

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
21-976

PHARMACOLOGY REVIEW



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-976
DATE RECEIVED BY CENTER:	12/23/2005
PRODUCT:	TMC 114, Darunavir, PREZISTA
INTENDED CLINICAL POPULATION:	HIV patients
SPONSOR:	Tibotec-Virco
REVIEW DIVISION:	Division of Antiviral Products (HFD-530)
PHARM/TOX REVIEWER:	James G. Farrelly, Ph.D. Pritam S. Verma, Ph.D.
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PROJECT MANAGER:	Elizabeth Thompson, M.S.

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The pharmacology/toxicology studies submitted to NDA 21-976 support the labeling for this submission and are sufficient for approval.

B. Recommendation for nonclinical studies

The sponsor has a Phase 4 commitment to complete the ongoing carcinogenicity studies in mice and rats.

C. Recommendations on labeling

The label should read:

Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis:

Long-term carcinogenicity studies of darunavir in rodents have not been completed. Darunavir, however, was tested negative in the in vitro Ames reverse mutation assay and in vitro chromosomal aberration assay in human lymphocytes, both tested in the absence and presence of metabolic activation system. Darunavir does not induce chromosomal damage in the in vivo micronucleus test in mice.

Impairment of Fertility:

There were no effects on fertility and early embryonic development with darunavir in rats and darunavir has shown no teratogenic potential in mice (in the presence or absence of ritonavir), rats and rabbits.

Pregnancy

Pregnancy Category B: Reproduction studies conducted with darunavir have shown no embryotoxicity or teratogenicity in mice, rats and rabbits. Because of limited bioavailability of darunavir in animals and/or dosing limitations, the plasma exposures (AUC values) were approximately 50% in mice and rats and 5% in the rabbit of those obtained in humans at the recommended clinical dose boosted with ritonavir.[139]

In the rat pre- and postnatal development study, a reduction in pup body weight gain was observed with darunavir alone or in combination with ritonavir during lactation. This was due to exposure of pups to drug substances via the milk. Sexual development, fertility or mating performance of offspring was not affected by maternal treatment with darunavir alone or in combination with ritonavir. The maximal plasma exposures achieved in rats were approximately 50% of those obtained in humans at the recommended clinical dose boosted with ritonavir.

There are, however, no adequate and well-controlled studies in pregnant women.

PREZISTA should be used during pregnancy only if the potential benefit justifies the potential risk.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

Three month study in mice:

The most relevant findings were in the hematological examinations. In both males and females there were essentially dose dependent decreases in red blood cell parameters and increases in bilirubin and reticulocyte measurements. The results are consistent with the studies in rats and indicate a tendency of rodents to exhibit the signs of anemia, a toxicity related to sulfonamide therapy. There were some toxic effects in the liver of females at the high dose (1000 mg/kg/day). This was shown as slight necrosis over controls and an increase in ALT.

Six month study in rats:

Effects on the hematopoietic system were seen with decreases (up to 11%) in RBC count, hemoglobin, and hematocrit and an increase in reticulocyte count occurring in animals given the high dose (500 mg/kg/day). Similar, but generally less marked changes in reticulocyte count occurred in animals given 100 mg/kg/day. There was an increase in bilirubin values which, together with the reticulocytes increases, indicated red blood cell turnover. Platelet count increased (up to 37%) in animals given 500 mg/kg. Less marked changes in platelet count occurred in animals given 100 mg/kg/day.

An increase in APTT occurred in animals given 100 and 500 mg/kg/day (up to 20% and 44%, respectively). Bilirubin was up as high as seven-fold and cholesterol was up to 74% higher while triglycerides were decreased in animals given 500 mg/kg/day. There was some effect at 100 mg/kg/day. Bilirubin increased slightly in males given 20 mg/kg/day at week 26 only. No other serum chemistry parameters were considered of consequence. At necropsy there was a slight increase in male kidney and spleen weight and male and female liver weight in animals given 100 and 500 mg/kg/day. The increase was confirmed to be a consequence of hepatocellular hypertrophy, an adaptive rather than a toxic response. Lipofuscin was present in the liver and kidney. Minimal hypertrophy and hyperplasia of the bile ducts was noted in most animals treated at 500 mg/kg/day.

Six month study in rats with darunavir/ritonavir (RTV):

Groups of male and female rats were administered darunavir/RTV via oral gavage at dose levels of 0 (vehicle control; PEG 400 in water), 20/50 (low), 100/50 (mid), 500/75 (high) or 1000/75 mg/kg/day (highest), or RTV alone (75 mg/kg/day) for 26 consecutive weeks. Thirty-three animals were found dead or were sacrificed prematurely during the study. In the main study, these animals were 2 (vehicle control), 10 (RTV alone), 3 (low), 4 (mid), 6 (high) and 3 (highest). Clinical signs: salivation, brown staining of the fur and hunched posture were noted in all groups other than the vehicle control. The incidence of salivation and hunched posture increased with increasing dose level of darunavir. Body weight and body weight gain: lower body weight gains were observed for male and females (high or highest). The deficits in total body weight gain were 16% and 12% for males and 8% and 9% for females for the high and highest dose levels, respectively. Treatment related disturbances of the hematological parameters consisted of several affected red blood cell parameters: lower hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and increased red cell distribution width, bilirubin levels and reticulocyte counts, all indicative of an increased blood cell turnover. Creatinine (males), bilirubin and cholesterol were increased (up to 18%, 3-fold and 3-fold, respectively) and triglycerides increased in females (2.4-fold) and decreased in males (73%) in

animals at the high and highest dose levels. Phospholipids were increased in both sexes (high and highest). An increase in ALT (2.9-fold) and AST (73%) was noted in males (RTV alone) and to a lesser extent, in groups treated with darunavir/RTV combination (up to 78%).

Six month study in dogs:

Groups of male and female dogs (4/sex/group) were administered darunavir via oral gavage at dose levels of 0 (vehicle control; PEG 400 in water), 30 (low), 60 (mid) or 120 (high) for a period of 26 consecutive weeks. Loose or liquid feces and vomiting were reported at an increased incidence in all treated animals (low, mid or high) in comparison to the vehicle controls. The incidence was greatest in the high dose group of animals. Salivation was present during and especially after dosing in the animals. Evidence of a drug related effect was restricted to the thymus only (high). Thymus involution was noted in animals from all groups including the vehicle control animals but the severity was only marginally higher in dogs at the high dose in comparison with the vehicle controls.

Twelve month study in dogs:

Groups of male and female dogs (4/sex/group) were administered darunavir via oral gavage at dose levels of 0 (vehicle control; PEG 400 in water), 30 (low), 60 (mid) or 120 (high) for a period of 12 months. Vomiting was increased in treated groups, particularly in animals at the two high doses. There was no effect on body weight, food consumption, ophthalmoscopy or EKG measurements. There was an increase in ALP at the high dose (2 male and 2 females) and the intermediate dose (2 females). Hematological and urinalysis parameters were unchanged. Liver weight was slightly higher in animals given the high and intermediate doses. Histopathological examination discovered increased hepatocellular pigment at all doses and vacuolation in both sexes at the two higher doses. Spleen weight of females at the high dose was decreased (36%) relative to controls in the absence of any histopathological change.

Genetic toxicology studies:

Darunavir was not mutagenic in an Ames Test, an in vitro mammalian chromosome aberration assay and an in vivo mammalian micronucleus test. Thus, it was not mutagenic in the full battery of ICH genetic toxicity tests.

Carcinogenicity studies:

Darunavir is presently being assayed in carcinogenicity tests in mice and rats. The studies will be completed as a Phase 4 commitment. The protocols for the studies were presented to the Executive Carcinogenicity Assessment Committee and received concurrence. The minutes of the Committee meeting are contained in the Appendix.

Reproductive toxicology studies:

Segment I

Darunavir, at doses up to 1000 mg/kg/day dosed to pregnant rats from gestation day (GD) 7 to 9 and from GD 13 to 19, did not induce any adverse events on any maternal and fetal parameters measured.

Segment II Rats

Pregnant Sprague Dawley rats were dosed with darunavir at 0 (PEG 400), 40, 200 and 1000 mg/kg/day between gestation days seven and 19. There was no evidence that TMC 114 was teratogenic in the dosed rats. Based on the fact that body weights and food consumption were reduced in the high dose dams, the maternal NOAEL was considered to be 200 mg/kg/day. There were no effects of darunavir treatment on embryo-fetal development at any of the doses administered. The embryo-fetal NOAEL was considered to be 1000 mg/kg/day.

Segment II Rabbits

Pregnant New Zealand white rabbits were dosed with darunavir at 0 (vehicle), 40, 200 and 1000 mg/kg/day between gestation days eight and 20. There were two deaths, 1 at 40 and 1 at 1000 mg/kg/day at which the cause was not determined. Two animals at the 1000 mg/kg/day dose aborted on gestation day 20 and were sacrificed but no relevant clinical signs were discerned. There were no differences in mean body weight among groups. Some high dose animals showed body weight gain decreases when compared to controls. There was a similar effect on food consumption during the treatment period with individual high dose animals showing a decrease compared to controls.

There was no evidence that darunavir was teratogenic in rabbits.

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.

Segment III Rats**Maternal Performance:**

Maternal treatment with darunavir 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 No Observed Adverse Effect Level (NOAEL) for maternal treatment was therefore considered to be 40 mg/kg/day.

When darunavir was administered together with Ritonavir at 1000+75/50 mg/kg/day similar observations were recorded but the effect was greater than with darunavir alone.

Maternal treatment with Ritonavir 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 greater than those in the combination groups.

Pup growth and Pup/F1 development:

Maternal administration of darunavir 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 in these groups. There was no

effect of maternal treatment on F1 developmental tests or mating and fertility at any dose level. The No Observed Effect Level (NOEL) for pup development after maternal treatment with the test article was therefore considered to be 40 mg/kg/day.

When darunavir was administered together with Ritonavir at 1000+75/50 mg/kg/day similar observations were recorded for bodyweight and developmental milestones but the effect was more pronounced than with darunavir alone. In addition, pup survival during lactation was reduced in comparison with the Controls. The reduced bodyweight gain during lactation was observed only in the group where dosing was continued until PND 14. In the group where dosing stopped at the end of gestation, bodyweight performance was comparable with the Controls. This confirms that this finding was directly related to administration to the mother 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 Ritonavir 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 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.

Local tolerance:

Local lymph node assay: In a T-lymphocyte proliferation assay, darunavir was studied to determine its potential to cause skin sensitization. Under the conditions of the study, darunavir was found to be negative to have the potential to cause skin sensitization.

Four week immunotoxicology study in rats:

Darunavir was not found to cause any immunological toxic response at doses ranging from 20 to 500 mg/kg/day. Similarly, for RTV alone or in combination (darunavir/RTV 100/50 mg/kg/day), no immunotoxicological response was observed.

B. Nonclinical safety issues relevant to clinical use

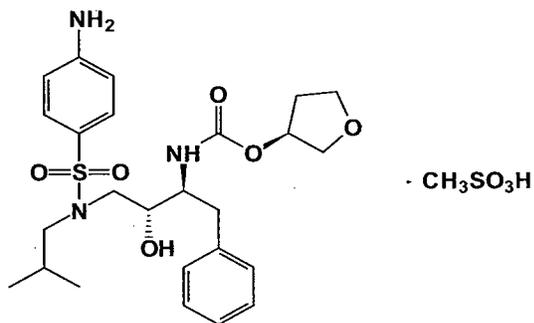
Darunavir has a classical sulfonamide structure and, as such, should be expected to elicit certain class-specific toxicities. In the clinic, the most common toxicities are disturbances to the urinary tract (crystalluria), hemolytic anemia and hypersensitivity reactions (usually manifested as rash). In a low percentage of patients, liver necrosis has been known to occur. Very few kidney toxicities were seen in nonclinical studies. In rats, there were some increases in kidney weight with some accumulation of brown pigment (lipofuscin). However, no related histology was seen. In rodents, darunavir had profound effects on the hematopoietic system with decreases in RBC counts as well as decreases in hemoglobin and hematocrit measurements with an increase in reticulocytes. These results with an increase in bilirubin measurements indicated increased red cell turnover, indicative of hemolytic anemia. In the mouse, darunavir showed hepatocellular hypertrophy and AST increases in the three month study. In the 12 month dog

study, darunavir elicited hepatocellular hypertrophy with an increase of pigments. On long-term dosing darunavir showed some hepatocellular vacuolation. It also induced an increase in alkaline phosphatase and a decrease in spleen weight.

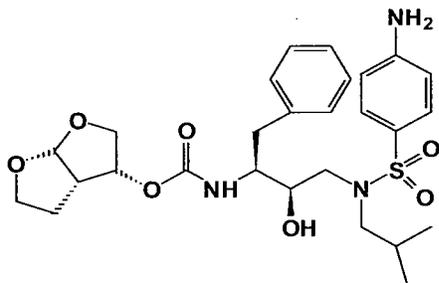
Comparison of the toxicity profile of agenerase and darunavir in rodents and dogs

The structure of agenerase and darunavir are extremely similar (see structures below).

