.1[a], 174[l]	37.0[c], 47.2[d]	35.7[c], 71.4[d]			35.7[j]
125			NA	61.4[i]			
150			47.1[k]	79.3[k]			

a TX-216-2041 (Week 4) b TX-216-2026 (Week 13) c TX-216-2004 (Day 27) d TX-216-2017 (Week 26) e TX-236-2001 (Day 90)
f TX-216-2024 (Day 90) g TX-216-2005 (Week 4) h TX-216-2016 (Week 39) i TX-216-2020 (GD 17) j TX-216-2021 (GD20)
k TX-216-2022 (Day 28) l TX-216-2030 (Week 29) m TX-216-2031 (Week 26)
NA = not applicable; ATV = atazanavir

2.6.7.4 Toxicology: Drug Substance-Darunavir[a]

Test Article: darunavir

Batch No.	Purity (%)	Specified Impurities (%)				Study No.	Type of Study
PROPOSED SPECIFICATION:	97.0 – 101.0[b]	不純物J* ! [redacted]	不純物K* ! [redacted]	不純物E* ! [redacted]	不純物L* ! [redacted]		
[redacted][c]	99.8	-	-	-	-	TMC114-NC101	Acute oral toxicity study with TMC114 in the Wistar rat.
						TMC114-NC104	Acute oral toxicity study with TMC114 in Wistar rat (Dose escalation rat, high dose).
						TMC114-NC102	Range finding oral toxicity study in male and female Beagle dogs.
						[redacted] 293063	Evaluation of the mutagenic activity of TMC114 in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat).
						[redacted] 294288	Evaluation of the ability of TMC114 to induce chromosome aberrations in cultured peripheral human lymphocytes.
[redacted][c]	99	-	-	-	-	TMC114-NC104	Acute oral toxicity study with TMC114 in Wistar rat (Dose escalation rat, high dose).
[redacted]	99.2	-	-	0.6	-	TMC114-NC111	Acute oral toxicity study with TMC114 in CD1 mouse.
						TMC114-NC110	Acute intravenous toxicity study with TMC114 in the Wistar rat.
						TMC114-NC109	Single and repeated dose intravenous toxicity of TMC114 in male and female Beagle dogs.
						TMC114-NC107	Subacute 14-day oral toxicity study with TMC114 by daily gavage in Wistar rat.
						TMC114-NC106	Subacute 14-day oral toxicity study with TMC114 by daily gavage in the Beagle dog.
						TMC114-NC114	Micronucleus test in bone marrow cells of the mouse with TMC114.
[redacted]	98.9	-	-	-	-	TMC114-NC129	The effects of TMC114 on fertility and early embryonic development in Sprague-Dawley rats.
						TMC114-NC127	Dose range finding study of prenatal developmental toxicity of TMC114 in Sprague-Dawley rats.
						TMC114-NC128	Study of the effect of TMC114 on embryo-fetal development in Sprague-Dawley rats.
						TMC114-NC125	Dose range finding study of prenatal developmental toxicity of TMC114 in New Zealand White rabbits.

[a] Data in this table is based on the information in the certificate of analysis included in the reports, information that became available after the studies were conducted can be found in Module 3.2.S; [b] calculated with reference to the anhydrous, ethanol-free substance; [c] No certificate of analysis in the report, data from the report.

*新薬承認情報提供時に置き換え

2.6.7.4 Toxicology: Drug Substance-Darunavir (Continued)[a]

Test Article: darunavir

Batch No.	Purity (%)	Specified Impurities (%)				Study No.	Type of Study
PROPOSED SPECIFICATION:	97.0 – 101.0[b]	不純物J* ! [redacted]	不純物K* ! [redacted]	不純物E* ! [redacted]	不純物L* ! [redacted]		
[redacted]	101.9 99.3 [d]	- < 0.05 [d]	- < 0.05 [d]	-	-	TMC114-NC127	Dose range finding study of prenatal developmental toxicity of TMC114 in Sprague-Dawley rats.
						TMC114-NC124	Multiple dose toxicity study of TMC114 in New Zealand White rabbits (pilot study).
[redacted]	97.7	< 0.05%	< 0.05%	-	-	TMC114-NC176	Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog (new drug substance batches).
						TMC114-NC170	Evaluation of the mutagenic activity of three batches of TMC114 ethanolate in the *Salmonella Typhimurium* reverse mutation assay tester strain TA98 and TA100.
[redacted]	102.3	< 0.05%	0.06	-	-	TMC114-NC130	Three-month oral (gavage) repeat dose toxicity study in Sprague-Dawley rat.
						TMC114-NC132	Six-month oral (gavage) repeat dose toxicity study in Sprague-Dawley rat.
						TMC114-NC131	Three month oral (gavage) repeat dose toxicity study in the Beagle dog.
						TMC114-NC129	The effects of TMC114 on fertility and early embryonic development in Sprague-Dawley rats.
						TMC114-NC128	Study of the effect of TMC114 on embryo-fetal development in Sprague-Dawley rats.
[redacted]	99.6	< 0.05%	< 0.05%	-	-	TMC114-NC132	Six-month oral (gavage) repeat dose toxicity study in Sprague-Dawley rat.
						TMC114-NC126	Study of the effect of TMC114 on embryo-fetal development in New Zealand White rabbits.
[redacted]	100.7	< 0.05%	< 0.05%	-	-	TMC114-NC133	Six month oral (gavage) repeat dose toxicity study in the Beagle dog.

[a] Data in this table is based on the information in the certificate of analysis included in the reports, information that became available after the studies were conducted can be found in Module 3.2.S; [b] calculated with reference to the anhydrous, ethanol-free substance; [d] Batch analysis.

*新薬承認情報提供時に置き換え

2.6.7.4 Toxicology: Drug Substance-Darunavir (Continued)[a]

Test Article: darunavir

Batch No.	Purity (%)	Specified Impurities (%)				Study No.	Type of Study
PROPOSED SPECIFICATION:	97.0 – 101.0[b]	不純物J* ! [redacted]	不純物K* ! [redacted]	不純物E* ! [redacted]	不純物L* ! [redacted]		
[redacted]	100.9	< 0.05%	< 0.05%	-	-	TMC114-NC141	Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in Wistar rat.
						TMC114-NC143	Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in Wistar rat.
						TMC114-NC146	6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in Wistar rat.
						TMC114-NC140	Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage in Beagle dog.
[redacted] (continued)						TMC114-NC144	Subacute 14-day oral toxicity study with TMC114 and ritonavir by daily gavage or capsules in Beagle dog.
						TMC114-NC150	Evaluation of the mutagenic activity of TMC114 in the *Salmonella typhimurium* reverse mutation assay in tester strain TA98 and TA100.
[redacted]	99.8 98.8[d]	-	- 0.10%[d]	- 0.09%[d]	-	TMC114-NC176	Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog (new drug substance batches).
						TMC114-NC170	Evaluation of the mutagenic activity of three batches of TMC114 ethanolate in the *Salmonella Typhimurium* reverse mutation assay tester strain TA98 and TA100.
[redacted]	98.1	-	0.1 0.07%[d]	<0.1 < 0.05%[d]	-	TMC114-NC157	Three-month repeated dose oral toxicity study in the Swiss mouse.
						TMC114-NC190	Two-week repeated dose oral toxicity study in the Swiss mouse.
						TMC114-NC189	Five-Day repeated dose oral toxicity study in the female rabbit.
						TMC114-NC187	Four-week immunotoxicity study by oral route (gavage) in rats.

[a] Data in this table is based on the information in the certificate of analysis included in the reports, information that became available after the studies were conducted can be found in Module 3.2.S; [b] calculated with reference to the anhydrous, ethanol-free substance; [d] Batch analysis.

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2.6.7.4 Toxicology: Drug Substance-Darunavir (Continued) [a]

Test Article: darunavir

Batch No.	Purity (%)	Specified Impurities (%)				Study No.	Type of Study
PROPOSED SPECIFICATION:	97.0 – 101.0 [b]	不純物J* ! [redacted]	不純物K* ! [redacted]	不純物E* ! [redacted]	不純物L* ! [redacted]		
[redacted]	99.1 98[d]	-	- 0.05%[d]	- 0.05%[d]	-	TMC114-NC176	Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog (new drug substance batches).
						TMC114-NC173	14-Day oral (gavage) bioavailability and tolerance study in the Beagle dog.
						TMC114-NC145	Twelve-month oral (gavage) toxicity study in Beagle dog including toxicokinetic sampling.
						TMC114-NC170	Evaluation of the mutagenic activity of three batches of TMC114 ethanolate in the *Salmonella typhimurium* reverse mutation assay tester strain TA98 and TA100.
[redacted]	97.5%	-	ND	0.16	ND	TMC114-NC196	Thirteen-week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats.
						TMC114-NC242	Two-week repeated dose oral toxicity study in the rat.
						TMC114-NC244	In vitro bacterial reverse mutation test with *Salmonella typhimurium*.
[redacted]	98.6	-	ND	0.21	ND	TMC114-NC175	Oral (gavage) development toxicity dose range finding study in the mouse.
						TMC114-NC172	Oral (gavage) developmental toxicity study in the mouse.
						TMC114-NC178	Oral (gavage) pre- and postnatal developmental toxicity and juvenile toxicity dose range finding study in the rat (juvenile tox)
[redacted]	98.7%	-	ND	0.23%	ND	TMC114-NC156	Oral (gavage) pre and post-natal developmental toxicity study in the rat
[redacted] ([redacted])	99.6	-	ND	0.24	ND	TMC114-NC227	Two-week repeated dose oral toxicity study in the rat.
						TMC114-NC228	Bacterial reverse mutation test.
						TMC114-NC194	Four-week toxicity study by oral route (gavage) in CB6F1-nonTgrasH2 mice.
[redacted]	97.3	-	-	0.04	-	TMC114-NC160	Two-week repeated dose intravenous toxicity study in the rat.
						TMC114-NC199	Two-week repeated dose intravenous toxicity study in the Beagle dog.

[a] Data in this table is based on the information in the certificate of analysis included in the reports, information that became available after the studies were conducted can be found in Module 3.2.S; [b] calculated with reference to the anhydrous, ethanol-free substance; [d] Batch analysis
ND = not detected

*新薬承認情報提供時に置き換え

2.6.7.4 Toxicology: Drug Substance-Darunavir (Continued)[a]

Batch No.	Purity (%)[b]	Specified Impurities (%)							Study No.	Type of Study
PROPOSED SPECIFICATION:	97%–101%	不純物C* / 不純物D* NMT [redacted]%	不純物B* NMT [redacted]%	不純物E* NMT [redacted]%	不純物A* NMT [redacted]%	不純物F* NMT [redacted]%	不純物G* NMT [redacted]%	不純物I* NMT [redacted]%		
[redacted] ([redacted])	96.7	-	-	0.23	-	-	-	-	TMC114-NC240	Juvenile tolerance study in rats
[redacted]	100.1	< 0.1	ND	0.1	0.1	ND	ND	-	TMC114-NC316	Primary skin irritation study in rabbits
									TMC114-NC330	Evaluation of skin sensitization potential in mice (LLNA).
[redacted]	99.6	< 0.1	0.1	0.1	0.1	< 0.1	< 0.1	-	TMC114-NC355	In vitro chromosome aberration test with TMC114 spiked with 2% impurities in human lymphocytes.
									TMC114-NC354	In vitro chromosome aberration test with TMC114 spiked with 2% impurities in human lymphocytes.
									TMC114-NC336	Ames test with TMC114 Spiked with 2% impurities in S. Typhimurium.
									TMC114-NC337	In vitro chromosome aberration test in cultured human lymphocytes.
									TMC114-NC338	2-Week repeated dose oral toxicity study in the Beagle dog.
									TMC114-NC359	Ames test with TMC114 spiked with 3% impurities in S. typhimurium.
									TMC114-NC357	In vitro chromosome aberration test with TMC114 spiked with 3% impurities in human lymphocytes.

*新薬承認情報提供時に置き換え

2.6.7.4 Toxicology: Drug Substance-Darunavir (Continued)[a]

Batch No.	Purity (%)[b]	Specified Impurities (%)							Study No.	Type of Study
PROPOSED SPECIFICATION:	97%–101%	不純物C* / 不純物D* NMT [redacted]%	不純物B* NMT [redacted]%	不純物E* NMT [redacted]%	不純物A* NMT [redacted]%	不純物F* NMT [redacted]%	不純物G* NMT [redacted]%	不純物I* NMT [redacted]%		
[redacted] ([redacted])	99.3	0.1	0.1	0.3	ND	-	-	-	TMC114-NC248	Range finding toxicity study in the juvenile rat by oral administration.
									TMC114-NC166	In vitro bovine corneal opacity-permeability eye irritation test.
									TMC114-NC162	1-month repeated dose mechanistic oral toxicity study in the rat.
									TMC114-NC158	Carcinogenicity study in the Rat.
									TMC114-NC159	Carcinogenicity study in the Mouse.
									TMC114-NC241	Toxicity study in the juvenile rat by oral administration
[redacted] ([redacted])	98.7	-	-	0.20	-	-	-	-	TMC114-NC158	Carcinogenicity study in the Rat.
									TMC114-NC159	Carcinogenicity study in the Mouse.
[redacted]	99.3	<0.05	<0.05	0.15	0.06	<0.05	0.0	0.06	TMC114-NC397	Pilot oral developmental toxicity study of TMC114 in the rat
									TMC114-NC399	Combined Pharmacokinetic/DRF Study in Female Minipigs
[redacted]	99.6	<0.05	ND	0.11	0.05	<0.05	ND	0.06	TMC114-NC398	Oral Developmental Toxicity Study of TMC114 in the Rat

[a] Data in this table is based on the information in the certificates of analysis available in the report; [b] calculated with reference to the anhydrous, ethanol-free substance
[c] 不純物C*/不純物D* = 不純物C* / 不純物D* ; [d] 不純物B* = 不純物B* ; [e] 不純物E* = 不純物E* = 不純物E* ; [f] 不純物A* = 不純物A* ; [g] 不純物F* = 不純物F* ;
[h] 不純物G* = 不純物G* ; [i] 不純物I* = 不純物I*
ND: not detected; - not defined

*新薬承認情報提供時に置き換え

2.6.7.4 Toxicology: Drug Substance-Cobicistat

Test Article: Cobicistat

Lot Number	Purity (% w/w)	Chiral Impurities (Normalized Area %)			Observed Impurities (Normalized Area %)													Type of Toxicity Study
		GS-9407	GS-9654	GS-9655	Total Impurities[c]	GS-492988	不純物CD*	GS-445739	GS-344433	不純物CB*	GS-9612	GS-9454	不純物CA*	GS-9390	GS-9397	不純物CC*	Any other impurity[d]	
Proposed Limit		!	!	!	!	!	!	!	!	!	!	!	!	!	!	!	!	
3168-121-23[a]																		IND Studies/Genetic[e]
3168-157-8[b]	[b]																	Repeat Dose, Reproductive, Immunotoxicity[f]
3168-171-16[a]																		Single/Repeat Dose, Developmental, Genetic[g]
9350-AC-2E crop 1[b]	[b]																	Carcinogenicity [h]
9350-AC-2E crop 2[b]	[b]																	Repeat Dose, Carcinogenicity [h]
3793-143-21[a]																		Repeat Dose, Carcinogenicity[k]
3793-143-22[a]																		Carcinogenicity[h]
3471-189-36[a]																		Peri/Postnatal Development[i]
4646-065-																		Local Tolerance,

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Test Article: Cobicistat

Lot Number	Purity (% w/w)	Chiral Impurities (Normalized Area %)			Observed Impurities (Normalized Area %)													Type of Toxicity Study
		GS-9407	GS-9654	GS-9655	Total Impurities[c]	GS-492988	不純物CD*	GS-445739	GS-344433	不純物CB*	GS-9612	GS-9454	不純物CA*	GS-9390	GS-9397	不純物CC*	Any other impurity[d]	
Proposed Limit		! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	! [redacted]	Impurity Qualification[j]
18[a]																		
4646-067-15[a]	[redacted]	[redacted]	0.25	0.25	3.9	0.25	0.26	0.20	0.23	0.26	0.23	0.20	0.27	0.37	0.28	0.40	0.27	Impurity Qualification[l]

a Neat Cobicistat [redacted] solid.

b Cobicistat (% w/w) in ethanol solution; reported purity by area norm %.

c Total impurities include all specified and unspecified impurities. Trace is reported when level detected is < [redacted]% and not included in total impurities calculation.

d Largest individual unspecified impurity observed.

e Used for study number(s): TX-216-2003, TX-216-2004, TX-216-2005, TX-216-2010, TX-216-2011, TX-216-2012.

f Used for study number(s): TX-216-2016, TX-216-2017, TX-216-2018, TX-216-2020, TX-216-2022, TX-216-2023, TX-216-2024, TX-216-2025, TX-216-2026, TX-236-2001.

g Used for study number(s): TX-201-2003, TX-201-2004, TX-201-2005, TX-201-2008, TX-216-2019, TX-216-2021.

h Used for study number(s): TX-216-2030, TX-216-2031, TX-216-2032.

i Used for study number(s): TX-216-2033.

j Used for study number(s): TX-216-2042, TX-216-2043, TX-216-2044, TX-216-2045.

k Used for study number(s): TX-216-2030, TX-216-2031, TX-216-2041.

l Used for study number(s): TX-216-2045.

*新薬承認情報提供時に置き換え

2.6.7.5. Single-Dose Toxicity-Darunavir

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/Formulation)	Batch no. (Purity)	Nominal Dose, mg/kg (Actual Dose, mg b.e./kg)	NOAEL (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mouse/CD-1 5M+5F/group	Oral/gavage (PEG400)	[redacted] (as base: 93.3% w/w; as ethanolate: 99.2% w/w)	Vehicle, 100, 300, 1000[a] (0, 93, 280, 933)	100 Based on mortalities	100: lethargy days 1 and 2 300: 1/5 M, found dead day 2, 1/5 M, killed day 2, lethargy days 1 and 2 1000: 1/5 M and 2/5 F, killed day 2, lethargy days 1 and 2 Distended or fluid-filled gastro-intestinal tract in 4/4 animals sacrificed before end of study	TMC114-NC111[f]/ 4.2.3.1
Rat/Wistar 5M+5F/group	Oral/gavage (PEG400)	Batch [redacted](as base: 94.2% w/w; as ethanolate: 99.8% w/w)	Vehicle, 20, 100, 500[a] (0, 19, 94, 471)	500	No TMC114-related adverse effects	TMC114-NC101[f]/ 4.2.3.1
Rat/Wistar 5M+5F/group	Oral/gavage (PEG400)	Batch [redacted](as base: 94.2% w/w; as ethanolate: 99.8% w/w) Batch [redacted](as base: 96.5% w/w)	Vehicle, 500, 1000, 1500, 2000[a] (Batch [redacted]: 0, 471, 942, 1413, 1884) (Batch [redacted]: 0, 455, 909, 1364, 1818)	2000	No TMC114-related adverse effects.	TMC114-NC104[f]/ 4.2.3.1
Rat/Wistar 5M+5F/group	Intravenous (50% PEG400, 10% ethanol, 40% saline v/v)	[redacted] (as base: 93.3% w/w; as ethanolate: 99.2% w/w)	Vehicle, 10, 20, 40[b] (0, 9, 19, 37)	40	No TMC114-related adverse effects. Several clinical signs related to ethanol in vehicle.	TMC114-NC110[f]/ 4.2.3.1
Dog/Beagle 2M+2F/group 1M+1F/group	Oral/gavage (PEG400)	Batch [redacted](as base: 94.2% w/w; as ethanolate: 99.8% w/w)	20→80→320[c] (19, 75, 301) 80[d, e] (75)	20	320: salivation (4/4, day 1), vomiting with mucus (2/4), vomiting with traces of blood (1/4) 80: abnormal posture and / or salivation (day 3)	TMC114-NC102[f]/ 4.2.3.1

(Continued)

2.6.7.5. Single-Dose Toxicity-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/ Group	Route/Method of Administration (Vehicle/Formulation)	Batch no. (Purity)	Nominal Dose, mg/kg (Actual Dose, mg b.e./kg)	NOAEL (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/Beagle 2M+2F/group	Intravenous (50% PEG400, 10% ethanol, 40% saline v/v)	[redacted] (as base: 93.3% w/w; as ethanolate: 99.2% w/w)	Vehicle→10→20→40 [c] (0, 9, 19, 37)	10	10: salivation (3/4) 20: salivation (4/4); comatose state (1/2 M, immediately after dosing). 40: salivation, abnormal posture, retching, shaking of the head, head tilt, vomiting of mucus (4/4).	TMC114-NC109 [f]/ 4.2.3.1
1M+1F/group			10 [d] (9)		Slight body weight loss (2/2, day 4)	

[a] Single dose followed by a 15 day observation period; [b] Single dose followed by a 16 day observation period; [c] Administered as single escalating doses on consecutive days followed by an observation period of 3 days; [d] Administered as a repeated dose for 3 consecutive days; [e] On the first day, 72 instead of 80 mg/kg was administered; [f] GLP compliant;

b.e. = base equivalent, MTD = maximum tolerated dose, PEG400 = polyethylene glycol 400

2.6.7.5. Single-Dose Toxicity-Cobicistat

Test Article: COBI

Species / Strain	Method of Administration (Vehicle)	Dose (mg/kg)	Gender / No. per Group	NOAEL (mg/kg)	Noteworthy Findings	Gilead Study No. (CRO Study No.) Location in CTD
Mouse/ CByB6F1-Tg (HRAS)2Jic (wild type)	Oral Gavage [10% (v/v) propylene glycol in 40 mM acetate buffer, pH 4.0]	30, 100, 300	24 M/F	100	No COBI-related clinical signs were observed at 30 or 100 mg/kg; hunched appearance, hypoactivity, and squinted eyes observed post-dose at 300 mg/kg. Blood collected at 1, 2, and 4 hours postdose as scheduled for the 300 mg/kg group. Due to moribundity, the 12 remaining M/F mice given 300 mg/kg were bled after the 4-hour postdose collection interval and subsequently euthanized. NOAEL = 100 mg/kg, C_{max} of 11.1 and 16.2 µg/mL and AUC_{last} of 109 and 129 µg•h/mL in males and females, respectively.	PC-216-2013 ([redacted]8221867) PC-216-2013-PK 4.2.2.2
Rat/ Sprague-Dawley	Oral Gavage (95% propylene glycol, 5% ethanol, pH adjusted to 2.3 with HCl)	0, 125, 250, 500	5 M/F toxicity group 3 M/F TK group	500	No test article related adverse findings. NOAEL = 500 mg/kg, $C_{1\ hr}$; 5.54 and 6.85 µg/mL in males and females, respectively.	TX-216-2003[a] ([redacted]6511-301) 4.2.3.1

a Indicates GLP compliant.

NOAEL = No-Observed-Adverse-Effect Level; M = male; F = female; TK = toxicokinetic; NA = not applicable

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Darunavir

Test article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (Purity)	Nominal Dose, mg/kg/day (Actual Dose, mg b.e./kg/day)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Wistar 10M+10F/ Group 5M+5F satellite/group	Oral/gavage (PEG400)	2 Weeks	[redacted] (as base: 93.3% w/w; as ethanolate: 99.2% w/w)	0 (vehicle), 40, 200, 1000, (0, 37, 187, 933)	40	No mortalities associated with TMC114; 5 accidental deaths associated with the blood collection procedure principal animals, 40: 1/10M and 1000: 2/10F (satellite animals, 40: 2/10F). 40: hepatocellular hypertrophy (1/10 M) 200: ↓ Hct (F, 2%), ↓ Hb (F, 5%), ↓ RBC (F, 2%), ↑ platelets (M, 10%; F, 23%), ↑ creatinine (F, 12%), ↑ liver wt (relative M, 13%; F, 22%), hepatocellular hypertrophy and vacuolation. 1000: ↓ Hct (F, 6%), ↓ Hb (F, 5%), ↓ RBC (F, 5%), ↑ platelets (M, 15%; F, 26%), ↑ creatinine (M, 15%; F, 21%), ↓ triglycerides (M, 41%), ↑ cholesterol (F, 30%), ↑ phospholipids (F, 20%), ↑ urinary Na^+ (M, 17%; F, 2.1-fold), ↑ urinary Cl^- (F, 81%), ↑ liver wt (relative M, 40%; F, 73%), hepatocellular hypertrophy and vacuolation; ↑ extramedullary hematopoiesis in spleen; ↑ thyroid follicular epithelial hypertrophy (F)	TMC114-NC107[b] /4.2.3.2
Rat, Sprague-Dawley 10M+10F /group (6M+6F satellite/group, except vehicle group)	Intravenous/ bolus (15% HP-β-CD in pyrogen free water)	2 Weeks	[redacted] (as ethanolate: 97.3% w/w)	0 (vehicle), 2.5, 10, 25 expressed as mg b.e./kg/day	25	No TMC114-related adverse effects	TMC114-NC160[b] /4.2.3.2

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Darunavir (Continued)

Test article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (Purity)	Nominal Dose, mg/kg/day (Actual Dose, mg b.e./kg/day)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Wistar 10M+10F /group (5M+5F satellite/group, except vehicle group)	Oral/gavage (TMC114: PEG400, RTV: PG)	2 Weeks	TMC114: [redacted], RTV: 79547VA (TMC114 as base: 93.1% w/w; as ethanolate: 100.9% w/w)	TMC114/RTV: 0/0 (vehicles), 0/50, 150/25, 500/25, 1000/50 (0/0, 0/50, 140/25, 466/25, 931/50)	Not established	No mortalities associated with TMC114; 3 accidental deaths associated with the dosing procedures: principal animals, 0/50: 1/10 M (satellite animals: 0/50: 1/5M and 500/25: 1/5 M). 0/50: ↓ Hct (F, 5%), ↑ phospholipids (F, .32%), ↑ creatinine (M, 32%), ↑ total globulins (F, 13%), ↑ liver wt (M_{rel}, 23% F_{rel}, 28%), hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis 150/25: ↓ RBC (F, 13%), ↓ Hb (F, 8%), ↓ Hct (F, 12%), ↑ phospholipids (F, 50%), ↑ cholesterol (F, 48%), ↑ creatinine (M, 19%), ↑ total globulins (F, 24%), ↑ liver wt (M_{rel}, 49%; F_{rel}, 62%), hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis, ↑ thyroid hypertrophy/hyperplasia (F) 500/25: ↓ RBC (F, 14%; M, 5%), ↓ Hb (F, 10%; M, 6%), ↓ Hct (F, 13%; M, 6%), ↑ platelets (F, 27%), ↑ bilirubin (M, 35%, F; 29%), ↑ phospholipids (F, 65%), ↑ cholesterol (F, 76%), ↑ creatinine (M, 20%), ↑ total globulins (M, 8%; F, 23%), ↑ liver wt (M_{rel}, 65%; F_{rel}, 94%), hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis, ↑ thyroid hypertrophy/hyperplasia (F) 1000/50:, ↓ RBC (F, 8%; M, 5%), ↓ Hb (F, 8%; M, 7%), ↓ Hct (F, 10%; M, 9%), ↑ RDW (M, 9%; F, 6%), ↑ platelets (M, 25%; F, 20%), ↑ bilirubin (M, 40%, F; 24%), ↑ phospholipids (M, 40%; F, 69%), ↑ cholesterol (F, 82%), ↑ creatinine (M, 50%), ↑ total protein (M, 8%; F, 13%), ↑ total globulins (M, 15%; F, 33%), ↑ liver wt (M_{rel}, 1.8-fold%;, F_{rel}, 2.1-fold%), hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis, ↑ thyroid hypertrophy/hyperplasia	TMC114-NC141[b] /4.2.3.2

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Darunavir (Continued)

Test article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (Purity)	Nominal Dose, mg/kg/day (Actual Dose, mg b.e./kg/day)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Wistar 5M+5F /group (5M+5F satellite/group)	Oral/gavage (TMC114: PEG400, RTV: PG)	2 Weeks	TMC114: [redacted], RTV: 81410VA (TMC114 as base: 93.1% w/w; as ethanolate: 100.9% w/w)	TMC114/RTV: 0/0 (vehicles), 0/75, 2000/0, 2000/75 expressed as mg b.e./kg/day	Not established	0/75: ↓ BW gain (F, 32%), ↓ RBC (M, 7%), ↓ Hb (M, 6%), ↑ platelets (M, 20%), ↓ PT (F, 7%), ↑ cholesterol (F, 64%), ↑ total globulin (F, 22%), ↑ liver wt (M_{rel}, 41%; F_{rel}, 48%), ↑ spleen wt (M_{abs}, 27%, M_{rel}, 22%), hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis (M) 2000/0: ↓ BW gain (F, 42%), ↓ RBC (M, 7%), ↓ Hb (M, 7%), ↓ Hct (M, 8%; F, 10%), ↑ platelets (M, 23%; F, 22%), ↑ cholesterol (F, 88%), ↑ phospholipids (F, 53%), ↑ total globulin (F, 26%), ↑ bilirubin (F, 2-fold), ↑ liver wt (M_{rel}, 59%; F_{rel}, 94%), ↑ hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis (M), ↑ thyroid follicular epithelial hypertrophy (F) 2000/75: ↓ BW (M, 7%; F, 12%), ↓ BW gain (M 32%; F 84%), ↓ food consumption (F, 20%), ↓ Hb (M, 8%), ↓ Hct (M, 9%), ↑ platelets (M, 36%; F, 57%), ↑ cholesterol (M, 2.1-fold; F, 2.8-fold), ↑ phospholipids (M, 2.1-fold; F, 2.3-fold), ↑ total protein (M, 12%; F, 13%), ↑ total globulin (F, 40%), ↑ bilirubin (F, 2-fold), ↑ liver wt (M_{rel}, 2.4-fold; F_{rel}, 2.5-fold), ↑ spleen wt (M_{rel}, 18%), ↑ hepatocellular hypertrophy and vacuolation, ↑ splenic erythropoiesis, ↑ thyroid follicular epithelial hypertrophy	TMC114-NC143[b] /4.2.3.2
Dog/Beagle 3M+3F/group	Oral/gavage (PEG400)	2 Weeks	[redacted] (TMC114 as base: 93.3% w/w; as ethanolate: 99.2% w/w)	0 (vehicle) 30, 60, 120, (0, 28, 56, 112)	120	No TMC114-related adverse effects	TMC114-NC106[b] /4.2.3.2

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Darunavir (Continued)

Test article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (Purity)	Nominal Dose, mg/kg/day (Actual Dose, mg b.e./kg/day)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/Beagle 1M+1F/group	Oral/gavage (PEG400)	2 Weeks 2 weeks washout period Single dose	[redacted] (TMC114 as base: 91.6% w/w; as ethanolate: 99.1% w/w)	360 120 (single dose) expressed as mg b.e./kg	Not determined	360: salivation, vomiting, loose or liquid feces, small white 'curd' like globules in vomitus and feces. Third eyelid visible (F), ↓ BW (M, 2%; F, 4%), ↑ creatinine (M, 25%, F, 21%) [c], ↑ bilirubin (M, 40%, F, 3.5-fold) [c] 120: salivation, third eyelid visible (F)	TMC114-NC173 /4.2.3.2
Dog/Beagle 3M+3F/group	Intravenous/bolus (15% HP-β-CD in pyrogen free water)	2 Weeks	[redacted] (as ethanolate: 97.3% w/w)	0 (vehicle), 3.12, 6.25, 12.5 expressed as mg b.e./kg/day	12.5	No TMC114-related adverse effects	TMC114-NC199[b] /4.2.3.2
Dog/Beagle 3M+3F/group	Oral/gavage (TMC114: PEG400, RTV: PG)	2 Weeks	TMC114: [redacted] RTV: 79547VA (TMC114 as base: 93.1% w/w; as ethanolate: 100.9% w/w)	TMC114/RTV: 0/10, 40/10, 120/10, 360/10 (0/10, 37/10, 112/10, 335/10)	Not established	0/10: vomiting, ↓ BW (F, 2/3) 40/10, vomiting, ↓ BW (M, 1/3; F, 3/3) 120/10: vomiting, ↓ BW (M, 2/3; F, 3/3), white particles in feces (M, 1/3) 360/10: vomiting, ↓ BW (M, 3/3; F, 3/3), white particles in feces (M, 2/3; F, 3/3)	TMC114-NC140[b] /4.2.3.2

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Darunavir (Continued)

Test article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (Purity)	Nominal Dose, mg/kg/day (Actual Dose, mg/b.e./kg/day)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/Beagle 3M+3F/group	Oral/capsule (twice daily)	2 Weeks	TMC114: [redacted] RTV: 81395VA, 82359VA	TMC114/RTV: 0/200, 240/200 expressed as mg b.e./kg/day	Not established	0/200: vomiting, abnormal posture, uncoordinated movements, ↓ BW (M, 12%; F, 20%), ↓ food consumption (M, 65%; F, 39%), ↓ heart rate (M, 34%; F, 11%), ↓ WBC (M, 16%; F, 33%), ↓ Hb (F, 8%), ↓ Hct (F, 8%), ↑ ALT (M, 6.9-fold; F, 2.4-fold), ↑ GLDH (M, 4.8-fold; F, 3.0-fold%), ↑ AST (M, 69%), ↑ GGT (M, 3.2-fold), ↑ ALP (M, 2.2-fold), ↓ LDH (M, 38%; F, 71%), ↑ urine volume (F, 2.4-fold), ↓ urinary Na^+ concentration (F, 83%), ↓ urinary K^+ concentration (F, 87%), ↑ liver wt, ↓ thymus wt 240/200: vomiting, abnormal posture and uncoordinated movements, ↓ BW (M, 16%; F, 20%), ↓ food consumption (M, 60%; F, 53%), ↓ heart rate (M, 35%; F, 24%), ↓ WBC (F, 20%), ↓ RBC (M, 6%), ↓ Hb (M, 6%; F, 6%), ↓ Hct (M, 6%; F, 5%), ↑ ALT (M, 2.85-fold; F, 4.4-fold), ↑ GGT (M, 82%; F, 2.3-fold), ↑ GLDH (M, 2.9-fold; F, 3.4-fold), ↓ CK (M, 47%; F, 36%), ↓ LDH (M, 53%; F, 38%), ↑ urine volume (M, 2.2-fold%, F, 2.5-fold), ↓ urinary Na^+ concentration (F, 89%), ↓ urinary K^+ concentration (F, 67%), ↑ liver wt, ↓ thymus wt	TMC114-NC144 /4.2.2.2

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Darunavir (Continued)

Test article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (Purity)	Nominal Dose, mg/kg/day (Actual Dose, mg/b.e./kg/day)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog, Beagle 3M+3F/group (continued)	Oral/gavage (PEG400)		TMC114: [redacted], RTV: 81410VA, 82359VA (TMC114 as base: 93.1% w/w; as ethanolate: 100.9% w/w)	TMC114/RTV: 120/10, 120/20 expressed as mg b.e./kg/day		120/10: vomiting, ↓ BW (M, 1%; F, 5%), ↓ RBC (M, 8%; F, 10%), ↓ Hb (M, 10%; F, 14%), ↓ Hct (M, 7%; F, 10%), ↓ LDH (M, 47%; F, 27%), ↑ urine volume (M, 2.5-fold), ↓ urinary Na^+ concentration (F, 65%), ↓ urinary K^+ concentration (F, 57%), ↓ thymus wt (F) 120/20: vomiting, ↓ BW (M, 1%; F, 5%), ↓ WBC (M, 27%; F, 16%), ↓ CK (M, 31%; F, 36%), ↓ LDH (M, 63%; F, 43%), ↑ urine volume (M, 80%), ↓ urinary Na^+ concentration (F, 66%), ↓ urinary K^+ concentration (F, 49%), ↓ thymus wt	TMC114-NC144 /4.2.2.2 (continued)

[a] - No Observed Adverse-Effect Level; [b] GLP compliant

↑ = increased, ↓ = decreased, abs = absolute, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, b.e. = base equivalent, BW = body weight, Ca^{2+} = Calcium, CK = creatine kinase, F = female, GGT = gamma glutamyl transferase, GLDH = glutamate dehydrogenase, Hb = hemoglobin, Hct = hematocrit, HP-β-CD = hydroxypropyl-β-cyclodextrin, K^+ = Kalium, LDH = lactate dehydrogenase, M = male, MCV = mean corpuscular volume, Na^+ = sodium, PEG400 = polyethylene glycol, PG = propylene glycol, PT = prothrombin time, RBC = red blood cell, RDW = red cell distribution width, rel = relative, WBC = white blood cell, wt = weight

2.6.7.6 Repeat-Dose Toxicity: Non-Pivotal Studies-Cobicistat

Test Article: Cobicistat

Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	No. per Group and Gender	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
Rat/ Sprague-Dawley	Oral Gavage (COBI Vehicle: 1.5% ethanol, 1.5% propylene glycol, 6% Labrasol, 6% Solutol (15% SEDDS), and water with 1 equivalent HCl); (RTV Vehicle: 5% ethanol, 50% propylene glycol, 45% water)	2 Weeks	0, 30, 100, 175[a], and 300 COBI 0 and 100 RTV	Main: 5 M TK: 3 M controls, 6 M treated	30 COBI (for 14 days) 175 COBI (for 8 days) 100 RTV (for 14 days)	100 mg/kg/day: erosion/ulceration of non-glandular stomach; increased liver weight and hepatocellular hypertrophy. 175 mg/kg/day: no observed adverse effects after 8 days treatment; increased liver weight and hepatocellular hypertrophy. 300 mg/kg/day: mortality (1/5 in main; 3/6 in TK group), body weight loss; moribund sacrifice of group on Day 6; erosion/ulceration of non-glandular stomach, hepatocellular hypertrophy. Decreases in total protein and marked decreases in CYP4A and CYP3A activity at 175 and 300 mg/kg/day. 100 mg/kg/day RTV: increased liver weight, hepatocellular hypertrophy. Marked decreases in CYP3A activity. NOAEL = 30 mg/kg/day COBI; Day 14 C_{max} 1.68 µg/mL, AUC_{0-t} 8.79 µg·h/mL NOAEL = 175 mg/kg/day COBI; Day 8 C_{max} 5.91 µg/mL, AUC_{0-t} 56.9 µg·h/mL NOAEL = 100 mg/kg/day RTV; Day 14 C_{max} 5.13 µg/mL, AUC_{0-t} 43.0 µg·h/mL	TX-216-2001 ([redacted] 6511-294) TX-216-2001-TK 4.2.3.2

Test Article: Cobicistat

Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	No. per Group and Gender	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
Rat/ Sprague-Dawley	Oral Gavage (95% propylene glycol/5% ethanol)	5 Days	COBI/ATV: 50/0, 0/150, 30/100, 50/150	5 F	COBI/ATV 50/0 or 30/100	Increased liver weights in animals given 50/0, 30/100 and 50/150 mg/kg/day COBI/ATV. COBI/ATV 0/150 mg/kg/day: ↑ ALT (3-fold) and AST (1.5-fold). COBI/ATV 50/150 mg/kg/day: ↑ alkaline phosphatase (1.8-fold) and total bilirubin (2.3-fold).	TX-216-2027 ([redacted] 814-390) TX-216-2027-TK 4.2.3.2
Dog/ Beagle	Oral Gavage (COBI Vehicle: 94.4% propylene glycol, 5% ethanol with 1.5 molar equivalents methane sulfonic acid); (RTV Vehicle: 46.6% propylene glycol, 34.6% ethanol, 18% water and 0.8% Cremophor EL)	7 Days	0, 50, 125, and 250 COBI 0 and 125 GS-017415 (RTV)	3 M	COBI # 50 RTV # 125	Emesis and salivation noted in all groups throughout the study with incidence and severity increasing with dose. ↓ body weight and food consumption in all groups. Moribund sacrifice in 1/3 and 2/3 males at 125 and 250 mg/kg/day COBI, respectively. GI discoloration, correlated with mucosal congestion/hemorrhage or erosion seen microscopically; ↓ thymus and spleen weights and ↑ adrenal weights; lymphocyte depletion/necrosis in all COBI and RTV groups. ↓ CYP1A activity at 50 mg/kg/day COBI and 125 mg/kg/day RTV, and marked decreases in CYP3A activity in all COBI and RTV groups. COBI 50 mg/kg/day, Day 7 C_{max} 16.6 µg/mL, AUC_{0-t} 276 µg·h/mL RTV 125 mg/kg/day, Day 7 C_{max} 24.0 µg/mL, AUC_{0-t} 199 µg·h/mL	TX-216-2002 ([redacted] 6511-308) TX-216-2002-TK 4.2.3.2

a Animals administered 175 mg/kg/day COBI for 8 days.