Agenerase:



Darunavir:



Both drugs are sulfonamides and differ in basic structure by only two carbons and one oxygen and one would expect their toxicity profiles to be similar.

Comparing the toxicity of agenerase and darunavir in the 12 month dog studies one will find that agenerase in the dog showed a lower hematocrit but also a decrease in reticulocytes. There was a decrease in the APPT and albumin levels and an increase in alkaline phosphatase. Agenerase administration showed hepatocellular hypertrophy with pigments in the liver. Darunavir elicited hepatocellular hypertrophy with an increase of pigments. On long-term dosing darunavir showed some hepatocellular vacuolation. It also induced an increase in alkaline phosphatase and a decrease in spleen weight. Thus, the toxicities were similar in the dog one year studies.

In rodents, both compounds induced cholesterol increases and triglyceride decreases. However, in rats, in six month studies, both compounds induced hepatocellular hypertrophy with increased

liver pigments but agenerase showed increased ALT and AST values with hepatocellular necrosis after long-term dosing. In the mouse, darunavir showed hepatocellular hypertrophy and AST increases in the three month study. In both the rat and mouse study, darunavir induced a decrease in red blood cells and red blood cell parameters (decreased hemoglobin and hematocrit) with an increase in reticulocytes and a profound increase in bilirubin. These effects, which signal red blood cell turnover, were not seen with agenerase.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-976

Review number: 1

Sequence number/date/type of submission: 000/12/23/05/New Chemical Entity

Information to sponsor: Yes () No (X)

Sponsor and/or agent: Tibotec-Virco

Manufacturer for drug substance: Tibotec-Virco

Reviewer name: James G. Farrelly and Pritam S. Verma

Division name: Division of Antiviral Products

HFD #: HFD-530

Review completion date:

Drug:

Trade name: PREZISTA

Generic name: Darunavir

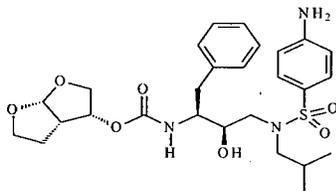
Code name: TMC 114

Chemical name: {3-[-amino-benzensulfonyl]-isobutyl-amino]-1-benzyl-2-hydroxy-propyl}-
carbamic acidhexahydro-furo[2,3-b]furan-3-yl ester ethanolate

CAS registry number: 313682-08-5

Molecular formula/molecular weight: $C_{27}H_{37}N_3O_7S.C_2H_5OH$; 593.724 (active moiety
+ ethanol) 547.656 (active moiety)

Structure:



Relevant INDs/NDAs/DMFs: IND 62,477

Drug class: Anti-HIV protease inhibitor

Intended clinical population: Treatment of HIV infection

Clinical formulation: Tablets containing 200 mg or 400 mg darunavir base formulated as darunavir ethanolate, microcrystalline cellulose, and magnesium stearate

Ritonavir: Ritonavir (Norvir) soft gelatin capsule (100 mg RTV/capsule, Abbott)

Route of administration: Oral

Disclaimer: Some graphical information was constructed by the sponsor unless cited otherwise.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Darunavir was investigated in a series of in vitro and in vivo safety pharmacology studies. There were no adverse effects of drug on in vitro cardiovascular electrophysiological parameters, and none observed on cardio-hemodynamic and ECG measurements in dogs given single oral doses up to 120 mg/kg. There were no relevant effects in rats on neurobehavior and motor activity, or on gastrointestinal and pulmonary safety at single oral doses up to 2000 mg/kg.

2.6.2.2 Primary pharmacodynamics

For primary pharmacodynamic effects, mechanism of action and drug activity, see the Microbiology review.

2.6.2.3 Secondary pharmacodynamics

Not applicable

2.6.2.4 Safety pharmacology

Neurological effects:

There were no adverse effects on neurobehavior and motor activity of rats assessed before treatment and over 24 hours after single oral administration up to 2000 mg/kg darunavir when compared with vehicle (PEG400) and a positive control substance (chlorpromazine hydrochloride).

Cardiovascular effects:

In vitro, darunavir at a concentration of 10 μ M, showed no significant effect on membrane potassium (K^+) current in an hERG assay. At concentrations up to 10 μ M, there were no effects, in vitro, on the electrophysiological cardiac action potential parameters in sheep isolated cardiac Purkinje fibers.

In vivo, single oral doses of 0 (PEG 400), 30, 60 or 120 mg/kg darunavir administered to conscious, telemetered dogs had no effect on cardio-hemodynamic and electrocardiogram (ECG) parameters. In addition, in 12-month repeat dose dog studies at doses up to 120 mg/kg/day, no treatment-related effects on heart rate or ECG morphology were seen. On day one of the repeat-dose study, at 120 mg/kg, mean TK values determined were C_{max} values for male and female dogs of 16.6 and 15.0 μ g/ml and AUCs of 69.4 and 53.4 μ g.h/ml.

Pulmonary effects:

Oral administration of darunavir had no acute effects on respiration in rats at doses up to 2000 mg/kg when compared with vehicle (40% aqueous PEG 400) and a methacholine, a positive control substance.

Gastrointestinal effects:

There was no effect on gastrointestinal transit time of a charcoal solution after oral administration of up to 2000 mg/kg darunavir when compared with vehicle (40% aqueous PEG400) and atropine, a positive control substance in rats.

Abuse liability:

None noted.

2.6.2.5 Pharmacodynamic drug interactions

Not applicable

2.6.3 PHARMACOLOGY TABULATED SUMMARY

See above

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

Results are shown in relevant studies

2.6.4.1 Brief summary

See above

2.6.4.2 Methods of Analysis

[See under individual study reviews]

2.6.4.3 Absorption

Since Caco-2 human colon tumor cells revealed a reasonably high transepithelial intestinal permeability, the proposed mechanism for absorption of darunavir was passive transcellular diffusion.

Based on observed t_{max} values (0.5 to 6 hours), darunavir absorption was rapid following oral administration in all tested species. The absolute oral bioavailability was 37 to 58% in rats. Bioavailability was higher in dogs ranging between 60 to 122%. The plasma clearance and the volume of distribution were moderate to high in rats and dogs and elimination was rapid in all species. The kinetics of darunavir was less than dose-proportional in mice, rats and dogs after single oral administration, especially at the high dose levels. Repeated oral dosing often resulted in a decrease in systemic exposure. The induction of metabolic enzymes could have contributed to the phenomenon. Darunavir was an inducer of CYP3A isoenzyme in rodents while UDP-GT activity was also induced in rats. However, in dogs, no decrease in exposure and enzymatic induction was observed upon repeated administration.

Intravenous administration was examined in an attempt to increase the exposure of darunavir in animal studies. This route was not successful due to the difficulty in finding a formulation into which a sufficiently high concentration of drug could be dissolved. Administration by dietary admixture resulted in systemic exposure levels comparable to those attained after gavage.

However, greater exposures could not be attained, even at very high levels of drug in the feed. The two above methods were abandoned by the sponsor.

The recommended clinical dose of darunavir/RTV for treatment of experienced HIV- infected patients is 600/100 mg b.i.d. At this dose level, C_{max} was approximately 10 $\mu\text{g/ml}$ while the estimated AUC_{0-24h} was 123 $\mu\text{g.h/ml}$.

2.6.4.4 Distribution

The tissue distribution of ^{14}C -darunavir in rats was extensive and rapid with the highest concentrations of radioactivity in the liver and adrenal gland. Except in melanin-rich tissues, no unusual retention or accumulation of radioactivity occurred. Even then, a gradual decrease of radioactivity levels occurred, showing the reversibility of binding. In pregnant rats, ^{14}C -darunavir was distributed to the placenta and fetus. Total radioactivity exposure in the fetus was about 13 to 27 % of that of maternal blood, while in placenta it was the same as in blood.

2.6.4.5 Metabolism:

Plasma protein binding was moderate to high both in humans (free fraction, 5%) and animals, ranging from 5% in the rat to 38% in the rabbit. In most species, binding was concentration dependent.

The metabolism of darunavir was extensive and qualitatively similar in all species, including humans. Metabolism studies were carried out mainly in rats, dogs and humans and, in general, discovered three types of expected Phase I metabolites, aromatic hydroxylation at the aniline moiety, carbamate hydrolysis and aliphatic hydroxylation at the isobutyl moiety. The major Phase I metabolic pathway in dogs and humans was carbamate hydrolysis. In rats, hydroxylation was the most prevalent. Glucuronidation was a minor pathway in all species tested. No unique human metabolites were found. Metabolic pathways proposed on the basis of in vitro studies were carried out using tissues from rats, dogs, mice and rabbits as well as humans. Since the metabolic profiles in the rat, dog and human, in vitro was very similar to those found in vivo, it was assumed that the in vitro profile in mice and rabbits would also mimic that which would be found in vivo (which may not necessarily be true).

The known in vivo pathways of metabolism for the dog, rat and human are shown in the following diagram:

2.6.4.6 Excretion:

¹⁴C- darunavir excretion was evaluated in three separate single dose gavage studies in male and female Sprague-Dawley rats at 40 mg/kg, in a biliary excretion study in male Sprague Dawley rats at the same dose level and in male dogs at 30 mg/kg. In the first study, darunavir was investigated alone and also in combination with RTV (25 mg/kg/day).

The major route of excretion of ¹⁴C- darunavir was via feces in both rats and dogs. In rats monitored over a 96-hour period, excretion in both sexes averaged 94%. In the first 24-hours, excretion of radioactivity in the feces was rapid and accounted for more 80 of the label. Co-administration with RTV in rats had no substantial effect on the excretion and the elimination rate of ¹⁴C- darunavir. In the dog, excretion was monitored over a 168-hour period, and 86% of the administered radioactivity was recovered in the. In the first 24 hour period, fecal excretion was rapid and accounted for 70%. Urinary excretion was limited representing 4.2% and 3.9% of the administered dose in rats and dogs.

2.6.4.6 Pharmacokinetic drug interactions

See the review by the member of the Biopharmaceutics team.

2.6.4.8 Other Pharmacokinetic Studies

2.6.4.9 Not applicable (see the individual toxicology studies)

2.6.4.10 Tables and figures to include comparative TK summary

See the individual toxicology studies

2.6.5 PHARMACOKINETICS TABULATED SUMMARY**2.6.6 TOXICOLOGY****2.6.6.1 Overall toxicology summary**

See under Summary of Nonclinical Findings (above)

2.6.6.2 Single-dose toxicity

Not reviewed. See attached reviews by Dr. Zhang.

2.6.6.3 Repeat-dose toxicity**Study title: 3-month Repeated Dose Oral Toxicity Study of Darunavir in the Swiss Mouse**

Key study findings: The most relevant findings were in the hematological examinations. In both males and females there were essentially dose dependent decreases in red blood cell parameters and increases in bilirubin and reticulocyte measurements. The results are consistent with the studies in rats and indicate a tendency of rodents to exhibit the signs of anemia, a toxicity known to be related to sulfonamide therapy.

There were some toxic effects in the liver of females at the high dose (1000 mg/kg/day). This was shown as slight necrosis over controls and an increase in ALT.

Study no.: TMC114 – NC157

Conducting laboratory and location: Johnson & Johnson Pharmaceutical Research & Development, a division of Janssen Pharmaceutical N.V. Global Preclinical Development, Beerse site Turnhoutseweg 30, 2340 Beerse, Belgium

Date of study initiation: October 14, 2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: 3510A/2003 —

Methods

Doses: 0, 150, 450 or 1000 mg/kg/day of darunavir in PEG 400 for three months. The high dose is essentially, a maximum feasible dose. Higher concentrations of the drug form a precipitate.

Species/strain: SPF albino Swiss CD-1 mice

Number/sex/group or time point (main study): 10

Route, formulation, volume, and infusion rate: gavage in PEG 400, at 10 ml/kg. Higher volumes per kilogram are toxic.

Satellite groups used for toxicokinetics or recovery: Thirty males and 30 females at each dose group

Age: five weeks old

Histopathology: Adequate Battery: yes (X), no ()—explain

Peer review: yes (), no (X)

Results

Mortality: Four deaths. One vehicle male and one vehicle female, one 150 mg/kg male and one 1000mg/kg female. All were gavage accidents.

Clinical signs: Soft feces in some animals in all groups was probably related to vehicle.

Body weights: No relevant body weight changes were noted up to the highest dose.

Food consumption: No change in regard to control in the low and high dose animals. At 450 mg/kg, there was a slight increase from week four leading to a slightly higher food consumption.

Ophthalmoscopy: Not done.

EKG: Not done.