M = male; F = female; NOAEL = No-Observed-Adverse-Effect Level; RTV = ritonavir, GS-017415; ATV = atazanavir; TK = toxicokinetics; ↑ = increased; ↓ = decreased

2.6.7.7.A Repeat-Dose Toxicity-Darunavir

Report Title: Three month oral (gavage) repeat dose toxicity study in the rat

Test Article: darunavir

Species/strain: Rat / Sprague-Dawley
Age/weight at first dose: 6-8 weeks / 145-216 g
Duration of dosing: 3 months
Duration of post-dose: 0 days

Administration route/method: Oral gavage
Doses: 0, 20, 100 and 500 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 95% w/w, as ethanolate 102.3% w/w)
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC114-NC130
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: 20 mg/kg based on the absence of any relevant hematological or histopathological changes

Toxicokinetics:

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:0**	**F:0**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Died or killed prematurely (accidental)	0	0	0	0	1	1	0	0
$AUC_{0-\infty}$ day 1 (µg.h/mL)	-	-	3.90	6.98	38.1	42.9	184	166
AUC_{0-24h} week 7 (µg.h/mL)	-	-	1.87	5.43	22.1°	29.6	83.8	83.0
AUC_{0-24h} week 13 (µg.h/mL)	-	-	2.10°	3.66°	27.4	28.2°	67.6	71.8

Noteworthy Findings:

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Died or killed prematurely (accidental)	1	1	0	1	1	2	0	2
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/wk)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology (week 13) $								
- RBC count (10^6/µL)	8.68	8.12	1.00	1.01	1.01	1.01	0.93***	0.93**
- Hemoglobin (g/dL)	16.5	15.8	0.99	1.01	0.98	1.01	0.88***	0.90***
- Hematocrit (%)	46.5	43.3	0.99	1.01	0.99	1.01	0.89***	0.91***
- Platelets (10^3/µL)	844	685	0.96	1.10	0.95	1.14	1.35**	1.46*
- Reticulocytes (%RBC)	2.0	2.2	1.00	1.00	1.10	1.32*	1.80***	1.45**
- APTT (sec)	12.5	10.1	1.02	0.99	1.08	1.17	1.35*	1.30

$ For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric); ° AUC_{0-8h}; # HPLC assay with reference to anhydrous solvent free substance; - No treatment related change; ^ Actual dose [redacted]: 0, 19, 95, 475 mg base equivalent/kg/day; es

2.6.7.7.A Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: Three month oral (gavage) repeat dose toxicity study in the rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Serum Chemistry (week 13) $								
- Bilirubin (µmol/L)	1	1	1.00	1.00	1.00	1.00	7.00***	2.00*
- Cholesterol (mmol/L)	2.7	2.8	0.96	1.00	0.89	1.04	1.26**	1.96***
- Triglycerides (mmol/L)	0.6	0.8	1.00	1.13	0.67***	0.88	0.33***	0.38***
Urinalysis&	-	-	-	-	-	-	-	-
Organ weights $								
- Liver (%)	3.93	3.32	0.95	1.03	1.02	1.18***	1.30***	1.75***
- Spleen (%)	0.21	0.28	0.97	1.04	1.03	1.10	1.16***	0.92
- Kidney (%)	0.64	0.68	1.03	1.02	1.04	1.03	1.11***	1.09*
Gross pathology (no. examined)	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
- Kidney, dark	0	0	0	0	0	0	2	0
- Liver, dark	0	0	0	1	0	0	7	5
Histopathology (no. examined)	**9**	**9**	**10**	**9**	**9**	**8**	**10**	**8**
Liver								
- hypertrophy, hepatocellular, diffuse								
minimal	0	0	0	0	0	0	9	7
- Brown pigment, hepatocellular, diffuse								
minimal	0	0	0	0	0	0	4	7
slight	0	0	0	0	0	0	2	0
Kidney								
- hyaline droplets, proximal tubules								
minimal	0	0	2	0	6	0	9	0
slight	0	0	0	0	0	0	1	0

$ For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric)
^ Actual dose 0, 19, 95, 475 mg base equivalent/kg/day
& No mean values calculated

2.6.7.7.A Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: Three month oral (gavage) repeat dose toxicity study in the rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Histopathology (no. examined)	**9**	**9**	**10**	**9**	**9**	**8**	**10**	**8**
Spleen								
- germinal centers (white pulp)								
minimal	2	1	1	1	1	1	6	1
Stomach								
- hyperkeratosis, focal (limiting ridge)								
minimal	1	0	1	0	4	0	3	0
slight	0	0	0	0	0	0	1	0
Thyroid gland								
- hypertrophy, follicular, diffuse								
minimal	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1

^ Actual dose 0, 19, 95, 475 mg base equivalent/kg/day

2.6.7.7.B Repeat-Dose Toxicity-Darunavir

Report Title: Six month oral (gavage) repeat dose toxicity study in the rat

Test Article: darunavir

Species/strain: Rat / Sprague-Dawley
Age/weight at first dose: 6-8 weeks / 133-212 g
Duration of dosing: 6 months
Duration of post-dose: 0 days
Vehicle/formulation: Polyethylene glycol 400

Administration route/method: Oral gavage
Doses: 0, 20, 100 and 500 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 95% w/w, as ethanolate102.3% w/w) and [redacted] (as base: 94% w/w, as ethanolate 99.6% w/w[#])

Testing facility: [redacted]
Study no.: TMC114-NC132
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: 20 mg/kg/day based on the absence of any relevant hematological and histopathological changes

Toxicokinetics:

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:0**	**F:0**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Died or killed prematurely (accidental)	-	-	0	1	1	0	0	1
$AUC_{0-\infty}$ day 1 (µg.h/mL)	-	-	3.34	6.46	28.1	44. 0	164	140
AUC_{0-24h} week 13 (µg.h/mL)	-	-	2.92	1.79	24.6	31.3	58.0	110
AUC_{0-24h} week 26 (µg.h/mL)	-	-	2.92	4.38	31.6	35.8	63.8	121

Noteworthy Findings:

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Died or killed prematurely (accidental)	1	4	4	2	0	3	1	4
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/wk)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology (week 26) [$]								
- RBC count (10^6/µL)	8.41	7.63	1.01	1.02	0.99	1.00	0.96*	0.96
- Hemoglobin (g/dL)	15.3	14.6	1.00	1.01	0.96**	0.97	0.92***	0.89***
- Hematocrit (%)	46.3	43.1	1.00	1.02	0.96*	0.97	0.90***	0.89***
- Platelets (10^3/µL)	803	832	1.14*	1.04	1.19*	1.19*	1.18*	1.37***
- Reticulocytes (%RBC)	2.6	2.8	1.19**	1.14	1.23**	1.00	1.38***	1.36***
- APTT (sec)	13.2	12.7	0.95	1.07	1.11	1.20***	1.37***	1.44***

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric); [#] HPLC assay with reference to anhydrous solvent free substance
^ Actual dose [redacted]: 0, 19, 95, 475 mg base equivalent/kg/day, [redacted]: 0, 19, 94, 470 mg base equivalent/kg/day;
- No treatment related changes

2.6.7.7.B Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: Six month oral (gavage) repeat dose toxicity study in the rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Serum Chemistry (week 26) $								
- Creatinine (µmol/L)	49	48	1.02	1.04	1.04	1.00	1.16***	1.19***
- Bilirubin (µmol/L)	1	1	2.00*	1.00	3.00***	2.00*	7.00***	4.00***
- Cholesterol (mmol/L)	2.9	3.1	1.00	0.97	1.21*	1.10	1.10*	1.74***
- Triglycerides (mmol/L)	0.6	0.9	1.17	1.11	0.83*	0.89	0.33***	0.44***
Urinalysis&	-	-	-	-	-	-	-	-
Organ weights $								
- Liver (%)	3.84	3.41	0.97	1.00	1.08***	1.14***	1.27***	1.71***
- Spleen (%)	0.18	0.22	1.01	1.03	1.08**	1.03	1.13***	1.02
- Kidney (%)	0.61	0.70	1.03	0.97	1.07***	1.02	1.13***	1.04
Gross pathology (no. examined)	**20**	**20**	**20**	**20**	**20**	**20**	**20**	**20**
- Kidney, dark	0	0	0	0	0	0	7	7
- Liver, dark	0	0	0	1	1	1	5	10
Histopathology (no. examined)	**19**	**16**	**16**	**18**	**20**	**17**	**19**	**16**
Kidney								
- pigment, brown, proximal tubules								
minimal	0	0	0	0	4	0	18	10
slight	0	0	0	0	0	0	0	4
moderate)	0	0	0	0	0	0	0	1
Liver								
- hypertrophy, hepatocellular, diffuse								
minimal	0	0	0	0	3	4	19	7
slight	0	0	0	0	0	0	0	9
- hypertrophy/hyperplasia bile ducts								
minimal	0	0	0	0	0	0	18	13

$ For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric)
^ Actual dose [redacted]: 0, 19, 95, 475 mg base equivalent/kg/day, [redacted]: 0, 19, 94, 470 mg base equivalent/kg/day
& No mean values calculated

2.6.7.7.B Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: Six month oral (gavage) repeat dose toxicity study in the rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**20**		**100**		**500**	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Histopathology (no. examined)	**19**	**16**	**16**	**18**	**20**	**17**	**19**	**16**
Liver (continued)								
- pigment, brown, hepatocellular, diffuse								
minimal	0	0	0	0	1	6	14	3
slight	0	0	0	0	0	0	4	5
moderate	0	0	0	0	0	0	0	7
marked	0	0	0	0	0	0	0	1
Spleen								
- hematopoiesis, erythroid								
minimal	4	3	4	3	8	5	11	8
slight	0	0	0	0	0	0	0	1

^ Actual dose [redacted]: 0, 19, 95, 475 mg base equivalent/kg/day, [redacted]: 0, 19, 94, 470 mg base equivalent/kg/day

2.6.7.7.C Repeat-Dose Toxicity-Darunavir

Report Title: 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat

Test Article: darunavir

Species/strain: Rat / Wistar
Age/weight at first dose: 6-8 weeks / 137-277 g
Duration of dosing: 6 months
Duration of post-dose: 0 days

Administration route/method: Oral gavage
Doses: 0, 0/75, 20/50, 100/50, 500/75 and 1000/75 mg TMC114/mg RTV/kg body weight/day
Test article batch (purity): [redacted] (as base: 93.1% w/w, as ethanolate 100.9% w/w)
Vehicle/formulation: Polyethylene glycol 400 (for TMC114) and propylene glycol (for RTV)

Testing facility: [redacted]
Study no.: TMC114-NC146
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: No NOAEL was established in this study.

Toxicokinetics: TMC114

Dose (mg base equivalent/kg/day)	0 (vehicle)		0/75		20/50		100/50		500/75		1000/75	
No. of animals	**M:2**	**F:2**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Died or killed prematurely (accidental/incidental)	1	1	0	0	0	1	0	0	0	0	1	0
Died or killed prematurely (cause of death not established)	0	0	0	0	0	0	0	0	0	1	0	0
AUC_{0-1} day 1 (µg.h/mL)	-	-	-	-	17.1	7.79*	66.3	60.5	194**	163**	179**	192
AUC_{0-24h} week 13 (µg.h/mL)	-	-	-	-	10.0*	20.5	70.9	67.2	191	294	164	210
AUC_{0-24h} week 26 (µg.h/mL)	-	-	-	-	11.2*	13.4	69.0	70.4	183	318	198	297

* AUC_{0-8h}; ** AUC_{0-24h}

Toxicokinetics: RTV

Dose (mg base equiv/kg/day)	0 (vehicle)		0/75		20/50		100/50		500/75		1000/75	
No. of animals	**M:2**	**F:2**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**	**M:5**	**F:5**
Died or killed prematurely (accidental/incidental)	1	1	0	0	0	1	0	0	0	0	1	0
Died or killed prematurely (cause of death not established)	0	0	0	0	0	0	0	0	0	1	0	0
AUC_{0-1} day 1 (µg.h/mL)	-	-	77.0	73.1	16.1*	19.6*	27.6	31.8	12.5	15.3	12.2	18.3
AUC_{0-24h} week 13 (µg.h/mL)	-	-	51.8	85.5	43.2	28.0	10.6*	13.6*	9.53	31.5	9.20	8.89
AUC_{0-24h} week 26 (µg.h/mL)	-	-	80.9	70.2	52.9	33.9	15.4	35.2	10.1	25.2	10.3	15.3

* AUC_{0-8h}; # HPLC assay with reference to anhydrous solvent free substance

2.6.7.7.C Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat **Test Article:** darunavir

Noteworthy Findings:

Dose (mg base equivalent/kg/day)	0 (vehicle)		0/75		20/50		100/50		500/75		1000/75	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Died or killed prematurely (accidental/incidental)	1	0	0	6	0	2	0	3	1	2	1	1
Died or killed prematurely (cause of death not established)	0	1	1	3	1	0	0	1	2	1	1	0
Clinical observations	-	-	-	-	-	-	-	-	-	-	-	-
Body weight (g)	603	328	0.96	1.03	1.03	1.02	1.02	1.04	0.90*	0.94	0.93	0.97
Body weight gain (%)	163	91	0.96	1.07	1.06	1.09	1.02	1.07	0.85	0.97	0.88	0.93
Food consumption (g/wk)	-	-	-	-	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-	-	-
Hematology (week 26) [$]												
- RBC count (10^12/L)	8.04	7.87	1.03	0.93*	1	0.98	0.99	0.95	0.97	0.91**	0.97	0.97
- Hemoglobin (mmol/L)	10.0	10.4	0.96	0.91**	0.94	0.96	0.95	0.91**	0.90**	0.84**	0.92**	0.88**
- Hematocrit (L/L)	0.40	0.42	0.99	0.92**	0.95	0.96	0.95	0.91**	0.90**	0.88**	0.92**	0.88**
- Reticulocytes (1/1000 RBC)	10	5	1	2.60**	0.90	1.60	1.10	1.80	1.30	2.40**	1.40	1.40
- Platelets (10^9/L)	773	902	1.08	0.90	1.27	0.99	1.41**	1.12	1.51**	1.22	1.63**	1.26
- APTT (sec)	15.0	13.8	0.91	0.99	0.91	1.08	0.99	1.04	0.81**	0.87*	0.94	0.93
- PT (sec)	13.2	13.8	1.03	0.95*	1	0.97	1.01	0.91**	1.01	0.89**	0.98	0.91**
Serum Chemistry (week 26) [$]												
- Creatinine (µmol/L)	43.2	47.3	1.08	1.07	1.02	0.99	1.11**	1	1.17**	1.07	1.18**	1.07
- Bilirubin (µmol/L)	3.4	4.1	0.71	0.76*	0.76	0.71*	0.71	0.71*	2.03	1.20	2.68**	1.24
- Cholesterol (mmol/L)	2.74	2.36	0.84	1.74*	1.05	1.56	0.84	1.78*	0.90	3.07**	1.29*	2.78**
- Phospholipids (mmol/L)	2.39	2.51	0.85	1.63*	1.05	1.40	0.87	1.57	1.11	2.68**	1.49**	2.51**
- Triglycerides (mmol/L)	1.00	0.49	0.66*	1.67	0.94	1.53	0.55**	1.35	0.27**	2.37*	0.47**	1.90
- ALT (U/L)	53.0	42.7	2.93**	1.13	1.96**	0.89	1.25	1.06	1.71	1.34*	1.78*	1.30
- AST (U/L)	89.3	66.9	1.81**	1.03	1.56*	0.85	0.96	0.84	1.08	1.11	1.27	1.22
Urinalysis [$]												
- Volume (mL)	15	9	1.47	1.78**	1.40	1.67	1.60	2.11**	1.80**	2.22**	2.07**	2.44**
- Sodium (mmol/L)	16	21.8	1.96	1.83*	1.28	1.52	2.16*	1.79*	2.68**	1.87*	2.07**	1.47
- Chloride (mmol/L)	19	27	1.95	1.44	1.05	1.37	2.11	1.52	2.26	1.93*	1.89	1.41

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric, Steel + Dunnett)

- No treatment related changes

2.6.7.7.C Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat **Test Article:** darunavir

Noteworthy Findings:

Dose (mg base equivalent/kg/day)	**0 (vehicle)**		**0/75**		**20/50**		**100/50**		**500/75**		**1000/75**	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Organ weights [$]												
- Liver (%)	2.54	2.52	1.36**	1.85**	1.33**	1.64**	1.55**	2.10**	2.20**	3.31**	2..21**	3.17**
- Spleen (%)	0.18	0.22	1.25**	1.39**	1.16*	1.23*	1.25**	1.15	1.34**	1.24*	1.25**	1.20
- Thyroids (%)	0.008	0.012	1.13	1.25	1	1.17	1.13	1.17	1.13*	1.42**	1.13*	1.42**
- Adrenals (%)	0.011	0.027	1.09	1.26**	1	1.04	1.09	1.07	1.64**	1.26**	1.46**	1.15
- Kidney (%)	0.64	0.71	1.13**	1.18**	1.09**	1.10*	1.09**	1.13**	1.19**	1.20**	1.17**	1.18**
Gross pathology (no. examined)	**19**	**19**	**19**	**16**	**19**	**18**	**20**	**16**	**17**	**17**	**18**	**19**
- Kidney, discoloration	0	0	0	0	0	0	1	1	8**	9**	12**	13**
- Liver, discoloration	0	1	0	0	1	1	2	5	8**	13**	5**	14**
Histopathology (no. examined)	**19**	**19**	**19**	**16**	**19**	**18**	**20**	**16**	**17**	**17**	**18**	**19**
Liver												
- multinucleated hepatocytes												
grade 1	0	0	9	2	8	0	13	1	6	3	7	7
grade 2	0	0	1	0	2	0	2	1	5	6	4	3
grade 3	0	0	0	1	1	0	0	0	0	1	2	1
grade 4	0	0	0	0	0	0	0	0	0	0	0	1
- single cell necrosis												
grade 1	0	1	6	1	5	0	4	1	7	6	12	9
grade 2	0	1	0	1	0	0	0	0	1	4	3	3
grade 3	0	0	0	0	0	0	0	0	0	0	0	0
grade 4	0	0	0	1	0	0	0	0	0	0	0	0
- hypertrophy, hepatocellular												
grade 1	0	0	16	10	8	9	15	10	5	2	0	7
grade 2	0	0	1	2	1	0	5	0	12	15	18	12
- vacuolation fine periportal												
grade 1	0	1	3	2	5	3	6	2	5	2	3	3
grade 2	0	0	0	1	0	0	1	0	3	2	1	0

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric, Fisher's exact test)

2.6.7.7.C Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in the rat **Test Article:** darunavir

Noteworthy Findings:

Dose (mg base equivalent/kg/day)	0 (vehicle)		0/75		20/50		100/50		500/75		1000/75	
No. of animals	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**	**M:20**	**F:20**
Histopathology (no. examined)	**19**	**19**	**19**	**16**	**19**	**18**	**20**	**16**	**17**	**17**	**18**	**19**
Kidney												
- nephropathy												
grade 1	7	3	11	6	14	5	11	8	10	9	6	10
grade 2	1	0	6	1	3	0	6	5	4	5	9	4
grade 3	0	0	0	0	0	0	0	0	1	1	0	0
Spleen												
- hematopoiesis												
grade 1	1	10	3	5	5	5	3	10	1	0	2	5
grade 2	15	4	15	6	13	4	15	3	10	10	5	4
grade 3	3	1	1	3	1	0	2	1	6	7	10	10
Pancreas												
- islet fibrosis/siderocytes												
grade 1	2	0	2	0	4	0	4	0	6	0	5	0
grade 2	0	0	1	0	0	0	2	0	1	0	4	0
Adrenal gland												
- cortical vacuolation												
grade 1	7	0	8	1	12	0	9	0	1	8	1	7
grade 2	11	0	9	0	7	0	9	0	8	1	7	4
grade 3	0	0	1	0	0	0	2	0	5	0	10	0
Thyroid gland												
- hypertrophy/hyperplasia												
grade 1	2	1	6	6	8	9	8	7	7	7	8	5
grade 2	0	0	4	6	4	4	7	7	5	6	2	5
grade 3	0	0	2	2	0	1	2	0	3	3	5	8

2.6.7.7.D Repeat-Dose Toxicity-Darunavir

Report Title: Three month oral (gavage) repeat dose toxicity study in the Beagle dog

Test Article: darunavir

Species/strain: Dog / Beagle
Age/weight at first dose: 6-7 months / 7.3 to 9.7 kg
Duration of dosing: 3 months
Duration of post-dose: 0 days

Administration route/method: Oral gavage
Doses: 0, 30, 60 and 120 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 95% w/w, as ethanolate 102.3% w/w)
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC114-NC131
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: 120 mg/kg body weight/day, as the small changes in blood chemistry and liver weight were considered not to be toxicologically significant in the absence of any histopathological changes

Toxicokinetics: AUC_{last} (µg.h/mL)

Nominal dose (mg/kg/day)^	0 (vehicle)		30		60		120	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
AUC_{0-1} day 1 (µg.h/mL)	-	-	12.3	13.7*	48.3	48.6	135	161
AUC_{last} day 29 (µg.h/mL)	-	-	10.1	6.55	25.8	20.7	55.4	53.9
AUC_{last} day 58 (µg.h/mL)	-	-	9.36	6.57	27.0	26.0	93.6	99.3
AUC_{last} day 86 (µg.h/mL)	-	-	10.8	8.43	33.3	24.3	109	96.2

* AUC_{last}

Noteworthy Findings:

Nominal dose (mg/kg/day)^	0 (vehicle)		30		60		120	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Died or killed prematurely (accidental)	0	0	2 (day 1, replaced)	0	0	0	0	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/wk)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-

HPLC assay with reference to anhydrous solvent free substance
^ Actual dose [redacted]: 0, 29, 57, 114 mg base equivalent/kg/day,
- No treatment related changes

2.6.7.7.D Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: Three month oral (gavage) repeat dose toxicity study in the Beagle dog **Test Article:** darunavir

Noteworthy Findings (Continued):

Nominal dose (mg/kg/day)^	**0 (vehicle)**		**30**		**60**		**120**	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Serum Chemistry (week 13) [$]								
- Creatinine (µmol/L)	70	58	1.00	1.10	1.06	1.21	1.16	1.31
- Cholesterol (mmol/L)	3.7	4.3	1.16	0.98	1.38	1.14	1.41	1.35
Urinalysis	-	-	-	-	-	-	-	-
Organ weights [$]								
- Liver (%)	2.97	3.51	1.07	0.93	1.09	1.04	1.20	1.14
Gross pathology (no. examined)	**4**	**4**	**6**	**4**	**4**	**4**	**4**	**4**
- incidental findings only	4	4	4	4	4	4	4	4
Histopathology (no. examined)	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
- incidental findings only	4	4	4	4	4	4	4	4

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown.
[^] Actual dose [redacted]: 0, 29, 57, 114 mg base equivalent/kg/day

2.6.7.7.E Repeat-Dose Toxicity-Darunavir

Report Title: Six month oral (gavage) repeat dose toxicity study in the Beagle dog

Test Article: darunavir

Species/strain: Dog / Beagle
Age/weight at first dose: 5-7 months / 8.2 to 11.5 kg
Duration of dosing: 6 months
Duration of post-dose: 0 days

Administration route/method: Oral gavage
Doses: 0, 30, 60 and 120 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 92.8% w/w, as ethanolate 100.1% w/w)
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC114-NC133
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: 120 mg/kg body weight/day, based on absence of histopathological findings

Toxicokinetics:

Nominal dose (mg/kg/day)^	0 (vehicle)		30		60		120	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
$AUC_{0-\infty}$ day 1 (µg.h/mL)	-	-	22.0	23.2	60.1	54.9	107	133
AUC_{last} week 13 (µg.h/mL)	-	-	20.0	19.9	44.3	51.8	84.8	78.2
AUC_{last} week 26 (µg.h/mL)	-	-	22.4	21. 8	44.0	53.1	70.3	97.3

Noteworthy Findings:

Nominal dose (mg/kg/day)^	0 (vehicle)		30		60		120	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Died or killed prematurely (accidental)	0	0	0	0	0	0	1	1 (day 8, replaced)
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/wk)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry (week 26) $								
- Creatinine (µmol/L)	67	61	1.24	1.25	1.16	1.34	1.43	1.41
Urinalysis	-	-	-	-	-	-	-	-
Organ weights	-	-	-	-	-	-	-	-
Gross pathology (no. examined)	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**5**
- incidental findings only	4	4	4	4	4	4	4	4
Histopathology (no. examined)	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
- incidental findings only	4	4	4	4	4	4	4	4

$ For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown; # HPLC assay with reference to anhydrous solvent free substance
^ Actual dose [redacted]: 0, 28, 56, 111 mg base equivalent/kg/day; - No treatment related changes

2.6.7.7.F Repeat-Dose Toxicity-Darunavir

Report Title: Twelve month oral (gavage) toxicity study in the Beagle dog including toxicokinetic sampling

Test Article: darunavir

Species/strain: Dog / Beagle
Age/weight at first dose: 5-7 months / 6.5 to 10.9 kg
Duration of dosing: 12 months
Duration of post-dose: 0 days

Administration route/method: Oral gavage
Doses: 0, 30, 60 and 120 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 91.6% w/w, as ethanolate 99.1 % w/w[#])
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC114-NC145
Location in CTD: 4.2.3.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: 30 mg/kg body weight/day, based on absence of any relevant histopathological findings and enzyme changes

Toxicokinetics:

Dose (mg base equivalent/kg/day)	0 (vehicle)		30		60		120	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
AUC_{0-1} day 1 (µg.h/mL)	-	-	23	17.9	57.8*	38.2*	69.4	53.4
AUC_{last} week 13 (µg.h/mL)	-	-	22.1	17.3	70.0	63.0	90.7	59.3
AUC_{last} week 26 (µg.h/mL)	-	-	37.9	20.3	72.8	43.2	74.3	79.2
AUC_{last} week 39 (µg.h/mL)	-	-	29.2	16.2	80.2	37.2	111	55.0
AUC_{last} week 52 (µg.h/mL)	-	-	31.6	21.2	71.4	50.3	130	100

* **AUC_{last}**

Noteworthy Findings:

Dose (mg base equiv/kg/day)	0 (vehicle)		30		60		120	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Died or killed prematurely (accidental)	0	1	0	0	1	0	0	0
Clinical observations	-	-	-	-	-	-	-	-
- Vomiting	-	-	+	-	+	+	++	+
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/wk)	-	-	-	-	-	-	-	-
Ophthalmoscopy	-	-	-	-	-	-	-	-
Electrocardiography	-	-	-	-	-	-	-	-
Hematology	-	-	-	-	-	-	-	-
Serum Chemistry (week 53) [$]								
- ALP (U/L)	74	93	1.16	0.71	1.07	1.10	1.41	1.45
Urinalysis	-	-	-	-	-	-	-	-
Organ weights [$]	-	-	-	-	-	-	-	-
- Liver (%)	3.04	2.98	0.93	1.10	1.05	1.16	1.13	1.22
- Spleen (%)	0.93	0.80	0.84	1.15	1.13	1.07	0.85	0.64

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown.; [#] HPLC assay with reference to anhydrous solvent free substance

2.6.7.7.F Repeat-Dose Toxicity-Darunavir (Continued)

Report Title: Twelve month oral (gavage) toxicity study in the Beagle dog including toxicokinetic sampling **Test Article:** darunavir

Noteworthy Findings (continued):

Dose (mg base equivalent/kg/day)	**0 (vehicle)**		**30**		**60**		**120**	
No. of animals	**M:0**	**F:0**	**M:4**	**F:4**	**M:4**	**F:4**	**M:4**	**F:4**
Gross pathology (no. examined)	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
- incidental findings only	4	4	4	4	4	4	4	4
Histopathology (no. examined)	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Liver								
- intracellular pigment deposits								
minimal	0	0	0	1	2	3	4	3
- vacuolation (minimal or slight)								
minimal	0	0	0	0	1	1	3	2
slight	0	0	0	0	0	0	1	0

2.6.7.7.G Repeat-Dose Toxicity-Cobicistat

Test Article: Cobicistat

Report Title: 4-week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 4-Week Recovery		**Gilead Study No. TX-216-2004** ([redacted] 6511-302)
Species/Strain: Rat/Crl:CD(SD)	**Duration of Dosing:** 4 Weeks	**Location in CTD:** 4.2.3.2
Initial Age: 8 Weeks	**Duration of Postdose**: 4 Weeks	**GLP Compliance**: Yes
Date of First Dose: [redacted]	**Method of Administration:** Oral gavage	**Lot Number:** 3168-121-23
Vehicle/Formulation: 15% SEDDS (1.5% ethanol, 1.5% propylene glycol, 6% Labrasol, 6% Solutol HS-15)		
Special Features: Hepatic enzyme analysis		
No Observed Adverse Effect Level: 50 mg/kg/day		

Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**20**		**50**		**100**	
Gender: No. of Animals (TK)	**M:15 (3)**	**F:15 (3)**	**M:10 (9)**	**F:10 (9)**	**M:10 (9)**	**F:10 (9)**	**M:15 (9)**	**F:15 (9)**	**M:15 (9)**	**F:15 (9)**
Toxicokinetics:										
C_{max} – Day 1 (µg/mL)	ND	ND	0.277	0.611	1.38	2.10	4.01	5.03	6.28	5.92
C_{max} - Day 27 (µg/mL)	ND	ND	0.436	0.949	1.32	1.66	3.59	5.11	3.79	4.69
AUC_{0-t} – Day 1 (µg.h/mL)	ND	ND	0.639	2.98	6.98	15.4	31.0	34.7	67.7	51.6
AUC_{0-t} – Day 27 (µg.h/mL)	ND	ND	1.23	3.05	5.76	7.48	21.2	24.8	37.0	35.7
Died or Sacrificed Moribund	None.									
Clinical Observations	Test article related oral/nasal discharge and skin/pelage changes at 100 mg/kg/day.									
Body Weight	No treatment-related findings.									
Food Consumption	No treatment-related findings.									
Ophthalmic Examinations	No treatment-related findings.									
Hematology										
Hemoglobin (g/dL)	16.2	15.8	–	–	–	–	–	15.0*	15.3*	14.7*

Dose (mg/kg/day)	0 (Vehicle)		10		20		50		100	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**	**M:15**	**F:15**
Hematocrit (%)	44.6	43.2	–	–	–	–	–	–	42.5*	–
Absolute Reticulocytes (E3/µL)	198	177	–	–	–	231*	–	232*	260*	269*
Platelet Count (E3/µL)	1132	1231	–	–	–	–	1269*	1458*	1440*	1443*
Clinical Chemistry										
Total Protein (g/dL)	6.6	7.2	–	–	–	–	7.0*	7.5*	7.4*	8.2*
Globulin (g/dL)	2.2	2.1	–	–	–	–	–	2.4*	2.5*	2.7*
Cholesterol (mg/dL)	65	63	–	–	–	–	–	88*	–	92*
Aspartate aminotransferase (U/L)	147	153	–	–	–	–	–	–	120*	124*
Calcium (mg/dL)	10.9	11.0	–	–	–	–	–	–	11.6*	11.8*
Chloride (mmol/L)	100	102	–	–	–	–	–	–	–	98*
Albumin (g/dL)	4.4	5.1	–	–	–	–	–	–	4.9*	5.5*
Urinalysis										
Urine Volume (mL)	19.2	9.0	–	–	–	–	–	28.4*	44.9*	40.1*
Specific Gravity	1.024	1.030	–	–	–	–	–	1.013*	1.008*	1.010*
pH	6.7	6.6	–	–	–	–	–	–	7.0*	7.0*
Organ Weight										
Liver (g)	11.1	7.65	–	–	–	–	13.8*	9.85*	16.6*	13.4*
Thyroid/Parathyroid (g)	0.0262	0.0228	–	–	–	–	–	–	–	0.0294*
Organ to Brain Weight										
Liver (%)	538	385	–	–	–	–	666*	508*	808*	675*
Organ to Body Weight										

Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**20**		**50**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**	**M:15**	**F:15**
Liver (%)	2.83	3.26	–	–	3.09*	–	3.58*	4.22*	4.52*	5.81*
Thyroid/Parathyroid (%)	0.0067	0.0097	–	–	–	–	–	–	–	0.0128*
Microscopic Observations – Final Phase Sacrifice										
Thyroid										
Hyperplasia/Hypertrophy/ Follicular Cell	0	0	0	0	0	0	6	3	10	10
Liver										
Hypertrophy, Hepatocellular	0	0	0	0	0	0	0	0	6	8
Hepatic Enzyme[a]										
CYP3A (% control)	NA	NA	–	–	–	210[b]	213[b]	502[b]	–	314[b]
Recovery Phase Sacrifice (Recovery Day 24)										
Gender: No. of Animals	**M: 5**	**F: 5**	**M: 0**	**F: 0**	**M: 0**	**F: 0**	**M: 5**	**F: 5**	**M: 5**	**F: 5**
Hematology										
Absolute Reticulocyte Count (E3/μL)	178	132	NA	NA	NA	NA	–	103*	–	94.2*
Clinical Chemistry										
Total Protein (g/dL)	6.7	7.2	NA	NA	NA	NA	–	–	–	7.8*
Organ Weight										
Liver (g)	12.5	6.90	NA	NA	NA	NA	–	8.05*	–	8.17*
Thyroid/Parathyroid (g)	0.0263	0.0189	NA	NA	NA	NA	–	0.0269*	–	–
Organ to Brain Weight										
Liver (%)	580	344	NA	NA	NA	NA	–	400*	–	411*

Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**20**		**50**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**	**M:15**	**F:15**
Organ to Body Weight										
Liver (%)	2.53	2.74	NA	NA	NA	NA	2.74*	−	−	3.25*
Microscopic Observations	No treatment-related findings.									

a Percent differences from control values noted after 4 Weeks dosing (final phase sacrifice). There were no notable changes in protein yield, total cytochrome P450 content, or CYP1A, CYP2B, CYP2E, CYP4A, and UDPGT activities. There were no notable findings after a 4 week recovery phase (recovery phase sacrifice).

b Indicates a notable change (≥ 2 fold increase or ≤ 50% compared to the control value).

GS-9350 = cobicistat; ND = none detected; TK = toxicokinetic; NA = not applicable; − = comparable to control group; M = male; F = female;
* = $p \leq 0.05$ (Levene's/ANOVA/Dunnett's/Welch's)

2.6.7.7.H Repeat-Dose Toxicity-Cobicistat

Test Article: Cobicistat

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 13-Week Recovery Period		**Gilead Study No.:** TX-216-2017 ([redacted] 6511-354)
Species/Strain: Rats/Crl:CD(SD)	**Duration of Dosing:** 26-Weeks	**Location in CTD:** 4.2.3.2
Initial Age: 7 Weeks	**Duration of Postdose:** 13-Weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Method of Administration:** Oral gavage	**Lot Number:** 3168-157-8
Vehicle/Formulation: 95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl		
Special Features: Thyroid function, hormone analysis, hepatic microsomal enzyme analysis, immunophenotyping, urine chemistry		
No Observed Adverse Effect Level: 30 mg/kg/day		

Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**30**		**100**	
Gender: No. of Animals (TK)	**M:15 (5)**	**F:15 (5)**	**M:10 (14)**	**F:10 (14)**	**M:10 (14)**	**F:10 (14)**	**M:15 (14)**	**F:15 (14)**
Toxicokinetics[a]:								
C_{max} - Day 1 (µg/mL)	ND	ND	0.132	0.854	1.65	2.83	3.72	4.97
C_{max} - Week 13 (µg/mL)	ND	ND	0.161	0.823	1.37	2.10	3.81	5.68
C_{max} - Week 26 (µg/mL)	ND	ND	0.797	1.32	1.93	3.99	4.40	7.67
AUC_{0-t} - Day 1 (µg.h/mL)	ND	ND	0.382	2.05	5.66	13.9	32.7	42.9
AUC_{0-t} - Week 13 (µg.h/mL)	ND	ND	0.651	2.00	8.23	10.4	29.9	51.6
AUC_{0-t} - Week 26 (µg.h/mL)	ND	ND	2.09	3.52	9.92	13.3	47.2	71.4
Died or Sacrificed Moribund	0	0	0	1	2	0	0	2[b]
Clinical Signs								
Clear Oral Discharge	0	0	0	0	5	1	13	9
Rough Haircoat	4	3	5	3	5	3	14	12
Body Weight – Week 26	No treatment-related findings.							

Test Article: Cobicistat

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 13-Week Recovery Period					**Gilead Study No.: TX-216-2017** ([redacted] 6511-354)			
Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**30**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**
Body Weight Change								
Weeks 1-26	706g	336g	–	–	–	–	-16%	-6.5%
Weeks 26-Recovery 12 (g[d])	20	34	NA	NA	NA	NA	63*	19
Food Consumption (%[c])								
Week 25	220	147	-2.3	1.4	-1.4	10	-12.3*	-5
Ophthalmic Examinations	No treatment-related findings.							
Hematology[d] (Week 26)								
Red Blood Cells (E6/µL)	9.10	7.85	–	–	–	–	8.57*	7.71
Hemoglobin (g/dL)	15.2	14.4	–	–	–	–	13.7*	13.2*
Hematocrit (%)	46.5	42.7	–	–	–	–	42.4*	40.4
MCV (fL)	51.2	54.4	–	–	–	–	49.5*	52.3*
MCH (pg)	16.7	18.3	–	–	–	–	16.0*	17.1*
Platelets (E3/µL)	1240	1217	–	–	1428*	–	1710*	1477*
Clinical Chemistry[d] (Week 26)								
Cholesterol (mg/dL)	107	103	–	–	–	134*	74*	139*
Total Protein (g/dL)	7.4	8.5	–	–	–	8.9*	8.1*	9.3*
Albumin (g/dL)	4.3	5.9	–	–	–	–	4.8*	–
Globulin (g/dL)	3.1	2.6	–	–	–	2.9*	–	3.3*
Calcium (mg/dL)	10.3	11.5	–	–	–	–	11.1*	12.0*

Test Article: Cobicistat

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 13-Week Recovery Period					**Gilead Study No.: TX-216-2017** ([redacted] 6511-354)			
Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**30**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**
GGT (U/L)	0	0	–	–	–	–	4*	2*
Sodium (mmol/L)	149	146	–	–	–	–	147*	145
Thyroid Function[d]								
T4 (ug/dL)								
Week 13	10.2	5.4	9.5	5.6	9.4	5.7	5.6*	5.3
Week 26	6.4	3.6	6.8	3.7	6.7	3.4	4.0*	3.1
TSH (uIU/mL)								
Week 13	0.189	0.113	0.169	0.130	0.279	0.266*	0.652*	0.562*
Week 26	0.342	0.178	0.235	0.314*	0.495	0.330	0.708*	0.971*
Hormone Analysis[d]								
Antidiuretic hormone (pg/mL)								
Week 13	56.2	NA	42.0*	NA	55.3	NA	61.4	NA
Week 26	30.4	45.1	27.1	48.1	51.2	49.8	39.5	77.1
Aldosterone (pg/mL)								
Week 13	149	NA	184	NA	201	NA	281*	NA
Week 26	50.9	133	69.0	164	74.6	122	87.8	127

Test Article: Cobicistat

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 13-Week Recovery Period							**Gilead Study No.:** TX-216-2017 ([redacted] 6511-354)	
Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**30**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**
Urine Chemistry[d] (Week 26)								
Urine Volume (mL)	11.5	6.7	9.8	5.7	17.6*	10.7*	27.6*	23.7*
Urine Osmolality (mOsm/kg)	1468	1372	1627	1581	1086	1115	633*	579*
Sodium Excretion (mmol)	0.23	0.13	0.37*	0.17	0.45*	0.21*	0.80*	0.1*
Urine Phosphorus (mg/dL)	192	202	227	238	134	165	98*	99*
Urine Calcium (mg/dL)	8.1	30.4	7.7	23.6	5.3	19.0*	4.9	13.2*
Urine Potassium (mg/dL)	115	108	135	137*	96	95	62*	52*
Urine Creatinine (mg/dL)	133	90.1	143	104	85.7*	66.3	50.3*	33.6*
Hematology (Recovery Week 13)	Comparable to control group.							
Clinical Chemistry[d] (Recovery Week 13)								
Cholesterol (mg/dL)	107	87	NA	NA	NA	NA	–	155*
Total Protein (g/dL)	6.5	7.5	NA	NA	NA	NA	–	8.3*
Albumin (g/dL)	3.9	5.0	NA	NA	NA	NA	–	5.6*
Urine Chemistry[d] (Recovery Week 13)	Comparable to control group.							
Immunophenotyping – Week 26	No treatment-related findings.							

Test Article: Cobicistat

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 13-Week Recovery Period					**Gilead Study No.: TX-216-2017** ([redacted] 6511-354)			
Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**30**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**
Organ Weights (Terminal Sacrifice)[d]								
Liver (g)	19.0	8.85	18.7	10.2	22.2	12.8*	24.2*	17.0*
Liver-to-body weight ratio (%)	2.86	2.89	2.97	3.21	3.54*	3.93*	4.50*	6.22
Liver-to-brain weight ratio (%)	833	444	841	508	984	639*	1105*	859*
Thyroid with Parathyroid (g)	0.036	0.029	0.039	0.032	0.047	0.031	0.040	0.033
Thyroid/Parathyroid-to-body weight ratio (%)	0.006	0.010	0.006	0.010	0.008*	0.010	0.007*	0.012
Thyroid/Parathyroid-to-brain weight ratio (%)	1.570	1.437	1.772	1.578	2.120	1.535	1.824	1.644
Organ Weights (Recovery Sacrifice)	No treatment-related findings.							
Macroscopic Observations	No treatment-related findings.							
Microscopic Observations								
Number of Animals Examined								
Unscheduled Sacrifice	NA	NA	NA	1	1	NA	NA	2
Terminal Sacrifice	10	10	10	9	9	10	10	8
Recovery Sacrifice	5	5	NA	NA	NA	NA	5	5
Liver								
Hepatocellular Hypertrophy[e]								
Unscheduled Sacrifice	NA	NA	NA	0	0	NA	NA	2
Terminal Sacrifice	0	0	5	7	9	10	10	8
Thyroid								
Follicular Cell Hypertrophy[e]								
Unscheduled Sacrifice	NA	NA	NA	0	0	NA	NA	1

Test Article: Cobicistat

Report Title: 26-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Rats with a 13-Week Recovery Period							**Gilead Study No.: TX-216-2017** ([redacted] 6511-354)	
Dose (mg/kg/day)	**0 (Vehicle)**		**10**		**30**		**100**	
Gender: No. of Animals	**M:15**	**F:15**	**M:10**	**F:10**	**M:10**	**F:10**	**M:15**	**F:15**
Terminal Sacrifice	0	0	0	1	0	1	10	7
Follicular Cell Carcinoma[e]								
Unscheduled Sacrifice	NA	NA	NA	0	0	NA	NA	0
Terminal Sacrifice	0	0	0	0	0	0	1	0
Microscopic Observations (Recovery)	Comparable to control group.							
Hepatic Microsomal Enzyme Activities (% of control)[f]								
CYP1A (% control)	NA	NA	120	173	166	229**	347**	238**
CYP2B/2C	NA	NA	85.5	97.4	71.2	170	36.7**	200**
CYP3A	NA	NA	97.7	130	154	479**	203**	787**

a Toxicokinetic data for metabolite GS-9612 is summarized in the report.

b Clinical signs observed prior to death in two 100 mg/kg/day females were isolated occurrences, though a relationship to treatment cannot be discounted.

c For controls, group means are shown in grams. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

d Group means are shown.

e Liver and thyroid changes considered secondary to hepatic microsomal enzyme induction and thyroid hormone changes.

f Mean percentages of control values after 26 Weeks dosing. There were no remarkable changes in protein yield, total cytochrome 450 content, or CYP2B, CYP2E, CYP4A, and UDPGT activities.

ND = none detected; TK = toxicokinetic; M = male; F = female; — = comparable to control group; NA = not applicable; MCH = Mean Corpuscular Hemoglobin; MCV = mean corpuscular volume; * = P≤0.05; ** = Indicates a notable change (≥2-fold increase, or ≥50% decrease) compared to the control values

2.6.7.7.I Repeat-Dose Toxicity-Cobicistat

Test Article: COBI + ATV

Report Title: 90-Day Oral Gavage Bridging Study with GS-9350 and Atazanavir in Rats with a 1-Month Recovery Period		**Gilead Study No.:** TX-216-2024 ([redacted] 6511-396)
Species/Strain: Rats/ Crl:CD(SD)	**Duration of Dosing:** 13 Weeks (92 days)	**Location in CTD:** 4.2.3.2
Initial Age: 7 Weeks	**Duration of Postdose:** 29 Days	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Method of Administration:** Oral gavage	**Lot Number:** 3168-157-8 (COBI); 3168-179-10 (ATV)
Vehicle/Formulation: 95% propylene glycol (PG) and 5% ethanol (EtOH)		
Special Features: Hepatic microsomal enzyme analysis		
No Observed Adverse Effect Level: 30/50 mg/kg/day COBI/ATV, alone or in combination		

Dose (mg/kg/day)	**0 (Vehicle)**		**30 (COBI High)**		**20 (ATV Low)**		**50 (ATV High)**		**30/20 (COBI/ ATV Low)**		**30/50 (COBI/ ATV High)**	
Gender: No. of Animals (TK)	**M:15 (3)**	**F:15 (3)**	**M:15 (6)**	**F:15 (6)**	**M:10 (6)**	**F:10 (6)**	**M:15 (6)**	**F:15 (6)**	**M:10 (6)**	**F:10 (6)**	**M:15 (6)**	**F:15 (6)**
Toxicokinetics: COBI												
C_{max} - Day 1 (µg/mL)	ND	ND	1.15	1.95	ND	ND	ND	ND	0.806	0.795	0.478	0.373
C_{max} - Day 90 (µg/mL)	ND	ND	2.99	2.59	ND	ND	ND	ND	0.862	2.11	1.23	1.66
AUC_{0-t} – Day 1 (µg.h/mL)	ND	ND	6.92	12.9	ND	ND	ND	ND	4.16	6.41	2.58	3.75
AUC_{0-t} – Day 90 (µg.h/mL)	ND	ND	10.8	12.8	ND	ND	ND	ND	4.58	11.2	6.10	6.34
Toxicokinetics: ATV												
C_{max} - Day 1 (µg/mL)	ND	ND	ND	ND	1.13	0.990	2.46	3.17	4.06	2.53	5.79	3.58
C_{max} - Day 90 (µg/mL)	ND	ND	ND	ND	1.31	2.40	3.33	5.23	2.99	3.93	6.75	7.79
$AUC_{(0-t)}$ - Day 1 (µg.h/mL)	ND	ND	ND	ND	2.92	4.97	12.0	17.6	32.9	27.0	50.4	48.0
$AUC_{(0-t)}$ - Day 90 (µg.h/mL)	ND	ND	ND	ND	7.94	11.7	15.4	31.6	30.8	30.6	44.3	45.9
Died or Sacrificed Moribund	1	0	0	0	0	0	0	0	0	0	0	0
Clinical Signs												
Discharge, Clear Oral	0	0	2	0	0	0	2	1	3	0	8	6

Test Article: COBI+ ATV

Report Title: 90-Day Oral Gavage Bridging Study with GS-9350 and Atazanavir in Rats with a 1-Month Recovery Period			**Gilead Study No.:** TX-216-2024 [redacted] 6511-396)									
Dose (mg/kg/day)	**0 (Vehicle)**		**30 (COBI High)**		**20 (ATV Low)**		**50 (ATV High)**		**30/20 (COBI/ ATV Low)**		**30/50 (COBI/ ATV High)**	
Gender: No. of Animals	**M:15**	**F:15**	**M:15**	**F:15**	**M:10**	**F:10**	**M:15**	**F:15**	**M:10**	**F:10**	**M:15**	**F:15**
Body Weight	No treatment-related findings.											
Food Consumption	No treatment-related findings.											
Ophthalmic Examinations	No treatment-related findings.											
Clinical Chemistry[a]												
Cholesterol (mg/dL)	74	80	–	–	94*	97	83	100*	88	112*	80	133*
Urinalysis[a]												
Urine volume (mL)	9.5	7.7	13.1	7.6	7.8	6.5	11.7	8.0	21.0	14.5	19.1	16.0
Organ Weights[a]												
Liver (g)	17.2	10.6	18.7	12.9	17.8	9.08	17.3	10.2	20.3*	12.2	18.1	12.5
Liver-to-body weight ratio (%)	3.13	3.70	3.52	4.30	3.20	3.13	3.25	3.39	3.75*	4.37	3.52	4.28
Liver-to-brain weight ratio (%)	757	525	843	648	796	448	790	506	903*	615	805	615
Macroscopic Observations	No treatment-related findings.											
Microscopic Observations	No treatment-related findings.											
Hepatic Microsomal Enzyme Activities (% control)[b]												
CYP3A	NA	NA	214**	564**	–	–	–	–	–	346**	163	259**
Number of Recovery Animals	5	5	5	5	0	0	5	5	0	0	5	5
Postdose Evaluation	No noteworthy findings.											

a Group Means are shown.

b Percent differences from control value noted after 13 Weeks dosing. There were no remarkable changes in protein yield, total cytochrome P450 content, or CYP1A, CYP2B, CYP2C, CYP2E, CYP4A, and UDPGT activities.

ATV = atazanavir; TK = toxicokinetic; M = male; F = female; ND = none detected; NA = not applicable; – = comparable to control group; *= P≤0.05; **= Indicates a notable change (≥ 2-fold increase) compared to the control value

2.6.7.7.J Repeat-Dose Toxicity-Cobicistat

Test Article: Cobicistat

Report Title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 4-Week Recovery Phase		**Gilead Study No. TX-216-2005** ([redacted] 6511-309)
Species/Strain: Dog/Beagle	**Duration of Dosing:** 4 Weeks	**Location in CTD:** 4.2.3.2
Initial Age: 6 Months	**Duration of Postdose**: 4 Weeks	**GLP Compliance**: Yes
Date of First Dose: [redacted]	**Method of Administration:** Oral gavage	**Lot Number:** 3168-121-23
Vehicle/Formulation: 95% propylene glycol, 5% ethanol, pH adjusted to 2.3 % 0.1 with HCl		
Special Features: Hepatic enzyme analysis		
No Observed Adverse Effect Level: 15 mg/kg/day		

Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**15**		**45/30[a]**	
Gender: No. of Animals	**M:5**	**F:5**	**M:3**	**F:3**	**M:5**	**F:5**	**M:5**	**F:5**
Toxicokinetics:								
C_{max} – Day 1 (µg/mL)	ND	ND	0.195	0.119	3.00	2.57	7.16	6.44
C_{max} - Day 15 (µg/mL)[b]	ND	ND	NA	NA	NA	NA	NA	6.93
C_{max} - Week 4 (µg/mL)[b]	ND	ND	1.14	0.739	3.52	4.46	7.05	6.04
AUC_{0-t} – Day 1 (µg.h/mL)	ND	ND	0.457	0.251	17.2	15.7	57.2	61.6
AUC_{0-t} – Day 15 (µg.h/mL)[b]	ND	ND	NA	NA	NA	NA	NA	53.8
AUC_{0-t} – Week 4 (µg.h/mL)[b]	ND	ND	4.39	2.18	22.9	34.7	89.0	53.6
Died or Sacrificed Moribund	1[c]	0	0	0	0	0[c]	0	0
Body Weight (%)[d]	8.4	8.3	5	&6	0	&7	&11	&7
Clinical Observations	Dose-related signs included salivation and emesis at ≥ 15 mg/kg/day in both sexes, with increased incidence in high dose group males and females.							

Test Article: Cobicistat

Report Title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 4-Week Recovery Phase					**Gilead Study No. TX-216-2005** ([redacted] 6511-309)			
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**15**		**45/30[a]**	
Gender: No. of Animals	**M:5**	**F:5**	**M:3**	**F:3**	**M:5**	**F:5**	**M:5**	**F:5**
Food Consumption	No treatment-related findings.							
Ophthalmic Examinations	No treatment-related findings.							
Electrocardiogram Examinations – Day 3								
PR Interval (msec)	81	78	85	90	91	102*	91	106*
Heart Rate (beats/minute)	131	134	147	127	157*	142	130	138
RR Interval (msec)	463	452	411	478	385	427	469	435
Electrocardiogram Examinations – Day 22								
PR Interval (msec)	77	79	86	99	85	92	100	95
Heart Rate (beats/minute)	129	128	130	114	135	143	95	131
RR Interval (msec)	471	492	470	527	455	424	659*	465
Blood Pressure Measurement	No treatment-related findings.							
Clinical Pathology - Day 25								
Clinical Chemistry								
Total bilirubin (mg/dL)	0.1	–	–	–	–	–	0.2	–
Alanine aminotransferase (U/L)	31	–	–	–	–	–	70*	–
Alkaline phosphatase (U/L)	95	–	–	–	–	–	148	–
Urinalysis								
Specific Gravity	1.046	1.029	1.037	1.011	1.026*	1.023	1.026*	1.021

Test Article: Cobicistat

Report Title: 4-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 4-Week Recovery Phase					**Gilead Study No. TX-216-2005** ([redacted] 6511-309)			
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**15**		**45/30[a]**	
Gender: No. of Animals	**M:5**	**F:5**	**M:3**	**F:3**	**M:5**	**F:5**	**M:5**	**F:5**
Anatomic Pathology - Final Phase Sacrifice								
Macroscopic Observations	No treatment-related findings.							
Organ Weight								
Absolute Liver/Gall bladder (g)	233	220	248	241	285	210	276	289
Liver-to-body (%)	2.79	2.62	2.80	3.21	3.24	2.73	3.44	3.70*
Liver-to-brain (%)	314	304	345	316	394	307	399	417*
Microscopic Observations								
Liver - Vacuolation, Hepatocyte								
Minimal	2	3	1	0	0	2	0	1
Slight	1	0	2	3	3	1	1	0
Moderate	1	0	0	0	0	0	2	2
Hepatic Enzymes[e]								
CYP3A (% control)	NA	NA	44.1[f]	60.7	44.0[f]	44.3[f]	20.0[f]	30.8[f]
Number of Recovery Animals	1	2	0	0	2	2	2	2
Postdose Evaluation	No noteworthy findings.							

a Group 4 females were dosed at 45 mg/kg/day on Days 1 through 10, dosing was suspended on Days 11 through 13, and dosing was resumed at 30 mg/kg/day beginning on Day 14.
b All animals were bled predose Day 1 and Week 4 of the dosing phase. In addition, Group 4 (45/30 mg/kg/day) females were bled Day 15 of the dosing phase. The Day 15 samples were collected to assess toxicokinetics after dosing resumed on Day 14 at the lower dose level of 30 mg/kg/day. Week 4 samples were collected after 8 days dosing at 30 mg/kg/day.
c Mortality due to dosing error, (N=1 for recovery group). Female animal replaced on Day 1.
d On Day 29 of the dosing phase. For controls, group means are shown in kilograms. For treated groups, percent of controls are shown.
e Values noted after 4 Weeks of dosing. There were no notable changes in protein yield, total cytochrome P450 content, or CYP1A, CYP2B, CYP2E, CYP4A, and UDPGT activities; and no notable findings after a 4 week recovery phase.
f Indicates a notable change (≥ 2 fold increase or ≤ 50% compared to the control value).
ND = none detected; NA = not Applicable; M = male; F = female; — = comparable to control group; * = $p \leq 0.05$

2.6.7.7.K Repeat-Dose Toxicity-Cobicistat

Test Article: Cobicistat

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period		**Gilead Study No.:** TX-216-2016 ([redacted] 6511-355)
Species/Strain: Dog/Beagle	**Duration of Dosing:** 13 or 39 Weeks	**Location in CTD:** 4.2.3.2
Initial Age: 7 Months	**Duration of Postdose:** 13 Weeks	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Method of Administration:** Oral gavage	**Lot Number:** 3168-157-8
Vehicle/Formulation: 95% propylene glycol (PG) and 5% ethanol (EtOH)		
Special Features: Thyroid function, immunophenotyping, urine chemistry		
No Observed Adverse Effect Level: 10 mg/kg/day		

Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**10**		**20**	
Gender: No. of Animals[a]	**M: 9 (6)**	**F: 9 (6)**	**M: 7 (4)**	**F: 7 (4)**	**M: 7 (4)**	**F: 7 (4)**	**M: 9 (6)**	**F: 9 (6)**
Toxicokinetics[b]								
C_{max} - Day 1 (µg/mL)	ND	ND	0.112	0.043	1.12	0.311	3.65	3.70
C_{max} - Week 13 (µg/mL)	ND	ND	1.26	0.724	3.40	2.47	4.46	6.45
C_{max} - Week 39 (µg/mL)	ND	ND	1.03	1.74	2.64	2.42	7.09	8.41
AUC_{0-t} - Day 1 (µg.h/mL)	ND	ND	0.248	0.116	5.25	1.14	26.4	30.8
AUC_{0-t} - Week 13 (µg.h/mL)	ND	ND	5.89	2.98	22.2	15.9	39.7	75.9
AUC_{0-t} - Week 39 (µg.h/mL)	ND	ND	5.27	9.12	19.6	16.9	71.3	99.7
Died or Sacrificed Moribund	None.							
Clinical Signs	Dose-related clinical signs included salivation, emesis, and fecal changes in both sexes at 20 mg/kg/day.							
Ophthalmic Examinations	No treatment-related findings.							

Test Article: Cobicistat

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period							**Gilead Study No.:** TX-216-2016 ([redacted] 6511-355)	
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**10**		**20**	
Gender: No. of Animals[a]	**M: 9 (6)**	**F: 9 (6)**	**M: 7 (4)**	**F: 7 (4)**	**M: 7 (4)**	**F: 7 (4)**	**M: 9 (6)**	**F: 9 (6)**
Body Weight (kg)[c]								
Week 10	10.3	8.5	-1.9	-2.4	-4.9	-7.1	-14.6*	-16.5*
Week 14	11.0	8.9	-3.6	-1.1	-8.2	-6.7	-13.6*	-13.5*
Week 40	11.2	9.0	0	-1.1	-11.6	-7.8	-9.8	-7.8
Recovery Week 14	Comparable to control group.							
Food Consumption (g)[c]								
Week 9	1625	1490	-4.6	-7.9	-3.3	-4.2	-32.4*	-26.6*
Week 13	1784	1615	-0.5	-0.7	-10.1	-1.5	-20.2*	-19.3*
Week 39	1622	1517	-21.8*	-24.7	-25.0*	-14.0	-24.0*	-43.0*
Recovery Week 13	1287	1671	NA	NA	NA	NA	-19.3	-22.5
Electrocardiogram Examinations	No treatment-related findings.							
Blood Pressure Measurements	No treatment-related findings.							
Hematology[d]								
Platelet (E3/µL)								
Week 13	437	435	–	–	–	–	–	608*
Week 39	480	521	–	–	–	–	–	747*
Recovery Week 13	Comparable to control group.							

Test Article: Cobicistat

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period							**Gilead Study No.: TX-216-2016** ([redacted] 6511-355)	
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**10**		**20**	
Gender: No. of Animals[a]	**M: 9 (6)**	**F: 9 (6)**	**M: 7 (4)**	**F: 7 (4)**	**M: 7 (4)**	**F: 7 (4)**	**M: 9 (6)**	**F: 9 (6)**
Coagulation[d]								
APTT (seconds)								
Week 13	16.1	17.5	–	–	–	15.7*	14.0*	16.2
Week 39	13.1	13.0	–	–	–	11.9	11.1*	11.6*
Recovery Week 13	Comparable to control group.							
Clinical Chemistry[d]								
Alkaline Phosphatase (U/L)								
Week 13	59	51	–	–	–	–	138*	107*
Week 39	48	37	–	–	–	–	81*	66*
Recovery Week 13	Comparable to control group.							
Total Protein (g/dL)								
Week 13	6.1	6.1	–	–	–	–	5.5*	5.6*
Week 39	6.4	6.2	–	–	–	–	5.6*	5.7
Recovery Week 13	Comparable to control group.							
Albumin (g/dL)								
Week 13	3.4	3.5	–	–	–	–	3.1*	–
Week 39	3.4	3.3	–	–	–	–	3.1	–
Recovery Week 13	Comparable to control group.							

Test Article: Cobicistat

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period							**Gilead Study No.: TX-216-2016** ([redacted] 6511-355)	
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**10**		**20**	
Gender: No. of Animals[a]	**M: 9 (6)**	**F: 9 (6)**	**M: 7 (4)**	**F: 7 (4)**	**M: 7 (4)**	**F: 7 (4)**	**M: 9 (6)**	**F: 9 (6)**
Globulin (g/dL)								
Week 13	–	2.6	–	2.5	–	2.3	–	2.2*
Week 39	–	2.9	–	2.6	–	2.8	–	2.5
Recovery Week 13	–	2.8	NA	NA	NA	NA	–	2.4
Thyroid Function	No treatment-related findings.							
Immunophenotyping	No treatment-related findings.							
Urine Chemistry[d, e]								
Urine Specific Gravity								
Week 13	–	1.044	–	1.054	–	1.050	–	1.030
Week 39	–	1.036	–	1.040	–	1.037	–	1.035
Recovery Week 13	Comparable to control group.							
Urine Volume (mL)								
Week 13	–	82.6	–	64.1	–	64.4	–	129
Week 39	–	110	–	107	–	102	–	107
Recovery Week 13	Comparable to control group.							
Urine Osmolality (mOsm/kg)								
Week 13	–	1792	–	2232	–	2054	–	1135
Week 39	–	1410	–	1620	–	1491	–	1360
Recovery Week 13	Comparable to control group.							

Test Article: Cobicistat

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period							**Gilead Study No.: TX-216-2016** ([redacted] 6511-355)	
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**10**		**20**	
Gender: No. of Animals[a]	**M: 9 (6)**	**F: 9 (6)**	**M: 7 (4)**	**F: 7 (4)**	**M: 7 (4)**	**F: 7 (4)**	**M: 9 (6)**	**F: 9 (6)**
Organ Weights[d]								
Interim Sacrifice – No. of Animals	3	3	3	3	3	3	3	3
Thymus (g)	15.5	–	9.84	–	11.3	–	6.97*	–
Thymus-to-body weight ratio (%)	0.15	–	0.10	–	0.12	–	0.08	–
Thymus-to-brain weight ratio (%)	19.0	–	12.2	–	13.3	–	9.11*	–
Terminal Sacrifice – No. of Animals	4	4	4	4	4	4	4	4
Thymus (g)	No treatment-related findings.							
Liver (g)	286	230	282	228	279	243	277	260
Liver-to-body weight ratio (%)	2.61	2.59	2.53	2.54	2.81	2.93	2.95	3.20*
Liver-to-brain weight ratio (%)	345	323	355	320	371	339	379	371
Macroscopic Observations	No treatment-related findings.							
Microscopic Observations								
Interim Sacrifice – No. of Animals	3	3	3	3	3	3	3	3
Thymic Involution	0	–	0	–	0	–	3	–
Adrenal Cortex Hypertrophy	0	0	1	0	0	0	3	1
Terminal Sacrifice – No. of Animals	4	4	4	4	4	4	4	4
Thymic Involution	No treatment-related findings.							
Adrenal Cortex Hypertrophy	No treatment-related findings.							
Liver, Diffuse Hepatocellular Hypertrophy	0	0	0	0	3	0	3	4

Test Article: Cobicistat

Report Title: 39-Week Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in Dogs with a 13-Week Interim Necropsy and a 13-Week Recovery Period							**Gilead Study No.: TX-216-2016** ([redacted] 6511-355)	
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**10**		**20**	
Gender: No. of Animals[a]	**M: 9 (6)**	**F: 9 (6)**	**M: 7 (4)**	**F: 7 (4)**	**M: 7 (4)**	**F: 7 (4)**	**M: 9 (6)**	**F: 9 (6)**
Recovery Sacrifice[f] – No. of Animals	2	2	NA	NA	NA	NA	2	2

a Numbers in parentheses refer to numbers per sex/group dosed for 39-Weeks (including n = 2 for recovery). Three/sex/group were euthanized after 13-Weeks administration.

b Toxicokinetic data for metabolite GS-9612 is summarized in the report.

c Group means are shown for control males and females; percent change from control are shown for remaining groups.

d Group means are shown.

e In high dose females, slightly higher urine volume, ↓ osmolality and lower specific gravity (primarily Weeks 13 and 26) was associated with slight to moderate decreases in urinary sodium, potassium, chloride, calcium and inorganic phosphorus urine concentrations and excretion rates (data summarized in the report).

f No noteworthy findings.

ND = none detected; M = male; F = female; NA = not applicable; — = comparable to control group; * = P≤0.05

2.6.7.8.A Genotoxicity: In Vitro-Darunavir

Report Title: Evaluation of the mutagenic activity of TMC114 in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat)

Test Article: Darunavir

Test: Bacterial reverse mutation test (Ames test)
Species/strain: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537; *Escherichia coli* WP_2uvrA
Metabolizing system: Aroclor induced rat liver S9 mix (assay # 1: 5% v/v, assay # 2: 10% v/v)
No. of independent assays: 2
No. of replicate cultures: 3

Treatment: Plate incorporation for 48 hours
Doses: 33, 100, 333, 1000, 3330 µg/plate (DRF doses)
Test article batch (purity): [redacted] (as base: 94.2% w/w, as ethanolate: 99.8% w/w)
Vehicle test article: DMSO
Vehicle positive controls: Saline, DMSO

Testing facility: [redacted]
Study no.: 293063
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes
Date start of study: [redacted]

Cytotoxic effects: None
Genotoxic effects: TMC114 ([redacted]) was not mutagenic in the *S. typhimurium* and *E. coli* reverse mutation assay under the experimental conditions described.

Noteworthy Findings

Metabolic Activation	Control, Test and Reference Articles	Dose Level (µg/plate)[a]	Assay # 1 Revertant Colony Counts (Mean ! SD)				
			TA1535	TA1537	TA98	TA100	WP_2uvrA
Without S9	DMSO	0.1 (mL)	14 ± 8	8 ± 3	20 ± 2	69 ± 18 (DRF)	10 ± 1 (DRF)
	TMC114	1000	16 ± 2	6 ± 1	19 ± 1	66 ± 9 (DRF)	12 ± 1 (DRF)
		3330[b]	19 ± 8	5 ± 1	22 ± 5	68 ± 3 (DRF)	11 ± 3 (DRF)
	Sodium azide	1	132 ± 14	-	-	-	-
	9-Aminoacridine	60	-	348 ± 89	-	-	-
	Daunomycine	4	-	-	692 ± 102	-	-
	Methylmethanesulfonate	650	-	-	-	880 ± 16 (DRF)	-
	4-Nitroquinoline N-oxide	10	-	-	-	-	74 ± 12 (DRF)
With S9 (5% v/v)	DMSO	0.1 (mL)	11 ± 1	6 ± 1	31 ± 5	83 ± 11 (DRF)	10 ± 3 (DRF)
	TMC114	1000	13 ± 6	6 ± 2	26 ± 5	83 ± 1 (DRF)	12 ± 4 (DRF)
		3330[b]	17 ± 5	5 ± 3	19 ± 4	78 ± 11 (DRF)	12 ± 6 (DRF)
	2-Aminoanthracene	1	216 ± 16	-	535 ± 64	1212 ± 77 (DRF)	-
		2.5		524 ± 132		-	-
		5	-	-	-	-	77 ± 7 (DRF)

[a] TMC114 as ethanolate; actual dose [redacted]: 0, 31, 94, 314, 942, 3137 µg base equivalent/plate; [b] Slight precipitate; - = Not applicable

2.6.7.8.A Genotoxicity: In Vitro-Darunavir (Continued)

Report Title: Evaluation of the mutagenic activity of TMC114 in the *Salmonella typhimurium* reverse mutation assay and the *Escherichia coli* reverse mutation assay (with independent repeat) **Test Article: D**arunavir

Metabolic Activation	Control, Test and Reference Articles	Dose Level (µg/plate)[a]	Assay # 2 Revertant Colony Counts (Mean ! SD)				
			TA1535	TA1537	TA98	TA100	WP_2uvrA
Without S9	DMSO	0.1 (mL)	17 ± 1	7 ± 2	26 ± 6	98 ± 3	15 ± 3
	TMC114	1000	20 ± 11	9 ± 2	24 ± 3	112 ± 10	10 ± 3
		3330[b]	19 ± 5	9 ± 5	26 ± 2	114 ± 18	14 ± 3
	Sodium Azide	1	164 ± 23	-	-	-	-
	9-Aminoacridine	60	-	239 ± 52	-	-	-
	Daunomycine	4	-	-	982 ± 31	-	-
	Methylmethanesulfonate	650	-	-	-	774 ± 63	-
	4-Nitroquinoline N-Oxide	10	-	-	-	-	502 ± 43
With S9 (10% v/v)	DMSO	0.1 (mL)	17 ± 1	7 ± 2	30 ± 5	98 ± 23	13 ± 3
	TMC114	1000	16 ± 7	5 ± 2	38 ± 2	121 ± 20	12 ± 1
		3330[b]	14 ± 7	5 ± 3	34 ± 4	105 ± 9	13 ± 5
	2-Aminoanthracene	1	233 ± 7	-	435 ± 39	634 ± 34	-
		2.5		189 ± 34		-	-
		10	-	-	-	-	252 ± 13

[a] TMC114 as ethanolate; actual dose [redacted]: 0, 31, 94, 314, 942, 3137 µg base equivalent/plate; [b] Slight precipitate; - = Not applicable

2.6.7.8.B Genotoxicity: In Vitro-Darunavir

Report Title: Evaluation of the ability of TMC114 to induce chromosome aberrations in cultured peripheral human lymphocytes

Test Article: Darunavir

Test: In vitro mammalian chromosome aberration test
Species/strain: Human peripheral lymphocytes
Metabolizing System: Aroclor induced rat liver S9 mix (1.8% v/v)
No of independent assays: 2
No of replicate cultures: 2
No of cells analyzed/culture: ≥ 100

Treatment: Assay # 1: 3 hours treatment (+/- S9), 24 hours fixation time.
Assay # 2: 24 hours treatment (- S9), 24 hours fixation; 48 hours treatment (- S9), 48 hours fixation; 3 hours treatment (+ S9), 48 hours fixation.
Doses: 33, 100, and 333 µg/mL
Test article batch (purity): [redacted] (as base: 94.2% w/w, as ethanolate: 99.8% w/w)
Vehicle test article: DMSO
Vehicle positive controls: Hank's balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}

Testing facility: [redacted]
Study No.: 294288
Location in CTD: 4.2.3.3.1
GLP Compliance: Yes
Date start of study: [redacted]

Cytotoxic effects: None
Genotoxic effects: TMC114 ([redacted]) was not clastogenic in human lymphocytes in vitro under the experimental conditions described.