Hematology: A dose-related decrease in red blood cells in males were noted throughout the study with decreases in hemoglobin and hematocrit values. Mean cell hemoglobin decreases were also seen in males at the two highest doses. Females also had decreases in red blood cell parameters but the results were somewhat lower than in the males. In both males and females, slight decreases in white blood cells were also noted. At the high dose, there was a pronounced increase in the number of reticulocytes in both males and females.

Clinical chemistry: At the low dose, females showed an increase in cholesterol and bilirubin. At the intermediate dose, increases in triglycerides were seen in males and females. Again, at the intermediate dose and higher, the males also showed the increase in cholesterol and bilirubin and the females showed an albumin increase. At the high dose, slight increases in calcium and albumin were seen in males. At this dose, females showed an increase in ALT levels and the males showed a decrease in alkaline phosphatase.

Urinalysis: not done

Gross pathology: At the two higher doses, swollen spleens were seen in a few males. At the higher doses swollen livers were noted. The liver effect was probably due to adaptive changes rather than to toxicity.

Organ weights: There were few events other than an increase in liver weights in males and females. In males, there were some increases in spleen and adrenal weights and in females at the high dose, an increase in relative thymus weights.

Histopathology: Adequate Battery: yes (X), no ()—explain
Peer review: yes (X), no ()

The only relevant histopathology results were noted in the liver. There was centrilobular hypertrophy seen in the males at the two higher doses and in females at the high dose. There was a slight accumulation of pigment in the effected animals. In the high dose females there was an increase in hepatocellular necrosis (over controls). Since there was an increase in ALT in females, this event was considered darunavir related.

Toxicokinetics:

On day 0 and day 86, blood samples (1.3 ml in EDTA) were taken at 1, 2, 4, 8 and 24h after dosing. For each sampling point/dose level, 3 male and 3 female mice of the satellite group were anesthetized with ether and blood samples were obtained from the carotid artery by decapitation. Samples were kept on ice.

The results of the analyses are shown in the following Table.

Time	Day	150 mg/kg/day				450 mg/kg/day				1000 mg/kg/day			
		Male		Female		Male		Female		Male		Female	
		0	86	0	86	0	86	0	86	0	86	0	86
1		5017	1733	7923	1980	9463	4787	12133	6317	11933	3160	29033	6797
2		2527	1777	3890	2890	7617	3660	14330	8447	7903	4177	19633	6000
4		931	567	1917	783	4117	3493	10763	6240	7207	3803	12300	4657
8		65.7	98.5 ⁷⁾	1201	141	50.5	690	6900	1877	2641	2490	11103	4600
24		<5.0	<5.0	<5.0	<5.0 ⁵⁾	51.1	11.3	12.4	7.46				
C _{max}	(ng/ml)	5017	1777	7923	2890	9463	4787	14330	8447	11933	4177	29033	6797
T _{max}	(h)	1	2	1	2	1	1	2	2	1	2	1	1
t _{1/2}	(h)	1.0	1.6	5.9	1.6	0.6	1.7	6.2	2.3	2.8	2.1	1.6	1.7
AUC _{0-8h}	(ng*h/ml)	10640	5811	21331	8153	28311	20658	78978	39642	49040	25630	116674	38905
AUC _{0-24h}	(ng*h/ml)	- ¹⁾	6035	- ¹⁾	8483	- ¹⁾	22359	- ¹⁾	45839	59545	32975	142788	50344
AUC _{0-∞}	(ng*h/ml)	10739	- ²⁾	- ³⁾	- ²⁾	28357	- ²⁾	- ³⁾	- ²⁾	59753	- ²⁾	142817	- ²⁾

Study title: Six Month Oral (Gavage) Repeat Dose Toxicity Study in the Rat

Key study findings: Darunavir was administered once daily in PEG 400, by gavage, for 6 months. One control group and three treated groups were given, 0 (vehicle), 20, 100 and 500 mg/kg/day. The dose volume was 5 ml/kg. Each group consisted of 20 male and 20 female Sprague-Dawley rats (called the principal group) together with 5 male and 5 female animals (treated groups only as satellites) for TK analysis. Observations 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) were made at regular intervals. Blood samples were collected on day 1 and during weeks 13 and 26 during the 24 hours after dosing for TK analysis. At necropsy, all principal group animals found dead or sacrificed were subject to macroscopic examination and specified organs were weighed. A specified group of tissues and organs were preserved for histological examination. Tissues and organs from principal group animals in the control and high dose group and a specified selection 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 darunavir, but there were a number of accidental deaths. There were no relevant clinical signs, or effects on body weight or food consumption. Effects on the hematopoietic system were seen with decreases (up to 11%) in RBC count, hemoglobin, and hematocrit and an increase in reticulocyte count occurring in animals given the high dose (500 mg/kg/day). Similar, but generally less marked changes in reticulocyte count occurred in animals given 100 mg/kg/day. There was an increase in bilirubin values which, together with the reticulocytes increases, indicated red blood cell turnover. Platelet count increased (up to 37%) in animals given 500 mg/kg. Less marked changes in platelet count occurred in animals given 100 mg/kg/day.

An increase in APTT occurred in animals given 100 and 500 mg/kg/day (up to 20% and 44%, respectively). Bilirubin was up as high as seven-fold and cholesterol was up to 74% above controls while triglycerides were decreased in animals given 500 mg/kg/day. There was some effect at 100 mg/kg/day. Bilirubin was slightly increased in males given 20 mg/kg/day at week 26. No other serum chemistry parameters were considered of consequence.

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

At necropsy there was a slight increase in male kidney and spleen weight and male and female liver weight in animals given 100 and 500 mg/kg/day. The increase was confirmed to be a consequence of hepatocellular hypertrophy, an adaptive rather than a toxic response. Lipofuscin

was present in the liver and kidney. 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.

Study no.: TMC 114-NC132

Conducting laboratory and location:

Date of study initiation: 9/1/2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: 86319 , 89025, _____

Methods

Doses: 0 (PEG 400), 20, 100 and 500 mg/kg/day

Species/strain: Sprague-Dawley rats

Number/sex/group or time point (main study): 20 males and 20 females

Route, formulation, volume, and infusion rate: Oral, in PEG 400 at 5 mg/kg

Satellite groups used for toxicokinetics or recovery: 5 males and 5 females

Age: 4 weeks

Weight: 192 g, males: 159 g, females

Sampling times: day 1, week 6, week 13 and week 26

Organ weights:

The following organs from all animals were weighed after trimming of fat and other contiguous tissue. Paired organs were weighed together.

Adrenals, brain, heart, kidneys, liver, ovaries, pituitary*, prostate, thymus, spleen, testes/epididymides, thyroids*, uterus

*Weighed after fixation.

Histopathology: Adequate Battery: yes (X), no ()—explain

Peer review: yes (X), no ()

With the exception of the testes, eyes, optic nerves and bone marrow smears, either whole organs or samples of the tissues listed below were preserved in 10% neutral buffered formalin:

Adrenals

all gross lesions

aorta

bone (sternum)

bone (femoro/tibial joint)

bone marrow smear
brain (3 sections)
caecum
cervix
colon
duodenum
eyes/optic nerves
heart
ileum with peyer's patches
jejunum
kidneys with ureter
larynx
liver
lungs (with mainstem bronchi)
mesenteric lymph node
esophagus
ovaries
pancreas
pituitary
prostate
rectum
salivary gland
sciatic nerve
seminal vesicles
site of mammary gland
skeletal muscle
skin
spinal cord (3 levels)
spleen
stomach
submandibular lymph node
testes /epididymides
thymus
thyroids/parathyroids
tongue
trachea
urinary bladder
uterus with oviduct
vagina

The testes, eyes and optic nerves were fixed in Davidson's fluid. Bone marrow smears were air-dried, fixed in anhydrous methanol and stained.

The above wet tissues from those animals detailed below were sent to the principal investigator,

~~_____~~ for processing to slides:

- i) All tissues from control and group 4 terminal kill animals.
- ii) All tissues from animals dying or killed during the study.
- iii) All gross lesions from all animals.

iv) Adrenals, liver, kidneys, salivary glands, spleen and thymus from all group 2 and 3 animals.

Results

Mortality:

There were 22 premature deaths during the study. Twenty of these animals were necropsied and 16 of these were confirmed as dosing accidents.

Nineteen were main study group animals and three were from the satellite groups. Of the 19 main study rats subjected to necropsy, five controls, three low dose, three intermediate dose and four high dose animals died as the result of dosing accidents.

The remaining 4 main study decedents were found dead in their cages having shown no previous clinical observations. No cause of death was found for any of these animals at necropsy or histopathology examination. However, due to the timing of these deaths early after dosing, it was considered highly likely that these were also dosing accidents.

Clinical signs:

There were no treatment related clinical signs noted during the dosing period.

Body weights:

There were no treatment related clinical signs noted during the dosing period.

Food consumption:

There were no treatment related clinical signs noted during the dosing period.

Ophthalmoscopy:

There were no treatment related clinical signs noted during the dosing period.

Hematology:

Week 6 - A reduction in red blood cell count, hemoglobin and hematocrit was seen at 500 mg/kg/day. The reduction was accompanied by reductions in mean cell volume in males, mean cell hemoglobin and mean cell hemoglobin concentration in females. There was also a slight increase in platelet count for 500 mg/kg/day males and 500 and 100 mg/kg/day females. Reticulocytes were increased in 100 and 500 mg/kg/day males and 500 mg/kg/day females, while activated partial thromboplastin time was increased in 500 mg/kg/day males and females.

Week 13 - In general, results were similar to those seen at week 6 with the exception that female APTT was unaffected.

Week 26 - Hematology parameters again reflected those seen at weeks 6 and 13. However, the changes in RBC, Hb and HCT were less marked. Reticulocytes were somewhat slightly increased in 20 mg/kg/day males. APTT was increased in 500 mg/kg/day males and females and also in 100 mg/kg/day females.

Clinical chemistry:

Week 6 - Creatinine levels were increased in 500 mg/kg/day females and to a lesser extent in males at this dose. Triglycerides were decreased in 500 and 100 mg/kg/day males and females while cholesterol levels were decreased in 500 and 100 mg/kg/day males but increased in 500 mg/kg/day females. Total protein and globulin were increased in 100 and 500 mg/kg/day females. There was a smaller increase in globulin in 20 mg/kg/day females. Potassium was increased in males at all doses but there was no dose response.

Week.13 - The 100 mg/kg/day males and both males and females at 500 mg/kg/day showed a decrease in triglycerides. At the same time, both sexes dosed at 500 mg/kg/day had increases in cholesterol. Creatinine was slightly increased in the high dose males and there was also a small increase in urea in these males. Bilirubin was increased in the high dose males but in the females to a lesser extent. Total protein and globulin were increased in 500 mg/kg/day females.

Week 26 - The 100 mg/kg/day males and 500 mg/kg/day males and females continued to show a decrease in triglycerides and 100 mg/kg/day males and 500 mg/kg/day males and females had increases in cholesterol. Urea and creatinine were increased in 500 mg/kg/day males and females and were increased in 500 mg/kg/day females but there was also an increase in urea in 100 mg/kg/day males. Bilirubin continued to be increased in 500 mg/kg/day males and females and there was also an increase in 100 mg/kg/day males and females and 20 mg/kg/day males. Total protein and globulin were increased in 100 and 500 mg/kg/day females.

Urinalysis:

Week 6 - There was a slight reduction in urine volume for males dosed at 500 mg/kg/day.

Week 13 - Urine volume was again slightly reduced in males dosed at 500 mg/kg/day.

Week 26 - Urine volume was lower in 500 mg/kg/day males and females as well as in 100 mg/kg/day females while osmolality was slightly higher than in control animals.

Gross pathology:

Kidneys were reported to be darkly discolored in 500 mg/kg/day males and females. Liver was also darkly discolored a few animals at all doses.

Organ weights:

Absolute and relative liver weights were increased at 100 and 500 mg/kg/day in both sexes, while absolute and relative spleen and kidney weights were increased at 100 and 500 mg/kg/day in males only.

Histopathology:

Vacuolation of the zona fasciculata was noted in adrenals of males dosed at 500 mg/kg/day and in a small number of males dosed at 100 mg/kg/day.

Brown pigment in the proximal tubules of the kidneys was seen in terminal sacrifice males and females treated at 500 mg/kg/day as well as in a few males dosed at 100 mg/kg/day.

Brown pigment was seen in hepatocytes of males and females treated at 500 mg/kg/day and also in a few animals at 100 mg/kg/day. Hypertrophy of hepatocytes was seen in all animals dosed

at 500 mg/kg/day and in a few males and females treated at 100 mg/kg/day. Minimal hypertrophy/hyperplasia of bile ducts was noted in most animals treated at 500 mg/kg/day. Small foci of altered hepatocytes were seen in animals treated at 500 mg/kg/day. The effect was more pronounced in males and some foci were seen at 100 mg/kg/day.