Noteworthy Findings:

Assay # 1: 3 hours treatment, 24 hours fixation

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Mitotic Index (% of control)	Aberrant Cells (Mean %) (- gaps)	Abs/Cell (- gaps)	Total Polyploid Cells
Without S9	DMSO	0.9% (v/v)	100	0	0	0
	TMC114	33	112	0	0	0
		100	115	2.5	0.025	0
		333[b]	119	0.5	0.005	0
	Mytomycin C	0.5	106	9.5***	0.105	0
With S9	DMSO	0.9% (v/v)	100	0.5	0.005	0
	TMC114	33	93	1	0.01	0
		100	99	1	0.01	0
		333[b]	88	3	0.035	0
	Cyclophosphamide	15	36	23.5***	0.265	0

[a] TMC114 as ethanolate, actual dose [redacted]: 31, 94, 314 µg base equivalent/mL; [b] Precipitation in culture medium; *** $p \leq 0.001$ (chi-square test); Abs = aberrations

2.6.7.8.B Genotoxicity: In Vitro-Darunavir (Continued)

Report Title: Evaluation of the ability of TMC114 to induce chromosome aberrations in cultured peripheral human lymphocytes **Test Article:** Darunavir

Assay # 2: 24 hours treatment, 24 hours fixation

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Mitotic index (% of control)	Aberrant Cells (Mean %) (- gaps)	Abs/Cell (- gaps)	Total polyploid cells
Without S9	DMSO	0.9% (v/v)	100	0	0	0
	TMC114	33	85	0.5	0.005	0
		130	50	0	0	0
		180	47	0	0	0
	Mytomycin C	0.2	74	18***	0.195	0

[a] TMC114 as ethanolate, actual dose [redacted]: 31, 94, 314 µg base equivalent/mL; *** $p \leq 0.001$ (chi-square test); Abs = aberrations

Assay # 2: 48 hours treatment, 48 hours fixation

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Mitotic index (% of control)	Aberrant Cells (Mean %) (- gaps)	Abs/Cell (- gaps)	Total polyploid cells
Without S9	DMSO	0.9% (v/v)	100	1.5	0.015	0
	TMC114	33	83	1.5	0.06	0
		130	71	1.5	0.015	0
		240	48	0.5	0.005	0
	Mytomycin C	0.1	67	26***	0.3	0

[a] TMC114 as ethanolate, actual dose [redacted]: 31, 94, 314 µg base equivalent/mL; *** $p \leq 0.001$ (chi-square test); Abs = aberrations

Assay # 2: 3 hours treatment, 48 hours fixation

Metabolic Activation	Control, Test and Reference Articles	Concentration (µg/mL)[a]	Mitotic index (% of control)	Aberrant Cells (Mean %) (- gaps)	Abs/Cell (- gaps)	Total polyploid cells
With S9	DMSO	0.9% (v/v)	100	1	0	0
	TMC114	33	97	0	0	0
		100	101	1	0.01	0
		333	79	0	0	0
	Cyclophosphamide	15	68[c]	21.5***	0.245	0

[a] TMC114 as ethanolate, actual dose [redacted]: 31, 94, 314 µg base equivalent/mL; [c] Cyclophosphamide was fixated after 24 hours; *** $p \leq 0.001$ (chi-square test); Abs = aberrations

2.6.7.8.C Genotoxicity: In Vitro-Cobicistat

Test Article: Cobicistat

Report Title: GS-9350 Salmonella-Escherichia coli/Mammalian-Microsome Reverse Mutation Assay		**Gilead Study No. TX-216-2010** ([redacted] 961679)
Test for Induction of: Reverse mutation in bacterial cells	**No. of Independent Assays:** 2	**Location in CTD:** 4.2.3.3.1
Strains: *Salmonella typhimurium* and *E. coli*	**No. of Replicate Cultures:** 3	**GLP Compliance:** Yes
Date of Treatment: [redacted]	**Vehicles for Test Article:** DMSO	**Lot Number:** 3168-121-23
Metabolizing System: Phenobarbital/5,6-Benzoflavone-induced rat liver S9		**Treatment:** Plate Incorporation
Vehicles for Positive Controls: Water for NaAz and 9AC, DMSO for all other positive controls		
Cytotoxic Effects: Yes		
Genotoxic Effects: None		

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation Test Revertants/Plate (Mean ! SD)				
			TA1535	TA1537	TA98	TA100	WP2 *uvrA*
Without Activation	DMSO	—	25 ! 8	12 ! 2	30 ! 2	128 ! 6	35 ! 5
	COBI	50	14 ! 4	12 ! 1	32 ! 7	141 ! 3	38 ! 2
		158	20 ! 5	10 ! 2	26 ! 3	142 ! 24	43 ! 5
		500	21 ! 6	11 ! 4	32 ! 6	139 ! 15	38 ! 4
		1581	22 ! 1	8 ! 6	20 ! 6	114 ! 30	34 ! 4
		5000	19[a] ! 1	8[a] ! 3	27[a] ! 4	106[a] ! 9	33[a] ! 10
	NaAz	0.5	349 ! 6			612 ! 32	
	9AC	50		159 ! 17			
	2NF	1			176 ! 10		
	NQO	0.5					144 ! 23

Metabolic Activation	Treatment	Dose Level (µg/plate)	Plate Incorporation Assay Revertants/Plate (Mean ! SD)											
			TA1535		TA1537		TA98		TA100		WP2 *uvrA*			
With Activation	DMSO	—	16	! 1	15	! 5	35	! 2	149	! 15	42	! 11		
	COBI	15.8		!	18	! 2								
		50	22	! 6	13	! 4	34	! 6	164	! 13	50	! 3		
		158	25	! 9	17	! 3	37	! 3	145	! 21	45	! 7		
		500	20	! 4	11	! 5	44	! 6	154	! 12	46	! 8		
		1581	25	! 7	10	! 2	41	! 8	92	! 11	44	! 2		
		5000	20[a]	! 5	8[a, b]	! 2	28[a]	! 4	83[a]	! 6	44[a]	! 22		
	2AA	5	272	! 11										
	2AA	15									299	! 12		
	BaP	5			97	! 7	369	! 23	1112	! 53				
Metabolic Activation	**Treatment**	**Dose Level (µg/plate)**	**Pre-incubation Assay Revertants/Plate (Mean ! SD)**											
Without Activation	DMSO	—	17	! 5	13	! 2	27	! 4	134	! 9	40	! 7		
	COBI	50	17	! 3	12	! 3	24	! 3	133	! 8	39	! 7		
		158	16	! 3	12	! 4	29	! 4	128	! 8	39	! 3		
		500	17	! 5	10	! 2	26	! 4	128	! 19	48	! 10		
		1581	14	! 6	10	! 2	20	! 2	124	! 6	47	! 4		
		5000	17	! 1	10	! 2	33	! 13	127	! 10	39	! 8		

Metabolic Activation	Treatment	Dose Level (µg/plate)	Pre-incubation Assay Revertants/Plate (Mean ± SD)				
			TA1535	TA1537	TA98	TA100	WP2 *uvrA*
Without Activation	NaAz	0.5	342 ± 50			633 ± 14	
	9AC	10		1197 ± 237			
	2NF	1			131 ± 21		
	NQO	0.5					1425 ± 70
With Activation	DMSO	—	26 ± 3	16 ± 2	39 ± 9	167 ± 7	49 ± 7
	COBI	15.8	–	16 ± 2	–	–	–
		50	26 ± 5	16 ± 2	42 ± 9	133 ± 8	57 ± 7
		158	20 ± 3	17 ± 4	39 ± 3	150 ± 7	52 ± 9
		500	27 ± 2	16 ± 3	45 ± 3	148 ± 11	56 ± 10
		1581	21 ± 1	9 ± 1	40 ± 1	105 ± 9	56 ± 7
		5000	25[a] ± 3	7[a, b] ± 1	35[a] ± 5	98[c] ± 8	52[c] ± 6
	2AA	5	166 ± 11				
	2AA	15					469 ± 32
	BaP	5		105 ± 19	360 ± 19	979 ± 45	

a Precipitate obscured assessment of background lawn.

b Toxic as indicated by low revertant colony counts, or incomplete/no background lawn.

c Precipitate.

DMSO = dimethyl sulfoxide; NaAz = sodium azide; 9AC = 9 Aminoacridine; 2NF = 2 nitrofluorene; NQO = 4 nitroquinoline N-oxide; 2AA = 2 aminoanthracene; BaP = benzo[a]pyrene; – = not tested;

2.6.7.8.D Genotoxicity: In Vitro-Cobicistat

Test Article: Cobicistat

Report Title: GS-9350 L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay		**Gilead Study No. TX-216-2011** [redacted] 961680)
Test for Induction of: Forward gene mutation at TK locus cells		**Location in CTD**: 4.2.3.3.1
Metabolizing System: Phenobarbital / 5,6-Benzoflavone-induced rat liver S9		**GLP Compliance**: Yes
No. of Replicate Cultures: 4 (vehicle); 2 (test article)	**No. of Cells Analyzed/Cultured**: Up to 1.2x10^{7}	**Lot Number:** 3168-121-23
Strains: L5178Y/TK$^{+/-}$	**Vehicles for Test Article**: DMSO	**No. of Independent Assays:** 2
Treatment: Without Activation, 3-hr treatment	**Vehicles for Positive Controls:** DMSO	**Date of Treatment**: [redacted]
Cytotoxic Effects: Dose-related decreases in relative total growth		
Genotoxic Effects: None		

Without Activation 3-hr Treatment	**Concentration (µg/mL)**	**Relative Survival (%)**	**Viable Plating Efficiency (%)**	**Relative Total Growth (%)**	**Mutant Frequency (10^{-6})**
DMSO	1% (v/v)	100	69	100	62
COBI	13.4	77	72	101	65
	16.8	85	67	103	86
	21.0	71	60	93	68
	26.2	83	73	95	62
	32.8	65	77	91	69
	41.0	74	55	66	66
	51.2	45	72	53	78
	64.0	21	44	13	108
	80.0	2	29	1	T
4-Nitroquinoline N-oxide	0.25	72	58	69	431[a]
Without Activation 24-hr Treatment	**Concentration (µg/mL)**	**Relative Survival (%)**	**Viable Plating Efficiency (%)**	**Relative Total Growth (%)**	**Mutant Frequency (10^{-6})**
DMSO	1% (v/v)	100	74	100	64
COBI	6.87	57	76	63	50
	8.59	47	83	60	44
	10.7	40	86	67	44
	13.4	47	82	63	48

Test Article: Cobicistat

Report Title: GS-9350 L5178Y TK$^{+/-}$ Mouse Lymphoma Forward Mutation Assay				**Gilead Study No. TX-216-2011** ([redacted] 961680)	
Without Activation 24-hr Treatment	**Concentration (µg/mL)**	**Relative Survival (%)**	**Viable Plating Efficiency (%)**	**Relative Total Growth (%)**	**Mutant Frequency (10^{-6})**
	16.8	37	76	46	73
	21.0	29	84	38	59
	26.2	16	58	16	56
	32.8	6	65	7	T
	41.0	1	60	1	T
4-Nitroquinoline N-oxide	0.10	45	79	50	487[a]
With Activation 3-hr Treatment	**Concentration (µg/mL)**	**Relative Survival (%)**	**Viable Plating Efficiency (%)**	**Relative Total Growth (%)**	**Mutant Frequency (10^{-6})**
DMSO	1% (v/v)	100	72	100	86
COBI	13.4	92	78	95	92
	16.8	106	65	79	110
	21.0	102	62	81	71
	26.2	96	67	85	63
	32.8	93	66	97	70
	41.0	80	71	101	59
	51.2	83	75	95	76
	64.0	43	61	46	72
	80.0	0.43	23	0.25	86a
Benzo[a]pyrene	1.0	38	46	34	1028[b]

a Positive response (> 125 mutants per 106 viable cells over vehicle control).

b Results shown to demonstrate that even for this excessively toxic dose level (RTG or RS < 10%), no substantial increase in the mutation frequency over the concurrent vehicle control was obtained. Note that the minimal reasonable dose interval of 1.25 was used for this treatment regimen.

T = no reportable results due to excessive toxicity (RTG or RS < 10%)

2.6.7.9.A Genotoxicity: In Vivo-Darunavir

Report Title: Micronucleus test in bone marrow cells of the mouse with TMC114 **Test Article:** darunavir

Test: Mammalian erythrocyte micronucleus test
Species/strain: Mouse / NMRI BR
Age/weight at first dose: 6-8 weeks / 31-33.6 g
Cells evaluated: Bone marrow polychromatic and normochromatic erythrocytes
No of cells analyzed/animal: 2000 polychromatic erythrocytes for micronuclei screening; 1000 normochromatic erythrocytes for bone marrow toxicity

Treatment: Single dose with sampling after 24 and 48 hours
Administration route/method: Oral intubation
Doses: 2000 mg/kg body weight
Test article batch (purity): [redacted] (as base: 93.3% w/w, as ethanolate: 99.2.0% w/w))
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC114-NC114
Location in CTD: 4.2.3.3.2
GLP Compliance: Yes
Date start of study: [redacted]

Toxic/Cytotoxic effects: None
Genotoxic effects: TMC114 (batch no. [redacted]) was not mutagenic in the micronucleus test under the experimental conditions described.
Evidence of exposure: No systemic exposure data are available for this study. At a dose of 1000 mg/kg, the $AUC_{0\text{-}24h}$ for the first day of treatment in a 3-month study in CD-1 mice (TMC114-NC157) was 59.8 µg.h/mL for males.

Noteworthy Findings

Control, Test and Reference Articles	Dose (mg/kg body weight)	Number of Animals and Gender	Sampling Time (Hours)	# MNPCEs per 2000 PCEs (Mean ± SD)	Ratio PCEs / NCEs (Mean ± SD)
Polyethylene glycol 400	-		24	2.4 ± 1.5	1.06 ± 0.03
TMC114[a]	2000	5M	24	2.0 ± 1.4	1.07 ± 0.02
	2000	5M	48	1.4 ± 2.2	1.02 ± 0.05
Cyclophosphamide	50	5M	48	25.4 ± 9.5**	0.37 ± 0.11

[a] TMC114 as ethanolate, actual dose [redacted] 1866 mg base equivalent/kg; ** significant difference from historical data of corresponding control group; $p \leq 0.01$ (Wilcoxon rank sum test)

M = male; MNPCEs = micronucleated polychromatic erythrocytes; PCEs = polychromatic erythrocytes; NCEs = normochromatic erythrocytes; # = number; SD = standard deviation

2.6.7.9.B Genotoxicity: In Vivo-Cobicistat

Test Article: Cobicistat

Report Title: GS-9350 Rat Micronucleus Test		**Gilead Study No.** TX-216-2012 ([redacted] 961681)
Test for Induction of: Micronuclei in Bone Marrow Immature Erythrocytes	**Treatment Schedule:** Single dose	**Location in CTD:** 4.2.3.3.2
Species/Strain: Rat, Sprague Dawley® Hsd:SD®	**Sampling Time:** 24 and 48 hours	**GLP Compliance:** Yes
Age: Approximately 8 Weeks	**Method of Administration:** Oral gavage	**Lot Number:** 3168-121-23
Vehicle/Formulation: 95% propylene glycol (PG)/5% ethanol (EtOH) (v/v), with addition of 0.005 moles HCl for every 100 mL of PG/EtOH		**No. of Cells Evaluated/Animal:** 2000
		Date of Treatment: [redacted]
Special Features: Toxicokinetic samples taken from satellite animals (n=3/sex/dose level) 1 hour post-dose		
Toxic/Cytotoxic Effects: Clinical signs of toxicity were observed in mid- and high-dose COBI treated animals; findings included decreased activity, partly closed eyes, soft feces, staining of fur, piloerection and shallow breathing.		
Genotoxic Effects: None		
Evidence of Exposure: COBI plasma levels at 1 hour postdose were in the range of 4370 to 8290 ng/mL (for males), and 4170 to 9050 ng/mL (for females)		

Test Article	Dose (mg/kg) M/F	No. of Animals	Sampling time (hours)	Mean % IE/(IE+ME)□[a, b]	Incidence mie Males	Incidence mie Females	Incidence mie Males & Females
Vehicle control	NA	5M+5F	24	44	2.0	1.8	1.9
COBI	212.5/125	5M+5F	24	41	1.8	0.8	1.3
COBI	425/250	5M+5F	24	41	1.6	1.4	1.5
COBI	850/500	5M+5F	24	38	1.4	1.4	1.4
Cyclophosphamide	20	3M+3F	24	37*	39.0**[c]	18.0**[c]	28.5
Vehicle control	NA	5M+5F	48	40	1.8	1.0	1.4
COBI	850/500	4M[d]+5F	48	41	1.3	1.2	1.2

a Significant trend across dose levels (Groups 1 to 4: 24 hour time point) : P ! 0.001 (one-sided probabilities test).
b Significant trend across dose orders (Groups 1 to 4: 24 hour time point) : P ! 0.001 (one-sided probabilities test).
c Analysis done using the following transformation: SQRT(2000*V + 0.5), where V is the proportion of mie.
d One male was found dead prior to scheduled sacrifice on Day 3 at 850 mg/kg.
NA = not applicable; M = male; F = female; mie = number of micronucleated cells observed per 2000 immature erythrocytes examined; IE = immature erythrocytes; ME = mature erythrocytes; %IE (IE+ME) = proportion of immature erythrocytes; * = P ! 0.01; ** = P ! 0.001 (one-sided probabilities test)

2.6.7.10.A Carcinogenicity-Darunavir

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse
Test Article: darunavir
Species/strain: Mouse / CD1
Administration route/method: Orally / gavage
Testing facility: J&J PRD
Age/weight at first dose: Approximately 6 weeks / 21 to 35 g
Doses: 0, 0, 150, 450, 1000 mg/kg/day
Study no.: TMC114-NC159
Test article batch: RWJPRI Lot No [redacted] and JJPRD Lot No [redacted]
Location in CTD: 4.2.3.4.1
Duration of dosing: 24 months
GLP compliance: Yes
Vehicle/formulation: Polyethylene glycol 400(PEG400)
Date start of study: [redacted]

No Observed Adverse Effect Level: < 150 mg/kg/day.

Toxicokinetics:

Dose (mg/kg/day)	**0 (PEG400)**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:9**	**F:9**	**M:9**	**F:9**	**M:15**	**F:15**	**M:15**	**F:15**	**M:15**	**F:15**
Died or killed prematurely										
AUC_{0-24h} Day 32 (µg.h/mL)	-	-	-	-	4.33	7.47	22.0	41.8 (1)	34.4	56.9
AUC_{0-24h} Day 188 (µg.h/mL)	-	-	-	-	6.23	7.79	14.8	40.8	48.1	63.8

Noteworthy Findings:

Dose (mg/kg/day)	**0 (PEG400)**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Died or killed prematurely	38	37	40	38	41	34	48	37	50 *	41
Mortality (treatment related)	0	0	0	0	0	0	0	0	15	0
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Body Weight[a]										
- Week 16	37.7	30.0	0.992	1.02	0.997	1.04 **	0.966 **	1.02	0.992	1.06 ***
- Week 52	40.4	33.0	0.970	1.01	0.998	1.02	0.936 **	0.994	0.955 *	1.04 *
- Week 93	40.4	34.8	0.983	0.991	0.983	1.02	0.928 *	0.980	0.891	0.986
- Week 96	40.2	35.1	0.988	0.974	0.985	1.02	0.933 *	0.980	0.933	0.991
- Week 104	-	34.1	39.5 (2)	0.997	39.0(2)	1.04	38.2(2)	1.02	-	1.02
Body Weight Gain[a]										
- Week 16	7.1	5.6	1.01	1.20 **	1.03	1.29 ***	0.915	1.23 ***	1.09	1.39 ***
- Week 52	9.9	8.6	0.919	1.13	1.00	1.12	0.818 *	1.06	0.889	1.17 **
- Week 93	9.7	10.3	0.969	1.06	0.990	1.11	0.814	1.02	0.649	0.99
- Week 96	9.5	10.7	0.989	0.981	1.00	1.10	0.832	1.00	0.842	1.00
- Week 104	-	9.8	8.9 (2)	1.03	9.1 (2)	1.15	8.6 (2)	1.16	-	1.07

-: no noteworthy findings; F: female; M: male; * p<0.05, ** p<0.01, *** p<0.001;
[a]: For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of Vehicle 1. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1; (1): AUC_{0-8h}; (2): Absolute value due to early termination vehicle 1 and high dose.

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400))		0 (PEG400)		150		450		1000	
No. of animals	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Food Consumption[a]										
- Week 52	36	35	0.972	0.943 *	1.03	0.971 *	0.972	0.914 **	1.06	1.00
- Week 96	37	35	0.973	0.943	1.00	1.00	1.05	0.971	1.05	0.971
- Week 104	-	35	35(2)	0.971	36(2)	1.00	38(2)	1.03	-	1.00
Ophthalmoscopy	-	-	-	-	-	-	-	-	-	-
Hematology[a,b]										
- Red blood cells (10^6/µL)	8.70	8.06	8.88	0.994	0.975	0.983	1.05	1.01	1.05	0.932 *
- Hemoglobin (g/dL)	13.3	12.7	13.7	1.00	0.942 +	1.00	1.02	0.992	0.962	0.906 ***
- Hematocrit (%)	41.3	39.5	42.6	1.00	0.960	0.995	1.00	0.992	0.959	0.911 ***
- Mean cell volume (fl)	48.1	49.2	48.1	1.01	0.988	1.01	0.956 ++	0.988	0.904 ***	0.978
- Mean cell haemoglobin (pg)	15.5	15.8	15.4	1.01	0.974 +	1.02	0.968 +	0.987	0.903 ***	0.968 *
- Reticulocytes (%)	4.4	3.4	2.9	0.941	1.24	0.882	0.931	1.06 *	0.727	1.35 ***
- Reticulocytes (10^3/µL)	325	260	249	0.945	1.16	0.907	1.02	1.10 **	0.891	1.29 ***
- Thrombocytes (10^3/µL)	1848	1297	1792	0.923	1.12 +	0.992	1.07	1.04	0.984	1.23 **
Clinical Chemistry[a,b]										
- Inorg. phosphorus (mg/dL)	6.8	7.2	6.4	0.986	1.05	1.01	1.27 +++	0.986	1.25 ***	1.06
- Cholesterol (mg/dL)	136	86	109	0.895	1.44 +++	1.13	1.39 ++	1.40 ***	2.04 ***	2.86 ***
- Urea nitrogen (mg/dL)	17.9	30.1	22.5	0.704 **	1.12 +	0.867	1.62 +	0.801	2.14 ***	1.13
- Total bilirubin (mg/dL)	0.17	0.13	0.15	1.00	1.00	1.15 **	1.00	1.62 ***	0.706 *	1.85 ***
- Aspartate aminotransferase (U/L)	149	169	160	0.805	1.53	0.793 *	1.05	0.911	1.40 **	2.86 *
- Alanine aminotransferase (U/L)	76	72	139	0.653	1.54	0.847	1.48 ++	1.25 *	2.20 ***	5.04 ***
- Gamma glutamyl transferase (U/L)	0	0	1	1 (2)	1.00	1 (2)	1.00	1 (2)	1 (2)	2 (2) **

-: no noteworthy findings; F: female; M: male

[a]: For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of Vehicle 1. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1.

[b]: For males only: are shown the group means for Vehicle 1 and 2, the multiples of Vehicle 2 for 150 and 450 mg/kg, the multiples of Vehicle 1 for 1000 mg/kg (due to termination males in week 97). Statistical significance versus Vehicles is based on actual data (not on the multiples of vehicle/baseline).

(2): Absolute value.

* p< 0.05, ** p<0.01, *** p< 0.001: significances versus Vehicle group 1 +p< 0.05, ++ p<0.01, +++ p< 0.001: significances versus Vehicle group 2

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400))**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Organ Weights[a,b]										
Final Body Weight	39.1	33.9	0.998	1.00	0.998	1.03	0.969	1.02	0.940	1.02
Liver	1.98	1.68	0.980	0.940	1.21 **	1.02	1.23 **	1.19 **	1.39 **	1.73 **
Kidneys	0.699	0.499	1.07	0.974	0.983	0.996	0.915	0.981	0.908	1.07
Mean % Body Weight										
Kidneys (%body)	1.79	1.48	1.07	0.966	0.983	0.959	0.939	0.953	0.966	1.05 *
Liver (%body)	5.08	4.93	0.978	0.943	1.22 **	1.00	1.29 **	1.17 **	1.49 **	1.69 **
Mean % Brain Weight										
Kidneys (%brain)	138	97.2	1.08	0.975	0.972	0.996	0.922	0.985	0.933	1.08 *
Liver (%brain)	390	329	0.989	0.941	1.21 **	1.02	1.25 **	1.19 **	1.43 **	1.74 **
Gross Pathology										
Animals Examined	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**
Heart										
- Enlargement	0	-	0	-	0	-	0	-	2	-
- Focus/area discolored	0	-	1	-	0	-	3	-	5	-
Kidneys										
- Discoloration: dark	0	0	2	0	1	0	4	4	6	5
Liver										
- Discoloration	0	1	0	0	0	0	0	0	4	0
- Discoloration: dark	1	1	0	1	1	3	12	10	39	38
- Focus/area discolored	2	3	2	0	5	2	8	5	18	10
- Mass	6	1	1	2	7	4	12	7	28	12
- Nodule	2	0	0	2	1	0	5	0	4	4
- Swollen	1	5	1	2	0	2	5	5	12	17

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400))**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Histopathology										
Epididymides (No. Examined)	**65**		**65**		**65**		**65**		**65**	
- Mineralization vascular	-		-		-		-		8	
grade 1									6	
grade 2									2	
Eyes (No. Examined)	55	54	52	57	53	58	43	52	47	53
- Scleral mineralization	-	-	-	-	-	-	1	-	17	8
grade 1							1		12	7
grade 2							0		5	1

-: no noteworthy findings; F: female; M: male

[a]: For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of Vehicle 1. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1.

[b]: For Vehicle 1 and 1000 mg/kg: data of week 97 presented since high dose group and vehicle group in males terminated in that week

*p< 0.05 ** p<0.01

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400))**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Histopathology (continued)										
Heart (No. Examined)	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**
- Atrial thrombosis	1	-	2	-	1	1	6	-	14	5
grade 1	0		0		0	1	1		2	1
grade 2	0		0		0	0	1		5	2
grade 3	1		1		0	0	3		4	2
grade 4	0		0		1	0	1		3	0
grade 5	0		1		0	0	0		0	0
- Mineralisation myocardium	1	-	1	1	-	-	4	2	9	4
grade 1	1		0	0			3	0	6	3
grade 2	0		1	1			0	1	1	1
grade 3	0		0	0			0	1	2	0
grade 4	0		0	0			1	0	0	0
- Mineralization vascular	5	1	-	-	2	1	1	1	12	6
grade 1	3	1			2	1	1	0	11	4
grade 2	2	0			0	0	0	1	1	2
Kidneys (No. Examined)	**65**	**64**	**63**	**62**	**65**	**64**	**62**	**62**	**62**	**62**
- Mineralization vascular	-	-	-	-	-	-	-	-	11	1
grade 1									11	1
- Nephrosis	1	-	3	-	4	-	9	5	11	6
grade 1	0		0		0		0	2	1	0
grade 2	1		1		1		1	2	3	4
grade 3	0		1		3		6	0	4	2
grade 4	0		1		0		2	1	3	0
- Tubular pigment	4	-	2	1	3	1	13	6	19	13
grade 1	1		0	0	0	0	1	0	2	4
grade 2	3		2	0	0	1	2	2	12	2
grade 3	0		0	1	3	0	8	4	2	4
grade 4	0		0	0	0	0	2	0	3	3

-: no noteworthy findings; F: female; M: male

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400))		0 (PEG400)		150		450		1000	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Histopathology (continued)										
Liver (No. Examined)	**65**	**65**	**64**	**63**	**65**	**64**	**62**	**63**	**62**	**61**
- Adenoma hepatocellular	4	-	1	-	8	1	16	8	38	16
- Carcinoma hepatocellular	1	-	-	-	3	1	5	1	7	3
- Hepatocholangiocellular carcinoma	-	-	-	-	-	-	-	-	1	-
- Hepatocholangiocellular adenoma	-	-	-	-	-	-	-	-	1	-
- Chronic inflammation	12	22	11	31	15	24	21	31	40	36
grade 1	7	17	9	21	3	14	7	16	8	11
grade 2	5	5	2	9	12	8	12	14	29	23
grade 3	0	0	0	1	0	2	2	1	3	2
- Hepatocyte hypertrophy	9	1	13	2	30	10	40	38	53	56
grade 1	3	1	1	2	8	8	4	2	3	0
grade 2	5	0	8	0	14	2	17	16	13	14
grade 3	1	0	3	0	8	0	17	18	30	26
grade 4	0	0	1	0	0	0	2	2	7	16
- Pigmented macrophage	15	12	11	15	32	18	50	48	61	58
grade 1	9	9	5	12	17	11	9	19	1	5
grade 2	5	2	4	3	11	7	30	26	10	35
grade 3	1	1	2	0	4	0	11	3	31	17
grade 4	0	0	0	0	0	0	0	0	19	1
- Pigmented hepatocyte	-	-	-	-	6	1	19	8	60	43
grade 1					5	1	10	6	19	26
grade 2					1	0	8	2	27	14
grade 3					0	0	1	0	13	3
grade 4					0	0	0	0	1	0

-: no noteworthy findings; F: female; M: male

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400)**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Histopathology (continued)										
Liver (No. Examined, continued)	**65**	**65**	**64**	**63**	**65**	**64**	**62**	**63**	**62**	**61**
- Focus clear cell	1	-	1	-	4	-	7	4	11	7
grade 1	0		1		2		2	2	7	4
grade 2	1		0		1		4	2	4	2
grade 3	0		0		1		1	0	0	1
- Focus eosinophilic cell	-	-	-	1	1	-	6	3	17	5
grade 1				0	1		5	1	9	4
grade 2				0	0		1	2	6	1
grade 3				1	0		0	0	2	0
- Focus basophilic cell	1	-	-	-	1	-	8	1	12	5
grade 1	1				0		6	1	7	4
grade 2	0				0		0	0	4	1
grade 3	0				1		2	0	1	0
- Oval cell hyperplasia	-	-	1	-	-	-	5	-	3	-
grade 1			1				1		2	
grade 2			0				3		1	
grade 3			0				1		0	
Lymph mesenteric (No. Examined)	**62**	**62**	**61**	**63**	**65**	**62**	**57**	**65**	**62**	**60**
- Hemopoiesis	2	-	-	-	1	1	1	1	1	7
grade 1	1				1	0	0	0	0	0
grade 2	1				0	1	1	1	1	5
grade 3	0				0	0	0	0	0	2

-: no noteworthy findings; F: female; M: male

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400)		0 (PEG400)		150		450		1000	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Histopathology (continued)										
Pancreas (No. Examined)	**64**	**65**	**63**	**63**	**64**	**64**	**60**	**64**	**63**	**64**
- Mineralization vascular	15	5	17	10	11	8	20	16	33	29
grade 1	6	4	8	5	5	4	6	7	8	7
grade 2	7	1	9	5	6	3	10	9	18	16
grade 3	2	0	0	0	0	1	4	0	7	6
Seminal vesicles (No. Examined)	**65**		**65**		**65**		**65**		**65**	
- Mineralization vascular	1		-		-		-		8	
grade 1	1								5	
grade 2	0								3	
Spleen (No. Examined)	**65**	**65**	**65**	**65**	**64**	**65**	**64**	**63**	**61**	**64**
- Hemopoiesis	8	20	3	16	9	21	14	21	16	33
grade 1	1	6	1	1	1	3	1	4	1	4
grade 2	5	4	1	10	4	8	4	9	8	16
grade 3	1	6	1	3	3	7	5	6	4	9
grade 4	1	4	0	2	1	3	4	2	3	4
Testes (No. Examined)	**65**		**65**		**65**		**65**		**65**	
- Pigment	1		-		-		1		33	
grade 1	1						1		24	
grade 2	0						0		9	
- Mineralization capsule	2		1		1		3		11	
grade 1	2		0		1		3		6	
grade 2	0		1		0		0		5	
- Mineralization vascular	2		-		3		2		23	
grade 1	1				2		0		16	
grade 2	1				1		2		7	
Tongue (No. Examined)	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**
- Vascular mineralization	1	-	-	-	-	-	3	2	11	8
grade 1	1						1	1	6	5
grade 2	0						2	1	5	3

-: no noteworthy findings; F: female; M: male

2.6.7.10.A Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Mouse **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400)**		**0 (PEG400)**		**150**		**450**		**1000**	
No. of animals	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**	**M:65**	**F:65**
Histopathology (continued)										
Nose (No. Examined)	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**	**65**
- Mineralization vibrissae	1	-	1	-	-	-	6	-	6	3
grade 1	0		1				1		1	1
grade 2	0		0				3		1	1
grade 3	1		0				2		3	1
grade 4	0		0				0		1	0

-: no noteworthy findings; F: female; M: male

2.6.7.10.B Carcinogenicity-Darunavir

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat

Test Article: darunavir

Species/strain: Rat / SPF Sprague-Dawley
Age/weight at first dose: Approximately 6 weeks/ 92 to 156 g
Duration of dosing: 24 months

Administration route/method: Orally / gavage
Doses: 0, 50, 150 or 500 mg/kg/day
Test article batch: RWJPRI Lot No [redacted] and JJPRD Lot No [redacted]
Vehicle/formulation: Polyethylene glycol 400 (PEG400)

Testing facility: J&J PRD
Study no.: TMC114-NC158
Location in CTD: 4.2.3.4.1
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: 50 mg/kg/day.

Toxicokinetics:

Dose (mg/kg/day)	0 (PEG400)		0 (PEG400)		50		150		500	
No. of animals	**M:3**	**F:3**	**M:3**	**F:3**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Died or killed prematurely	0	0	0	0	1	0	0	0	0	0
AUC_{0-24h} Day 31 (µg.h/mL)	-	-	-	-	15.9	16.8	50.1	39.4	133	102
AUC_{0-24h} Day 184 (µg.h/mL)	-	-	-	-	17.1	24.9	50.4	51.7	90.3	89.5

Noteworthy Findings:

Dose (mg/kg/day)	0 (PEG400)		0 (PEG400)		50		150		500	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Died or killed prematurely	44	49	47	45	39	49	38	42	42	43
Mortality (treatment related)	0	0	0	0	0	0	0	3	1	7
Clinical Observations										
- Pale mucosae/skin	4/70	1/70	3/70	2/70	7/70	2/70	14/70 *	2/70	16/70 **	2/70
- Feces, soft	68/70	52/70	58/70 **	58/70	49/70 ***	55/70	33/70 ***	42/70	28/70 ***	33/70 **
- Excessive Salivation	43/70	20/70	48/70	25/70	70/70 ***	70/70 ***	70/70 ***	70/70 ***	70/70 ***	69/70 ***
- Blood loss from the oral cavity	0/70	0/70	0/70	0/70	1/70	0/70	3/70	0/70	3/70	2/70
- Blood on tray	16/70	7/70	17/70	10/70	28/70 *	7/70	31/70 *	6/70	38/70 ***	12/70
- Excessive salivation, discolored red	2/70	0/70	2/70	1/70	33/70 ***	7/70 *	34/70 ***	21/70 ***	37/70 ***	16/70 ***
- Bloody fur	19/70	6/70	14/70	10/70	35/70 **	6/70	38/70 **	16/70 *	44/70 ***	20/70 **

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400))		0 (PEG400)		50		150		500	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Body Weight[a]										
- Week 104 (g)	596	386	1.03	1.15 **	0.983	1.16 *	0.978	1.03	0.896 ***	0.909 *
Body Weight Gain[a]										
- Week 104 (g)	469	269	1.03	1.23 **	0.983	1.23 *	0.981	1.05	0.870 ***	0.885 *
Food Consumption[a]										
- Total (g)	18213	14272	1.02	1.02	0.961 *	1.01	0.967	0.987	0.966 *	0.986
Ophthalmoscopy	-[c]	-[c]	-[c]	-[c]	[b]	[b]	[b]	[b]	-[c]	-[c]

[a]: At end of dosing period. For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of vehicle/baseline are shown. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1.
[b]: Ophtalmoscopy was not performed in animals given 50 or 150 mg/kg, [c]: only in the first 20 surviving animals.
-: no noteworthy findings; F: female; M: male; * $p<0.05$, ** $p<0.01$, *** $p<0.001$,

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400))		0 (PEG400)		50		150		500	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Hematology and coagulation[a]										
Week 53										
- Prothrombin Time (sec)	15.3	12.5	0.974	1.02	0.997	1.01	1.03	1.03	1.07 **	1.01
- Fibrinogen (mg/dL)	201	150	0.968	1.01	0.998	1.04	1.05 *	1.05	1.01	1.10
- Hemoglobin (g/dL)	16.3	14.8	0.982	1.00	0.957 **	1.01	0.933 ***	0.973	0.902 ***	0.905 ***
- Hematocrit (%)	47.8	42.6	0.981	1.01	0.954 **	1.00	0.946 **	0.974	0.908 ***	0.913 ***
- Mean cell volume (fL)	51.9	55.0	0.979 *	1.00	0.961 **	0.984	0.954 **	0.969 *	0.923 ***	0.924 ***
-Mean cell haemoglobin (pg)	17.7	19.1	0.983	1.00	0.966 *	0.990	0.944 ***	0.969 **	0.921 ***	0.916 ***
Week 105-106										
- Prothrombin Time (sec)	16.1	13.8	0.944 *	0.998	0.999	0.991	1.01	0.999	1.06 *	1.01
- Fibrinogen (mg/dL)	208	171	1.03	1.08	1.04	0.957	1.03	1.05	1.03	1.16 **
- Hemoglobin (g/dL)	15.6	13.9	1.00	1.00	0.994	1.01	0.962 *	1.01	0.885 ***	0.914 **
- White blood cells (10^3/µL)	9.1	8.1	1.04	0.901	1.13	0.926	1.10	0.914	1.31 ***	0.864
- Hematocrit (%)	45.0	39.8	0.991	1.02	0.996	1.02	0.971	1.01	0.896 ***	0.935 **
- Mean cell volume (fl)	53.4	58.2	1.00	1.02	0.994	0.993	0.951 ***	0.978 *	0.933 ***	0.916 ***
- Mean cell haemoglobin (pg)	18.5	20.2	1.02	1.01	0.989	0.980 *	0.941 ***	0.975 **	0.914 ***	0.901 ***
- Reticulocytes (10^3/µL)	163	182	0.939	1.25	1.10	1.23	1.31 **	1.04	1.84 ***	1.37 **
- Thrombocytes (10^3/µL)	1213	1085	0.978	1.07	1.11 **	1.14 *	1.21 ***	1.14	1.31 ***	1.32 ***
- Neutrophils (10^3/µL)	3.30	3.70	1.06	0.805	1.18	0.827	1.06	0.914	1.45 **	0.816
- Lymphocytes (10^3/µL)	5.27	3.87	1.03	0.987	1.09	1.02	1.11	0.904	1.22 *	0.904
- Eosinophils (10^3/µL)	0.14	0.10	0.929	0.900	0.857	0.900	0.857	1.00	0.643 ***	0.700

[a]: For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of vehicle/baseline are shown. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1.
-: no noteworthy findings; F: female; M: male; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400))		0 (PEG400)		50		150		500	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Clinical Chemistry[a]										
Week 53										
- Total protein (g/dL)	7.6	7.9	0.987	0.987	1.00	1.01	1.01	1.09 ***	1.01	1.06 ***
- Week 105-106										
- Sodium (mmol/L)	146	143	1.00	1.00	0.993	1.00	0.993 *	0.993 *	0.986 **	0.993 **
- Potassium (mmol/L)	4.6	3.9	1.04	1.05 *	1.09 **	1.08	1.11 ***	1.13 ***	1.15 ***	1.18 ***
- Calcium (mg/dL)	10.7	11.1	1.02	1.00	1.01	1.02	1.03 *	1.05 *	1.02	1.06 **
- Total protein (g/dL)	7.3	8.0	1.01	0.975	1.03	1.01	1.07 ***	1.06 **	1.06 *	1.08 **
- Albumin (g/dL)	3.8	4.5	1.03	1.02	1.03	1.11 *	1.05	1.13 ***	1.00	1.09 *
- Cholesterol (mg/dL)	102	112	1.34 *	0.920	1.00	1.05	0.980	1.24 **	1.09	1.67 ***
- Urea nitrogen (mg/dL)	18.3	16.1	0.918	1.03	0.995	0.944	1.18 **	1.01	1.13 ***	1.30 ***
- Triglycerides (mg/dL)	89	119	1.55 *	1.15	0.955	0.908	0.775 *	0.672 *	0.539 ***	0.546 **
- Creatinine (mg/dL)	0.27	0.27	1.15	1.04	1.04	1.00	1.04	1.04	1.19 *	1.30 *
- Total bilirubin (mg/dL)	0.17	0.20	1.18	0.850	1.18 **	0.750 **	1.88 ***	1.00	3.00 ***	1.45 ***
- Alkaline phosphatase (U/L)	55	32	0.909	0.938	0.927	0.688	1.09	0.875	1.49 **	1.03
- Aspartate aminotransferase (U/L)	179	181	0.972	0.867	1.03	0.823	1.01	0.762 *	1.20	1.46 *
- Alanine aminotransferase (U/L)	41	35	0.829 *	0.886	0.829	0.914	0.927	0.829	1.76 **	1.66
- Gamma glutamyl transferase (U/L)	2	1	0.500	1.00	2.00	1.00	5.50 ***	4.00	28.0 ***	29.0 ***
Urinalysis[a]										
Week 105-106										
- Volume (mL)	13.9	12.7	0.935	1.09	1.05	1.08	1.10	1.22 *	1.19	1.37 *
- Proteins (Score)	2.68	1.52	1.03	0.855	1.06	1.15	1.03	1.62 *	1.09	1.65 *
- Ketones (Score)	0.04	0.62	3.50	0.726	3.50	0.726	3.25	0.468	9.25 **	0.435
- Granular casts (Score)[b]	0.00	0.17	0.00	0.29	0.11	0.00	0.06	0.14	0.23 *	0.22
- Color (Score)	1.04	0.95	1.10	1.16	1.03	1.05	1.74 ***	1.01	2.53 ***	1.38

[a]: For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of vehicle/baseline are shown. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1.

[b]: absolute values

-: no noteworthy findings; F: female; M: male; * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400))**		**0 (PEG400)**		**50**		**150**		**500**	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Organ Weights[a,b]										
Terminal sacrifice group/Versus Vehicle 1										
Liver (mean Weight)	13.11	10.18	1.18 *	1.13	1.11	1.16	1.38 **	1.33 **	1.92 **	1.59 **
Liver (mean % Body Weight)	2.41	2.91	1.12	0.976	1.13 *	0.993	1.41 **	1.30 **	2.15 **	1.78 **
Liver (mean % Brain Weight)	562.7	488.9	1.19	1.15	1.15	1.15	1.41 **	1.34 **	1.99 **	1.62 **
Gross Pathology										
Terminal sacrifice group, including deaths. Animals Examined	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
Kidneys										
- Discoloration: dark	1	0	0	0	0	0	4	0	15	11
- Irregular surface	0	0	1	0	0	0	0	3	0	8
- Swollen	2	0	1	1	5	0	0	1	4	6
Liver										
- Discoloration: dark	1	0	0	0	4	0	13	24	25	59
- Focus/area discolored	10	9	10	10	12	18	24	14	27	24
- Mass	3	1	1	0	2	0	11	2	2	9
- Nodule	2	0	0	0	0	0	1	1	4	0
- Swollen	1	1	3	3	3	2	1	5	3	3

[a]: At end of dosing period. For Vehicle 1, group means are shown. For Vehicle 2 and treated groups, multiples of vehicle/baseline are shown. Statistical significance is based on actual data (not on the multiples of vehicle/baseline) and computed versus Vehicle 1.

[b]: Both absolute and relative weights differed from controls in the direction indicated.

-: no noteworthy findings; F: female; M: male; * $p<0.05$, ** $p<0.01$.

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400))**		**0 (PEG400)**		**50**		**150**		**500**	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Histopathology										
Adrenal glands (no. examined)	**70**	**69**	**70**	**69**	**70**	**69**	**70**	**70**	**69**	**70**
- Vacuolization	3	-	6	-	-	-	-	-	21	-
grade 1	3		6						2	
grade 2	0		0						1	
Bone marrow femur (no. examined)	**70**	**70**	**70**	**69**	**70**	**70**	**70**	**70**	**70**	**70**
- Decreased cellularity	15	-	10	-	-	-	3	-	2	-
grade 1	14		8				3		2	
grade 2	1		2				0		0	
- Increased cellularity	2	-	3	-	-	-	-	-	17	-
grade 1	2		3						17	
grade 2	0		0						0	
- Increased fat	8	-	8	-	-	-	1	-	0	-
grade 1	8		8				1		0	
- Prominent erythropoiesis term	0	-	0	-	-	-	-	-	7[c]	-
grade 1	0		0						7	
Bone marrow sternum (no. examined)	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**68**	**70**
- Decreased cellularity	9	-	4	-	-	-	2	-	2	-
grade 1	8		3				2		2	
grade 2	1		1				0		0	
- Increased cellularity	3	-	3	-	-	-	-	-	17	-
grade 1	3		3						17	
grade 2	0		0						0	
- Prominent erythropoiesis term	0	-	0	-	-	-	-	-	4[c]	-
grade 1	0		0						4	
Lungs (no. examined)	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
- Foamy macrophages	-	16	-	15	-	-	-	-	-	34
grade 1		14		15						27
grade 2		2		0						7

[c]: Only retained in terminal rats (4/7 bone marrow femur; 3/4 bone marrow sternum), -: no noteworthy findings; F: female; M: male.

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400)**		**0 (PEG400)**		**50**		**150**		**500**	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Histopathology (continued)										
Kidney (no. examined)	**70**	**70**	**70**	**70**	**70**	**70**	**69**	**70**	**70**	**70**
- Chronic progressive nephropathy	-	18	-	13	-	-	-	36	-	41
grade 1		15		10				21		21
grade 2		2		2				13		12
grade 3		1		1				2		8
- Mineralization cortex	25	-	21	-	-	-	-	-	6	-
grade 1	24		20						6	
grade 2	1		1						0	
- Mineralization pelvis	-	62	-	62	-	-	-	-	-	48
grade 1		39		44						39
grade 2		23		17						9
grade 3		0		1						0
- Pigmentation tubules	2	3	1	3	-	-	7	21	40	61
grade 1	2	3	1	3			6	21	37	41
grade 2	0	0	0	0			1	0	3	20
Liver (no. examined)	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
- Hepatocellular tumors: # of rats	5	2	1	0	2	1	17	5	33	18
- Adenoma Benign	5	2	0	0	1	1	16	5	25	15
- Carcinoma Malignant	0	0	1	0	1	0	4	0	19	7
- Degeneration cystic	5	-	5	-	12	-	15	-	16	-
grade 1	5		4		12		12		16	
grade 2	0		1		0		3		0	

-: no noteworthy findings; F: female; M: male.

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	**0 (PEG400)**		**0 (PEG400)**		**50**		**150**		**500**	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Histopathology (continued)										
Liver (no. examined, continued)	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
- Focus of cellular alteration basophilic	15	41	28	42	-	-	-	-	38	42
grade 1	15	34	27	34					27	26
grade 2	0	7	1	6					11	15
grade 3	0	0	0	2					0	1
- Focus of cellular alteration eosinophilic	21	14	23	28	33	-	46	40	48	53
grade 1	19	13	21	26	32		22	32	18	24
grade 2	2	1	2	2	1		18	7	20	18
grade 3	0	0	0	0	0		6	1	10	11
- Hyperplasia oval cell	-	0	-	0	-	-	-	-	-	11
grade 1		0		0						10
grade 2		0		0						1
- Hypertrophy hepatocellular	0	2	1	6	4	-	26	32	40	58
grade 1	0	2	1	6	4		25	31	38	51
grade 2	0	0	0	0	0		1	1	2	7
- Pigmentation Kupffer cell(s) and/ or macrophages	7	9	4	8	-	-	22	39	39	64
grade 1	7	9	4	8			22	37	33	20
grade 2	0	0	0	0			0	1	6	37
grade 3	0	0	0	0			0	1	0	7
- Pigmentation hepatocyte	0	0	0	1	-	-	7	33	27	66
grade 1	0	0	0	1			7	29	24	14
grade 2	0	0	0	0			0	4	3	46
grade 3	0	0	0	0			0	0	0	6
- Vacuolization hepatocellular	15	14	9	26	-	-	22	34	31	45
grade 1	11	13	8	20			19	28	24	31
grade 2	3	1	1	5			3	6	7	13
grade 3	1	0	0	1			0	0	0	1

-: no noteworthy findings; F: female; M: male.

2.6.7.10.B Carcinogenicity-Darunavir (Continued)

Report Title: 24-Month Repeated Dose Oral Carcinogenicity Study of TMC114 in the Rat **Test Article:** darunavir

Noteworthy Findings (Continued):

Dose (mg/kg/day)	0 (PEG400)		0 (PEG400)		50		150		500	
No. of animals	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**	**M:70**	**F:70**
Histopathology (continued)										
Thyroid gland (no. examined)	**70**	**70**	**69**	**70**	**70**	**70**	**70**	**70**	**70**	**69**
- Adenoma C-cell Benign	4	4	1	1	4	3	3	3	5	5
- Adenoma follicular cell Benign	0	1	0	1	1	1	4	1	5	0
- Hyperplasia follicular cell	2	-	0	-	-	-	-	-	6	-
grade 1	1		0						5	
grade 2	1		0						1	
- Hypertrophy follicular cell	0	0	1	1	-	-	3	4	10	8
grade 1	0	0	1	1			3	4	10	8
- Increased small follicles	11	4	5	2	-	-	16	15	31	34
grade 1	11	4	5	2			15	15	29	34
grade 2	0	0	0	0			1	0	2	0
- Inspissated colloid	7	3	8	2	-	-	28	13	35	43
grade 1	7	3	8	2			28	13	34	43
grade 2	0	0	0	0			0	0	1	0
Pituitary glands (no. examined)	**70**	**70**	**69**	**70**	**70**	**70**	**68**	**69**	**69**	**70**
- Vacuolated cells pars distalis	4	-	2	-	-	-	8	-	13	-
grade 1	4		2				8		13	
Spleen (no. examined)	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**	**70**
- Extramedullary hematopoiesis	11	-	7	-	-	-	-	-	23	-
grade 1	10		6						18	
grade 2	0		1						5	
grade 3	1		0						0	
- Pigmentation	-	53	-	54	-	-	-	60	-	63
grade 1		31		30				16		25
grade 2		20		23				39		33
grade 3		2		1				5		5

-: no noteworthy findings; F: female; M: male.

2.6.7.10.C Carcinogenicity-Cobicistat

Test Article: Cobicistat

Report Title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Mice		**Gilead Study No. TX-216-2030** ([redacted] 8203827)
Species/Strain: Mouse/Crl:CD1(ICR)	**Duration of Dosing:** 96 weeks for males; 100 weeks for females	**Location in CTD:** 4.2.3.4.1
Initial Age: 6 to 7 weeks	**Method of Administration:** Oral gavage	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Vehicle:** 95% PG/5% EtOH (v/v), pH 2.3 [Weeks 1−13]; 95% PG/5% EtOH (v/v) [Weeks 14 −25]; 10% PG in 90% 40mM acetate buffer (v/v), pH 4.0 [Week 26−termination]	**Lot Number:** [redacted] and [redacted]

Basis for High Dose Selection: The high dose in males was selected based on attainment of the MTD at 50 mg/kg/day in a 3-month toxicity study in mice. For females, a dose of 200 mg/kg/day exceeded the MTD in a 2-week study, whereas a 50 mg/kg/day dose did not reach the MTD in the 3-month toxicity study in mice.

Special Features: Satellite groups of animals added for toxicokinetic evaluation on Day 1 and during Week 29.

Daily Dose (mg/kg/day)	**0 (Water Control)**		**0 (Vehicle Control)**		**5**	**10**	**15**	**30**	**50**	**100**
Gender: No. of Animals (TK)	M:11	F:11	M:11	F:11	M:49	F:49	M:49	F:49	M:49	F:49
Toxicokinetics										
C_{max} - Day 1 (!g/mL)	ND	ND	ND	ND	0.194	2.00	3.34	7.07	9.35	16.8
C_{max} - Week 29 (!g/mL)	ND	ND	ND	ND	2.31	7.88	4.42	13.3	10.1	21.6
AUC_{0-t} - Day 1 (!g·h/mL)	ND	ND	ND	ND	0.600	4.26	14.3	36.0	53.5	100
AUC_{0-t} - Week 29 (!g·h/mL)	ND	ND	ND	ND	4.05	13.9	12.4	72.1	75.0	174
Daily Dose (mg/kg/day)	**0 (Water Control)**		**0 (Vehicle Control)**		**5**	**10**	**15**	**30**	**50**	**100**
Gender: No. of Animals (Main)	M:60	F:60	M:60	F:60	M:60	F:60	M:60	F:60	M:60	F:60
Died/Sacrificed Moribund	24	37	40	37	36	41	33	45	47	46

Test Article: Cobicistat

Report Title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Mice							**Gilead Study No. TX-216-2030** ([redacted] 8203827)			
Terminal Sacrifice	36	23	20	23	24	19	27	15	13	14
Survival (%)[a]										
Week 52	92	98	82	90	80	85	81	82	83	75
Week 91	69	56	39	59	50	48	50	42	40	NA[b]
Termination[c]	61	40	36[d]	40	44	35	47	28	29	30[e]
Body Weight (%)[f]										
Week 54	↑4.7	↑12.0*	44.6 g	32.6 g	↑1.1	0.0	↑0.7	↓0.3	↓4.3	↑0.3
Final Assessment[g]	↑4.6	↑10.9*	43.9 g	36.8 g	↓2.5	↓7.3	↓0.2	↓6.0	↓5.9	NA[b]
Food Consumption (%)[f]										
Week 53	↓5.2	↑2.9	36.5 g	34.4 g	↓4.1	↓4.9	↓8.2*	↓9.6	↓13.7*	↓3.2
Final Assessment[g]	↑1.2	↑10.2	34.4 g	35.2 g	↓0.6	↓5.4	↓1.7	↓10.2	↓2.0	NA[b]
Neoplastic Lesions	-	-	-	-	-	-	-	-	-	-
Clinical Observations										
Feces, Few	9	7	25	13	18	22	23	15	24	15
Hypoactive	8	8	13	10	12	13	9	14	14	12
Audible Respiration	0	1	7	5	4	5	5	9	9	9
Irregular Respiration	5	7	7	2	7	7	5	7	12	4
Labored Respiration	4	9	10	9	9	7	3	12	11	10
Daily Dose (mg/kg/day)	**0 (Water Control)**		**0 (Vehicle Control)**		**5**	**10**	**15**	**30**	**50**	**100**
Gender: No. of Animals (Main)	M:60	F:60	M:60	F:60	M:60	F:60	M:60	F:60	M:60	F:60
Hunched Posture	9	9	13	9	6	18	7	11	18	16
Thin	1	6	3	4	2	3	3	3	5	10

Test Article: Cobicistat

Report Title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Mice							**Gilead Study No. TX-216-2030** ([redacted] 8203827)			
Clinical Pathology	-	-	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-
Histopathology (Non-neoplastic Lesions)										
Number of Animals Examined	60	60	60	60	60	60	60	60	59	60
Nasal Turbinates, Infiltrates, Neutrophils, Airway	10	8	25	28	29	37	30	41	50	46
Liver, Pigment, Kupffer Cells	3	14	1	10	2	2	0	7	21	31
Liver, Hypertrophy, Hepatocellular	4	2	8	2	7	1	8	0	23	9

a Percent adjusted survival = (survival/[60-no. of accidental deaths]) x 100

b The 100 mg/kg/day female group was terminated during Week 88.

c Terminal sacrifice was Week 95 for the 50 mg/kg/day male group, Week 97 for the remaining male groups, Week 88 for the 100 mg/kg/day female group, and Week 100 for the remaining female groups.

d Significant increase in mortality in vehicle control males versus the water control group (p=0.0033 by Cox-Tarone test and p=0.0023 by Gehan-Breslow test)

e Significant positive trend in mortality occurred in COBI-treated groups compared with the vehicle control group ($0.0040 < p < 0.0050$ by the Cox-Tarone and $p = 0.0052$ by the Gehan-Breslow tests). The high-dose females (100 mg/kg/day) exhibited a significant increase in mortality ($p = 0.0067$ by the Cox-Tarone and $p = 0.0047$ by the Gehan-Breslow tests) versus the vehicle control group.

f For vehicle controls, group means are shown. For water control and treated groups, percent differences from controls are shown. Statistical significance is based on actual data not on the percent difference.

g For body weight, the final assessment was Week 94 for males and Week 98 for females. For food consumption, the final assessment was Week 93 for males and Week 97 for females.
− = Comparable to control animals or no treatment-related findings; ND = None detected; MTD = maximum tolerated dose; M = Male; F = Female; PG = propylene glycol; EtOH = ethanol; * = $p \leq 0.05$ (Dunnett's t-test); ↑ = increased versus vehicle control group; ↓ = decreased versus vehicle control group

2.6.7.10.D Carcinogenicity-Cobicistat

Test Article: Cobicistat

Report Title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Rats		**Gilead Study No. TX-216-2031** ([redacted] 8203828)
Species / Strain: Rat / Crl:CD(SD)	**Duration of Dosing:** 97 weeks for males; 102 weeks for females	**Location in CTD:** 4.2.3.4.1
Initial Age: 6 to 7 weeks	**Method of Administration:** Oral gavage	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Vehicle:** 95% propylene glycol (v/v)/5% ethanol (v/v), pH 2.3 ± 0.1 with hydrochloric acid	**Lot Numbers:** [redacted] and [redacted]

Basis for High Dose Selection: Dose levels were based on mortality (females) and decreases in body weight gain (males) at 100 mg/kg/day in a 26-week oral gavage toxicity study in rats.

Special Features: Satellite groups of animals added for toxicokinetic evaluation on Day 1 and during Week 26.

Daily Dose (mg/kg/day)	**0 (Water)**		**0 (Vehicle)**		**10**	**5**	**25**	**15**	**50**	**30**
Gender: No. of Animals (TK)	M:6	F:6	M:6	F:6	M:12	F:12	M:12	F:12	M:12	F:12
Toxicokinetics										
C_{max} - Day 1 (!g/mL)	ND	ND	ND	ND	0.037	0.017	0.544	1.05	3.00	2.18
C_{max} - Week 26 (!g/mL)	ND	ND	ND	ND	0.430	0.725	1.75	1.75	2.42	3.23
AUC_{0-t} - Day 1 (!g·h/mL)	ND	ND	ND	ND	0.090	0.038	2.57	3.88	20.8	12.0
AUC_{0-t} - Week 26 (!g·h/mL)	ND	ND	ND	ND	1.31	0.807	9.08	6.38	22.7	19.9

Test Article: Cobicistat

Report Title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Rats					**Gilead Study No. TX-216-2031 ([redacted] 8203828)**					
Daily Dose (mg/kg/day)	**0 (Water)**		**0 (Vehicle)**		**10**	**5**	**25**	**15**	**50**	**30**
Gender No. of Animals (Main)	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Died/Sacrificed Moribund	32	40	45	45	40	37	31	43	28	35
Terminal Sacrifice	33	25	20	20	25	28	34	22	37	30
Survival (%)[a]	51	38	31[b]	31	38	45	52[c]	34	57[c]	46
Body Weight (%)[d]										
Week 54	↓3.2	↓0.0	810 g	400 g	↓2.5	↓1.0	↓6.8*	↑7.0	↓6.4*	↑3.8
Termination[e]	↓5.9	↓4.0	834 g	505 g	↓2.2	↓3.8	↓12.0*	↓8.1	↓8.9	↑0.8
Body Weight Gain (%)[d,f]	↓9.5	↓5.8	631 g	326 g	↓4.0	↓6.1	↓16.5*	↓12.3	↓12.8*	↑0.3
Food Consumption (%)[e] Week 53	↑5.0	↑4.4	219 g	158 g	↓0.9	↑0.6	↓4.1	↑5.7	↓1.4	↑1.3
No. of Animals with Notable Neoplastic Lesions										
Thyroid										
Adenoma, Follicular Cell[g]	3	0	0	0	1	0	5	2	15[++]	6
Carcinoma, Follicular Cell[h]	0	0	0	0	0	0	1	1	5	2
Adenoma and Carcinoma (combined), Follicular Cell[i]	3	0	0	0	1	0	6[+]	3	20[++]	8[++]
Clinical Observations	-	-	-	-	-	-	-	-	-	-
Clinical Pathology	-	-	-	-	-	-	-	-	-	-
Gross Pathology	-	-	-	-	-	-	-	-	-	-

Test Article: Cobicistat

Report Title: 104-Week Oral Gavage Carcinogenicity Study with GS-9350 in Rats							**Gilead Study No. TX-216-2031 ([redacted] 8203828)**			
Daily Dose (mg/kg/day)	**0 (Water)**		**0 (Vehicle)**		**10**	**5**	**25**	**15**	**50**	**30**
Gender No. of Animals (Main)	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65	M:65	F:65
Histopathology (Non-neoplastic Lesions)										
Thyroid										
Hypertrophy, Follicular Cell	5	1	1	1	3	3	17	9	25	16
Hyperplasia, Follicular Cell	3	0	2	0	3	1	3	4	5	4
Liver										
Karyocytomegaly, Hepatocyte	1	4	0	3	1	3	8	7	13	8
Hypertrophy, Hepatocyte, Centrilobular	6	1	5	0	16	0	25	0	27	12

a At termination (Week 98 for males and Week 103 for females). Percent adjusted survival = (survival/[65-no. of accidental deaths]) x 100

b Significant increase in mortality rate compared with the water control group ($p = 0.0137$ by Cox-Tarone and $p = 0.0175$ by Gehan-Breslow tests)

c Significant negative trend in mortality in males ($0.0020 \leq p \leq 0.0022$ by Cox-Tarone and $p = 0.0078$ by Gehan-Breslow tests), with 25 and 50 mg/kg/day exhibiting significantly lower mortality ($p = 0.0056$ by Cox-Tarone and $p = 0.0045$ by Gehan-Breslow tests at 25 mg/kg/day and $p = 0.0054$ by Cox-Tarone and $p = 0.0152$ by Gehan-Breslow tests at 50 mg/kg/day, respectively) versus vehicle control.

d For vehicle controls, group means are shown. For water controls and treated groups, percent differences from vehicle controls are shown. Statistical significance is based on actual data, not on the percent difference.

e Week 98 for males and Week 102 for females.

f Over the treatment period (Weeks 1−98 for males and Weeks 1−102 for females).

g For males and females, a significant positive trend for thyroid follicular cell adenoma (common tumors) was present ($p = 0.0011$ for males and $p = 0.0060$ for females), with a significant increase in pairwise comparison for males at 50 mg/kg/day. The effect in females at 30 mg/kg/day was not significant.

h For males, thyroid follicular cell carcinoma incidences showed a significant positive trend for common tumors ($p = 0.0011$).

i For males, combined thyroid follicular cell adenoma and carcinoma incidences exhibited a significant positive trend for common tumors ($p = 0.0000$), with significant increases at 25 and 50 mg/kg/day ($p = 0.0102$ and $p = 0.0000$, respectively). For females, combined follicular cell adenoma and carcinoma incidences showed a significant positive trend for common tumors ($p = 0.0003$), with a significant increase at 30 mg/kg/day ($p = 0.0059$).

− = Comparable to control animals or no treatment-related findings; ND = None detected; M = Male; F = Female; * = $p \leq 0.05$ (Dunnett's t-test); $^{+}$ = $p \leq 0.05$; $^{++}$ = $p \leq 0.01$; ↑ = increased versus vehicle control group; ↓ = decreased versus vehicle control group

2.6.7.10 Carcinogenicity: Non-Pivotal-Darunavir

Species/Strain Sex/No. Per Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg b.e./kg/day)	Batch no. (purity)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Mouse/CD-1 5M + 5F	Oral/gavage (PEG400)	2 weeks	Vehicle, 50, 150, 450, 1000	[redacted] (as base: 92% w/w; as ethanolate: 98.1% w/w)	NE	Four accidental deaths (M, 150 mg/kg/day; 2M, 450 mg/kg/day; F, 1000 mg/kg/day) 50: ↓ RBC (M, 4%); ↓ Hb (M, 5%), ↓ Hct (M, 6%), ↑ reticulocytes (F, 26%), hepatocellular centrilobular hypertrophy (M, 3/5) 150: ↓ RBC (M, 5%); ↓ Hb (M, 5%), ↓ Hct (M, 4%), ↑ bilirubin (M, 95%; F, 69%), dark liver (M, 1/5) , hepatocellular centrilobular hypertrophy (M, 2/5; F, 2/5) 450: ↓ RBC (M, 4%); ↓ Hb (M, 5%), ↓ Hct (M, 6%), ↑ reticulocytes (F, 21%), ↑ cholesterol (M, 36%; F, 46%), ↑ bilirubin (M, 4.9-fold; F, 6.1-fold%), ↑ liver wt (M, 20%; F, 36%), dark liver (M, 1/5), hepatocellular centrilobular hypertrophy (M, 2/5; F, 5/5), hepatocellular diffuse hypertrophy (M, 1/5; F, 2/5) 1000: ↓ RBC (M, 7%; F, 8%); ↓ Hb (M, 8%; F, 6%), ↓ Hct (M, 8%; F, 7%), ↑ reticulocytes (M, 20%; F, 24%), ↑ cholesterol (M, 61%; F, 27%), ↑ bilirubin (M, 7.9-fold; F, 9-fold), ↑ liver wt (M, 50%; F, 53%), dark liver (M, 4/5), swollen liver (M, 5/5; F, 1/5), hepatocellular centrilobular hypertrophy (M, 4/5; F, 3/5), hepatocellular diffuse hypertrophy (M, 3/5; F, 2/5), splenic red pulp hyperplasia (M, 5/5)	TMC114-NC190/ 4.2.3.4.1
Mouse/CB6F1-nonTgrasH2 10M + 10F (12M + 12F satellite/vehicle group, 15M + 15F satellite/dosed group)	Oral/gavage (PEG400),	4 weeks	Vehicle, 150, 450, 1000	[redacted] (as base: 92% w/w; as ethanolate: 99.6% w/w)	NE	Two deaths (F, 450 mg/kg/day; F, satellite control, accidental) 150: ↓ BW gain (M, 33%), ↑ reticulocytes (M, 30%; F, 25%), ↑ cholesterol (M, 26%; F, 10%), 450: ↓ BW gain (M, 29%), ↑ reticulocytes (M, 18%; F, 10%), ↑ creatinine (M, 24%), ↑ bilirubin (M, 3-fold; F, 2-fold), ↑ cholesterol (M, 61%; F, 40%), ↑ liver wt (M, 30%; F, 16%), hepatocellular hyperthrophy (M, 10/10; F, 9/10) 1000: ↓ BW gain (M, 38%), ↑ reticulocytes (M, 2.2-fold; F, 20%), ↑ creatinine (M, 47%; F, 28%), ↑ bilirubin (M, 10-fold; F, 8-fold), ↑ cholesterol (M, 96%; F, 90%), ↑ liver wt (M, 64%; F, 47%), hepatocellular hyperthrophy (M, 10/10; F, 10/10)	TMC114-NC194[b]/ 4.2.3.4.3

[a] - No Observed Adverse-Effect Level; [b] GLP compliant

b.e. = base equivalent, BW = body weight, F = female, Hb = hemoglobin, NE = not established; Hct = hematocrit, PEG = polyethylene glycol, RBC = red blood cell, wt = weight

2.6.7.10 Carcinogenicity: Non-Pivotal-Darunavir (Continued)

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Swiss mouse

Test Article: darunavir

Species/strain: Mouse / CD-1

Administration route/method: Oral gavage

Testing facility: J&J PRD

Age/weight at first dose: 6-7 weeks

Doses: 0, 150, 450, 1000 mg/kg/day

Study No.: TMC114-NC157

Duration of dosing: 3 months

Test article batch (purity): [redacted] (as base: 92% w/w#, as ethanolate: 98.1% w/w)

Location in CTD: 4.2.3.4.1

GLP Compliance: yes

Duration post-dose: 0 days

Vehicle/formulation: Polyethylene glycol 400

Date of start of study: [redacted]

No Observed Adverse Effect Level: The NOAEL was not established in this study but at 150 mg/kg/day, the changes in serum and the hematopoietic system were minimal

Toxicokinetics:

Daily dose (mg base equivalent/kg)	0(Vehicle)		150		450		1000	
No. of Animals	**M:0**	**F:0**	**M:30**	**F:30**	**M:30**	**F:30**	**M:30**	**F:30**
Died or killed prematurely (accidental)	-	-	1	1	0	0	0	0
$AUC_{0-\infty}$ day 1 (µg.h/mL)	-	-	10.7	21.3*	28.4	79*	59.8	143
AUC0-24h day 87 (µg.h/mL)	-	-	6.03	8.48	22.4	45.8	33	50.3

* AUC_{0-8h}

Noteworthy Findings

Daily dose (mg base equivalent/kg)	0(Vehicle)		150		450		1000	
No. of animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Died or killed prematurely (accidental)	1	1	1	0	0	0	0	1
Clinical obervations	-	-	-	-	-	-	-	-
Body weight (g)	-	-	-	-	-	-	-	-
Food consumption (g/wk)	-	-	-	-	-	-	-	-
Hematology (week 13) [$]								
- Red Blood Cells (10^6/µL)	9.03	8.96	0.95*	0.99	0.95*	0.96	0.93*	0.93*
- Hemoglobin (g/dL)	14.3	13.5	0.93**	1.03	0.90***	1.01	0.88***	0.96
- Hematocrit (%)	46.0	44.9	0.94**	1.02	0.92**	0.98	0.91***	0.95*
- Reticulocytes (%)	2.8	2.6	1.07	1.19	1.07	1.19	1.36***	1.46***
Serum Chemistry (week 13)[$]								
- Cholesterol (mg/dL)	123	76	1.09	1.21	1.25*	1.37*	1.63***	2.11***
- Total Bilirubin (mg/dL)	0.12	0.10	1.08	1.20*	1.25	1.70***	1.92***	2.30***
- Alanine Aminotransferase (U/L)	67	47	1.52	1.32	1.57	1.91	1.06	1.77*

[$] For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

*-p<0.05, ** -p<0.01, *** -p<0.001; - No treatment related change

2.6.7.10 Carcinogenicity: Non-Pivotal-Darunavir (Continued)

Report Title: 3-Month Repeated Dose Oral Toxicity Study in the Swiss mouse **Test Article**: darunavir

Noteworthy Findings

Daily dose (mg base equivalent/kg)	0 (Vehicle)		150		450		1000	
No. of animals	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**	**M:10**	**F:10**
Organ Weights[$]								
- Spleen (%)	0.23	0.37	1.08	0.99	1.29	0.97	1.44**	1.05
- Liver (%)	5.22	5.36	1.08	1.04	1.31***	1.19***	1.54***	1.41***
Gross Pathology (no. examined)	10	10	10	10	10	10	10	10
- Liver, dark	0	0	-	-	8**	-	10***	6*
Histopathology (no. examined)	10	10	10	10	10	10	10	10
Liver								
- Hepatocytic hypertrophy,centrilobular								
score 1	0	0	-	-	7	-	6	4
score 2	0	0	-	-	3	-	3	1
score 3	0	0	-	-	0	-	1	0
- Hepatocytic necrosis, multifocal								
score 1	0	0	-	-	-	-	1	0
score 2	0	0	-	-	-	-	0	2
- Single cell necrosis								
score 1	4	1	2	3	4	4	6	3
score 2	0	1	0	0	0	0	1	2
- Pigmentation, diffuse (hepatocytes)								
score 1	0	0	-	-	3	-	7	1
score 2	0	0	-	-	1	-	0	1
Spleen								
- Pigmentation (hemosiderine)								
score 1	6	-	-	-	6	-	3	-
score 2	2	-	-	-	3	-	7	-
score 3	0	-	-	-	0	-	0	-

[$] For controls, group means are shown. For treated groups, multiples of control/baseline are shown. Statistical significance is based on actual data (not on the multiples of control/baseline).

*-p<0.05, ** -p<0.01, *** -p<0.001

- No treatment related changes

2.6.7.10 Carcinogenicity: Non-Pivotal-Darunavir (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats **Test article:** darunavir

Species/Strain: Rats/Sprague-Dawley
Age/ weight at first dose: 7 Weeks/ 151 – 270 g
Duration of dosing: 1 or 3 months
Duration post-dose: 0 days
Administration route/method: Oral/dietary admixture
Intended doses: 0, 260, 1040, 2600 mg/kg/day
Test article batch (purity): [redacted] (as base: 93%, as ethanolate: 97.5%)
Vehicle/Formulation: SSNIFF R/M-H powdered maintenance diet
Testing Facility: [redacted]
Study no.: TMC114-NC196
Location in CTD: 4.2.3.4.3
GLP compliance: Yes
Date start of study: [redacted]
No Observed Effect Level: not established and considered to be lower than 260 mg/kg/day.

Toxicokinetics

Nominal Daily Dose (mg/kg)^	0 (Control)		260		1040		2600	
Number of Satellite Animals	**M:3**	**F:3**	**M:6**	**F:6**	**M:6**	**F:6**	**M:6**	**F:6**
Died or killed prematurely	0	0	0	2	0	0	0	0
$AUC_{0\text{-}24h}$ week 1 (µg.h/mL)	-	-	24.8	21.9	53.2	62.4	65.1	95.2
$AUC_{0\text{-}24h}$ week 4 (µg.h/mL)	-	-	26.9	23.5	54.4	60.8	76.1	113
$AUC_{0\text{-}24h}$ week 13 (µg.h/mL)	-	-	23.9	30.8	47.4	66.4	72.2	123

Noteworthy Findings

Nominal Daily Dose (mg/kg)^	0 (Control)		260		1040		2600	
Number of Principal Animals	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
Clinical observations								
- Emaciated appearance	0	0	-	-	-	-	4	9
Body Weight (g) [a]								
Day 91	554	290	0.94	0.97	0.85	0.85	0.72	0.76
Body Weight Change (g) [a]								
Days 1 to 91	318	117	0.90	0.94	0.76	0.61	0.53	0.37
Food Consumption (g) [a]								
Days 1 to 91	29.1	21.2	0.94	0.92	0.88	0.82	0.80	0.77
Ophthalmoscopy	-	-	-	-	-	-	-	-
Hematology [a]								
- Red Blood Cell Count (T/L)	8.20	7.60	0.98	0.96**	0.95**	0.95**	0.95**	0.96*
- Hemoglobin (g/dL)	15.4	15.1	0.96	0.93**	0.91**	0.86**	0.87**	0.85**
- Packed Cell Volume (L/L)	0.42	0.41	0.98	0.93**	0.93**	0.88**	0.90**	0.88**

2.6.7.10 Carcinogenicity: Non-Pivotal-Darunavir (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats **Test article:** darunavir

Noteworthy Findings (Continued)

Nominal Daily Dose (mg/kg)	**0 (Control)**		**260**		**1040**		**2600**	
Number of Principal Animals (Second set)	**M: 20**	**F: 20**	**M: 20**	**F: 20**	**M: 20**	**F: 20**	**M: 20**	**F: 20**
Hematology [a] (continued)								
- Platelet Count (G/L)	913	876	1.09	1.15*	1.28**	1.27**	1.34**	1.38**
- Prothrombin Time (s)	15.1	15.8	1	0.89**	1	0.84**	0.99	0.84**
- Activated Partial Thromboplastin Time (s)	19.9	15.3	1.06	0.97	1.03	0.84**	0.94	0.83**
- Fibrinogen (g/L)	2.8	1.95	1.1**	1.22**	1.09*	1.33**	1.04	1.35**
- Reticulocyte Count (p.1000)	28	22	1.18	1.23	1.68**	1.36*	1.54**	1.68**
Serum Chemistry								
- Glucose Level (mmol/L)	8.16	7.43	0.85**	0.89*	0.76**	0.85**	0.78**	0.77**
- Triglyceride Level (mmol/L)	0.61	0.38	0.49**	0.76*	0.36**	0.66**	0.38**	0.74**
- Urea (mmol/L)	4.5	5.9	1.18*	1.10	1.38**	1.29**	1.38**	1.46**
- Total Bilirubin (µmol/L)	4	4	1	1	1.5**	1.25**	1.75**	1.5**
- Cholesterol Levels (mmol/L)	1.2	1.7	1.25*	1.29**	1.75**	2.06**	2.16**	2.58**
Urinalysis[&]	-	-	-	-	-	-	-	-
Organ weights (g)[a,b]								
- Liver	13.81	7.35	1.14*	1.36**	1.34**	1.63**	1.27**	1.68**
- Thymus	0.24630	0.25675	1.11	0.90	0.87	0.64**	0.75**	0.54**
Gross pathology (Number Examined)	20	20	20	20	20	20	20	20
Liver								
- Enlargement	0	0	3	4	12	10	8	12
- Coloration	0	0	1	0	5	15	9	16
Kidneys								
- Coloration	0	0	0	0	12	10	18	14
Thymus								
- Reduced in Size	1	1	0	1	4	9	7	11
Histopathology (Number Examined)	20	20	20	20	20	20	20	20
Liver								
- Hepatocellular Hypertrophy								
grade 1	0	0	0	6	0	1	0	0
grade 2	0	0	14	6	12	8	11	9
grade 3	0	0	1	0	8	11	8	11

2.6.7.10 Carcinogenicity: Non-Pivotal-Darunavir (Continued)

Report Title: 13-week toxicity study with 1-month interim kill by oral route (dietary admixture) in rats **Test article:** darunavir

Noteworthy Findings (Continued)

Nominal Daily Dose (mg/kg)	**0 (Control)**		**260**		**1040**		**2600**	
Number of Principal Animals (Second set)	**M: 20**	**F: 20**	**M: 20**	**F: 20**	**M: 20**	**F: 20**	**M: 20**	**F: 20**
Histopathology (Number Examined)	20	20	20	20	20	20	20	20
Liver								
- Vacuolated Hepatocytes								
grade 1	0	0	0	0	0	0	0	0
grade 2	4	3	3	3	4	5	10	9
grade 3	1	0	1	0	0	1	4	1
- Yellowish Pigments in Hepatocytes/ Kuppfer's Cells								
grade 1								
grade 2	0	0	0	0	5	1	1	0
grade 3	0	0	0	0	3	12	10	12
	0	0	0	0	0	4	4	6
Kidneys								
- Brownish Pigments in the Cortical Tubular Epithelium								
grade 1								
grade 2	0	0	0	0	0	0	0	0
grade 3	0	0	0	0	7	6	7	14
	0	0	0	0	8	5	13	4
Thyroids								
- Morphological Changes								
grade 1	10	11	7	6	0	5	3	2
grade 2	6	3	9	7	13	10	8	9
grade 3	4	0	2	0	7	5	7	9
grade 4	0	0	0	0	0	0	2	0
Thymus								
- Lymphoid Depletion								
grade 1	6	2	0	4	4	10	4	5
grade 2	1	0	0	1	3	3	4	11
grade 3	0	0	0	0	1	1	4	3

[a] For controls, group means are shown. For treated groups, multiples of controls are shown.
[b]: both absolute and relative weights differed from controls in the direction indicated; difference from control for absolute organ weights are given
*: $p<0.05$ **: $p<0.01$ (Dunnett + Dunn's tests); -: No noteworthy findings; [&] No mean values calculated
[^] Actual dose [redacted]: 242, 967, 2418 mg base equivalent/kg/day

2.6.7.10 Carcinogenicity: Non-Pivotal Studies-Cobicistat

Test Article: Cobicistat

Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	No. per Group and Gender	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
Mouse/ Hsd:ICR (CD-1[#])	Oral Gavage (95% propylene glycol, 5% ethanol, pH adjusted to 2.3 with HCl)	2 Weeks	0, 10, 30, 100 and 300/200 [a]	Main Study: 5 M/F TK: 6 M/F controls, 36 M/F treated	100	Test article-related mortality at 300/200 mg/kg/day. Dose-related clinical signs: squinted eyes and hypoactivity generally noted at ≥ 30 mg/kg/day. Increases in ALT and AST activity at dose levels of ≥ 30 mg/kg/day. Dose-related increases in liver weights in both sexes at 100 and 300/200 mg/kg/day. Hepatocellular hypertrophy and hepatocellular vacuolation in males at ≥ 100 mg/kg/day, and females at 300/200 mg/kg/day. Notable to marked increases in CYP2B and CYP3A activity at ≥ 30 mg/kg/day, accompanied by increases in total microsomal cytochrome P450 content. NOAEL = 100 mg/kg/day; Day 14 C_{max} 12.0 and 13.9 µg/mL, AUC_{0-t} 134 and 75.7 µg·h/mL in males and females, respectively.	TX-216-2025 ([redacted] 6511-435) 4.2.3.4.1
Mouse/ CD-1	Oral Gavage (95% propylene glycol and 5% ethanol v/v, pH adjusted to 2.3 and pH-unadjusted)	2 Weeks	M: 0, 5, 15, 50; F: 0, 10, 30, 100	24 M/F	M = 50 F=100	Higher incidence of gavage-related mortality in pH-adjusted vehicle groups compared to pH-unadjusted vehicle groups (12 versus 2 deaths, respectively). A dose-dependent increase in struggling during dosing was noted with both vehicles. There were no other test article-related adverse effects. NOAEL Day 14 AUC_{0-t} of 51.0 and 54.8 µg·h/mL in males and 151 and 170 µg·h/mL in females, in pH-adjusted and pH-unadjusted vehicles, respectively.	TX-216-2032[b] ([redacted] 8216084) 4.2.3.4.1

Test Article: Cobicistat

Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	No. per Group and Gender	NOAEL (mg/kg/day)	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
Mouse/CByB6F1-Tg (HRAS)2Jic (wild type)	Oral Gavage (10% v/v propylene glycol in 40 mM acetate buffer, pH 4.0)	4 Weeks	0, 10, 30, 100	Main: 10 M/F TK: 4 M/F controls, 21 M/F treated	100	No COBI-related changes in clinical signs, body weight, food consumption, thyroid hormone level, organ weight or macroscopic parameters were observed. Minor changes in liver and kidney weight and microscopic findings in liver were not considered adverse. NOAEL = 100 mg/kg/day, Week 4 C_{max} 11.0 and 13.4 µg/mL, AUC_{0-t} of 65.3 and 90.1 µg·h/mL for males and females, respectively.	TX-216-2041[b] ([redacted] 8235071) 4.2.3.4.1

a Due to animal deaths in the high dose group at 300 mg/kg/day, the high dose animals were not dosed on Dosing Phase Day 2. Beginning on Dosing Phase Day 3, the high dose level was reduced to 200 mg/kg/day.

b Indicates GLP compliant.