Minimal diffuse acinar hypertrophy was present in the salivary glands of males and females (more pronounced in females) at 500 mg/kg/day.

Extramedullary hematopoiesis of erythroid precursors was seen in the spleen of both treated and control animals but the incidence was slightly greater in animals dosed at 500 mg/kg/day.

Involution of the thymus was seen in treated and control animals but with slightly greater severity in animals treated at 500 mg/kg/day.

Study title: 6-Month oral toxicity study with TMC114 and ritonavir by daily gavage in rats

Key study findings: Groups of male and female rats administered darunavir/RTV via oral gavage at dose levels of 0 (vehicle control; PEG 400 in water), 20/50 (low), 100/50 (mid), 500/75 (high) or 1000/75 mg/kg/day (highest), or RTV alone (75 mg/kg/day) for 26 consecutive weeks. Results: 33 animals were found dead or were sacrificed prematurely during the study. In the main study, these animals were 2 (vehicle control), 10 (RTV alone), 3 (low), 4 (mid), 6 (high) and 3 (highest). In the satellite group, these animals were 2 (vehicle control), 0 (RTV alone), 1 (low), 0 (mid), 1 (high) and 1 (highest). Clinical signs: salivation, brown staining of the fur and hunched posture were noted in all groups other than the vehicle control. The incidence of salivation and hunched posture increased with increasing dose level of darunavir. Body weight and body weight gain: lower body weight gains were observed for male and females (high or highest). The deficits in total body weight gain were 16% and 12% for males and 8% and 9% for females for the high and highest dose levels, respectively. Hematology: treatment related disturbances of the hematological parameters consisted of several affected red blood cell parameters: lower hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and increased red cell distribution width, bilirubin levels and reticulocyte counts, all indicative of an increased blood cell turnover. Clinical chemistry: creatinine (males), bilirubin and cholesterol were increased (up to 18%, 3-fold and 3-fold, respectively) and triglycerides increased in females (2.4-fold) and decreased in males (73%) in animals at the high and highest dose levels. Phospholipids were increased in both sexes (high and highest). An increase in ALT (2.9-fold) and AST (73%) was noted in males (RTV alone) and to a lesser extent, in groups treated with darunavir/RTV combination (up to 78%).

A NOAEL was not established in the study. Main findings were noted in the liver (hepatocyte hypertrophy, vacuolation, multinucleated hepatocytes and single cell necrosis of multinucleated hepatocytes, histiocytosis), erythrocyte parameters and spleen (extramedullary hematopoiesis), thyroid gland (hypertrophy and/or hyperplasia) and kidney (nephropathy and pigmentation of cortical tubules). These findings were observed mainly with darunavir/RTV combination treatment and to some extent with RTV treatment alone. In general, these findings increased with increasing dose of darunavir. Findings in pancreas (increased incidence/severity of islet fibrosis/siderocytes in males), adrenals (increased severity of cortical vacuolation) and brown pigmentation of hepatocytes was only observed after treatment with darunavir.

In the 6-month darunavir/RTV combination study in rats, a dose level of 20/50 mg/kg/day was the lowest dose level utilized. At the 20/50 mg/kg/day, based on the body surface area factor, an

equivalent dose for darunavir in humans would be 3.25 mg/kg/day or 195 mg/day for a 60 kg person. At the low dose, exposures (steady state AUC and Cmax) of darunavir were 11.2 (male) and 13.4 (female) $\mu\text{g}\cdot\text{hr}/\text{ml}$ and 1.66 (male) and 1.95 (female) $\mu\text{g}/\text{ml}$ in rats.

Study no.: TMC 114-NCI146-TSR

Volume # and page #: 1 and 1-1003

Conducting laboratory and location: _____

Date of study completion: March 15, 2004

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: TMC 114 (AA-5977-batch-5-01; _____ and ritonavir (85407VA)

Methods

Doses: Groups of male and female rats administered darunavir/RTV combination via oral gavage at dose levels of 0 (vehicle control; PEG 400 in water), 20/50 (low), 100/50 (mid), 500/75 (high) or 1000/75 mg/kg/day (highest), or RTV alone (75 mg/kg/day) for 26 consecutive weeks according to an experimental design shown in the following Table.

Experimental design of the oral (gavage) 6-month toxicology study in rats

Group	Dose level (mg/kg/day)		Number of animals			
	darunavir	RTV	Main study		Satellite for toxicokinetics	
			Male	female	male	female
1. Vehicle control	0	0	20	20	2	2
2. Ritonavir alone	0	75	20	20	5	5*
3. Low	20	50	20	20	5	5
4. Mid	100	50	20	20	5	5
5. High	500	75	20	20	5	5
6. Highest	1000	75	20	20	5	5

* = these animals were also necropsied and subjected to histopathological examination because 9 main females of vehicle control 2 had died intercurrently during the study.

Species/strain: male and female rats; strain: Wistar CrI: (WI) BR (outbred, SPF-quality)

Number/sex/group or time point (main study): See above table

Route, formulation, volume, and infusion rate: oral gavage; TMC114 = 4 ml/kg; ritonavir = 1.25 ml/kg

Satellite groups used for toxicokinetics: See above table

Age: 7 weeks

Weight: 181-227 g for males and 137-211 g for females

Sampling times: Plasma concentrations of darunavir/RTV were determined at 1, 2, 4, 8 and 24 hr on day first and weeks 13 and 26 the study.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Ophthalmoscopy: weeks 1, 6 and 26

Hematology: weeks 6, 13 and 26

Clinical chemistry: weeks 6, 13 and 26

Urinalysis: weeks 6, 13 and 26

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: See table below

Histopathology: Table, Adequate Battery: yes; Peer review: yes

Table
Rat tissues, weighed, preserved and examined

Organ name	Weighed	Preserved	Examined microscopically
adrenal glands	X	X	X
aorta (thoracic)		X	X
Bone marrow smear (rib)		X	
Bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X
Brain (medulla, pons, cerebrum and cerebellum)	X	X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes with optic nerve		X	X
Harderian gland		X	X
Heart	X	X	X
Kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
Liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
Lymph nodes (mesenteric, mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
Ovaries	X	X	X
Pancreas		X	X
pituitary gland		X	X

prostate gland		X	X
Salivary glands submandibular)		X	X
seminal vesicles		X	X
Skeletal muscle (biceps femoris)		X	X
Skin		X	X
Small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumber)		X	X
Spleen	X	X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid,/parathyroid glands	X, thyroid	X	X
Trachea		X	X
Turbinates (skull)		X	
urinary bladder		X	X
uterus (body/horns) with cervix		X	X
Vagina		X	X
All gross lesions		X	X

Results

Mortality: 33 animals were found dead or were sacrificed prematurely during the study. In the main study, these animals were 2 (vehicle control), 10 (RTV alone), 3 (low), 4 (mid), 6 (high) and 3 (highest). In the satellite group, these animals were 2 (vehicle control), 0 (RTV alone), 1 (low), 0 (mid), 1 (high) and 1 (highest). The majority of these deaths were associated with dosing error. The cause of death was not evident in 12 animals. The high mortality in females (RTV alone) was the reason why female satellites in this group were necropsied and examined macro- and microscopically.

Clinical signs: salivation, brown staining of the fur and hunched posture were noted in all groups other than the vehicle control. The incidence of salivation and hunched posture increased with increasing dose level of darunavir.

Body weight and body weight gain: lower body weight gains were observed for male and females (high or highest). The deficits in total body weight gain were 16% and 12% for males and 8% and 9% for females for the high and highest dose levels, respectively. These deviations were related to darunavir exposure and were treatment related.

Food consumption: absolute and relative food consumption was significantly lower for males and female (high or highest) and for females (RTV alone) in the first week of treatment.

Ophthalmology: no treatment related abnormalities were noted.

Hematology: treatment related disturbances of the hematological parameters consisted of several affected red blood cell parameters: lower hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin and increased red cell distribution width, bilirubin levels and reticulocyte counts, all indicative of an increased blood cell turnover. This was confirmed by the microscopic findings (increased extramedullary hematopoiesis (high and highest) and macrophage aggregates (low or mid) in treated animals. Maximum severity was noted at the highest dose level. Treatment related increase in the platelet count and a decreased in the prothrombin time were observed. When compared to RTV treatment alone, darunavir treatment resulted, in general, in an increased in effects on erythrocyte parameters, increased platelet counts and further decreased in prothrombin time.

Clinical chemistry: creatinine (males), bilirubin and cholesterol were increased (up to 18%, 3-fold and 3-fold, respectively) and triglycerides increased in females (2.4-fold) and decreased in males (73%) in animals at the high and highest dose levels. Phospholipids were increased in both sexes (high and highest). An increase in ALT (2.9-fold) and AST (73%) was noted in males (RTV alone) and to a lesser extent, in groups treated with darunavir/RTV combination (up to 78%).

Urinalysis: urine volume was higher (all treated groups) as a result of the treatment sodium and chloride excretion was increased in all treated groups.

Gross pathology: compared to the vehicle control, the following drug related changes were seen:

Liver enlargement: observed at low (3 males), mid (10 males and 8 females), high (15 males and 17 females) and highest (17 males and 18 females). The microscopic correlate was hepatocellular hypertrophy.

Liver discoloration (red brown): observed at high (8 males and 13 females) and highest (5 males and 14 females). The microscopic correlate was pigmentation of the hepatocytes.

Kidney discoloration (red brown): observed at high (8 males and 9 females) and highest (12 males and 13 females). The microscopic correlate was brown pigmentation of the cortical tubules.

Similar changes (liver enlargement, liver discoloration, kidney discoloration) in decedent animals in these groups were also considered drug related.

Effect of ritonavir alone compared to the vehicle control, there was liver enlargement in two females in this group.

Organ weights: compared to the vehicle control, the following drug related statistically significant deviations were seen:

Liver: absolute and relative weights in both sexes (low, mid, high or highest), with a dose related trend.

Thymus: lower absolute and relative weights in females (mid, high or highest); lower absolute and relative weights in males (high or highest).

Terminal body weights: slightly lower in males and females (high or highest), not always statistically significant.

Kidney: in males higher absolute and relative weights (low or mid), and higher relative weights (high or highest); in females higher absolute weights (mid) and higher relative weights (low, mid, high or highest).

Thyroid: higher relative weights (high or highest) in both sexes, in females higher absolute weights (highest).

Adrenals: higher absolute and relative weights in males (high or highest); higher relative weights in females (high).

Spleen: in males higher absolute and relative weights (low, mid or high) and higher relative weights (highest); in females higher absolute weights (low) and higher relative weights (low or high).

Effect of ritonavir alone compared to the vehicle control, the following drug related statistically significant deviations were seen:

Liver: higher absolute and relative weights in both sexes,

Spleen: higher absolute and relative weights in both sexes,

Thymus: lower absolute and relative weights in both sexes,

Kidneys: higher absolute and relative weights in females and higher relative weights in males.

Histopathology:

Low dose: compared to the vehicle control, there was an increase in incidence/severity of the following drug related findings:

Thyroid: hypertrophy and/or hyperplasia of follicular cells,

Kidney: nephropathy (males),

Spleen: macrophages aggregates.

Compared to ritonavir alone, there was an increase in incidence/severity of the following drug related findings:

Mesenteric lymph node: macrophage aggregates.

Mid dose: compared to the vehicle control, there was an increase in incidence/severity of the following drug related findings:

Thyroid: hypertrophy and/or hyperplasia of follicular cells,

Liver: brown pigmented hepatocytes,

Pancreas: islet fibrosis/siderocytes (males only),
Kidney: nephropathy (males),
Spleen: macrophages aggregates.

Compared to ritonavir alone, there was an increase in incidence/severity of the following drug related findings:

Liver: fine periportal hepatocytes vacuolation (males),
Mesenteric lymph node: macrophage aggregates.

High dose: compared to the vehicle control, there was an increase in incidence/severity of the following drug related findings:

Thyroid: hypertrophy and/or hyperplasia of follicular cells,
Liver: brown pigmented hepatocytes, pigmented Kupffer cells
Pancreas: islet fibrosis/siderocytes (males only),
Kidney: brown pigment in cortical tubules (females), nephropathy (males),
Spleen: hematopoiesis,
Adrenals: cortical vacuolation.

Compared to ritonavir alone, there was an increase in incidence/severity of the following drug related findings:

Thyroid: hypertrophy and/or hyperplasia of follicular cells,
Liver: multinucleated hepatocytes, single cell necrosis (of multinucleated hepatocytes), hepatocytes hypertrophy, fine periportal hepatocytes vacuolation (males),
Kidney: nephropathy (females), brown pigment in cortical tubules (males)
Mesenteric lymph node: macrophage aggregates.

Highest dose: compared to the vehicle control, there was an increase in incidence/severity of the following drug related findings:

Liver: brown pigmented hepatocytes,
Pancreas: islet fibrosis/siderocytes (males only),
Kidney: brown pigment in cortical tubules (females),
Spleen: hematopoiesis,
Adrenals: cortical vacuolation.
Mesenteric lymph node: macrophage aggregates

Compared to ritonavir alone, there was an increase in incidence/severity of the following drug related findings:

Thyroid: hypertrophy and/or hyperplasia of follicular cells,
Liver: multinucleated hepatocytes, single cell necrosis (of multinucleated hepatocytes), hepatocytes hypertrophy,
Kidney: nephropathy (females), brown pigment in cortical tubules (males).

Effect of ritonavir alone compared to the vehicle control, increase in incidence/severity of the following ritonavir related findings:

Lung: alveolar macrophages,
Thyroid: hypertrophy and/or hyperplasia of follicular cells,
Stomach: dilated/basophilic glands (females),

Liver: multinucleated hepatocytes, single cell necrosis (of multinucleated hepatocytes), hepatocyte hypertrophy, fine periportal hepatocytes vacuolation, histiocytosis (males),
Mesenteric lymph node: macrophage arrogates,
Kidneys: nephropathy (especially males), brown pigment in critical tubules (males),
Bone marrow: hypercellularity (males).

Preneoplastic and neoplastic findings: liver: foci of cellular alteration and focal hyperplasia were occasionally recorded in treated animals, but were not seen in animals from the vehicle control group.

The following neoplasms were present: vehicle controls (one male, skin fibrosarcoma), low (one male, malignant nephroblastoma; one male, skin squamous cell carcinoma), mid (one female, malignant lymphoma) and highest (one male, malignant lymphoma, one male, pituitary, pars anterior carcinoma).

Toxicokinetics: systemic exposures, expressed as C_{max} and AUC values are given in the tables below. The toxicokinetic analysis indicated that combining darunavir with RTV resulted in a dose proportional increase in exposure of darunavir for the 20/50 and 100/50 mg/kg/day dosing regimens. Less than dose proportional increase in exposure of darunavir 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 darunavir compared to administration of RTV alone.

Mean toxicokinetic parameters of darunavir after repeated oral administration of darunavir/RTV for 6 months in rats

Darunavir/RTV Dose (mg/kg/day)	Sampling period	Toxicokinetic parameters			
		C _{max} (µg/ml)		AUC (µg*hr/ml)	
		male	female	male	female
20/50	Day 1	2.0	1.54	17.1	7.79
	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	163
	Week 26	11.5	26.6	183	318
1000/75	Day 1	9.74	10.8	179	192
	Week 26	14.4	24.8	198	297

Mean toxicokinetic parameters of ritonavir after repeated oral administration of darunavir/RTV for 6 months in rats

Darunavir/RTV Dose (mg/kg/day)	Sampling period	Toxicokinetic parameters			
		C _{max} (µg/ml)		AUC (µg*hr/ml)	
		male	female	male	female
0/75	Day 1	6.61	7.76	77	73.1
	Week 26	9.6	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	2.1	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	1.3	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

Conclusion: A NOAEL was not established in the study. Main findings were noted in the liver (hepatocyte hypertrophy, vacuolation, multinucleated hepatocytes and single cell necrosis of multinucleated hepatocytes, histiocytosis), erythrocyte parameters and spleen (extramedullary hematopoiesis), thyroid gland (hypertrophy and/or hyperplasia) and kidney (nephropathy and pigmentation of cortical tubules). These findings were observed mainly with darunavir/RTV combination treatment and to some extent with RTV treatment alone. In general, these findings increased with increasing dose of darunavir. Findings in pancreas (increased incidence/severity of islet fibrosis/siderocytes in males), adrenals (increased severity of cortical vacuolation) and brown pigmentation of hepatocytes were only observed after treatment with darunavir.

Study title: Six month oral (gavage) repeat dose toxicity study of darunavir in the beagle dog

Key study findings: Groups of male and female dogs (4/sex/group) administered darunavir via oral gavage (2ml/kg) at dose levels of 0 (vehicle control; PEG 400 in water), 30 (low), 60 (mid) or 120 (high) for a period of 26 consecutive weeks. Clinical signs: loose or liquid feces and vomiting were reported at an increased incidence in all treated animals (low, mid or high) in comparison to the vehicle controls. The incidence was greatest in the high dose group of animals. The salivation was present during and especially after dosing in the animals. Histopathology: evidence of drug related effect was restricted to the thymus only (high). Thymus involution was noted in animals from all groups including the vehicle control animals but the severity was marginally higher in dogs at the high dose in comparison with the vehicle controls.

Thus, the treatment was associated with a marginal increase (high) in severity of thymic involution with slight to marked involution recorded in these animals in comparison to the vehicle controls. Marked severity in the thymus was noted to be dose related; thus, a NOAEL could not be established in the study. There were no other histopathological changes of

toxicological significance noted in the study. Except for the thymus changes, a dose level of 120 mg/kg/day may be considered the NOAEL. At the 120 mg/kg/day dose level, based on the body surface area factor, an equivalent dose for darunavir in humans would be 65 mg/kg/day or 3.9 g/day for a 60 kg person. At the NOAEL, exposures (steady state AUC and C_{max}) of darunavir were 70.3 (male) and 97.3 (female) µg*hr/ml and 19.8 (male) and 26 (female) µg/ml in dogs.

Study no.: TMC114-NC145

Volume # and page #: 1 and 1-460

Conducting laboratory and location: _____

Date of initiation: July 17, 2001

Date of study completion: January 30, 2002

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: AA-5977-batch-4-01; _____

Methods

Doses: Groups of male and female dogs (4/sex/group) administered darunavir via oral gavage (2ml/kg) at dose levels of 0 (vehicle control; PEG400 in water), 30 (low), 60 (mid) or 120 (high) for a period of 26 consecutive weeks.

Species/strain: male and female beagle dogs

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: oral gavage; 2 ml/kg

Satellite groups used for toxicokinetics: none

Age: 5-7 months

Weight: 8-11 kg

Sampling times: Plasma concentrations of darunavir/RTV were determined at 1, 2, 4, 8, 12 and 24 hr on day first and weeks 13 and 26 the study.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Ophthalmoscopy: week 25

Electrocardiography: week 25

Hematology: weeks 6, 13 and 26

Clinical chemistry: weeks 6, 13 and 26

Urinalysis: weeks 6, 13 and 26

Gross Pathology: all dogs found dead were necropsied as soon as possible.

Organ weights: listed in the table below

Histopathology: Table, Adequate Battery: yes; Peer review: yes

Dog tissues, weighed, preserved and examined

Organ name	Weighed	Preserved	Examined microscopically
adrenal glands	X	X	X
aorta (thoracic)		X	X
Bone marrow smear (rib)		X	
Bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X
Brain (medulla, pons, cerebrum and cerebellum)	X	X	X
Epididymides	X	X	X
Esophagus		X	X
Eyes with optic nerve		X	X
Gall bladder	X	X	x
Heart	X	X	X
	X		

Kidneys		X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
Liver	X	X	X
lungs (with mainstem bronchi)	X	X	X
Lymph nodes (mesenteric, mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
Ovaries	X	X	X
Pancreas		X	X
pituitary gland	X	X	X
prostate gland	X	X	X
Salivary glands submandibular)	X	X	X
seminal vesicles		X	X
Skeletal muscle (biceps femoris)		X	X
Skin		X	X
Small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumber)		X	X
Spleen	X	X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid,/parathyroid glands	X, thyroid	X	X
Trachea		X	X
Turbinates (skull)		X	

urinary bladder		X	X
uterus (body/horns) with cervix	X	X	X
Vagina		X	X
All gross lesions		X	X

Results

Mortality: one male and one female both from the high dose group were sacrificed during the study. The female was sacrificed on day 5 of the study; 25-min after dosing, the female was prostrate and had labored breathing and salivation. The male was sacrifice on day 40 of the study. The sponsor considered these deaths were caused by complications of the dosing procedure.

Clinical signs: loose or liquid feces and vomiting were reported at an increased incidence in all treated animals (low, mid or high) in comparison to the vehicle controls. The incidence was greatest in the high dose group of animals. The salivation was present during and especially after dosing in the animals.

Body weight and body weight gain: no effect of treatment was noted.

Food consumption: no effect of treatment was noted.

Ophthalmology: no treatment related abnormalities were noted.

Electrocardiology: no treatment effect on heart rates or ECG parameters.

Hematology: there were no treatment related changes in any hematology parameters studied.

Clinical chemistry: creatinine level was increased from week 6 in the low, mid or high dose animals. The levels increased slightly in some animals and it was not a clinically progressive change.

Urinalysis: there were no treatment related changes in any urinalysis parameter studied.

Gross pathology: there was no evidence of an effect of treatment.

Organ weights: relative liver weight of one female (high) was slightly increased in comparison to the vehicle controls. There were no histopathological correlates in the liver of the female.

Histopathology: evidence of drug related effect was restricted to the thymus only (high). Thymus involution was noted in animals from all groups including the vehicle control animals but the severity was marginally higher in dogs at the high dose in comparison with the vehicle controls (See table below).

Incidence and severity of thymus involution in dogs

Dosage groups (mg/kg/day)	Thymus – involution incidence and severity			
	Minimal	Slight	Moderate	Marked
0 (vehicle control)	1/8	3/8	4/8	0/8
30 (low)	2/8	4/8	1/8	1/8
60 (mid)	1/8	2/8	3/8	2/8
120 (high)	0/7	1/7	3/7	3/7

Deaths: one male and one female (high) were killed before the scheduled termination date. The cause of death was attributed to the clinical condition of these animals.

Toxicokinetics: systemic exposures, expressed as C_{max} and AUC values are given in the tables below. The toxicokinetic analysis indicated that combining darunavir with RTV resulted in a dose proportional increase in exposure of darunavir for the 20/50 and 100/50 mg/kg/day dosing regimens. Less than dose proportional increase in exposure of darunavir 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 darunavir compared to administration of RTV alone.

Mean toxicokinetic parameters of darunavir after repeated oral administration for 6 months in dogs

Darunavir Dosage (mg/kg/day)	Sampling period	Toxicokinetic parameters			
		C _{max} (µg/ml)		AUC (µg*hr/ml)	
		male	female	male	female
30	Day 1	9.69	10.9	22	23.2
	Week 26	7.91	11.4	22.4	21.8
60	Day 1	18.1	17.6	60.1	54.9
	Week 26	15	16.5	44	53.1
120	Day 1	23.4	29	107	133
	Week 26	19.8	26	70.3	97.3

Conclusion: The treatment was associated with a marginal increase (high) in severity of thymic involution with slight to marked involution recorded in these animals in comparison to the vehicle controls. The slight effects in the thymus were noted to be dose related; thus, a NOAEL could not be established in the study. There were no other histopathological changes of toxicological significance noted in the study.

Except for the minimal changes in the thymus, a dose level of 120 mg/kg/day may be considered the NOAEL. At the 120 mg/kg/day dose level, based on the body surface area factor, an equivalent dose for darunavir in humans would be 65 mg/kg/day or 3.9 g/day for a 60 kg person. At the NOAEL, exposures (steady state AUC and C_{max}) of darunavir were 70.3 (male) and 97.3 (female) µg*hr/ml and 19.8 (male) and 26 (female) µg/ml in dogs.

Study title: Twelve month oral gavage toxicity study in the dog

Key study findings:

Darunavir, formulated in PEG 400 was administered by gavage, once daily, for at least twelve months. Two control groups and three treated groups were given, 0 (vehicle), 0 (untreated), 30, 60 and 120 mg/kg/day. The treated animals, including the vehicle controls were administered a dose volume of 1.33 ml/kg. Each group but the untreated one consisted of 4 male and 4 female Beagle dogs. There were two males and two females in the untreated group. Clinical signs, body weight and food consumption were recorded at regular intervals while ophthalmoscopic examination, electrocardiography and clinical laboratory investigations (hematology, blood chemistry and urinalysis) were carried out at week 13, 26 and 52. Toxicokinetic analyses were carried on blood samples collected on day one and during week 13, 26, 39 and 52 during the 24 hours after dosing for toxicokinetic analysis. All animals found dead or sacrificed during the study were subject to macroscopic examination 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.

One male at 60 mg/kg/day was sacrificed during week 20 as a result of traumatic injury. One vehicle control female was sacrificed during week 41 owing to complications of intubation. Vomiting was increased in treated groups, particularly in animals at the two high doses. There was no effect on body weight, food consumption, ophthalmoscopy or EKG measurements. There was an increase in ALP at the high dose (2 male and 2 females) and the intermediate dose (2 females). Hematological and urinalysis parameters were unchanged. Liver weight was slightly higher in animals given the high and intermediate doses. Histopathological examination discovered increased hepatocellular pigment at all doses and vacuolation in both sexes at the two higher doses. Spleen weight of females at the high dose was decreased (36%) relative to controls in the absence of any histopathological change. The NOAEL was considered to be 30 mg/kg/.