M = male; F = female; NOAEL = No-Observed-Adverse-Effect Level; RTV = ritonavir, GS-017415; ATV = atazanavir; TK = toxicokinetics; ↑ = increased; ↓ = decreased

2.6.7.10 Carcinogenicity: Non-Pivotal Studies-Cobicistat (Continued)

TestArticle: Cobicistat

Report Title: 3-Month Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CD-1 Mice		**Gilead Study No. TX-216-2026** ([redacted]6511-436)
Species/Strain: Mouse/ Hsd:ICR(CD-1#)	**Duration of Dosing:** 92 Days	**Location in CTD:** 4.2.3.4.1
Initial Age: 7 Weeks	**Duration of Postdose**: None	**GLP Compliance**: Yes
Date of First Dose: [redacted]	**Method of Administration:** Oral gavage	**Lot Number:** 3168-157-8
Vehicle/Formulation: 95% propylene glycol (PG), 5% ethanol (EtOH) with 1.5 eq. HCl, pH adjusted to 2.3 with HCl		
Special Features: Hepatic microsomal enzyme analysis		
No Observed Adverse Effect Level: 5 mg/kg/day (males); 50 mg/kg/day (females)		

Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**15**		**50**	
Gender: No. of Animals (TK [a])	**M:15 (8)**	**F:15 (8)**	**M:15 (40)**	**F:15 (40)**	**M:15 (40)**	**F:15 (40)**	**M:15 (40)**	**F:15 (40)**
C_{max} - Day 1 (µg/mL)	ND	ND	0.378	0.436	3.61	4.46	8.94	11.4
C_{max} - Week 13 (µg/mL)	ND	ND	0.236	0.256	3.53	2.73	8.48	11.4
AUC_{0-t} - Day 1 (µg.h/mL)	ND	ND	0.470	1.07	7.57	9.15	32.5	46.4
AUC_{0-t} - Week 13 (µg.h/mL)	ND	ND	0.931	1.19	16.3	11.3	46.2	60.1
Died or Sacrificed Moribund	5	1	1	2	3	1	1	1
Clinical Observations								
Audible Respiration	9	6	7	7	7	4	8	6
Irregular Respiration	5	1	3	4	5	4	6	2
Struggling During Dosing	3	5	8	7	10	10	10	12
Body Weight	No treatment-related findings.							
Body Weight Change	No treatment-related findings.							

TestArticle: Cobicistat

Report Title: 3-Month Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CD-1 Mice					**Gilead Study No. TX-216-2026 ([redacted] 6511-436)**			
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**15**		**50**	
Gender: No. of Animals (TK [a])	**M:15 (8)**	**F:15 (8)**	**M:15 (40)**	**F:15 (40)**	**M:15 (40)**	**F:15 (40)**	**M:15 (40)**	**F:15 (40)**
Food Consumption	No treatment-related findings.							
Ophthalmic Examinations	No treatment-related findings.							
Clinical Chemistry[b]								
Alanine aminotransferase (U/L)	37[c]	36	–	–	128	67	414	236
Aspartate aminotransferase (U/L)	70[c]	121	–	–	232	147	676	290
Cholesterol (mg/dL)	175[c]	110	–	–	222	164*	216	161*
Organ Weights								
Liver (g)	1.67	1.37	1.62	1.43	1.73	1.50	2.08*	1.71*
Liver-to-body weight ratio (%)	4.63	4.62	4.63	4.77	4.99	5.05	5.60*	5.63*
Liver-to-brain weight ratio (%)	334	270	328	278	350	291	406*	336*
Microscopic Observations – Unscheduled Deaths [a]								
Number of Animals Examined	5	1	1	2	3	1	1	1
Death – Gavage-related	3	1	1	2	1	1	0	0
Death – Undetermined	2	0	0	0	2	0	1	1
Microscopic Observations – Terminal Sacrifice [a]								
Number of Animals Examined	11	15	14	15	14	15	14	15
Liver								
Hypertrophy, Hepatocellular	0	0	0	0	0	0	7	0
Increased Vacuolation	0	0	0	0	0	0	3	1

Test Article: Cobicistat

Report Title: 3-Month Oral Gavage Toxicity and Toxicokinetic Study with GS-9350 in CD-1 Mice					**Gilead Study No. TX-216-2026** ([redacted] 6511-436)			
Dose (mg/kg/day)	**0 (Vehicle)**		**5**		**15**		**50**	
Gender: No. of Animals[a]	**M:15**	**F:15**	**M:15**	**F:15**	**M:15**	**F:15**	**M:15**	**F:15**
Hepatic Microsomal Enzyme Activities[d]								
CYP2B (% control)	NA	NA	98.0	98.1	77.0	175	339**	300**
CYP3A (% control)	NA	NA	105	115	142	194	365**	247**

a Due to unscheduled deaths on study, toxicokinetic animals (one male and one female control, two females at 5 mg/kg/day, two males and one female at 15 mg/kg/day, and one female at 50 mg/kg/day) were treated as toxicity animals for clinical pathology assessment at scheduled sacrifice and other terminal endpoints (terminal sacrifice, necropsy, organ weights, bone marrow smears, tissue preservation, and histopathology).

b Group Means are shown.

c n=3/control group; males excluded from statistical analysis.

d Percent differences from control value noted after 13 Weeks dosing. There were no notable changes in protein yield, total cytochrome P450 content, or CYP1A, CYP2E, CYP4A, and UDPGT activities.

ND = none detected; NA = not applicable; TK = toxicokinetics; M = male; F = female; − = comparable to control group; * = $p \leq 0.05$; ** = Indicates a notable change (≥2-fold increase) compared to the control value

2.6.7.11 Reproductive and Developmental Toxicity: Non-Pivotal Studies-Darunavir

Test Article: Darunavir

Species/Strain Sex/No. Per Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Nominal dose, mg/kg/day (Actual dose, mg b.e./kg/day)	Batch no. (purity)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 6 pregnant F/group	Oral/gavage (PEG400)	Day 7 to 9 and Day 13 to 19 of gestation[a]	40, 200, 1000, vehicle [redacted] 38, 188, 939, vehicle) ([redacted]: 39, 193, 965, vehicle)	[redacted] (as base: 93.9% w/w; as ethanolate: 101.9% w/w) and [redacted] (as base: 96.5% w/w; as ethanolate: 98.9% w/w)	No effect on the evaluated maternal or fetal parameters	TMC114-NC127[b]/ 4.2.3.5.2
Rat/Sprague-Dawley 8 F/group	Oral/gavage (PEG400)	GD6-17	Vehicle, 1000/0, 2000/0 or 1000/100	[redacted]	Salivation, ↓ body weight and food consumption	TMC114-NC397/ 4.2.3.5.2
Rabbit/New Zealand White 2 F/group	Oral/gavage (1% CMC and 0.2% Tween 80 in deionized water),	250, 500 mg/kg/day: single dose (day 1) 1000 mg/kg/day: day 1 and days 3 to 7	250, 500, 1000 (235, 470, 939)	[redacted] (as base: 93.9% w/w; as ethanolate: 101.9% w/w)	No adverse findings	TMC114-NC124/ 4.2.3.5.2
Rabbit/New Zealand White 6 pregnant F/group	Oral/gavage (1% CMC and 0.2% Tween 80 in deionized water),	Day 8 to 20 of gestation	40, 200, 1000, vehicle (39, 193, 965, vehicle)	[redacted] (as base: 96.5% w/w; as ethanolate: 98.9% w/w)	No effect on the evaluated maternal or fetal parameters	TMC114-NC125[c]/ 4.2.3.5.2
Rabbit/New Zealand White 4 F/group	Oral/gavage (TMC114: 0.5% (w/v) aqueous hydroxypropyl methylcellulose and 0.2% (w/v) Tween 80; RTV: PG)	5 days	TMC114/RTV: 0/40, 200/40[c], 1000/40, 1000/0, vehicle Expressed as mg b.e/kg/day	TMC114: [redacted] (as base: 92% w/w; as ethanolate: 98.1% w/w)	1 Death (1000/0: 1/4 F) unrelated to treatment No treatment related effects	TMC114-NC189/ 4.2.3.5.2

2.6.7.11 Reproductive and Developmental Toxicity: Non-Pivotal Studies-Darunavir (Continued)

Test article: Darunavir

Species/Strain Sex/No. Per Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Nominal dose, mg/kg/day (Actual dose, mg b.e./kg/day)	Batch no. (purity)	Noteworthy Findings	Study No./ Location in CTD
Mouse/CD-1 10 F/group	Oral/gavage (PEG400)	Day 6 to 15 of gestation	150, 450, <u>1000</u>, vehicle Expressed as mg b.e/kg/day	[redacted] (as base: 93% w/w; as ethanolate: 98.6%)	No effect on the evaluated maternal or fetal parameters	TMC114-NC175/ 4.2.3.5.2
Minipigs/ 3 non-pregnant females/group	Oral/gavage (PEG400)	1 day	Vehicle, 1000/0, 2000/0 or 1000/100	[redacted]	vomiting, passivity and soft/watery feces	TMC114-NC399/ 4.2.3.5.2
		6 days	Vehicle, 500/0, 1000/0 or 500/100		vomiting, passivity and soft/watery feces	
		7 days	Vehicle, 250/0, 500/0 or 250/50		vomiting, passivity and soft/watery feces	

[a] no dosing on gestation days 10 to 12 because of lack of test article; [b] 3 F where incorrectly dosed with TMC125 (750 mg base eq/kg) on day 3; they were redosed with TMC114/RTV in the early afternoon; [c] GLP compliant
NOAEL is underlined

b.e. = base equivalent, F = female, PEG = polyethylene glycol, CMC = carboxymethylcellulose, RTV = ritonavir, GD = gestation day, NOAEL = no observed adverse effect level

2.6.7.11 Reproductive and Developmental Toxicity: Non-Pivotal Studies-Cobicistat

Test Article: Cobicistat

Species / Strain	Method of Administration (Vehicle / Formulation)	Dosing Period	Dose (mg/kgday)	No. Per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
Rat/ Sprague-Dawley	Oral Gavage (95% propylene glycol/5% ethanol with 1.5 eq. HCl, pH 2.3)	GD 6-17	0, 25, 75, 200	8 Pregnant Females (Tox) 3-9 Pregnant Females (TK)	200 mg/kg/day: Deaths; weight loss; decreased food consumption; clinical signs (thin and hunched appearance, tremors, hypoactivity, squinted eyes, clear oral discharge, irregular respiration, rough or brown haircoat, cold to the touch, and/or few or no feces). !25 mg/kg/day: Slight increases in early resorptions and a corresponding decrease in live fetuses. Therefore, a dose of greater than 75 mg/kg/day but less than 200 mg/kg/day was recommended as the high dose for a definitive developmental toxicity study in rats.	TX-216-2018[a] ([redacted] 6511-356) 4.2.3.5.2
Rabbit/ NZW	Oral Gavage (95% propylene glycol/5% ethanol with 1.5 eq. HCl, pH 2.3)	GD 7-20	0, 50, 100, 175, 300	5 Pregnant Females (Tox) 3 Pregnant Females (TK)	!175 mg/kg/day: Deaths, clinical signs (appearance, hypoactivity and few or no feces), weight loss; decreased food consumption. 100 mg/kg/day: one abortion on GD 26, weight loss; decreased food consumption. Based on these results, doses of 25, 50 and 100 mg/kg/day GS-9350 were recommended for the definitive developmental toxicity study in rabbits.	TX-216-2019[a] ([redacted] 6511-358) 4.2.3.5.2

a Indicates GLP compliant.

TK = toxicokinetic; GD = gestation day; NZW = New Zealand White

2.6.7.12.A Reproductive and Developmental Toxicity- Fertility and Early Embryonic Development to Implantation- Darunavir

Report Title: The effects of TMC114 on fertility and early embryonic development in Sprague Dawley rats

Test Article: darunavir

Species/strain: Rat / Sprague-Dawley
Age/weight at first dose: 6-8 weeks / 201-287 g
Duration of dosing: M: 12 weeks before mating until necropsy; **F:** 2 weeks prior to mating up to GD14-16
Duration of post-dose: 0 days
Day of mating: Gestation Day (GD) 1
Day of c-section: GD14-16

Administration route/method: Oral gavage
Doses: 0, 40, 200 and 1000 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 96.5% w/w, as ethanolate 98.9% w/w[#]) and [redacted] (as base: 95% w/w, as ethanolate 102.3% w/w)
Vehicle/formulation: Polyethylene glycol 400

Testing facility: [redacted]
Study no.: TMC114-NC129
Location in CTD: 4.2.3.5.1
GLP compliance: Yes
Date start of study: [redacted]

Special features: study design based on ICH 4.1.3
No Observed Adverse Effect Level: No Observed Adverse Effect Level (NOAEL) was 200 mg/kg based on the body weight effect
Toxicokinetics: Not measured

Noteworthy Findings:

Nominal Daily dose (mg/kg) [^]	**0 (vehicle)**		**40**		**200**		**1000**	
No. of animals	**M:25**	**F:25**	**M:25**	**F:25**	**M:25**	**F:25**	**M:25**	**F:25**
Died or killed prematurely (accidental)	0	0	1	0	0	0	0	1
Died or killed prematurely (cause of death not established)	1	0	0	1	2	0	1	0
Clinical observations	-	-	-	-	-	-	-	-
Body weight (g) – 12 weeks[$]	495.1	-	1.00	-	1.08*	-	0.93*	-
Body weight (g) – 2 weeks (mating) [$]	-	262.3	-	1.07	-	1.04	-	0.98
Body weight (g) – 2 weeks (pregnancy) [$]	-	327.4	-	1.00	-	1.01	-	0.94*
Body weight gain (g) – 2 weeks (pregnancy) [$]	-	56.7	-	1.00	-	1.04	-	0.84*
Food intake (g/wk) – 12 weeks [$]	24.3	-	1.00	-	1.08	-	1.03	-
Food intake (g/wk) – 2 weeks (mating) [$]		19.4		1.01		1.03		0.95
Food intake (g/wk) – 2 weeks (pregnancy) [$]		23.3		0.97		1.00		0.97
Vaginal cytology	-	-	-	-	-	-	-	-
Organ weights (male sex organs)	-	-	-	-	-	-	-	-

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric)
- No noteworthy findings
[#] HPLC assay with reference to anhydrous solvent free substance
[^] Actual dose: 0, 39, 193 and 965 [redacted]), or 0, 38, 190 and 950 ([redacted]) mg base eq./kg body weight/day

2.6.7.12.A Reproductive and Developmental Toxicity- Fertility and Early Embryonic Development to Implantation- Darunavir

Report Title: The effects of TMC114 on fertility and early embryonic development in Sprague Dawley rats **Test Article:** darunavir

Noteworthy Findings:

Nominal Daily dose (mg/kg) ^	**0 (vehicle)**		**40**		**200**		**1000**	
No. of animals	**M:25**	**F:25**	**M:25**	**F:25**	**M:25**	**F:25**	**M:25**	**F:25**
Gross pathology (no. examined)	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
No relevant findings	-	-	-	-	-	-	-	-
Histopathology (no. examined)	**24**	**25**	**0**	**0**	**0**	**0**	**24**	**24**
No relevant findings	-	-	-	-	-	-	-	-
Sperm data								
sperm	-		-		-		-	
morphology	-		-		-		-	
motility	-		-		-		-	
spermatids	-		-		-		-	
Mean no. days prior to mating	2.6		2.7		2.7		2.7	
No. males that mated	24		24		23		25	
No of females mated		23		24		25		23
Mean no. oestrus cycles/days		1.8		1.6		1.7		1.7
No. females sperm-positive		25		25		25		25
No. pregnant females		23		24		25		23
No. aborted or with total resorption of litter		0		0		0		1
No. females with live fetuses		23		24		25		22
Mean no. corpora lutea		18		17		17		15*
Mean no. implantations		16		16		16		14*
Mean % preimplantation loss		8.8		9.0		5.3		4.2
Mean no. live conceptuses		16		15		15		14
Mean no. resorptions		0.7		0.9		0.9		0.6
No dead conceptuses		0		0		0		0
Mean % postimplantation loss		4.5		5.6		5.8		4.4

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric)

- No noteworthy findings

^ Actual dose: 0, 39, 193 and 965 ([redacted]), or 0, 38, 190 and 950 ([redacted]) mg base eq./kg body weight/day

2.6.7.12.B Reproductive and Developmental Toxicity- Fertility and Early Embryonic Development to Implantation- Cobicistat

Test Article: Cobicistat

Report Title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats		**Gilead Study No. TX-216-2023** ([redacted] 6511-360)
Species / Strain: Rat/CD (SD)	**Method of Administration:** Oral gavage	**Location in CTD:** 4.2.3.5.1
Duration of Dosing: F: 2 Weeks prior to mating, through GD 7. **M:** 28 days prior to mating, and throughout the mating phase through the day prior to termination (at least 10 Weeks prior to sacrifice).		
Initial Age: 9 Weeks	**Day of Mating:** GD 0	**GLP Compliance:** Yes
Date of First Dose: Males: [redacted]; **Females:** [redacted]	**Day of C-Section:** GD 13 **Special Features:** None	**Lot Number:** 3168-157-8 **Design Similar to ICH 4.1.1:** Yes
Vehicle/Formulation: 95% propylene glycol and 5% ethanol with 1.5 eq. HCl (pH 2.3)		
NOAEL: F_0 Males and Females = 30 mg/kg/day **NOEL:** F_1 Litters (embryo/fetal viability) = 100 mg/kg/day; F_0 Male and Female reproductive performance and competency = 100 mg/kg/day		

Dose (mg/kg/day)	**0 (Vehicle)**	**10**	**30**	**100**
No. Males	22	22	22	22
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations:				
Clear Oral Discharge	0	3	19	22
Alopecia	2	2	5	9
Body Weight (%)[a]				
Day 28	454	0	-1.7	-3.0
Day 49	531	-0.9	-3.8	-7.2*
Day 52	538	-1.0	-3.7	-7.9**
Day 71	585	-0.5	-3.9	-6.6*
Body Weight Change (%)[a]				
Dose (mg/kg/day)	**0 (Vehicle)**	**10**	**30**	**100**
Day 0-3	21.9	-5.0	-14.2	-33.3**
Day 42-45	13.0	-26.9*	-33.8**	-36.9**
Day 0-71	281	-1.4	-8.2	-13.5*

Test Article: Cobicistat

Report Title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats			**Gilead Study No. TX-216-2023** ([redacted]6511-360)	
Food Consumption (%)[a]				
Day 0-7	33.1	0.0	-3.0	-12.1**
Days 14-21	32.5	+2.2	-1.8	-6.5*
Days 0-28	33.1	+1.8	-1.5	-7.3*
Necropsy Observations	No treatment-related findings.			
Organ Weights[a]				
Liver (absolute)	23.3	+2.9	+1.9	+24.4**
Liver (% body weight)	3.98	+3.7	+6.0	+33.5**
Thyroid (absolute)	0.0257	-2.3	-2.3	+16.3*
Thyroid (% body weight)	0.0044	-1-1	+2.3	+25.6**
Mean No. Days to Mating Confirmation	2.36	2.32	2.41	2.41
No. of Males that Mated	22	22	22	22
No. of Fertile Males	22	22	22	20
Sperm Motility Data	No treatment-related findings.			
Sperm Morphology Data	No treatment-related findings.			
Sperm Count	No treatment-related findings.			

Test Article: COBI

Report Title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats			**Gilead Study No. TX-216-2023** ([redacted] 6511-360)	
Dose (mg/kg/day)	**0 (Vehicle)**	**10**	**30**	**100**
No. Females	**22**	**22**	**22**	**22**
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations				
Premating				
Salivation	0	0	4	21
Alopecia	0	1	1	5
Gestation				
Salivation	0	0	3	17
Alopecia	1	2	1	8
Premating Body Weight (%)				
Body Weight (%)[a]				
Day 14	247	-0.3	+0.6	-1.1
Body Weight Change (%)[a]				
Days 0-3	5.4	0.0	+13.9	-38.9
Days 0-14	29.3	-2.7	+4.4	-11.6
Gestation Body Weight (%)[a]				
Body Weight Change				
Days 0-3	17.7	-4.5	-5.1	-29.9**
Days 0-13	68.5	-2.2	-2.8	-6.0
Premating Food Consumption (%)[a]				
Days 0-7	21.6	-0.5	-2.3	-15.3**
Days 7-14	22.1	-1.4	-0.5	-10.4**
Days 0-14	21.8	-0.9	-1.4	-12.8**

Test Article: COBI

Report Title: Oral Gavage Study of Fertility and Early Embryonic Development to Implantation with GS-9350 in Rats			**Gilead Study No. TX-216-2023** ([redacted] 6511-360)	
Dose (mg/kg/day)	**0 (Vehicle)**	**10**	**30**	**100**
Females				
Gestation Food Consumption (%)[a]				
Days 0-3	25.1	-1.6	-3.6	-13.5**
Days 3-7	26.5	+1.9	-3.4	-15.1**
Days 0-13	27.6	+0.4	-2.2	-5.1
Necropsy Observation	No treatment-related findings.			
Organ Weights[a]				
Liver (absolute)	14.2	-3.1	+0.7	+19.3**
Liver (% body weight)	4.52	-2.9	+0.9	+22.6**
Thyroid (absolute)	0.0.194	+4.1	-4.6	+13.9
Thyroid (% body weight)	0.0062	+3.7	-5.0	+16.2*
Mean No. Estrous Cycles/14 days	2.82	2.77	2.95	2.68
No. of Females in Prolonged Diestrus (4 or more continual days)	2	1	1	2
Mean No. Days to Mating Confirmation	2.36	2.32	2.41	2.41
No. of Females Sperm Positive (confirmed to have mated)	22	22	22	22
No. of Pregnant Females	22	22	22	20
Mean No. Corpora Lutea	15.5	16.2	15.5	15.4
Mean No. Implantations	15.0	15.2	15.0	15.0
Mean % Preimplantation Loss	2.9	6.5	3.0	2.2
Mean No. Live Conceptuses	14.0	14.4	14.1	14.0
Mean No. Resorptions	1.0	0.8	0.9	1.1
No. Dead Conceptuses	0	0	0	0
Mean % Postimplantation Loss	6.2	5.1	6.1	6.8

a For controls, group means are shown in grams. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

GD = gestation day; * = $p<0.05$; ** = $p<0.01$ (Dunnett's Test)

2.6.7.13.A Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir

Report Title: Study of the effects of TMC114 on embryo-fetal development in Sprague Dawley rats **Test Article:** Darunavir

Species/strain: Rat / Sprague-Dawley
Age/weight at first dose: 8-10 weeks / 217-270 g
Duration of dosing: Gestation days (GD) 7-19
Day of mating: GD1
Day of c-section: GD22
Vehicle/formulation: Polyethylene glycol 400
Special features: study design based on ICH 4.1.3

Administration route/method: Oral gavage
Doses: 0, 40, 200 and 1000 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 96.5% w/w, as ethanolate 98.9% w/w#) and [redacted] (as base: 95% w/w, as ethanolate 102.3% w/w#)

Testing facility: [redacted]
Study no.: TMC114-NC128
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: The maternal NOAEL is 190 mg base equivalent/kg based on the body weight effect. The embryo/fetal NOAEL is 1000 mg /kg, and there is no evidence that TMC114 is teratogenic in rats

Toxicokinetics: Not measured

Noteworthy Findings

Nominal Daily Dose (mg/kg) ^	**0 (vehicle)**	**40**	**200**	**1000**
No. of animals	**F:25**	**F:25**	**F:25**	**F:25**
Maternal Data:				
Died or killed prematurely (accidental)	0	0	0	0
Died or killed prematurely (cause of death not established)	0	0	0	1
Clinical observations	-	-	-	-
Body weight (g) – GD19 $	326	1.00	1.03	0.95*
Body weight (g) – GD22 $	369	1.00	1.04	0.99
Body weight gain (g) – GD1-GD22 $	152	1.00	1.10	0.98
Food consumption (g/GD7-GD19) $	91.6	0.96	1.05	0.90*
Gross pathology (no. examined)	25	25	24	25
No relevant findings	-	-	-	-
No. pregnant females	25	25	25	25
No. aborted or with total resorption of litter	0	0	0	0

$ For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric); # HPLC assay with reference to anhydrous solvent free substance
^ Actual dose: 0, 39, 193 and 965 ([redacted]), or 0, 38, 190 and 950 ([redacted]) mg base eq./kg body weight/day; - No noteworthy findings

2.6.7.13.A Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Study of the effects of TMC114 on embryo-fetal development in Sprague Dawley rats **Test Article:** Darunavir

Noteworthy Findings (Continued)

Nominal Daily Dose (mg/kg) ^	**0 (vehicle)**	**40**	**200**	**1000**
No. of animals	**F:25**	**F:25**	**F:25**	**F:25**
No. females with live fetuses	25	25	24	24
Mean no. corpora lutea	16	16	16	17
Mean no. implantations	13	13	13	14
Mean % preimplantation loss	17	20	13	22
Mean no. live conceptuses	13	13	13	13
Mean no. resorptions	0	0	0	0.3
Mean % postimplantation loss	0.3	0	0	1.8
Litter Data:				
No. litters evaluated	25	25	24	24
No. live fetuses	334	316	320	321
Mean male fetuses (%)	56	52	50	49
Mean fetal bodyweight (g)	5.4	5.6	5.7*	5.4
Fetal abnormalities				
Gross external defects – variations (%)	0	0	0	0
Gross external defects – malformations (%)	0.6	0.3	0.3	0
Visceral body defects – variations (%)	3.1	3.2	2.6	2.6
Visceral body defects – malformations (%)	0.6	0	0	0
Bouin's head defects – variations (%)	0	0.6	0	0
Bouin's head defects – malformations (%)	0	2.6	3.2	2.6
Skeletal defects – variations (%)	8.7	4.3	7.3	7.8
Skeletal defects – malformations (%)	5.8	4.3	1.2	1.2
Total fetal defects (%)	8.4	6.3	7.2	7.2
Total litters affected (%)	56	36	52	50

* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric)

^ Actual dose: 0, 39, 193 and 965 ([redacted]), or 0, 38, 190 and 950 ([redacted]) mg base eq./kg body weight/day

2.6.7.13.B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir

Report Title: Study of the effects of TMC114 on embryo-fetal development in Sprague Dawley rats

Test Article: darunavir

Species/strain: Rat/SPF Sprague-Dawley
Age/weight at pairing: about 9 weeks/ 212 - 285 g
Duration of dosing: GD6-17
Day of mating: GD0
Day of c-section: GD21

Administration route/method: Oral/gavage
Doses: 0/0, 600/100, 1000/100 mg/kg/day RTV/DRV
Test article batch: [redacted]
Vehicle/formulation: darunavir (DRV): Solution in polyethylene glycol 400 (PEG400) + ritonavir (RTV: propylene glycol (PG)

Testing facility: J&J PRD
Study no.: TMC114-NC398/TOX9639
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted]

Special features: None
No Observed Adverse Effect Level: 600/100 mg/kg/day DRV/RTV

Toxicokinetics:

Doses in mg/kg/day	600/100 DRV/RTV	1000/100 DRV/RTV
No. of animals	**6**	**6**
C_{max1} (µg/ml) GD6	9.8	12
C_{max2} (µg/ml) GD6	22	35
AUC_{0-24h} (µg.h/ml) GD6	280	423
C_{max1} (µg/ml) GD11	11	13
C_{max2} (µg/ml) GD11	14	23
AUC_{0-24h} (µg.h/ml) GD11	202	283
C_{max1} (µg/ml) GD12	9.7	35
C_{max2} (µg/ml) GD12	20	24
AUC_{0-24h} (µg.h/ml) GD12	241	312
C_{max2} (µg/ml) GD17	17	23
C_{max12} (µg/ml) GD17	14	24
AUC_{0-t} (µg.h/ml) GD17	261	219

GD = gestation day; DRV= darunavir; RTV = ritonavir

(continued)

2.6.7.13.B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Study of the effects of TMC114 on embryo-fetal development in Sprague Dawley rats **Test Article:** darunavir

Noteworthy Findings

Dose (mg/kg/day)	**0 (PEG400)**	**600 DRV/100 RTV**	**600 DRV/100 RTV**	**1000 DRV/100 RTV**	**1000 DRV/100 RTV**
Administration period (GD)	**GD6-17**	**GD-6-11**	**GD12-17**	**GD-6-11**	**GD12-17**
No. of animals	**24**	**24**	**24**	**24**	**24**
No. Died or Sacrificed Moribund [a,b]	0/24	0/24	1/24	2/24	1/24
No. Aborted or with Total Resorption of Litter [c]	0	1	0	0	0
No. Pregnant [a,c]	23/24	23/24	22/23	21/22	22/23
Clinical Observations [a,b]					
Feces, pale	0/24	23/24***	13/24***	17/24***	15/24***
piloerection	0/24	-	-	4/24*	5/24*
Feces, reduced amount	0/24				5/24*
Body Weight Gain (g) [c,d]					
(GD 6 - 7)	3.8	-5.9***	-	-1.2	-
(GD 8- 9)	7.2	11.3*	-	11.6	-
(GD 10 - 11)	10.8	8.4	13.8	5.3	13.0
(GD 12 - 13)	7.3	-6.6*	-4.5***	-3.0**	-2.9**
(GD 14 - 15)	18.9	-	10.0*	-	17.0
(GD 16 - 17)	26.6	-	17.7**	-	15.1**
(GD 18 - 20)	49.4	51.0	31.5*	52.4	23.5**
Corrected Mean Maternal Weight Gain [c,e]	19.6	20.0	2.2**	22.0	-2.9**
Food Consumption (g) [c,f]					
(GD6 – 9)	81	54***	104***	56***	100***
(GD10 – 11)	45	32***	54***	33***	52***
(GD12 – 13)	48	37***	23***	40**	24***
(GD14 – 17)	100	114***	61***	119***	63***
(GD18 – 20)	93	98*	68***	96	63***

[a] Number of positive animals / total number of animals. For the number pregnant, this is the number of pregnant rats / total number of animals alive at the time of c-section.
[b] Statistical analysis method used: Fisher Exact test; [c] Statistical analysis method used: Mann-Whitney U test.
[d] Finding recorded during the interval shown. Group means are show; [e] Corrected mean maternal body weight gain = body weight change (Day 21 minus Day 6) minus the gravid uterus weight.; GD = gestation day; DRV= darunavir; RTV = ritonavir; - No noteworthy findings * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

2.6.7.13.B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Oral Developmental Toxicity Study in the Rat **Test Article:** darunavir

Dose (mg/kg/day)	**0 (PEG400)**	**600 DRV/100 RTV**	**600 DRV/100 RTV**	**1000 DRV/100 RTV**	**1000 DRV/100 RTV**
Administration period (GD)	**6-17**	**6-11**	**12-17**	**6-11**	**12-17**
No. of animals	**24**	**24**	**24**	**24**	**24**
Noteworthy Findings (Continued)					
Litters: No. of Litters Evaluated [b]	23	23	22	21	22
Mean No. Corpora Lutea of Pregnancy [d]	16.7	15.8	16.9	17.2	17.9
Mean No. Implantations [d]	14.4	13.9	14.9	14.0	15.5
Mean % Preimplantation Loss [d]	11.13	7.45	10.63	18.68	10.48
No. Live Fetuses [b]					
Mean No. Live Fetuses [b]	14.0	13.3	14.2	13.2	15.0
Mean No. Early Resorptions [b]	0.35	0.61	0.64	0.71	0.50
Mean No. Late Resorptions [b]	0.04	0.00	0.05	0.00	0.00
Mean No. Total Resorptions [b]	0.39	0.61	0.68	0.71	0.50
No. Dead Fetuses [b]	-	-	-	-	-
Mean % Postimplantation Loss [b]	2.53	8.31	4.22	7.95	3.18
Mean Fetal Body Weight (g) [b]	5.71	5.31*	4.79***	5.24**	4.48***
Fetal Sex Ratios [b, d]	-	-	-	-	-
Fetal Anomalies: [a]					
Gross External	-	-	-	-	-
Visceral Anomalies	-	-			-
Thymus: incompletely descended					
No. Fetuses (%) [e]	7	31***	14	28***	13
No. Litters (%)	7	17**	9	15**	9

[a] Statistical analysis method used: Fisher Exact probability test.
[b] Statistical analysis method used: Mann-Whitney U test.
[c] Skeletal examinations were conducted in 50% of fetuses.
[d] expressed as % male fetuses
[e] Mean % fetuses affected per litter.

- No noteworthy findings, GD = gestation day; DRV= darunavir; RTV = ritonavir
* - p<0.05, ** - p<0.01, *** - p<0.001

2.6.7.13.B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Oral Developmental Toxicity Study in the Rat **Test Article:** darunavir

Dose (mg/kg/day)	**0 (PEG400)**	**600 DRV/100 RTV**	**600 DRV/100 RTV**	**1000 DRV/100 RTV**	**1000 DRV/100 RTV**
Administration period (GD)	**6-17**	**6-11**	**12-17**	**6-11**	**12-17**
No. of animals	**24**	**24**	**24**	**24**	**24**
Noteworthy Findings (Continued)					
Litters (Continued)**:**					
Fetal Anomalies (continued):[a]					
Skeletal Anomalies [b]					
Supra-occipital bone: incomplete ossification					
No. Fetuses (%) [c]	1	-	-	5*	-
No. Litters (%)	1	-	-	5*	-
Cervical vertebra(e) centrum not ossified ≥ 4					
No. Fetuses (%)[c]	32	65***	45*	64***	59***
No. Litters (%)	12	18*	17*	19**	16
Ventral tubercle: not ossified					
No. Fetuses (%)[c]	29	45**	16	36*	31
No. Litters (%)	12	17	9	12	14
Thoracic vertebra(e): centrum bipartite					
No. Fetuses (%)[c]	0	6**	3	8***	3
No. Litters (%)	0	5*	2	4*	3
Thoracic vertebra(e): centrum not ossified					
No. Fetuses (%)[c]	0 (0)	-	-	6**	-
No. Litters (%)	0 (0)	-	-	2	-
Lumbar vertebr(e): centrum dumbbell shaped					
No. Fetuses (%)[c]	0 (0)	-	-	3*	-
No. Litters (%)	0 (0)	-	-	1	-

[a] Statistical analysis method used: Fisher Exact probability test.
[b] Skeletal examinations were conducted in 50% of fetuses.
[c] Mean % fetuses affected per litter.