Study no.: TMC 114-NC 145

Conducting laboratory and location:

Date of study initiation: 1/14/2003

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: Lot 3510A/2004

Methods

Doses: 0 (PEG 400), 30, 60, 120 mg/kg/day, or not dosed

Species/strain: Beagle dog

Number/sex/group or time point (main study): 4 males and 4 females per group with 2 males and 2 females in the untreated group

Route, formulation, volume, and infusion rate: Oral gavage in PEG 400 at 1.33 ml/kg

Satellite groups used for toxicokinetics or recovery: None

Age: 5-7 months old

Weight:

Sampling times:

Unique study design or methodology (if any):

Histopathology: Adequate Battery: yes (X), no ()—explain

Peer review: yes (X), no ()

Results

Mortality: Animals were observed twice a day. One mid dose male was sacrificed at week 20. At necropsy, all indications led to the conclusion that the animal was compromised due to trauma probably from a fall or rough contact with another dog. One control female was sacrificed at week 41 due to lesions best described as gavage error. No other animals were sacrificed other than at the scheduled necropsy.

Clinical signs: Animals were observed twice a day. The three common observations were vomiting, loose stools and salivation. These occurred in all dose groups and were not necessarily dose related.

Body weights: Body weights were recorded weekly and on the day of autopsy and were comparable across dose groups.

Food consumption: Food consumption was recorded daily. There were no treatment related abnormalities.

Ophthalmoscopy: Animals were examined at the start of the study and at weeks 26 and 52. There were no treatment related abnormalities.

EKG: EKGs were recorded at the start of the study and two hours after dosing on weeks 26 and 52. There were no treatment related abnormalities.

Hematology, clinical chemistry and urinalysis: Taken prestudy and at weeks 13, 26 and 52. There were no treatment related abnormalities in hematology measurements. There was an increase in alkaline phosphatase evaluations in some females at the mid and high dose groups. There were no treatment related abnormalities in urinalysis measurements.

Gross pathology: Carried out during necropsy. There were no treatment related abnormalities.

Organ weights:

The following organs from all animals were weighed after trimming of fat and other contiguous tissue. Paired organs were weighed together: Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, thymus, salivary glands (mandibular), spleen, testes/epididymides, thymus, thyroids and uterus.

Spleen weights were reduced somewhat in high dose females while the mean liver weights were increased in high dose males and females.

Histopathology: Adequate Battery: yes (X), no ()—explain. Peer review yes Minor hepatocellular liver pigment changes were seen at all doses and vacuolation of hepatocytes was seen at the two high doses.

Toxicokinetics: Toxicokinetics during 12-month dosing in dogs is shown in the following table

TMC 114 toxicokinetics after repeated p.o. administration 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 AUC₀₋₂₄ after single dose (day 1) or AUC₀₋₂₄ after repeated dose

2.6.6.4 Genetic toxicology

Study title: In Vitro Bacterial Reverse Mutation Assay, Ames Test

Key findings: It was concluded that darunavir was not mutagenic under the conditions of this assay.

Study no.: █████ 293063

Conducting laboratory and location:

██████████
██████████
██████████
██████████

Date of study initiation: 5/25/2000

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Batch 6, █████

Methods

Strains/species/cell line: Salmonella typhimurium strains TA98, 100, 135 and 1537 and E. coli strain WP_{2uvrA}

Doses used in definitive study: Up to 5000µg per plate

Basis of dose selection: Dose range-finding study

Negative controls: Saline and DMSO

Positive controls: With activation, Sodium azide, 9-aminoacridine, daunomycine, MMS and 4nitroquinoline-N-oxide. Without activation, 2-aminoanthracene.

Incubation and sampling times: 48 hours

Results

At the highest level tested, darunavir precipitated on the plates 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 twofold increase in the number of revertant colonies in any of the tester strains in the absence or presence of S9-metabolic activation. The experiments were repeated with the same results.

Study title: In Vitro Mammalian Chromosome Aberration Assay

Key findings: It was concluded that darunavir was not clastogenic in human lymphocytes under the conditions of this assay.

Study no.: 294288

Conducting laboratory and location:

Date of study initiation: 5/24/2000

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: 6, _____

Methods

Strains/species/cell line: Cultured human lymphocytes from healthy male volunteers.

Doses used in definitive study: 33, 100 and 333µg darunavir per ml of culture medium with and without S9 from Aroclor-1254 induced rats

Basis of dose selection: Dose range-finding assay

Negative controls: DMSO

On day 12 at one, three and five hours after dosing and on day 25 up to eight 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 darunavir exposure.

Results

F₀ in-life: Two dams were found dead (one given vehicle and one given 200 mg/kg/day). Based on clinical signs, the animal that died at 200 mg/day was probably a dosing accident. The cause of death was not determined for the control animal. During lactation, owing to the death of the complete litter in one vehicle animal and one dosed at 1000 mg/kg/day, or failure to produce milk in a 40 mg/kg/day animal, three animals were sacrificed. In dams given 1000 mg/kg/day, body weight gain was lower than controls after the start of dosing and during days one to seven of lactation while food consumption was slightly reduced between days six and nine of gestation.

F₁ physical development: There was no effect of maternal treatment on parturition or the numbers of pups born. There was lower pup body weight gain to day six of lactation in groups given 200 and 1000 mg/kg/day. Maternal treatment did not affect pup survival up to start of pup direct dosing on day 12 postpartum. On day 12 postpartum, all pups given 500 and 1000 mg/kg/day at the start of direct dosing of the pups died or were sacrificed prematurely owing to adverse clinical signs on day 12 or 13. Of the 16 pups directly dosed with 40 mg/kg/day, five exhibited adverse clinical signs and were sacrificed although there was no effect on body weight among these animals. The same happened to six of the pups dosed at 200 mg/kg/day. The deaths were considered to be darunavir associated.

F₁ Kinetics and drug distribution: Pups of dams dosed at 40, 200 and 1000 mg/kg/day from gestation day six to day seven of lactation were indirectly exposed to darunavir via the dam's milk and the exposure seemed to increase with increasing dose levels. Up to 0.61 µg/ml was detected in pups whose mothers received darunavir at 1000mg/kg/day. Exposure was higher after a single direct dose of 1000 mg/kg/day to juvenile rats on day 12 than on day 25 of age. At day 12 of age, exposure, expressed as AUC_{0-5h}, 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. AUC_{0-5h} at day 26, was 59.6 to 102 µg.h/ml in plasma, 371 to 604 µg.h/g in liver and 4.7 to 7.1 µg.h/g in brain. The exposure data indicate that exposure to darunavir in juvenile animals who were directly dosed, was dose and age dependent. It is likely that maturation of the liver enzymes in the pups influenced the elimination of darunavir. Brain distribution was assumed to be influenced by the maturation of the blood-brain barrier.

Study title: Oral (gavage) pre- and post-natal developmental toxicity study in the rat

Key study findings:

Maternal performance:

Treatment of pregnant rats with darunavir at 1000 mg/kg/day throughout the gestation and lactation periods brought about some clinical signs and reduced bodyweight gain and food consumption. There was a only a single interval of lower bodyweight gain and reduced mean food intake at 200 mg/kg/day, so that the NOAEL was considered to be 40 mg/kg/day although the value could easily be considered 200 mg/kg/day.

Similar observations (clinical signs, lower body weight and food consumption) were recorded when darunavir was administered together with ritonavir at 1000+75/50 mg/kg/day, but the effect was greater than that with darunavir alone.

A dose level of 75/50 mg/kg/day of ritonavir alone administered to pregnant females was associated with maternal clinical signs and lower mean food consumption and bodyweight gain but the values were greater than those in the combination groups.

Pup growth and Pup/FI development:

Darunavir alone had no effect upon pup survival during lactation at dose levels up to and including 1000 mg/kg/day when administered to pregnant rats. At 1000 mg/kg/day, an overall lower mean pup bodyweight throughout lactation and FI maturation was seen. At 200 and 1000 mg/kg/day, there was a slight delay in the acquisition of the developmental milestones pinna detachment and eyes open. This was most likely due to the lower body weights in the pups. FI developmental tests or mating and fertility were not affected by maternal treatment at any dose level. Treatment of the dams at 40 mg/kg/day was considered to be the NOEL for pup development effects.

Similar effects on body weight and developmental milestones were seen when darunavir was administered together with ritonavir at 1000+75/50 mg/kg/day. In the latter case, the effects were greater than those seen with darunavir alone. Additionally, pup survival during lactation was reduced. Importantly, the reduced bodyweight gain during lactation was observed only in the group where dosing was continued until post natal day 14. When dosing was stopped at the end of gestation, bodyweight performance was comparable with the controls. This confirms that this finding was directly related to administration to the mother during lactation and not resulting from in utero exposure.

During FI post-weaning, lower mean body weights were recorded but there was no effect on developmental tests or the fertility and mating performance of the FI animals.

Ritonavir administered to pregnant female rats 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 developmental milestones. During FI post-weaning, lower mean body weights were recorded but there was no effect on developmental tests or the fertility and mating performance of the FI animals.

Study no.: TMC 114-NC156

Conducting laboratory and location:

Date of study initiation: 20 December, 2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: TMC 114: 03PO484,

Ritonavir: 21350VA and 22353VA, not given

Methods

Doses: 0 (Vehicle control PEG , 40/0 TMC 114/ritonavir in propylene glycol, 200/0, 1000/0, 1000/75 (50), 1000/50 or 0/75 mg/kg/day (since the drug is the ethanolate, a correction factor of 1.0753 has been used to calculate the milligram equivalents. I have shortened the dosing to mg/kg/day, meaning mg/kg/day throughout the review).

Species/strain: Sprague Dawley rat, CD (SD) IGS BR VAF PLUS

Number/sex/group: See following Table:

The following table summarises the groups and dose levels used during the study:

Group number	Colour code	Number of mated females (a)	Animal identification numbers	Dose level (mg eq/kg/day) TMC114 / Ritonavir
1	White	25	1 – 25	Vehicle control
2	Green	25	26 – 50	40/0
3	Yellow	25	51 – 75	200/0
4	Purple	25	76 – 100	1000/0
5a	Red	12	101 – 112	1000 / 75 (50)
5b	Red	13	113 – 125	1000 / 50
6	Pink	25	126 – 150	0 / 75 (50)

(a) = expected to give 20 pregnant per group.

() = indicates dose level reduced due to poor clinical condition of the dams.

Route, formulation, volume, and infusion rate: Oral gavage, 10 ml/kg for darunavir and 1.25 ml/kg for ritonavir.

Study design and parameters and endpoints evaluated:

Three groups of mated female Sprague Dawley rats were dosed with darunavir by gavage, once daily from day six of gestation until day 20 of lactation, at dose levels of 40, 200 and 1000 mg/kg/day (groups 2 to 4 above) at a dose volume of 10 ml/kg. Two groups (5a and 6), were dosed with 1000 mg/kg darunavir /75 mg/kg/day ritonavir or 75 mg/kg/day ritonavir (the ritonavir dose was reduced to 50 mg/kg/day at day 0 of lactation through day 14 of lactation in group 5a and from day 0 of lactation through day 20 of lactation in group 6). An additional

group (5b) was dosed with 1000 mg/kg/day darunavir /50 mg/kg/day ritonavir from day six of gestation until day 20 of lactation.

The females were allowed to litter and total litter size and numbers of each sex recorded. Pups were examined and weighed on days 1, 4, 7, 14 and 21 of lactation. Righting reflex, eye opening, startle response and light reflex of pupils were recorded on days 3, 5, 15 and 21 of lactation.

F₀ females in all groups but 4 and 5a were sacrificed on day 21 of lactation and examined macroscopically and the number of implantation scars determined. The pups of litters in groups remained with their mothers until they reached a weight of 40 to 50 g, then weaned. The mothers were examined macroscopically and the number of implantation scars determined.

At one week after the beginning of weaning, 20 male and 20 females were randomly selected from all of the litters other than group 5a (at least one animal from each litter). The selected animals were allowed to mature untreated and the effects on growth, development, behavior and reproductive performance were recorded. Ten males and 10 females from group 5b were tested in the E-maze and rotarod. Thirteen males and thirteen females from group 5a were tested for E-maze learning, memory and mating ability only.

The following table gives the breakdown of dosing for groups 5 (a and b) and 6, since it is somewhat complex.

Group	Animal Numbers	Dose TMC114 and/or Ritonavir (mg.eq/kg/day)	Days	Dose TMC114 and/or Ritonavir (mg.eq/kg/day)	Days
5a	101 - 112	1000 + 75	6G - 21G ± 1	1000 + 50	0L - 14L ± 1
5b	113 - 125	1000 + 50	6G - 21G	0	21G - 20L
6	126 - 137	75	6G - 21G ± 1	50	0L - 20L
6	138 - 150	50	6G - 21G	50	0L - 20L

Results for darunavir

F₀ in-life: There were no parental mortalities considered related to drug. There were no effects at 40 mg/kg/day and no clinical signs at 200 mg/kg/day. At 1000 mg/kg/day, clinical signs such as piloerection during gestation and frequent grooming throughout the study were recorded. Frequent salivation was considered to be related to vehicle (propylene glycol). Lower mean body weight was seen at 200 and 1000 mg/kg/day and lower food intake was seen at these doses. There was no effect of treatment throughout gestation, parturition, pregnancy and pup survival to weaning.

F₀ necropsy: There were no macroscopic effects on the mothers or pups not selected for subsequent testing.

F₁ physical development: There were no clinical signs or litter deaths related to treatment with darunavir. There was mean body weight lowering on pups in the 1000 mg/kg/day group at birth reflecting the effect of darunavir on the mothers. Reduced mean body weights during lactation

resulted in lower body weights at weaning. There were lower percentages of pups with ear openings at day three of lactation and with eyes opened at day seven of lactation at 200 as well as 1000 mg/kg/day

F₁ behavioral evaluation: No effects on learning and memory or auditory acuity or locomotion parameters.

F₁ reproduction: No effect on sexual maturity or copulation or fertility indices. All pregnancy parameters (corpora lutea, implantations and live embryos and preimplantation loss) were normal.

Results for darunavir/ritonavir

F₀ in-life: At 1000 mg/kg/day/75(50) mg/kg/day less frequent grooming was seen along with piloerection, decreased activity, partially closed eyes and excessive salivation (probably due to vehicle). Lower body weight gains was seen in the 1000/75 group during gestation but improved when the ritonavir was lowered to 50 during lactation. Dosing was discontinued on lactation day 14 and weight gain increased compared to controls after that. Food intake was lower than animals in groups 5a and 5b when compared to those animals administered darunavir at 1000 mg/kg/day alone. When dosing was discontinued at lactation day 14, the food intake was similar to those animals receiving darunavir alone. There was no effect on gestation, parturition or pregnancy data in the mothers.

F₀ necropsy: No effects

F₁ physical development: In group 5a, pup survival through day 14 of lactation was lower than pups born to mothers administered darunavir alone. Two complete litters died in this group and there was a higher incidence of pups found dead or missing. The dead pups usually showed no milk in their stomachs. In group 5b, survival between lactation days seven and 21 was reduced.

Pup body weights in groups 5a and b were decreased compared to controls. This was due to the reduced body weight of the mothers in the same groups. In group 5b where dosing was discontinued at the beginning of lactation, the body weight increased at a rate greater than the controls and animals in group 4 (1000 mg/kg/day of darunavir alone). In group 5a, compared to controls, there was a lower percent of pups with ears or eyes open, showing a righting reflex or exhibiting a normal startle reflex. Group 5b only had a deficit of ears open compared to controls. When one examines the pup bodyweight gain from day one to day 21 of lactation, the controls gained 45.3 grams while the pups from group 5b, the group not dosed during lactation, gained 46.6 grams. In contrast, group 5a the group dosed through day 14 of lactation, the value was 24.6 grams, confirming that a toxic effect was found on the pups when the mothers were dosed during lactation.

F₁ behavioral evaluation: No effect on learning and memory, auditory acuity or locomotion parameters was seen.

F₁ reproduction: No effect on sexual maturity or copulation or fertility indices. All pregnancy parameters (corpora lutea, implantations and live embryos and preimplantation loss) were normal.

Results for ritonavir alone

F₀ in-life: Clinical sign of less grooming than controls was seen. There were some slight effects on body weight gain but little effect on body weight gain as the study progressed. There were some slight effects on food consumption early in the gestation phase and lactation phase but the effects went away as the phases progressed so that by the end of gestation and lactation, the treated animals were eating in a similar manner to controls. There was no effect on gestation and parturition data in the mothers. Pup survival was lower than that of the controls.

F₀ necropsy: No effects on mothers.

F₁ physical development: Mean pup body weight was similar to controls up to day one of lactation. However, during lactation, mean body weight gains were lower than controls but the deficit was made up during untreated development. There were lower percentages of pups with ear openings at day three of lactation and with eyes opened at day seven of lactation in the treated animals compared to controls.

F₁ behavioral evaluation: No effect on learning and memory, auditory acuity or locomotion parameters was seen.

F₁ reproduction: No effect on sexual maturity or copulation or fertility indices. All pregnancy parameters (corpora lutea, implantations and live embryos and preimplantation loss) were normal.

2.6.6.7 Local tolerance**Local lymph node assay (TMC114-NC245)**

Key Findings: In a T-lymphocyte proliferation assay, darunavir was studied as 0 [vehicle control, dimethyl formamide (DMF)], 10%, 25% or 50% (w/v) levels to determine its potential to cause skin sensitization. Under the conditions of the study, darunavir was found to be negative to have the potential to cause skin sensitization

A sample of darunavir was assessed for the potential to cause skin sensitization using the mouse Local Lymph Node Assay (LLNA). The assay determines the level of T-lymphocyte proliferation in the lymph nodes draining the site of chemical application by measuring the amount of radiolabeled thymidine incorporated into the dividing cells. Darunavir was applied as 0 (vehicle control, DMF), 10%, 25% or 50% (w/v) levels and tritiated thymidine was administered to measure its incorporation into dividing cells at a proximal lymph node.

Results: are shown in the table below. Thymidine did not significantly incorporate into the dividing cells as shown as a ratio to the vehicle control after administration of darunavir.

Incorporation of radiolabel thymidine in mouse lymph nodes

Darunavir concentrations	Radiolabel incorporation (DMP/lymph node)	Darunavir/vehicle control ratio
0, Vehicle control (DMF)	243	N/A
10% (low)	360	1.5
25% (mid)	341	1.4
50% (high)	369	1.5

Conclusion: under the conditions of the study, darunavir was found to be negative when tested in the LLNA assay and is unlikely to have the potential to cause skin sensitization.

2.6.6.8 Special toxicology studies

4-Week immunotoxicity study with Darunavir and ritonavir by daily gavage in rats

Key study findings: Groups of male and female rats administered (via oral gavage) darunavir/RTV combination at dose levels of 0 (vehicle control; purified water), 0 (vehicle control; PEG 400 in purified water) 20/0 (low), 100/0 (mid), 500/0 (high), 0/50 (RTV alone) or 100/50 (darunavir/RTV) mg/kg/day for 4 consecutive weeks. The antibody response to the T-cell dependent antigen Keyhole Limpet Hemocyanin (KLH) was evaluated by subcutaneous administration of KLH (2.5 mg) on day 22, followed by ELISA determination of IgM level in serum samples taken at necropsy.

In the 6-month darunavir/RTV combination study in rats, a dose level of 20/50 mg/kg/day was the lowest dose level utilized. At the 20/50 mg/kg/day, based on the body surface area factor, an equivalent dose for darunavir in humans would be 3.25 mg/kg/day or 195 mg/day for a 60 kg person. At the low dose, exposures (steady state AUC and C_{max}) of darunavir were 11.2 (male) and 13.4 (female) µg*hr/ml and 1.66 (male) and 1.95 (female) µg/ml in rats.

Study no.: TMC114-NC187

Conducting laboratory and location: 

Date of study completion: September 8, 2005

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: TMC 114 (3510A/2003;  pure) and ritonavir (07687VA)

Methods

Doses: Groups of male and female rats administered darunavir/RTV combination via oral gavage at dose levels of 0 (vehicle control; purified water), 0 (vehicle control; PEG 400 in purified water) 20/0 (low), 100/0 (mid), 500/0 (high), 0/50 (RTV alone) or 100/50 (darunavir/RTV) mg/kg/day for 4 consecutive weeks according to an experimental design shown in Table 1. The antibody response to the T-cell dependent antigen Keyhole Limpet Hemocyanin (KLH) was evaluated by subcutaneous administration of KLH (2.5 mg) on day 22, followed by ELISA determination of IgM level in serum samples taken at necropsy.

Results: darunavir was not found to cause any immunological response at doses ranging from 20 to 500 mg/kg/day under conditions of the study. Similarly, for RTV alone or in combination (darunavir/RTV 100/50 mg/kg/day), no immunotoxicological response was observed. A dose level of 500 mg/kg/day may be considered the NOAEL for producing immunological response in rats. At the 500 mg/kg/day, based on the body surface area factor, an equivalent dose for darunavir in humans would be 81 mg/kg/day or 4.8 g/day for a 60 kg person. At the 500 mg/kg/day dose level (high), exposures (steady state AUC and C_{max}) of darunavir were 102 (male) and 84.4 (female) µg*hr/ml and 13.6 (male) and 9.96 (female) µg/ml in rats.

Experimental design of the oral (gavage) 4-week immunotoxicology study in rats

Group	Dose level (mg/kg/day)		Number of animals			
	darunavir	RTV	Main study		Satellite for toxicokinetics	
			Male	female	male	female
1. Vehicle control (purified water)	0	0	8	8	0	0
2. vehicle control (PEG400)	0	0	8	8	0	0
3. Low	20	0	8	8	6	6
4. Mid	100	0	8	8	6	6
5. High	500	0	8	8	6	6
6. RTV alone	0	50	8	8	6	6
7. Darunavir/RTV	100	50	8	8	6	6

Species/strain: male and female rats; strain: Sprague-Dawley Crl: CD (SD) IGS BR

Number/sex/group or time point (main study): See table above

Route, formulation, volume, and infusion rate: oral gavage; 5 ml/kg

Satellite groups used for toxicokinetics: See table above

Age: 6 weeks

Weight: 184-228 g for males and 154-197 g for females

Sampling times: Plasma concentrations of darunavir/RTV were determined at 1, 2, 4, 8 and 24 hr on day 28 the study.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: day 29

Clinical chemistry: day 29

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: listed in the table below

Histopathology: Table 2, Adequate Battery: yes; Peer review: yes

Rat tissues, weighed, preserved and examined

Organ name	Weighed	Preserved	Examined microscopically
Liver	X	X	
Lymph nodes: popliteal and mesenteric): paracortex, medulla, cortex		X	X
Bone marrow (sternum, femur)		X	X
Thymus (cortex, medulla)	X	X	X
Spleen	X	X	X
Peyer's patches (ileum: follicles, interfollicular area)		X	X
Thyroid,/parathyroid glands	X	X	

Results

Mortality: no deaths.

Clinical signs: ptyalism was observed with higher incidence (low, mid or high) or darunavir/RTV treated animals.

Body weight and body weight gain: no effect of the treatment.

Food consumption: no changes

Hematology: when compared to the control groups, changes in RBC parameters were seen as follows:

-slightly lower mean cell volume in males, slightly mean cell hemoglobin in males, slightly lower mean cell hemoglobin concentration in females and slightly higher reticulocyte counts in males and females at the high dose level.

Myelogram: when compared to controls, the major changes noted (high) consisted of slightly lower M/E ratio (up to 35%) in males and females; in females, this was due to higher total number of erythroid elements as well as the number of different erythroblasts and normoblasts.

Response to T-cell dependent antigen: the immune response of the treated animals as measured by IgM production was not affected as measured by an ELISA assay.

Gross pathology: enlarged liver was noted in animals RTV alone or darunavir/RTV or the high dose groups.

Organ weights: higher liver weights were noted in animals RTV alone or darunavir/RTV or at the mid or high dose groups.

Histopathology: no changes were noted.

Toxicokinetics: systemic exposures, expressed as C_{max} and AUC values are given in Tables 3 and 4. The toxicokinetic analysis indicated that darunavir was rapidly absorbed and C_{max} values were observed between 1 and 2 hr. When increasing the dose from low to mid, there was a slightly more than dose proportional in AUC values (6 to 6.4-fold increase). C_{max} values increased dose proportionally in males, but less than dose proportionally in females. When increasing the dose level from mid to high, there was a less than dose proportional increase in both C_{max} and AUC values in both males and females.