GD = gestation day; DRV= darunavir; RTV = ritonavir
* - p<0.05, ** - p<0.01, *** - p<0.001

(continued)

2.6.7.13.B Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Oral Developmental Toxicity Study in the Rat **Test Article:** darunavir

Dose (mg/kg/day)	**0 (PEG400)**	**600 DRV/100 RTV**	**600 DRV/100 RTV**	**1000 DRV/100 RTV**	**1000 DRV/100 RTV**
Administration period (GD)	**6-17**	**6-11**	**12-17**	**6-11**	**12-17**
No. of animals	**24**	**24**	**24**	**24**	**24**
Noteworthy Findings (Continued)					
Litters (Continued)**:**					
Fetal Anomalies (continued):[a]					
Skeletal Anomalies (continued)[b]					
Sternum bones: 6th incomplete ossification					
No. Fetuses (%)[c]	0	15***	8**	10***	21***
No. Litters (%)	0	8***	6**	5**	7**
Sternum bones: 5th not ossified					
No. Fetuses (%)[c]	0 (0)	7**	2	3*	7**
No. Litters (%)	0 (0)	5*	2	3	5*
Sternum bones: 5th rudimentary					
No. Fetuses (%)[c]	6	13*	12	7	25***
No. Litters (%)	4	7	8	5	11*
Sternum bones:rudimentary other than 5th					
No. Fetuses (%)[c]	0	3	1	3*	5*
No. Litters (%)	0	2	1	3	3
Metatarsal bones: not ossified >1					
No. Fetuses (%)[c]	3	27***	22***	26***	37***
No. Litters (%)	2	11**	12***	12***	11**
Metatarsal bones: 1 not ossified					
No. Fetuses (%)[c]	2	8*	10**	11**	5
No. Litters (%)	2	5	5	5	5
Total Affected Fetuses (Litters)					
No. Fetuses (%)[c]	22 (41.1)	27 (57.5)			30 (62.2)
No. Litters (%)	11 (91.7)	9 (90.0)			6 (100.0)

[a] Statistical analysis method used: Fisher Exact probability test.
[b] Skeletal examinations were conducted in 50% of fetuses.
[c] Mean % fetuses affected per litter.; * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$

2.6.7.13.C Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir

Report Title: Study of the effect of TMC114 on embryo-fetal development in New Zealand White rabbits **Test Article:** Darunavir

Species/strain: Rabbit / New Zealand White
Age/weight at first dose: 8-10 weeks / 3109-4418 g
Duration of dosing: Gestation days (GD) 8-20
Day of mating: GD1
Day of c-section: GD30
Special features: study design based on ICH 4.1.3

Administration route/method: Oral intubation
Doses: 0, 40, 200 and 1000 mg/kg body weight/day
Test article batch (purity): [redacted] (as base: 94% w/w, as ethanolate: 99.6% w/w)
Vehicle/formulation: CMC/Tween 80

Testing facility: [redacted]
Study no.: TMC114-NC126
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: The maternal and embryo/fetal NOAEL is 1000 mg/kg based on the absence of any adverse effects.

Toxicokinetics:

Nominal Daily dose (mg/kg) ^	**1000**
No. of animals	**F:3**
Maternal plasma concentration (range) (µg/mL)*	0.089 – 1.52
Fetal plasma concentration (range) (µg/mL)	NQ – 0.065

* at approximately 2 hours after dosing; NQ not quantifiable

Noteworthy Findings:

Nominal Daily dose (mg/kg) ^	**0 (vehicle)**	**40**	**200**	**1000**
No. of animals	**F:20**	**F:20**	**F:20**	**F:20**
***Maternal Data*:**				
Died or killed prematurely (accidental)	0	0	0	0
Died or killed prematurely (cause of death not established)	0	1	0	1
Clinical observations	-	-	-	-
Body weight (g) – GD20[$]	3970	0.98	0.98	0.95
Body weight (g) – GD30[$]	4057	0.98	0.98	0.97
Food consumption (g/GD7-19)	-	-	-	-
Gross pathology (no. examined)	20	19	20	19
No relevant findings	-	-	-	-

[$] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric); [#] HPLC assay with reference to anhydrous solvent free substance
^ Actual dose [redacted]: 0, 38, 188, 940 mg base eq./kg/day; - No noteworthy changes

2.6.7.13.C Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Study of the effect of TMC114 on embryo-fetal development in New Zealand White rabbits **Test Article:** Darunavir

Noteworthy Findings (Continued)

Nominal Daily dose (mg/kg) ^	**0 (vehicle)**	**40**	**200**	**1000**
No. of animals	**F:20**	**F:20**	**F:20**	**F:20**
No. pregnant females	17	19	19	19
No. aborted or with total resorption of litter	0	0	0	2
No. females with live fetuses	17	18	19	16
Mean no. corpora lutea	11	12	11	11
Mean no. implantations	9	9	9	9
Mean % preimplantation loss	16	20	18	18
Mean no. live conceptuses	8	9	8	9
Mean no. resorptions – early	0.3	0.4	0.1	0.3
Mean no. resorptions – late	0.1	0	0.5	0.1
Mean % postimplantation loss	4.7	4.1	6.6	3.5
***Litter Data*:**				
No. litters evaluated	17	18	19	16
No. live fetuses	142	162	156	137
Mean male fetuses (%)	54	52	46	48
Mean fetal bodyweight (g)	41.3	40.4	41.0	41.6
Fetal abnormalities				
Gross external defects – variations (%)	0	0	0	0
Gross external defects – malformations (%)	0	0.6	0	0
Visceral body defects – variations (%)	0	0	0	0
Visceral body defects – malformations (%)	0.7	1.2	0.6	0.7
Skeletal defects – variations (%)	19.7	26.5	22.4	21.9
Skeletal defects – malformations (%)	14.8	14.2	14.7	13.9

Absolute numbers given. Statistical significance based on actual data.
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$ (ANOVA +/- non-parametric)
^ Actual dose [redacted]: 0, 38, 188, 940 mg base eq./kg/day

2.6.7.13.D Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir

Report Title: Oral (gavage) developmental toxicity study in the mouse **Test Article:** Darunavir

Species/strain: Mouse / CD-1
Age/weight at first dose: ~10 weeks / 25-35 g
Duration of dosing: GD6 – GD15
Day of mating: GD1
Day of c-section: GD18

Administration route/method: Oral gavage
Doses: 0 (vehicle), 150/0, 450/0, 1000/0, 1000/50 and 0/50 mg/kg TMC114/RTV
Test article batch (purity): [redacted] (as base 93 w/w, as ethanolate 98.6% w/w[#])
Vehicle/formulation: For TMC114, polyethylene glycol 400. For RTV, propylene glycol

Testing facility: [redacted]
Study no.: TMC114-NC172
Location in CTD: 4.2.3.5.2
GLP compliance: Yes
Date start of study: [redacted]

Special features: study design based on ICH 4.1.3
No Observed Adverse-Effect Level: F_0 Females (dams): 1000 mg/kg/day based on no maternal toxicity and **F_1 Litters:** 1000 mg/kg/day based on the absence of any relevant changes

Toxicokinetics[b] TMC114

Daily Dose (mg base equivalent/kg/day)	0 (vehicle)	150/0	450/0	1000/0	1000/50	0/50
No. of animals	F:8	F:10	F:10	F:10	F:10	F:10
Died or killed prematurely (accidental)	0	0	0	0	1	1
$AUC_{0-\infty}$ GD6 (µg.h/mL)	-	24.2	69.8**	122	158	-
AUC_{0-24h} GD15 (µg.h/mL)	-	12.5	27.6**	63.9	81.6	-

Toxicokinetics[b] RTV

Daily Dose (mg base equivalent/kg/day)	0 (vehicle)	150/0	450/0	1000/0	1000/50	0/50
No. of animals	F:8	F:10	F:10	F:10	F:10	F:10
Died or killed prematurely (accidental)	0	0	0	0	1	1
AUC_{0-24h} GD6 (µg.h/mL)	-	-	-	-	41.6*	37**
AUC_{0-24h} GD15 (µg.h/mL)	-	-	-	-	13.1	42.4**

* due to high volume (9.21 µg/mL) for one animal at 24 hours; ** AUC_{0-8h}
[a] includes two females that littered, [b] includes one female that littered , GD = gestation day
[#] HPLC assay with reference to anhydrous solvent free substance

2.6.7.13.D Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Oral (gavage) developmental toxicity study in the mouse **Test Article:** Darunavir

Noteworthy Findings

Daily Dose (mg base equivalent/kg/day)	**0 (vehicle)**	**150/0**	**450/0**	**1000/0**	**1000/50**	**0/50**
No. of animals	F:30	F:30	F:30	F:30	F:30	F:30
Maternal data						
Died or killed prematurely (accidental)	0	0	1	1	2	0
Died or killed prematurely (cause of death not established)				1		
No. pregnant (%)	25 (83.3)	30 (100)	30 (100)	27 (90.0)	28 (93.3)	28 (93.3)
With live fetuses at scheduled kill	25	30 [a]	28 [b]	25	26 [b]	27

2.6.7.13.D Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Darunavir (Continued)

Report Title: Oral (gavage) developmental toxicity study in the mouse **Test Article:** Darunavir

Noteworthy Findings (Continued)

Daily Dose (mg base equivalent/kg/day)	**0 (vehicle)**	**150/0**	**450/0**	**1000/0**	**1000/50**	**0/50**
No. of animals	F:30	F:30	F:30	F:30	F:30	F:30
Body weight (g)[c]						
Day 6-15 body weight gain	17.0	0.98	0.99	1.01	0.95	0.84
Food consumption (g/GD6-GD15)[c]	22.3	0.94	0.90	1.05	0.89	1.07
Mean no. implantations	14.2	14.6	14.1	14.4	13.8	13.3
Number of early fetal deaths	13	18	21	28	19	29
Number of late fetal deaths	3	12	8	5	14	5
Mean % post-implantation Loss	4.8	8.2	10.6	9.5	9.7	11.6
Mean number live fetuses per female	13.5	13.4	13.1	13.1	12.4	12.0
Litter data						
No. litters evaluated	25	28	27	25	25	27
Mean fetal weight (g)	1.42	1.39	1.38	1.41	1.36	1.38
Mean placental weight	0.125	0.119	0.119	0.127	0.129	0.129
Fetal Anomalies (external/visceral/skeletal)	-	-	-	-	-	-

[c] For controls, group means are shown. For treated groups, the multiples of vehicle/baseline are shown.
[d] = statistically analysed; group 1 vs 2-6 *p<0.05 **p<0.01 (Cochran Armitage Trend test and Fisher's Exact Test)
[e] Number of litter
[f] Percentage of fetuses affected
- No treatment related changes

2.6.7.13.E Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Cobicistat

Test Article: Cobicistat

Report Title: Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rats		**Gilead Study No.** TX-216-2020 ([redacted] 6511-357)
Species / Strain: Rat/Crl:CD(SD)	**Duration of Dosing:** GD 6-17	**Location in CTD:** 4.2.3.5.2
Initial Age: 11 to 12.5 Weeks	**Day of Mating:** GD 0	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Day of C-Section:** GD 20	**Lot Number:** 3168-157-8
Special Features: None	**Method of Administration:** Oral gavage	**Design Similar to ICH 4.1.3** Yes
No Observed Adverse Effect Level: F_0 Females = 50 mg/kg/day **No Observed Effect Level:** F_1 Litters = 50 mg/kg/day	**Vehicle/Formulation:** 95% propylene glycol and 5% ethanol with 1.5 eq. HCl (pH 2.3)	

Dose (mg/kg/day)		**0 (Vehicle)**	**25**	**50**	**125**
Dams:	No. of Animals - Toxicokinetics	3	9	9	9
	C_{max} (µg/mL) – GD 6	ND	1.43	2.69	5.06
	C_{max} (µg/mL) – GD 17	ND	0.922	1.65	4.19
	AUC_{0-t} (µg.h/mL) – GD 6	ND	9.95	27.1	57.9
	AUC_{0-t} (µg.h/mL) – GD 17	ND	6.66	14.8	61.4
	No. Pregnant	24	25	25	25
	No. Died or Sacrificed Moribund	0	0	0	0
	No. Aborted or with Total Resorption of Litter	0	0	0	0
	Clinical Observations				
	Clear oral discharge	0	5	11	20
	Thin; Audible/irregular respiration; hypoactivity; alopecia; rough haircoat; fecal changes	Transiently observed findings in animals given 125 mg/kg/day only.			
	Necropsy Observations	No treatment-related findings.			
	Body Weight GD 18 (%)[a]	341	+0.2	+0.09	-15**
	Food Consumption GD 6-18 (%)[a]	26.6	-1.5	-4.9*	-34**
	Mean No. Corpora Lutea	14.5	14.3	14.5	14.6
	Mean No. Implantations	13.8	13.0	13.5	13.4
	Mean % Preimplantation Loss	4.3	10.0	6.2	8.0

Test Article: Cobicistat

Report Title: Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rats				**Gilead Study No. TX-216-2020 (** [redacted] 6511-357)	
Litters:	No. Litters Evaluated	24	25	25	25
Dose (mg/kg/day)		**0 (Vehicle)**	**25**	**50**	**125**
Litters:	No. Live Fetuses	13.3	12.5	12.8	12.2
	Mean No. Resorptions	0.5	0.5	0.7	1.2
	No. Dead Fetuses	0	0	0	0
	Mean % Postimplantation Loss	3.2	4.4	5.1	9.6
	Mean Fetal Body Weight (g)	3.78	3.90	3.83	3.28**
	Fetal Sex Ratios (% males)	46.5	48.5	49.5	47.2
	Fetal Anomalies	No treatment-related Gross or Visceral Anomalies.			
	Skeletal Anomalies				
	Spinal column: Less than 4 cauda vertebrae ossified				
	No. Fetuses (%)	39 (24)	34 (22)	40 (25)	67 (45**)
	No. Litters (%)	13 (54)	14 (56)	15 (60)	19 (76)
	Sternebrae: 5th sternebra unossified				
	No. Fetuses (%)	39 (24)	37 (24)	47 (29)	58 (39**)
	No. Litters (%)	16 (67)	14 (56)	19 (76)	18 (72)
	Sternebrae: 6th sternebra unossified				
	No. Fetuses (%)	4 (2.5	1 (0.6)	6 (3.7)	21 (14**)
	No. Litters (%)	2 (8.3)	1 (4.0)	4 (16)	10 (40**)
	Sternebrae: Other sternebra unossified				
	No. Fetuses (%)	1 (0.6)	2 (1.3)	2 (1.2)	16 (11**)
	No. Litters (%)	1 (4.2)	2 (8.0)	2 (8.0)	8 (32**)
	Sternebrae: Other sternebra incomplete ossification				
	No. Fetuses (%)	4 (2.5)	7 (4.5)	8 (5.0)	16 (11**)
	No. Litters (%)	3 (13)	3 (12)	6 (24)	10 (40**)

a At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

GD = gestation day; ND = none detected; * = $p<0.05$; ** = $p<0.01$ (Data from Dams evaluated using Dunnett's Test; Data from Litters evaluated using Fisher Exact Test)

2.6.7.13.F Reproductive and Developmental Toxicity-Effects on Embryo-Fetal Development- Cobicistat

Test Article: COBI

Report Title: Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rabbits		**Gilead Study No. TX-216-2021** ([redacted] 6511-359)
Species / Strain: Rabbit/Hra:(NZW)	**Duration of Dosing:** GD 7 to 20	**Location in CTD:** 4.2.3.5.2
Initial Age: 5 Months	**Day of Mating:** GD 0	**GLP Compliance:** Yes
Date of First Dose: [redacted]	**Day of C-Section:** GD 29	**Lot Number:** 3168-171-16
Special Features: None	**Method of Administration:** Oral gavage	**Design Similar to ICH 4.1.3** Yes
No Observed Adverse Effect Level: F_0 Females = 100 mg/kg/day **No Observed Effect Level:** F_1 Litters = 100 mg/kg/day	**Vehicle/Formulation:** 95% propylene glycol and 5% ethanol with 1.5 eq. HCl (pH 2.3)	

Dose (mg/kg/day)		**0 (Vehicle)**	**25**	**50**	**100**
Dams:	No. of Animals - Toxicokinetics	3	3	3	3
	C_{max} (µg/mL) – GD 7	ND	0.037	0.190	2.75
	C_{max} (µg/mL) – GD 20	ND	0.717	4.59	3.02
	AUC_{0-t} (µg.h/mL) – GD 7	ND	0.042	0.237	10.6
	AUC_{0-t} (µg.h/mL) – GD 20	ND	1.59	19.2	35.7
	No. Pregnant	18	18	15	18
	No. Died or Sacrificed Moribund	1	0	2	0
	No. Aborted or with Total Resorption of Litter	0	0	1	0
	Clinical Observations	No treatment-related findings.			
	Necropsy Observations	No treatment-related findings.			
	Body Weight GD 21 (%)[a]	3484.5	-2	+1	-3
	Food Consumption GD 7-21 (%)[a]	147.0	+8	-9	-16
	Mean No. Corpora Lutea	9.6	8.5	9.6	9.6
	Mean No. Implantations	9.1	7.9	9.1	9.1
	Mean % Preimplantation Loss	4.3	5.4	4.9	5.0

Test Article: COBI

Report Title: Oral Gavage Study for Effects on Embryo-fetal Development with GS-9350 in Rabbits	**Gilead Study No. TX-216-2021** ([redacted] 6511-359)

Dose (mg/kg/day)		**0 (Vehicle)**	**25**	**50**	**100**
Litters:	No. Litters Evaluated	17	18	14	18
	No. Live Fetuses	8.9	7.7	7.9	8.4
	Mean No. Resorptions	0.2	0.3	1.1	0.2
	No. Dead Fetuses	0	0	0	9
	Mean % Postimplantation Loss	1.6	3.2	11.3	6.4
	Mean Fetal Body Weight (g)	39.7	39.1	41.4	39.5
	Fetal Sex Ratios (% males)	38.8	46.7	48.6	52.3
	Fetal Anomalies	No treatment-related Gross, Visceral or Skeletal Anomalies.			

a At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

GD = gestation day; ND = none detected; * = $p<0.05$; ** = $p<0.01$ (Dunnett's Test)

2.6.7.14.A Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir: non-GLP study

Species/Strain Sex/No. Per Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Nominal dose, mg/kg/day (Actual dose, mg b.e./kg/day)	Batch no. (purity)	NOAEL[a] (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/ Sprague-Dawley 6 F/group	Oral/gavage (PEG400)	F: Day 6 of gestation to day 7 of lactation F1[a]: day 12 to day 25 of age[e]	40, 200, 1000, vehicle, vehicle Expressed as mg b.e/kg/day	[redacted] (as base: 93% w/w; as ethanolate: 98.6%)	Females: 200 F1: 40 Not established	2 Dams found dead (F, vehicle; F, 200 mg/kg) *Females:* 40: Salivation (6/6) 200: Salivation (6/6), ↓ pup BW gain (M, 29%; F, 22%) 1000: Salivation (6/6), ↓ activity with low posture (6/6), ↓ BW gain (40%, GD 6-9; 71%, LD 1-4), ↓ food consumption (18%, GD 6-9), ↓ pup BW gain (M, 27%; F, 31%) *F1dosing:* 40: mortalities 5/16 All distended abdomen, unsteady gait from LD 15 200: mortalities 6/16 All distended abdomen unsteady gait from LD 15	TMC114-NC178/ 4.2.3.5.3

[a] Pups were dosed once only on Day 12 of age at 1000 mg/kg/day and following deaths and adverse findings the remaining animals were sent to necropsy on Day 12 of age. Another set of pups were dosed once only on Day 12 of age at 500 mg/kg/day and following deaths the remaining animals were sent to necropsy on Day 13 of age. No data presented for these groups.

b.e. = base equivalent, BW = body weight, F = female, PEG = polyethylene glycol, GD = gestation day, LD = lactation day

2.6.7.14.B Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat **Test Article(s):** Darunavir

Species/strain: Crl:CD (SD) IGS BR VAF PLUS
Initial Age: 9 – 10 Weeks
Duration of Dosing: GD6 to LD20
Day of Mating: GD0
Vehicle/Formulation: TMC114: PEG 400, **Ritonavir:** solution in propylene glycol
Administration route/method: Oral gavage
Doses: 0/0, 40/0, 200/0, 1000/0, 1000/75 (50), 1000/50, 0/75 (50) mg base equivalent/kg/day TMC114/Ritonavir
Test article batch (purity): [redacted] (as base: 93% w/w; as ethanolate 98.6% w/w)
Litters Culled: Yes
Testing facility: [redacted]
Study no.: TMC114-NC156
Location in CTD: 4.2.3.5.3
GLP Compliance: Yes
Date of First Dose: [redacted]

Special Features: study design based on ICH 4.1.3
NOAEL for TMC114 administered alone: F0 Females: 40 mg base equivalent/kg/day; **F1 Males:** 40 mg base equivalent/kg/day; **F1 Females:** 40 mg base equivalent/kg/day

Daily Dose TMC114 (mg base equivalent/kg/day)	Control	40	200	1000
F0 Females: No. of Animals	**F: 25**	**F: 25**	**F: 25**	**F: 25**
No. Pregnant	23	22	21	23
No. Died or Sacrificed Moribund	0	0	1	1
No. Aborted or With Total Resorption of Litter	0	0	0	0
Clinical Observations				
Excessive salivation post-dose	-	-	-	x
Hairstaining	-	-	-	x
Piloerection	-	-	-	x
Necropsy Observations	-	-	-	-
Gestation Body Weight [a]				
Day 6	249	1.02	1.02	1.02
Day 20	367	1.02	1.04	0.97
Day (6-20) gain	118	1.02	1.08	0.87
Lactation Body Weight [a]				
Day 1	288	1.02	1.03	0.94*
Day 21	315	1.04	1.04	0.95*
Day (1-21)	27	1.19	1.07	1.00
Gestation Food Consumption [a]				
Day (6-9)	26	1.00	0.92	0.85***
Day (9-12)	28	1.00	1.00	0.89**
Day (12-15)	28	1.04	1.07	0.96
Day (15-20)	30	1.03	1.07	0.93

[a] = For Controls, group means are shown. For treated groups, noteworthy findings are expressed as multiples of control; GD = gestation day, LD = lactation day, x = sign present
Statistically significant from Controls: * p ! 0.05 ** p ! 0.01 *** p ! 0.001; - = no noteworthy findings

2.6.7.14.B Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat **Test Article(s):** Darunavir

Noteworthy Findings (Continued)

Daily Dose TMC114 (mg base equivalent/kg/day)	**Control**	**40**	**200**	**1000**
F0 Females: no. of animals	**F: 25**	**F: 25**	**F: 25**	**F: 25**
Lactation Food Consumption [a]				
Day (1-4)	39	1.13	1.05	0.85**
Day (4-7)	49	1.04	1.00	0.76***
Day (7-10)	59	0.98	0.90	0.71***
Day (10-14)	68	1.01	0.93*	0.69***
Mean Duration of Gestation (days)	21.2	21.2	21.0	21.1
Abnormal Parturition	-	-	-	-
F1 Litters (Preweaning) **No. Litters Evaluated**	**23**	**22**	**21**	**22**
Mean No. of Implantations	13	13	13	13
Mean No. Pups/Litter	12.1	12.3	12.3	12.5
Mean live birth index (%)	99.7	99.7	98.9	100.0
Postnatal Survival to Day 4 (%)	98.5	98.9	98.7	97.9
Postnatal Survival to Weaning – Day 21 (%)	98.9	94.3	93.5	94.3
No. of Total Litter Losses	0	0	0	0
Change in Pup Body Weights (g) [a]				
Males Day (1-21)	46.1	1.01	0.96	0.62***
Females Day (1-21)	45.3	1.01	0.96	0.64 ***
Pup Sex Ratios (% male)	55.7	47.8	49.6	44.0
% pups with ears open on Day 3	24.4	19.8	10.6	11.6 *
% pups with eyes open on Day 15	84.4	77.8	64.9**	28.5***
Pup Clinical Signs	-	-	-	-
Pup Necropsy Observations	-	-	-	-
F1 Males (Postweaning) **No. Evaluated Postweaning**	**20**	**20**	**20**	**20**
No. Died or Sacrificed Moribund	1	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-

[a] = For Controls, group means are shown. For treated groups, noteworthy findings are expressed as multiples of control; [b] = Sensory function – Preyer reflex, Motor Activity – Rotarod, Learning and memory - E-maze; Statistically significant from Controls: * p ! 0.05 ** p ! 0.01 *** p ! 0.001; - = no noteworthy findings

2.6.7.14.B Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat **Test Article(s):** darunavir

Daily Dose TMC114 (mg base equivalent/kg/day)	Control	40	200	1000
F1 Males (Postweaning) (Continued): No evaluated	**20**	**20**	**20**	**20**
Body Weight Change (g) [a]				
Week 1	81	1.05	0.99	0.78***
Week 11	484	1.10	1.05	0.92**
Preputial Separation (Day of age)	45.1	43.2	43.3	45.0
Pup Development and Learning Post Weaning[b]	-	-	-	-
No. of Males that Mated	19	20	20	20
No. of Fertile Males	17	20	20	20
F1 Females (Postweaning)				
No. Evaluated Postweaning	**20**	**20**	**20**	**20**
No. Died or Sacrificed Moribund	1	0	0	0
Clinical Observations	-	-	-	-
Necropsy Observations	-	-	-	-
Premating Body Weight Change [a]				
Week 1	74	1.05	1.01	0.82***
Week 7	246	1.03	1.01	0.95
Gestation Body Weight Change [a]				
Day 0	256	1.02	1.00	0.93*
Day 7	287	1.03	1.03	0.96
Day 13	317	1.04	1.04	0.97
Day (0-7) gain	31	1.16*	1.29**	1.19**
Day (7-13) gain	30	1.07	1.07	1.07
Mean Age of Vaginal Patency (days)	35.2	33.6	34.4	34.9
Pup Development and Learning Post Weaning[c]	-	-	-	-
No. of Females Sperm-Positive	19	20	20	20
No. of Pregnant Females	18	20	20	20
Mean No. Corpora Lutea	16.3	16.5	16.8	17.2
Mean No. Implantations	15.6	16.0	15.6	16.0
Mean % Preimplantation Loss	4.3	3.2	7.2	5.9
Mean % Postimplantation Loss	5.0	4.3	8.0	2.3
Mean Number Live Embryos Per Female	14.8	15.3	14.6	15.6

[a] = For Controls, group means are shown. For treated groups, noteworthy findings are expressed as multiples of control; [b] = Sensory function – Preyer reflex, Motor Activity – Rotarod, Learning and memory - E-maze; Statistically significant from Controls: * p ! 0.05 ** p ! 0.01 *** p ! 0.001; - = no noteworthy findings

2.6.7.14.B Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat **Test Article(s):** Darunavir

Daily Dose TMC114/Ritonavir (mg base equivalent/kg/day)	**Control**	**1000/0**	**1000/75 (50)**	**1000/50**	**0/75 (50)**
F0 Females: No. of Animals	**F: 25**	**F: 25**	**F: 12**	**F: 13**	**F: 25**
No. Pregnant	23	23	11	11	18
No. Died or Sacrificed Moribund	0	1	1	1	0
No. Aborted or with Total Res. Of Litter	0	0	0	0	0
Clinical Observations					
Excessive salivation post-dose	-	x	x	x	x
Hairstaining	-	x	x	x	-
Piloerection	-	x	x	x	x
Necropsy Observations	-	-	-	-	-
Gestation Body Weight [a]					
Day 6	249	1.02	0.99	1.03	1.00
Day 20	367	0.97	0.86**	0.92	0.98
Day (6-20) gain	118	0.87	0.50	0.67	0.96
Lactation Body Weight [a]					
Day 1	288	0.94*	0.86***	0.93	0.95
Day 21	315	0.95*	1.01	1.07	1.00
Day (1-21)	27	1.00	2.56	2.56	1.44
Gestation Food Consumption [a]					
Day (6-9)	26	0.85***	0.73***	0.77***	0.85***
Day (9-12)	28	0.89***	0.68***	0.82**	0.89
Day (12-15)	28	0.94	0.64***	0.86*	0.93
Day (15-20)	30	1.03	0.67***	0.77**	0.87
Lactation Food Consumption [a]					
Day (1-4)	39	0.85***	0.85**	1.08	0.92
Day (4-7)	49	0.76***	0.80***	1.00	0.90**
Day (7-10)	59	0.71***	0.68***	0.92	0.83***
Day (10-14)	68	0.69***	0.65***	0.96	0.81***
Mean Duration of Gestation (days)	21.2	21.1	21.4	21.1	21.2
Abnormal Parturition	-	-	-	-	-

[a] = For Controls, group means are shown. For treated groups, noteworthy findings are expressed as multiples of control; x = sign present
Statistically significant from Controls: * p ! 0.05 ** p ! 0.01 *** p ! 0.001; - = no noteworthy findings

2.6.7.14.B Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat **Test Article(s):** Darunavir

Noteworthy Findings (Continued)

Daily Dose TMC114/Ritonavir (mg base equivalent/kg/day)	Control	1000/0	1000/75 (50)	1000/50	0/75 (50)
F1 Litters (Preweaning): No. evaluated:	**23**	**22**	**10**	**10**	**18**
Mean No. of Implantations	13	13	14	13	14
Mean No. Pups/Litter	12.1	12.5	12.9	12.2	12.8
Mean live birth index (%)	99.7	100.0	98.4	98.3	98.5
Postnatal Survival to Day 4 (%)	98.5	97.9	71.2 **	95.5	99.5
Postnatal Survival to Weaning – Day 21 (%)	98.9	94.3	93.8	74.5 ***	84.7 **
No. of Total Litter Losses	0	0	2	0	
Change in Pup Body Weights (g) [a]					
Males (Day 1 to 21)	46.1	0.62***	0.53***	1.01	0.81***
Females (Day 1 to 21)	45.3	0.64***	0.54***	1.03	0.83***
Pup Sex Ratios (% male)	55.7	44.0	46.6	47.1	46.6
% of pups with startle response Day 15	100.0	99.4	43.3***@@@	100.0	98.6
% of pups with eyes open Day 15	84.4	28.5***	8.9***	91.3@@@	72.9
Pup Clinical Signs	-	-	-	-	-
Pup Necropsy Observations	-	-	-	-	-
F1 Males (Postweaning): No. evaluated:	**20**	**20**	**13**	**20**	**20**
No. Died or Sacrificed Moribund	1	0	0	0	0
Clinical Observations	-	-	-	-	-
Necropsy Observations	-	-	-	-	-
Body Weight Change [a]					
Week 1	81	0.78***	-	-	0.86
Week 3	193	0.83	0.72***	0.98	0.91*
Week 11	484	0.92**	0.87***	1.01	0.98
Preputial Separation (Day of age)	45.1	45.0	46.6	43.6	44.2
Pup Development and Learning Post Weaning [b]	-	-	-	-	-
No. of Males that Mated	19	20	13	20	18
No. of Fertile Males	17	20	13	20	18

[a] = For Controls, group means are shown. For treated groups, noteworthy findings are expressed as multiples of control; [b] = Sensory function – Preyer reflex, Motor Activity – Rotarod, Learning and memory - E-maze; Statistically significant from Controls: * p ! 0.05 ** p ! 0.01 *** p ! 0.001; Statistically significant from Group dosed at 1000 mg/kg/day TMC114: @@@ p ! 0.001; - = no noteworthy findings

2.6.7.14.B Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Darunavir (Continued)

Report Title: Oral (Gavage) Pre- and Post-natal Developmental Toxicity Study in the Rat **Test Article(s): D**arunavir

Noteworthy Findings (Continued)

Daily Dose TMC114/Ritonavir (mg base equivalent/kg/day)	Control	1000/0	1000/75 (50)	1000/50	0/75 (50)
F1 Females (Postweaning)					
No. Evaluated Postweaning	**20**	**20**	**13**	**20**	**20**
No. Died or Sacrificed Moribund	1	0	0	0	0
Clinical Observations	-	-	-	-	-
Necropsy Observations	-	-	-	-	-
Premating Body Weight Change [a]					
Week 1	74	0.82***	-	0.99	0.91
Week 3	151	0.90	0.78***	1.01	0.94
Week 7	246	0.95	0.88	1.02	0.97
Gestation Body Weight Change [a]					
Day 0	256	0.93*	0.87***	1.01	0.97
Day 7	287	0.96	0.91**	1.04	1.00
Day 13	317	0.97	0.93*	1.04	1.00
Day (0-7) gain	31	1.19**	1.16	1.29**	1.23*
Day (7-13) gain	30	1.07	1.17	1.03	1.03
Mean Age of Vaginal Patency (days)	35.2	34.9	-	34.4	33.9
Pup development and Learning Post Weaning [b]	-	-	-	-	-
No. of Females Sperm-Positive	19	20	13	20	20
No. of Pregnant Females	18	20	13	20	20
Mean No. Corpora Lutea	16.3	17.2	15.7	17.0	17.1
Mean No. Implantations	15.6	16.0	15.4	14.8	16.2
Mean % Preimplantation Loss	4.3	5.9	2.1	12.5	4.4
Mean % Postimplantation Loss	5.0	2.3	7.1	5.7	5.4
Mean Number Live Embryos Per Female	14.8	15.6	14.3	14.0	15.3

[a] = For Controls, group means are shown. For treated groups, noteworthy findings are expressed as multiples of control; [b] = Sensory function – Preyer reflex, Motor Activity – Rotarod, Learning and memory - E-maze; Statistically significant from Controls: * p ! 0.05 ** p ! 0.01 *** p ! 0.001; - = no noteworthy findings

Footnotes

For comparison of Groups control to 1000mg/kg/day, all parameters with statistical significance are significantly different from the Control, Williams' Test.

For comparison of Groups 1000/75 (50), 1000/50 and 0/75 (50) mg/kg/day against Control, parameters with statistical significance are significantly different from the Control, Dunnett's Multiple Comparison Test with the exception of the following: Post natal survival weaning – Day 21 of lactation, Wilcoxon Rank sum Test.

For comparison of Groups 1000/75 (50) and 1000/50 against Group 1000/0 mg/kg/day: parameters with statistical significance are significantly different from the Control, Dunnett's Multiple Comparison Test with the exception of the following: Post natal survival weaning – Day 21 of lactation, Wilcoxon Rank sum Test, F1 male and female bodyweight Week 1, Students T-Test.

2.6.7.14.C Reproductive and Developmental Toxicity- Effects on Pre- and Postnatal Development, Including Maternal Function- Cobicistat

Test Article: COBI

Report Title: An Oral Pre and Postnatal Development Study, Including a 28-Day Juvenile Toxicity Evaluation, of GS-9350 in the Rat		**Gilead Study No. TX-216-2033** ([redacted] 902310)
Species / Strain: Crl:CD(SD)	**Duration of Dosing:** F_0 GD 7- PND 20, 21, 22 (depending on the day of necropsy) or GD 7-24 (rats that did not deliver a litter) F_1 Juvenile Toxicity PND 22-49	**Location in CTD:** 4.2.3.5.3
F_0 Initial Age: 77 to 84 Days at start of treatment	**Day of Mating:** GD 0	**GLP Compliance:** Yes
F_0 Date of First Dose: [redacted]	**Method of Administration:** Oral Gavage	**Lot Number:** 3471-189-36
NOAEL: F_0 Females: 30 mg/kg/day F_1 Males and Females: 75 mg/kg/day F_1 Juvenile Toxicity: 75 mg/kg/day	**Litters Culled:** PND 4	**Design Similar to ICH 4.1.2?** Yes
Vehicle: 10% (v/v) propylene glycol in 90% (v/v) 40 mM acetate buffer (pH 4)		**Special Features:** None

Dose (mg/kg/day)		**0 (Vehicle)**	**10**	**30**	**75**
F_0 Females:	No. of Animals (TK)	4	8	8	8
	GS-9350 C_{max} (µg/mL) PND 10[a]	ND	0.389	1.14	1.85
	GS-9350 AUC_{0-t} (µg•h/mL) PND 10[a]	ND	1.92	6.41	9.93
	Plasma:Milk Ratio PND 10, 2 hours postdose[a]	ND	0.819	0.560	0.627
	No. Pregnant	22	23	23	24
	No. Died or Sacrificed Moribund	0	0	0	0
	No. with Total Resorption of Litter	1	0	0	0
	Clinical Observations - Gestation				
	Salivation	0	0	1	0
	Wet fur muzzle	0	0	2	3
	Wet fur lower jaw	0	0	2	5
	Clinical Observations - PND				
	Salivation	0	0	1	5
	Wet fur muzzle	0	0	1	3
	Wet fur lower jaw	0	0	1	13
	Necropsy Observations	No treatment-related findings.			

Test Article: COBI

Report Title:	An Oral Pre and Postnatal Development Study, Including a 28-Day Juvenile Toxicity Evaluation, of GS-9350 in the Rat			**Gilead Study No. TX-216-2033** ([redacted] 902310)	
F_0 Females:	Body Weight Gains (%)[b]				
	GD 6 to 21	147.8g	1.6	3.4	-15.5*
	PND 0 to 21	25.4g	9.8	16.5	44.9
	Food Consumption (%)[c]				
	GD 6 to 18	359.4g	-1.0	-8.3*	-21.1*
	PND 0 to 12	599.8g	0.6	-0.1	-7.0*
	Mean Duration of Gestation (days)	21.7	21.6	21.8	21.9
F_1 Litters: (preweaning)	No. Litters Evaluated	21	23	23	24
	Mean No. of Implantations per delivered litter	14.4	14.6	14.2	13.8
	Mean No. Liveborn Pups/Litter	13.2	13.4	13.2	12.2
	Mean No Dead Pups/Litter	0.1	0.1	0.3	0.2
	Postnatal Survival to Day 4[a]	99.7	98.9	99.6	99.7
	Postnatal Survival to Weaning[a]	99.4	99.5	99.5	100.0
	No. of Total Litter Losses	0	0	0	0
	Change in Pup Body Weights	No treatment-related findings.			
	Litter size (PND 0)[a]	13.2	13.4	13.2	12.2
	Pup Sex (% Male - PND 0)[a]	49.54	48.10	45.53	53.56
F_1 Litters: (preweaning)	Pup Clinical Signs	No treatment-related findings.			
	Pup Necropsy Observations	No treatment-related findings.			
	Pup reflex development (righting reflex, negative geotaxis, and auricular startle response)	No treatment-related findings.			
F_1 Males (postweaning)	No. Evaluated Postweaning Per Litter	24	24	24	24
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	No treatment-related findings.			
	Body Weight Change	No treatment-related findings.			
	Food Consumption	No treatment-related findings.			
	Necropsy Observations	No treatment-related findings.			
	Mean Age of Preputial Separation (PND)	41.8	42.0	41.9	41.7
	Sensory Function	No treatment-related findings.			
	Motor Activity	No treatment-related findings.			

Test Article: COBI

Report Title:	An Oral Pre and Postnatal Development Study, Including a 28-Day Juvenile Toxicity Evaluation, of GS-9350 in the Rat				**Gilead Study No. TX-216-2033** ([redacted] 902310)
	Learning and Memory	No treatment-related findings.			
	Mean No. Days Prior to Mating	3.4	3.1	2.6	3.5
	No. of Males that Mated	21	19	23	24
	No. of Fertile Males	19	18	21	23
F_1 Females (postweaning)	No. Evaluated Postweaning Per Litter	24	24	24	24
	No. Died or Sacrificed Moribund	0	0	0	0
	Clinical Observations	No treatment-related findings.			
	Premating and Gestating Body Weight Change	No treatment-related findings.			
	Premating and Gestating Food Consumption	No treatment-related findings.			
	Necropsy Observations	No treatment-related findings.			
	Mean Age of Vaginal Patency (PND)	32.8	32.1	32.5	32.8
	Sensory Function	No treatment-related findings.			
	Motor Activity	No treatment-related findings.			
	Learning and Memory	No treatment-related findings.			
	Mean No. Days Prior to Mating	3.4	3.1	2.6	3.5
	No. of Females Sperm-Positive	21	19	23	24
	No. of Pregnant Females	19	18	21	23
	Mean No. Corpora Lutea	18.7	19.5	19.5	19.0
	Mean No. Implantations	15.0	17.3	18.1	17.0
	Mean % Preimplantation Loss	20.83	13.88	7.17	10.40
F_2 Litters	Mean No. Live Embryos/Litter	14.1	16.1	17.6	16.3
	Mean No. Resorptions	0.8	1.3	0.5	0.7
	No. of Litters with Dead Embryos	1	1	0	0
	Mean No. Dead Embryos/Litter	0.1	0.1	0.0	0.0
	Mean % Postimplantation Loss	5.01	7.14	2.52	4.18

Test Article: COBI

Report Title:	An Oral Pre and Postnatal Development Study, Including a 28-Day Juvenile Toxicity Evaluation, of GS-9350 in the Rat	**Gilead Study No. TX-216-2033** ([redacted] 902310)							
Dose (mg/kg/day)		**0 (Vehicle)**		**10**		**30**		**75**	
F_1 Juvenile Toxicity Evaluation	Gender: No. TK Animals	M:6	F:6	M:24	F:24	M:24	F:24	M:24	F:24
	Toxicokinetics, PND 22[a]								
	GS-9350 C_{max} (µg/mL)	ND	ND	0.119	0.183	1.68	1.70	3.65	3.08
	GS-9350 AUC_{0-t} (µg·h/mL)	ND	ND	0.327	0.483	9.13	6.53	32.1	18.6
	Toxicokinetics, PND 49[a]								
	GS-9350 C_{max} (µg/mL)	ND	ND	0.071	0.349	1.10	1.89	3.16	3.08
	GS-9350 AUC_{0-t} (µg·h/mL)	ND	ND	0.303	1.84	4.99	8.49	20.6	21.2
	No. Evaluated	10	10	10	10	10	10	10	10
	No. Died or Sacrificed Moribund	0	0	0	0	0	0	0	0
	Body Weight Gains (%)[b] PND 21 to 49	264.0 g	159.5 g	5.6	1.1	1.8	2.8	-10.1	-1.1
	Food Consumption (%)[c] PND 35 - 49	423.8 g	313.1 g	6.1	2.0	1.8	3.9	-10.0	0.13
	Clinical Observations								
	Salivation	1	0	0	0	2	3	4	7
	Fur staining red (lower jaw)	0	0	0	0	1	1	5	5
	Clinical Pathology (hematology, coagulation, urinalysis)	No treatment-related findings.							
	Thyroid stimulating hormone (ng/mL)[a]	0.94	0.74	1.27	1.24	1.94	1.26	1.26	1.80[#]
	Thyroxine (µg/dL)[a]	4.70	2.88	4.44	2.82	4.24	2.49	3.17**	2.43
	Triiodothyronine (ng/dL)[a]	83.18	88.44	73.57	75.56	68.76	64.18*	60.14*	64.3
	Necropsy Observations	No treatment-related findings.							
	Organ Weights[b]								
	Liver	9.71g	6.57g	12.0[#]	9.2	12.2	20.9**	11.8[#]	38.9
	Parathyroid/Thyroid	0.02022	0.01534	2.4	12.8	10.4	12.6	-4.9	13.8

Test Article: COBI

Report Title:	An Oral Pre and Postnatal Development Study, Including a 28-Day Juvenile Toxicity Evaluation, of GS-9350 in the Rat						**Gilead Study No. TX-216-2033** ([redacted] 902310)		
	Histopathology								
	Hepatocellular hypertrophy	0	0	0	0	0	0	0	9
	Thyroid follicular cell hypertrophy	0	0	0	0	0	5	3	7
	Hepatocellular vacuolation	0	3	0	3	0	7	1	6

a Data represent mean value.

b For controls, group means are shown (grams). For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

c For controls, group means are shown (grams/day). For treated groups, percent differences from controls are shown. Statistical significance is based on actual data (not on the percent differences).

GD = gestation day; PND = Post natal day; LD = lactation day; ND = none detected; M = male; F = female

* = $P \leq 0.05$ (Dunnett); ** = $P \leq 0.01$ (Dunnett); # = $P \leq 0.05$ (Dunn)

2.6.7.15.A Studies in Juvenile Animals-Darunavir

Test Article: darunavir

Species/Strain Sex/No. Per Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Dose mg b.e./kg/day (Age)	Batch No.	NOAEL (mg b.e./kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 1 dam with minimum litter size of 10 pups/group (minimum litter size of 12 for satellite groups)	Oral/Gavage (PEG400)	Single dose (PND 5, 8 and 11)	20, 40, 80 (5 days), 40, 80, 120 (8 days), 40, 80, 160 (11 days)	[redacted] ([redacted])	No NOAEL	1 to 5 deaths in all dose groups. Plasma toxicokinetic parameters were determined, C_{max} between 2.7 and 55.9 µg/mL and $AUC_{0-\infty}$ between 11 and 242 µg.h/mL for doses of 20 to 160 mg/kg. Brain and liver profiles similar to plasma profile.	TMC114-NC240[a]/ 4.2.3.5.4
Juvenile Rat / Sprague-Dawley 6/sex/group (12/sex/group satellite)	Oral/Gavage (PEG400/PG)	2 Weeks (PND 23 to 36)	20/0, 100/0, 500/0, 500/50, 0/50 darunavir / RTV	[redacted]	100/0	No deaths, increased platelets in 500/0 (F only) and 500/50, enlarged livers in 500/50 (3/6 F only). Day 23: C_{max} 0.4 to 11 µg/mL and $AUC_{0-\infty}$ 1.1 to 122 µg.h/mL for doses of 20/0 to 500/0 mg/kg.	TMC114-NC248/ 4.2.3.5.4

[a] GLP

b.e. = base equivalent, F = female, M = male, NOAEL = No Observed Adverse Effect Level, PEG = polyethylene glycol, PND = postnatal day, PG = propylene glycol, RTV = ritonavir

2.6.7.15.B Studies in Juvenile Animals-Darunavir

Report Title: TMC114 toxicity study in the juvenile rat by oral (gavage) administration **Test Article:** Darunavir

Species/strain: Rat / Sprague-Dawley
Age/weight at first dose: 23 days / approximately 55-59 g
Duration of dosing: 28 days
Duration of post-dose: 25 days

Administration route/method: Oral / gavage
Doses: 0, 40, 200, 500 mg TMC114/kg body weight/day, 500/75 TMC114/mg RTV/kg body weight/day, and 75 mg RTV/kg body weight/day
Test article batch: [redacted] lot [redacted]
Vehicle/formulation: Polyethylene glycol 400 (for TMC114) and propylene glycol for RTV

Testing facility: [redacted]
Study no.: TMC114-NC241
Location in CTD: 4.2.3.5.4
GLP compliance: Yes
Date start of study: [redacted]

No Observed Adverse Effect Level: No NOAEL was established in this study owing to the liver changes at 40/0 mg/kg/day and higher doses.

Toxicokinetics: TMC114

Dose TMC114/RTV (mg base equivalent/kg/day)	0/0 (vehicle)		40/0		200/0		500/0		500/75		0/75	
No. of animals	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**
Died or killed prematurely (accidental/incidental)	0	0	0	0	0	0	0	0	0	0	0	0
$AUC_{0-\infty}$ Day 23 of age (µg.h/mL)	-	-	7.50	8.51	61.5	73.9	144	173	254 [a]	228 [a]	-	-
AUC_{0-24h} Day 50 of age (µg.h/mL)	-	-	6.38	10.8	26.5	39.8	71.3	73.9	135	171	-	-

[a] AUC_{0-24h}, M = male; F = female; RTV = ritonavir

Toxicokinetics: RTV

Dose TMC114/RTV (mg base equivalent/kg/day)	0/0 (vehicle)		40/0		200/0		500/0		500/75		0/75	
No. of animals	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**	**M:12+12**	**F:12+12**
Died or killed prematurely (accidental/incidental)	0	0	0	0	0	0	0	0	0	0	0	0
$AUC_{0-\infty}$ Day 23 of age (µg.h/mL)	-	-	-	-	-	-	-	-	11.9[a]	7.88[a]	29.3	30.1
AUC_{0-24h} Day 50 of age (µg.h/mL)	-	-	-	-	-	-	-	-	4.96	7.31	25.8[b]	40.7

[a] AUC_{0-24h}, [b] AUC_{0-8h}, M = male; F = female; RTV = ritonavir

2.6.7.15.B Studies in Juvenile Animals-Darunavir (Continued)

Report Title: TMC114 toxicity study in the juvenile rat by oral (gavage) administration **Test Article:** Darunavir

Dose TMC114/RTV (mg base equivalent/kg/day)	**0/0 (vehicle)**		**40/0**		**200/0**		**500/0**		**500/75**		**0/75**	
No. of animals (main + recovery)	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**
Died or killed prematurely (accidental/incidental)	0	0	0	0	0	0	0	1	0	0	1	1
Clinical observations	-	-	-	-	-	-	-	-	-	-	-	-
Body weight (g) (Day 51 of age) [c]	280	186	0.99	1.04	0.99	1.04	0.95**	1.04*	0.92**	1.04*	0.96	1.02
Body weight gain (g) (Day 23-51 of age) [c]	221	130	0.99	1.05	0.99	1.06*	0.93**	1.06*	0.90**	1.05*	0.95*	1.02
Food consumption (g/week)	-	-	-	-	-	-	-	-	-	-	-	-
Sexual maturation (time to preputial separation (M) or vaginal opening (F)	-	-	-	-	-	-	-	-	-	-	-	-
Auditory startle response (pre-pulse inhibition)	-	-	-	-	-	-	-	-	-	-	-	-
Morris water maze performance (trial time, failed trials, sector entered)	-	-	-	-	-	-	-	-	-	-	-	-
Hematology (Day 51 of age) [c]												
- Hemoglobin (g/dL)	13.7	13.4	0.99	1.01	0.97*	0.99	0.95**	0.96	0.93**	0.95**	0.99	1.01
- Hematocrit (L/L)	0.43	0.41	0.99	1.01	0.97	0.99	0.95**	0.97	0.93**	0.97	1.00	1.02
- Reticulocytes (%)	6.07	4.12	1.01	1.01	1.13	1.23*	1.27**	1.37**	1.32**	1.58**	1.11	1.13
- Platelets (10^9/L)	1090	1109	0.97	1.04	1.19**	1.11	1.34**	1.21**	1.27**	1.34**	1.09	1.05
- Prothrombin time (sec)	13.9	15.0	1.04	1.04	1.02	0.97	1.08**	0.97	1.14**	0.97	1.06**	0.98
Hematology (recov. Day 75 of age)[c]												
- Reticulocytes (%)	2.94	3.08	1.03	0.99	1.13	1.06	1.20**	0.92	1.10	0.78**	1.16*	0.87
- Platelets (10^9/L)	1036	1068	0.93	1.00	1.01	1.05	1.12*	1.14	1.02	1.13*	1.06	1.01
Serum (Day 51 of age) [c]												
- Cholesterol (mmol/L)	2.27	2.25	0.89*	0.96	0.74**	0.93	0.69**	1.02	1.07	1.27**	0.88*	1.03
- Triglycerides (mmol/L)	1.79	0.83	0.63**	0.88	0.32**	0.42**	0.15**	0.24**	0.27**	0.33**	0.88	0.77
Serum (recov. Day 75 of age)[c]												
- Cholesterol (mmol/L)	1.86	2.00	0.94	1.06	1.00	1.14*	1.05	1.15**	1.03	1.14*	1.01	1.05

[c] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data.
* $p \leq 0.05$, ** $p \leq 0.01$ (compared with controls; Bartlett's test followed by parametric, Healey/Williams or non-parametric, Healey/Shirley)
- No treatment related changes; M = male; F = female; recov. = recovery

2.6.7.15.B Studies in Juvenile Animals-Darunavir (Continued)

Report Title: TMC114 toxicity study in the juvenile rat by oral (gavage) administration **Test Article:** Darunavir

Dose TMC114/RTV (mg base equivalent/kg/day)	**0/0 (vehicle)**		**40/0**		**200/0**		**500/0**		**500/75**		**0/75**	
No. of animals (main + recovery)	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**
Organ weights (Day 51 of age) [c]												
- Adrenals (%)	0.02	0.03	1.03	0.98	1.04	1.06	1.25**	0.99	1.45**	1.13*	1.06	1.06
- Liver (%)	5.38	5.07	0.98	1.02	1.06*	1.16**	1.16**	1.44**	1.44**	1.74**	1.05*	1.14**
- Spleen (%)	0.25	0.26	1.09	1.00	1.11*	1.00	1.15**	1.02	1.20**	1.07	1.11*	1.11*
Organ weights (recovery Day 75 of age) [c]												
- Liver (%)	4.39	4.3	0.99	1.02	1.02	1.05	1.04	1.10**	1.05	1.16**	1.03	1.04
Gross pathology (no. examined) (Day 51 of age)	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**14**	**15**	**15**	**14**	**15**
- Liver, accentuated liver lobular pattern	0	0	0	0	0	4	2	11	4	12	0	1
Gross pathology (no. examined) (recovery Day 75 of age)	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**15**	**14**
- Macroscopic observations	-	-	-	-	-	-	-	-	-	-	-	-
Histopathology (no. examined) (Day 51 of age)	**15**	**15**	**15** [d]	**15** [d]	**15** [d]	**15** [d]	**15**	**14**	**15**	**15**	**14**	**15**
Liver (no. examined)	15	15	15	15	15	15	15	14	15	15	14	15
- hepatocyte vacuolation, periportal	0	0	0	2	2	11	2	14	10	11	0	3
minimal	0	0	0	2	2	5	2	4	6	8	0	3
slight	0	0	0	0	0	4	0	7	4	3	0	0
moderate	0	0	0	0	0	2	0	3	0	0	0	0
-hepatocyte hypertrophy, centrilob.	0	0	0	0	7	9	0	10	1	14	5	1
minimal	0	0	0	0	5	4	0	5	1	3	4	0
slight	0	0	0	0	2	5	0	5	0	11	1	1
- hepatocyte hypertrophy, diffuse	0	0	0	0	2	0	15	0	15	0	0	0
minimal	0	0	0	0	2	0	0	0	0	0	0	0
slight	0	0	0	0	0	0	15	0	15	0	0	0

[c] For vehicle group means are shown. For treated groups, the multiples of vehicle/baseline are shown. Statistical significance based on actual data;
[d] Liver and abnormal tissues; * $p \leq 0.05$, ** $p \leq 0.01$ (compared with controls; Bartlett's test followed by parametric, Healey/Williams or non-parametric, Healey/Shirley); - No treatment related changes; centrilob. = centrilobular

2.6.7.15.B Studies in Juvenile Animals-Darunavir (Continued)

Report Title: TMC114 toxicity study in the juvenile rat by oral (gavage) administration **Test Article:** Darunavir

Noteworthy Findings:

Dose TMC114/RTV (mg base equivalent/kg/day)	**0/0 (vehicle)**		**40/0**		**200/0**		**500/0**		**500/75**		**0/75**	
No. of animals (main + recovery)	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**	**M:15+15**	**F:15+15**
Histopathology (no. examined) (Day 51 of age) continued	**15**	**15**	**15** [d]	**15** [d]	**15** [d]	**15** [d]	**15**	**14**	**15**	**15**	**14**	**15**
Thyroid (no. examined)	15	15	15	15	15	15	15	14	15	15	14	15
- follicular cell hypertrophy	0	0	0	0	0	0	3	1	6	6	5	1
minimal	0	0	0	0	0	0	0	0	0	0	1	0
slight	0	0	0	0	0	0	3	1	5	6	4	1
moderate	0	0	0	0	0	0	0	0	1	0	0	0
Histopathology (no. examined) (recovery Day 75 of age)	**15**	**15**	**15** [d]	**15** [d]	**15** [d]	**15** [d]	**15**	**15**	**15**	**15**	**15**	**14**
Liver (no. examined)	15	15	15	15	15	15	15	15	15	15	15	14
- hepatocyte vacuolation, periportal	0	0	0	1	0	1	0	5	0	2	0	0
minimal	0	0	0	1	0	1	0	4	0	1	0	0
slight	0	0	0	0	0	0	0	1	0	1	0	0
- hepatocyte hypertrophy, centrilobular	0	0	0	0	0	0	1	0	2	7	0	1
minimal	0	0	0	0	0	0	0	0	1	3	0	1
slight	0	0	0	0	0	0	1	0	1	4	0	0
Thyroid (no. examined)	15	15	0	0	0	0	15	15	15	15	15	14
- follicular cell hypertrophy	0	0	0	0	0	0	1	0	1	0	0	0
minimal	0	0	0	0	0	0	0	0	0	0	0	0
slight	0	0	0	0	0	0	1	0	1	0	0	0

Statistical significance based on actual data.
* $p \leq 0.05$, ** $p \leq 0.01$ (compared with controls; Bartlett's test followed by parametric, Healey/Williams or non-parametric, Healey/Shirley)
- No treatment related changes
[d] Liver and abnormal tissues

2.6.7.15. Studies in Juvenile Animals-Cobicistat

Studies in juvenile animals for COBI are described in Tabulated Summary 2.6.7.14.

2.6.7.16 Local Tolerance- Darunavir

Test Article: Darunavir

Type of Study	In Vitro System or Species/Strain	Route/Method of Administration	Gender and No. per Group	Dose (Batch) (Reference / Controls)	Noteworthy Findings	Study No./ Location in CTD
Skin sensitization						
Local lymph node assay	Mouse	Topical		10%, 25% or 50% w/v in DMF	Unlikely to cause skin sensitization	TMC114-NC245[a]/ 4.2.3.6
Skin Tolerance. In Vitro/In Vivo Test						
Mouse skin sensitization/irritation (local lymph node assay with addition of local irritation)	Mouse/CBA/J	Topical 25 µL/ear applied to ears for 3 consecutive days.	4F	2.5, 5, 10, 25 or 50% w/v darunavir in DMF, vehicle, HCA (positive control) ([redacted])	No noteworthy lymph proliferation occurred. Darunavir did not induce delayed contact hypersensitivity in the mouse local lymph node assay. No cutaneous reactions and no increase in ear thickness occurred.	TMC114-NC330[a]/ 4.2.3.6
Skin Tolerance. In Vivo Tests						
Primary skin irritation	Rabbit/New Zealand white	4 hour topical application	1M + 2F	0.5 g as 1% w/w darunavir solution ([redacted])	No skin reactions, staining of skin or corrosive effects were observed. Classified as "non-irritant".	TMC114-NC316[a]/ 4.2.3.6
Ocular Tolerance. In Vitro Tests						
Bovine corneal opacity-permeability eye irritation assay	In vitro	---	---	20% w/w darunavir in saline ([redacted])	No increase in opacity and no increase in permeability. Darunavir considered a "non-irritant".	TMC114-NC166/ 4.2.3.6

[a] GLP compliant

DMF = dimethylformamide; HCA = α-hexylcinnamaldehyde

2.6.7.16 Local Tolerance- Cobicistat

Test Article: Cobicistat

Type of Study	Species / Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name & Study No.) Location in CTD
Local lymph node assay	Mouse/CBA, Ca	Topical (4:1 v/v acetone:olive oil)	3 Days	2.5, 5, 10% w/v	5 F	COBI did not show the potential to cause skin sensitization.	TX-216-2042[a] ([redacted] 001) 4.2.3.6
BCOP	Bovine Cornea	In Vitro (prepared in 0.9% saline)	Opacity = 4 hrs; Permeability = 90 min.	20% w/w	≥ 3 Corneas	COBI elicited an In Vitro Irritancy Score of -1.6 ± 0.0 and was predicted to be a noncorrosive/nonsevere eye irritant.	TX-216-2043[a] ([redacted] 002) 4.2.3.6
Dermal Irritation	Rabbit/NZW	Dermal (moistened in 0.5 mL purified water)	4 Hours	0.5 g	3 F	The Primary Irritation Index (PII) was calculated to be 1.0 and COBI was classified as "mildly-irritating" (PII > 0 – 2.0) to rabbit skin.	TX-216-2044[a] ([redacted] 003) 4.2.3.6

a Indicates GLP compliant.
BCOP = bovine corneal opacity and permeability; NZW = New Zealand White; F = female; w/w = weight/weight

2.6.7.17.A Other Toxicity Studies: Immunotoxicity-Darunavir

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 8M + 8F (main) 6M + 6F (satellite)	Oral/gavage (TMC114: PEG400; RTV: propylene glycol)	4 Weeks	[redacted] (TMC114 as base: 92% w/w, as ethanolate: 98.1% w/w) 07687VA (RTV: 99.2%)	0 (vehicle), 00 (water), 20/0, 100/0, 500/0, 0/50, 100/50 TMC114/RTV	No relevant effects on immune function at doses up to 500 mg/kg. NOAEL – 20 mg/kg 100/0 ↑ liver wt (M_{rel}, 8%, F_{rel}, 17%), 500/0: ↓ Hb (F, 7%), ↑ reticulocytes (M, 1.75-fold; F, 2-fold), ↓ M/E (M, 24%, F, 35%), ↑ liver wt (M_{rel}, 51%, F_{rel}, 72%), hepatocellular hypertrophy 0/50: ↑ liver wt (M_{rel}, 37%, F_{rel}, 36%), hepatocellular hypertrophy 100/50: ↑ liver wt (M_{rel}, 55%, F_{rel}, 62%), hepatocellular hypertrophy	TMC114-NC187[a]/ 4.2.3.7.2

[a] GLP compliant

↑ = increased, ↓ = decreased, F = female, Hb = hemoglobin, M = male, M/E = myeloid/erythroid, PEG = polyethylene glycol, RTV = ritonavir, rel = relative, wt = weight

2.6.7.17.B Other Toxicity Studies: Mechanistic Studies-Darunavir

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg /day)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 20/sex/group (main animals) 6/sex/group (satellite animals) 3/sex/group (satellite animals vehicle group)	Oral/Gavage (PEG400)	28/29 days	[redacted]	Vehicle, 50, 500	No mortality Vehicle: *liver*: prominent lobular pattern (2/20 (M)), vacuolization (M, 2/19; F, 4/19); *pituitary gland*: vacuolated cells pars distalis (M, 10/19), TSH positivity (F, 19/19 – 11/19 grade 2); *thyroid glands*: hypertrophy follicular cells (M, 1/19; F, 1/19), small follicles (M, 1/19) 50: excessive salivation (M, 7/20; F, 5/20); ↑ T4 (Week 1: M, 20%), ↑ kidney weight (M, 9%); ↑ liver weight (M/F, 10%); liver: dark discoloration (M, 2/20; F, 1/20), prominent lobular pattern (M/F, 5/20), swollen (M, 2/20), hepatocellular hypertrophy (F, 1/19). 500: excessive salivation (M, 18/20; F, 15/20); ↑ K^+ (F, 7%); ↑ Ca^{2+} (M/F, 5%); ↑ inorganic phosphorus (F, 4%); ↑ total protein (M, 8%; F, 10%); ↑ albumin (F, 4%); ↓ glucose (M, 15%); ↑ cholesterol (M, 13%; F, 68%); ↓ triglycerides (M, 34%); ↑ urea nitrogen (M/F, 15%); ↑ total bilirubin (M, 23%); ↑ GGT (M, 1; F, 3)[b] ↑ T4 (Week 1: M, 35%; F, 43%), ↑ TSH (Week 1: M, 80%; F, 2.1-fold – Day 28/29: M, 62%: F, 2.5-fold); ↑ kidney weight (M, 9%; F, 8%); ↑ liver weight (M, 51%; F, 76%); ↑ thyroid glands weight (M, 15%); *liver*: dark discoloration (M, 17/20; F, 18/20), prominent lobular pattern (M, 11/20; F, 17/20), swollen (M, 17/20; F, 16/20), dense staining of cytoplasm (M, 14/20; F, 18/20), hepatocellular hypertrophy (M/F, 20/20), vacuolization (M, 9/20; F, 8/20); *pituitary gland*: vacuolated cells pars distalis (M, 15/20), TSH positivity (F, 19/19 – 12/19 grade 3); *thyroid glands*: hypertrophy follicular cells (M, 11/19; F, 17/19), small follicles (M, 5/19; F, 4/19)	TMC114-NC162[a]/ 4.2.3.7.3

[a] GLP compliant; [b] absolute values

ALP: alkaline phosphatase; AST: aspartate aminotransferase; b.e.: base equivalent; Ca^{2+}: calcium; F: female; GGT: gamma glutamyl transferase; K^+: potassium; M: male; PEG400: polyethylene glycol 400; T4: thyroxine; TSH: thyroid stimulating hormone

2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
S. typhimurium TA98, TA100	In vitro	---	[redacted] (TMC114 as base 93.1% w/w; as ethanolate: 100.9% w/w)	3, 10, 33, 100, 333, 1000, 3330, 5000 #g/plate	No evidence of mutagenic properties	TMC114-NC150[a]/ 4.2.3.7.7
S. typhimurium TA98, TA100	In vitro	---	[redacted] (TMC114 as base 90.5% w/w; as ethanolate: 97.7% w/w), [redacted] (TMC114 as base 92.1% w/w; as ethanolate: 99.8% w/w), [redacted] (TMC114 as base 91.6% w/w; as ethanolate:99.1% w/w)	3, 10, 33, 100, 333, 1000, 3330, 5000 #g/plate	No evidence of mutagenic properties	TMC114-NC170[a]/ 4.2.3.7.7
Dog/Beagle 2M + 2F/group; vehicle: 1M +1F	Oral/gavage (PEG400)	2 Weeks	[redacted] (TMC114 as base 90.5% w/w; as ethanolate: 97.7% w/w), [redacted] (TMC114 as base 92.1% w/w; as ethanolate: 99.8% w/w), [redacted] (TMC114 as base 91.6% w/w; as ethanolate:99.1% w/w)	0 (vehicle), 120 [g]	No relevant difference in toxicological profile between the batches of TMC114	TMC114-NC176[a]/ 4.2.3.7.7

2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
S. typhimurium TA98, TA1537, TA100, TA1535, TA102	In vitro (DMSO)	---	[redacted] (TMC114 as base: 92% w/w, as ethanolate: 99.6% w/w), 23175957 (不純物A*), 23175964 (不純物B*)	0 (vehicle), 312.5, 625, 1250, 2500, 5000 #g/plate (1st and 2nd experiment) b 0 (vehicle), 312.5, 625, 1250, 1875, 2500, 3750 #g/plate (3rd experiment) b	No evidence of mutagenic properties c	TMC114-NC228[a]/ 4.2.3.7.6

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2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 10M + 10F/group	Oral/gavage (PEG400)	2 Weeks	[redacted] (TMC114 as base: 92% w/w, as ethanolate: 99.6% w/w)	0 (vehicle), 20, 20 [d], 500, 500 [d]	No relevant difference in toxicological profile between the spiked and non-spiked batches of TMC114 500: ↓ Hb (F, 6%), ↓ Hct (M, 5%; F, 6%), ↑ reticulocytes (M, 33%; F, 46%), ↑ thrombocytes (M, 24%; F, 21%), ↑ cholesterol (M, 29%; F, 71%), ↓ triglycerides (M, 39%), ↑ GGT (M, 2-fold; F, 2-fold), ↑ liver wt (M_{abs}, 39%; F_{abs}, 65%), hepatocellular hyperthrophy and vacuolation (M, 10/10; F, 10/10), splenic erythropoiesis (F, 7/10), thyroid follicular hyperthrophy (M, 10/10; F, 10/10). microvacuolated adrenal zona fasciculata cells (M, 5/10; F, 8/10), 500 [d]: ↓ Hb (F, 5%), ↓ Hct (M, 4%; F, 5%), ↑ reticulocytes (M, 23%; F, 49%), ↑ thrombocytes (M, 12%; F, 28%), ↑ cholesterol (M, 29%; F, 62%), ↓ triglycerides (M, 27%), ↑ GGT (M, 3-fold; F, 2-fold), ↑ liver wt (M_{abs}, 50%; F_{abs}, 65%), hepatocellular hyperthrophy and vacuolation (M, 10/10; F, 10/10), splenic erythropoiesis (F, 7/10), thyroid follicular hyperthrophy (M, 10/10; F, 10/10), microvacuolated adrenal zona fasciculata cells (M, 5/10; F, 7/10)	TMC114-NC227[a]/ 4.2.3.7.6

2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Mammalian chromosomal aberration – human peripheral lymphocytes	In vitro (DMSO)	+/-S9 4h –S9 20h	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity[d] 0 (vehicle), 25 to 1000 µg/mL	No evidence of clastogenic properties in 2 independent assays with adequate test system.	TMC114-NC355[a]/ 4.2.3.7.6
S. typhimurium TA98, TA1537, TA100, TA1535, TA102	In vitro (DMSO)	---	[redacted] (TMC114 as base: 93% w/w, as ethanolate:97.5% w/w), [redacted] (不純物C* / 不純物D* : 95.8%)	0 (vehicle), 78.13, 156.25, 312.5, 625, 1250, 2500, 5000 #g/plate [e]	No evidence of mutagenic properties [f].	TMC114-NC244[a]/ 4.2.3.7.6

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2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague-Dawley 10M + 10F/group	Oral/gavage (PEG400)	2 Weeks	[redacted](TMC114 as base: 93% w/w, as ethanolate:97.5% w/w), [redacted] 不純物C* / 不純物D* : 95.8%)	0 (vehicle), 20, 20 [e], 500, 500 [e]	No relevant difference in toxicological profile between the spiked and non-spiked batches of TMC114 500: ↓ Hb (F, 5%), ↓ Hct (M, 4%; F, 5%), ↑ reticulocytes (M, 24%; F, 50%), ↑ thrombocytes (F, 25%), ↑ cholesterol (F, 29%), ↓ triglycerides (M, 44%; F, 28%), ↑ urea nitrogen (M, 21%), ↑ GGT (M, 1-fold; F, 3-fold), ↑ liver wt (M, 39%; F, 63%), hepatocellular hypertrophy (M, 9/10; F, 10/10), high follicular thyroid epithelium (M, 9/10; F, 9/10), ↑ erythropoiesis (F, 10/10) 500 [e]: ↓ Hb (F, 6%), ↓ Hct (F, 7%), ↑ reticulocytes (F, 69%), ↑ thrombocytes (F, 26%), ↑ cholesterol (F, 44%), ↓ triglycerides (M, 56%; F, 36%), ↑ urea nitrogen (M, 35%), ↑ GGT (M, 2-fold; F, 3-fold), ↑ liver wt (M, 30%; F, 59%), hepatocellular hypertrophy (M, 10/10; F, 10/10), high follicular thyroid epithelium (M, 10/10; F, 9/10), ↑ erythropoiesis (F, 10/10)	TMC114-NC242[a]/ 4.2.3.7.6

*新薬承認情報提供時に置き換え

2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Mammalian chromosomal aberration – human peripheral lymphocytes	In vitro (DMSO)	+/-S9 5h +S9 4h –S9 20h	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity 0 (vehicle), 25 to 1000 µg/mL [e]	No evidence of clastogenic properties in 2 independent assays with adequate test system.	TMC114-NC354[a]/ 4.2.3.7.6
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity 0 (vehicle), 78.13 to 5000 µg/plate [h]	No evidence of mutagenic properties in 2 independent assays with adequate test system.	TMC114-NC359[a]/ 4.2.3.7.6
Mammalian chromosomal aberration – human peripheral lymphocytes	In vitro (DMSO)	+/-S9 4h –S9 20h	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity 0 (vehicle), 50 to 1000 µg/mL [h]	No evidence of clastogenic properties in 2 independent assays with adequate test system.	TMC114-NC357[a]/ 4.2.3.7.6
S. typhimurium TA98, TA100, TA102, TA1535, TA1537 +/- S9	In vitro (DMSO)	---	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity 0 (vehicle), 78.13 to 5000 µg/plate	No evidence of mutagenic properties in 2 independent assays with adequate test system.	TMC114-NC336[a]/ 4.2.3.7.6
Mammalian chromosomal aberration – human peripheral lymphocytes	In vitro (DMSO)	+/-S9 3h –S9 20h	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity 0 (vehicle), 2.5 to 500 µg/mL	No evidence of clastogenic properties in 2 independent assays with adequate test system.	TMC114-NC337[a]/ 4.2.3.7.6

2.6.7.17.C Other Toxicity Studies: Impurities-Darunavir (Continued)

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no. (purity)	Doses (mg base equivalent/kg/day)	Noteworthy Findings	Study No./ Location in CTD
Dog/Beagle 3/sex/group	Oral/Gavage (PEG400)	2 weeks	TMC114 [redacted] spiked	TMC114 spiked with 1% of each impurity 0 (vehicle), 120 (ref TMC114), 120 (spiked TMC114)	No relevant difference in toxicological profile between the spiked and non-spiked batches of TMC114.	TMC114-NC338[a]/ 4.2.3.7.6

[a] GLP compliant,
[b] TMC114 spiked with 1% of 不純物A* and 1% of 不純物B* ,
[c] 1st experiment (incorporation method) no precipitate, 2nd and 3rd experiment (pre-incubation method) precipitation from 1875 µg/plate (TA1537 and TA98), from 2500 µg/plate (TA1535, TA102) and from 5000 µg/plate (TA100),
[d] TMC114 spiked with 1% of 不純物A* and 1% of 不純物B* ,
[e] TMC114 containing 1% of a racemate of 2 isomers: 不純物C* / 不純物D* ,
[f] dose-related increase in precipitation from 2500 or 5000 µg/plate onwards,
[g] dose level corrected for purity,
[h] TMC114 spiked with 1% of 不純物E* , 1% of 不純物F* and 1% of 不純物G* ,
[i] TMC114 spiked with 1% of 不純物H* and 1% of 不純物I* ;

↑ = increased, ↓ = decreased, abs = absolute , DMSO = dimethylsulfoxide, F = female, GGT = gamma glutamyl transferase , Hb = hemoglobin, Hct = hematocrit, M = male, M/E = myeloid/erythroid, PEG = polyethylene glycol, RTV = ritonavir, rel = relative, wt = weight

2.6.7.17.D Other Toxicity Studies: Other Studies-Darunavir

Test Article: darunavir

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Batch no.	Doses	Noteworthy Findings	Study No./ Location in CTD
Human platelets	In vitro	---	---	TMC114: 6, 50 #M Lopinavir: 10, 50 #M Tipranavir: 30, 50 #M	TMC114: no direct effect on human platelets, no effect on collagen-, PAF- (only at 6 #M TMC114) or ADP-induced platelet aggregation; inhibited PAF-induced platelet aggregation at 50 #M TMC114. Lopinavir: no direct effect on human platelets, no effect on collagen- or PAF-induced platelet aggregation; stimulated ADP-induced platelet aggregation. Tipranavir: no direct effect on human platelets, no effect on PAF- or ADP-induced platelet aggregation; inhibited collagen-induced platelet aggregation.	TMC114-NC394/ 4.2.3.7.7

ADP = adenosine diphosphate; PAF = platelet activating factor

2.6.7.17.E Other Toxicity Studies: Immunotoxicity-Cobicistat

Test Article: Cobicistat

Species/ Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
Rat/Sprague-Dawley	Oral Gavage	4 Weeks	0, 20, 50, 150	8 M/F	↓ in body weight gain and/or food consumption, ↑in liver and thyroids weights, and lymphoid depletion of germinal centers in the spleen observed at 50 and/or 150 mg/kg/day. NOAEL = 20 mg/kg/day in males and females (Week 4 C_{max} 0.492 and 1.42 µg/mL, AUC_{0-t} of 2.33 and 5.69 µg.h/mL, respectively). NOEL: for T-cell dependent antibody response [TDAR]: 20 mg/kg/day for females (Week 4 AUC_{0-t} 5.69 µg.h/mL); 50 mg/kg/day for males (Week 4 AUC_{0-t} 11.2 µg.h/mL).	TX-216-2022[a] ([redacted] 6511-351) 4.2.3.7.2

[a] Indicates GLP compliant

↓ = decrease; ↑ = increase; F = female; M = male; NOAEL = no observed adverse effect level; NOEL = no observed effect level

2.6.7.17.F Other Toxicity Studies: Impurities-Cobicistat

Test Article: Cobicistat

Species/ Strain	Method of Administration (Vehicle / Formulation)	Duration of Dosing	Dose (mg/kg/day)	Gender and No. per Group	Noteworthy Findings	Gilead Study No. (CRO Name and Study No.) Location in CTD
In Silico	NA	NA	NA	NA	No structural matches identified in DEREK; therefore, no prediction for carcinogenicity, chromosome damage, or mutagenicity of COBI process intermediates/impurities.	TX-216-2046 TX-216-2054 4.2.3.7.6
Rat/Sprague-Dawley	Oral Gavage	4 Weeks	0, 100[b], 30[c], 100[c]	10 F	NOAEL = 100 mg/kg/day for both lots of COBI. Day 28 C_{max} 5.83 and 3.96 µg/mL, respectively, and AUC_{0-t} 40.3 and 35.9 µg.h/mL for lots A and B, respectively).	TX-216-2045[a] ([redacted] 8241408) 4.2.3.7.6
S. typhimurium and *E. coli*	In Vitro	NA (Pre-incubation and Plate Incorpor-ation)	1.58 to 5000 µg/plate	NA	Four COBI impurities/degradants 不純物CA* , 不純物CB* , 不純物CC* and 不純物CD*) when tested in the Bacterial Reverse Mutation Assay (Ames) using both plate incorporation and pre-incubation methods did not show any evidence of genotoxic activity.	TX-216-2052 ([redacted] 964147) 4.2.3.7.6
Mouse lymphoma L5178Y $TK^{+/-}$ cells	In Vitro	3 Hours ± S9 and 24 hours without S9	2.5 to 2510 µg/mL	NA	Four COBI impurities/degradants (不純物CA* , 不純物CB* , 不純物CC* and 不純物CD*) did not show any evidence of genotoxic activity in the in vitro mouse lymphoma forward mutation assay.	TX-216-2053 ([redacted] 964148) 4.2.3.7.6

a Indicates GLP compliant; b GS-9350, HPLC purity = 99.4%; c GS-9350, HPLC purity = 95.1% (spiked with impurities);
d GS-9350/ 不純物CE* non-degraded crushed tablets; e GS-9350/ 不純物CE* degraded crushed tablets.
M = male; F = female; NA = not applicable;

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