Mean toxicokinetic parameters of darunavir after repeated oral administration of darunavir/RTV for 4-week in rats

Darunavir/RTV Dose (mg/kg/day)	Sampling period	Toxicokinetic parameters			
		C _{max} (µg/ml)		AUC (µg*hr/ml)	
		Male	female	male	female
20/0 (low)	Week 4	0.75	1.71	4.2	4.68
100/0 (mid)	Week 4	3.87	6.54	25.2	30
500/0 (high)	Week 4	13.6	9.96	102	84.4
100/50 (darunavir/RTV)	Week 4	6.62	6.63	59	57.7

Mean toxicokinetic parameters of ritonavir after repeated oral administration of darunavir/RTV for 4-week in rats

Darunavir/RTV Dose (mg/kg/day)	Sampling period	Toxicokinetic parameters			
		Cmax (µg/ml)		AUC (µg*hr/ml)	
		male	female	male	female
0/50	Week 4	3.43	5.45	21.8	37.3
100/50	Week 4	1.92	2.7	14.4	20.1

Conclusion: darunavir was not found to cause any immunological response at doses ranging from 20 to 500 mg/kg/day under conditions of the study. Similarly, for RTV alone or in combination (darunavir/RTV 100/50 mg/kg/day), no immunotoxicological response was observed. A dose level of 500 mg/kg/day may be considered the NOAEL for producing immunological response in rats. At the 500 mg/kg/day, based on the body surface area factor, an equivalent dose for darunavir in humans would be 81 mg/kg/day or 4.8 g/day for a 60 kg person. At the 500 mg/kg/day dose level (high), exposures (steady state AUC and Cmax) of darunavir were 102 (male) and 84.4 (female) µg*hr/ml and 13.6 (male) and 9.96 (female) µg/ml in rats.

2.6.6.9 Discussion and Conclusions

See body of review

2.6.6.10 Tables and Figures

See body of review

**Appears This Way
On Original**

2.6.7 TOXICOLOGY TABULATED SUMMARY

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The pharmacology/toxicology studies submitted to NDA 21-976 support the labeling for this submission and are sufficient for approval.

Unresolved toxicology issues (if any):

None. The carcinogenicity studies in rats and mice will be completed as part of the Phase 4 commitments.

Recommendations:

Approve

Suggested labeling:

See above under labeling

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

Attachment #1

Appended to this review is the original review for IND 62,477 that was the product of Hao Zhang, M.D., the original reviewer of this drug.

PHARMACOLOGY/TOXICOLOGY COVER SHEET

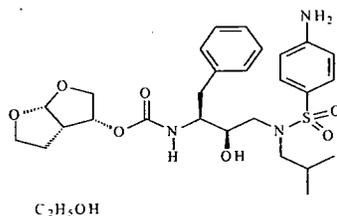
IND# 62477 Serial No: 000 (IT)
Date of submission: 12/19/2002
Information to sponsor: Yes (X)
Sponsor: Tibotec-Virco USA, 2505 Meridian Parkway, Suite 350, Durham, NC 27713;
 Telephone: 919-313-2672

Manufacturer for drug substance:

Division name: Division of Antiviral Drug Products
HFD #: HFD-530
Review completion date: 2/26/2003
Reviewer name: Hao Zhang, M.D

Drug:

Trade name: None
Generic name (list alphabetically): None
Code name: TMC114
Chemical name: {3-[-amino-benzensulfonyl]-isobutyl-amino]-1-benzyl-2-hydroxy-propyl}-
 carbamic acidhexahydro-furo[2,3-b]furan-3-yl ester ethanolate
CAS registry number: 313682-08-5
Molecular formula/molecular weight: C₂₇H₃₇N₃O₇S.C₂H₅OH; 593.724 (active moiety +
 ethanol) 547.656 (active moiety)
Structure:



Relevant INDs/NDAs/DMFs: None
Drug class: Anti-HIV protease inhibitor
Indication: Treatment of HIV infection
Clinical formulation: An oral solution contains TMC114 active moiety

Ritonavir: Ritonavir (Norvir) soft gelatin capsule (100 mg RTV/capsule, Abbott)

Route of administration: Oral

Proposed clinical protocol: The clinical study proposed in this IND (TMC-114-C133; in the United States) is an open, randomized, one way, cross-over trial to assess the effect of TMC114 oral solution in combination with ritonavir on the pharmacokinetics of atorvastatin, as well as atorvastatin lactone and 2- and 4-hydroxy-atorvastatin. Safety and tolerability will also be evaluated. Sixteen healthy volunteers will receive the following treatments: treatment A - 40 mg atorvastatin q.d. for 4 days, and treatment B - 300 mg TMC114/100 mg ritonavir b.i.d. for 9 days and from day 4 to day 7, 10 mg atorvastatin q.d. for 14 days.

Previous clinical experience: In Europe, eight clinical phase I studies (TMC114-C101, TMC114-C102, TMC114-C104, TMC114-105, TMC114-106, TMC114-C110, TMC114-C112, TMC114-C207) with TMC114 alone or with TMC114 in combination with RTV have been conducted in healthy volunteers and HIV infected patients. In Study TMC-114-C207, healthy volunteers and HIV infected patients have been treated with up to 900mg TMC114/100mg RTV daily for 14 days. In this study, co-administration of 100 mg or 200 mg RTV daily increases the C_{min} and AUC values of TMC114 substantially. TMC114 treatment-related adverse events were seen, including rash, vomiting, diarrhea, as well as increases in LDL, cholesterol, bilirubin and glucose.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Introduction and Drug history:

TMC114 is a novel HIV protease inhibitor, which is being developed by the sponsor for the treatment of HIV infection. In vitro, TMC114 has demonstrated potent in vitro activity against wild type strains of HIV-1. Additionally, it is potent against PI-resistant strains of HIV-1. _____

_____ However, data from a Phase I healthy volunteer trial (TMC114-C112) suggested that the co-administration of TMC114 with low-dose RTV resulted in a more favorable pharmacokinetic profile of TMC114. Therefore, the sponsor wishes to develop TMC114 in combination with RTV. The present IND contains 32 non-clinical pharmacology and toxicology study reports in support of the proposed clinical study.

Non-clinical pharmacology/toxicology studies reviewed within this submission:

Safety Pharmacology

1. *Neuro-behavior and motor activity in rats (Study No.: TMC114-NC116)*
2. *Pulmonary safety study in rats (Study No.: TMC114-NC117)*
3. *Gastrointestinal safety study in rats (Study No.: TMC114-NC120)*
4. *Cardiovascular safety study (hERG) (Study No.: TMC114-NC103)*
5. *Cardiovascular safety in purkinje fibers (Study No.: TMC114-NC105)*
6. *Cardiovascular safety study (telemetry) in dogs (Study No.: TMC114-NC108)*

Toxicology

Multiple-dose studies

Rat

Positive controls: Mitomycin C for assays not using S9 activation and Cyclophosphamide for those using S9.

Incubation and sampling times: 3, 24 and 48 hours in the absence of S9 activation and 3 hours in the presence of S9 activation.

Results

The test was carried out in duplicate. The number of cells with aberrations in the solvent controls were within the historical control range for the laboratory. The positive control chemicals both produced a statistically significant increase in the frequency of aberrant cells. Both in the absence and presence of an S9 activation system, there was no induction of a significant increase in the number of cells with chromosomal aberrations.

Study outcome: 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-activation (from Aroclor-induced rat liver S9-mix) in two repeated experiments. The vehicle control and appropriate positive control articles confirmed the adequacy of the test system. It was concluded that darunavir was not clastogenic in human lymphocytes under the conditions of this assay.

Study title: In Vivo Mammalian Micronucleus Test

Key findings: It was concluded that darunavir was not genotoxic under the conditions of this assay.

Study no.: 303874

Conducting laboratory and location:

Date of study initiation: 9/27/2000

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Batch CS00/071A/RP2, _____

Methods

Strains/species/cell line: NMRI BR mice (SPF)

Doses used in definitive study: 2000 mg/kg by oral gavage at 10 mg/kg dissolved in polyethylene glycol

Basis of dose selection: Dose range-finding study in 2 males and 2 female mice

Negative controls: polyethylene glycol

Positive controls: Cyclophosphamide

Incubation and sampling times: Vehicle control was examined at 24 hours. The 2000 mg/kg sample in the treated group was examined at 24 and 48 hours. The positive control (50 mg/kg cyclophosphamide) was examined at 48 hours. All the time points were carried out on 5 males. The number of micronucleated polychromatic erythrocytes was counted in 2000 polychromatic erythrocytes. The ratio of polychromatic to normochromatic erythrocytes was determined in the first 1000 erythrocytes. Micronuclei were only counted in polychromatic erythrocytes.

Results No increase in the frequency of micronucleated cells was observed in the polychromatic erythrocytes of the bone marrow of animals tested with darunavir. 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 darunavir was not genotoxic under the conditions of this assay.

2.6.6.5 Carcinogenicity

Carcinogenicity studies in mice and rats are underway and will be completed as a Phase 4 commitment.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Dose range-finding study of prenatal developmental toxicity of darunavir in Sprague Dawley rats

Key study findings: Darunavir, at doses up to 1000 mg/kg/day dosed to pregnant rats from gestation day (GD) 7 to 9 and from GD 13 to 19, did not induce any adverse events on any maternal and fetal parameters measured. The NOAEL was considered to 1000 mg/kg/day.

Study no.: TMC 114-NC127

Conducting laboratory and location:

Date of study initiation: 4/12/2001

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: AA-5977-Batch-1-01, _____ and CS00/092X/002, _____

Methods

Doses: 0 (PEG 400), 40, 200 and 1000 mg/kg/day

Species/strain: Sprague Dawley rat

Number/sex/group: 6 mated females per group

Route, formulation, volume, and infusion rate: oral gavage, in PEG 400 at 10 ml/kg
 Satellite groups used for toxicokinetics: NA but samples were taken on day 7 and day 19
 for TK measurements (0.5, 1, 2, 4, 6 and 8 hours on three animals per time point)
 Study design: Animals were dosed on GD 7-9 and 13-19.

Results

Mortality: There were no deaths in the study

Clinical signs: Only minor incidental signs

Body weight: There was no effect on body weights, body weight changes, gravid uterine weights or adjusted total body weight changes.

Food consumption: There were no significant changes among groups

Toxicokinetics: The TK parameters are shown in the following Table:

TMCI14 Dose (mg/kg/day)	Sampling Day	C _{max}	AUC ^a
		(µg/mL)	(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-∞} after single dose (day 1) or AUC_{last} after repeated dose, ^b AUC_{last}

Exposures are approximately 50% of that measured in the clinic.

Necropsy: All findings were considered unrelated to drug

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): One female was not pregnant and a total litter resorption was observed in one high-dose rat. There were no treatment effects on pregnancy rate, number of corpora lutea, number or type of implantations, pre- or post-implantation loss, intrauterine deaths or number of live fetuses (excluding the one litter in which resorption had taken place).

There were no significant differences on fetal body weights or sex among the groups.

Embryofetal development

Study title: Study of the effect of darunavir on embryo-fetal development in Sprague Dawley rats

Key study findings: Pregnant Sprague Dawley rats were dosed with darunavir at 0 (PEG 400), 40, 200 and 1000 mg/kg/day between gestation days seven and 19. There was no evidence that darunavir was teratogenic in the dosed rats. Based on the fact that body weights and food consumption were reduced in the high dose dams, the maternal NOAEL was considered to be 200 mg/kg/day. There were no effects of darunavir treatment on embryo-fetal development at any of the doses administered. The embryo-fetal NOAEL was considered to be 1000 mg/kg/day.

Study no.: TMC 114-NC128

Conducting laboratory and location:

Date of study initiation: 6/21/2001

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: CS00/092X/002, _____ and AA-5977-Batch-2-01, _____

Methods

Doses: 0 (PEG 400), 40, 200 and 1000 mg/kg/day

Species/strain: Sprague Dawley rat

Number/sex/group: 25 mated females per group

Route, formulation, volume, and infusion rate: oral gavage, in PEG 400 at 10 ml/kg

Satellite groups used for toxicokinetics: NA. No toxicokinetic evaluations were performed in this study, however, the same strain of animals and the dose levels were used in study TMC114-NC127 (see above study review) and these TK data are considered representative of values expected in this present study.

Study design: TMC 114 formulated in PEG400 was administered once daily, by gavage, during the period of GD7 to GD19. Regular observations were made for clinical signs, body weight and food consumption. Dams were sacrificed and necropsied on GD22. 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. 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.

Results

Mortality: There was one death at the high dose, but the cause was not identified.

Clinical signs: There were no relevant clinical signs.

Body weight and food consumption: Body weights and food consumption were lower (5% and 10%) than controls in animals given 1000 mg/kg/day. Body weight gain was also reduced (53% lower GD7-GD10 and 15% lower GD7-GD19).

Toxicokinetics: See previous study

Necropsy: There was no effect on gross pathology

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): There was no effect on 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.

Study title: Study of the effect of darunavir on embryo-fetal development in New Zealand white rabbits

Key study findings: Pregnant New Zealand white rabbits were dosed with darunavir at 0 (vehicle), 40, 200 and 1000 mg/kg/day between gestation days eight and 20. There were two deaths, 1 at 40 and 1 at 1000 mg/kg/day at which the cause was not determined. Two animals at the 1000 mg/kg/day dose aborted on gestation day 20 and were sacrificed but no relevant clinical signs were discerned. There were no differences in mean body weight among groups. Some high dose animals showed body weight gain decreases when compared to controls. There was a similar effect on food consumption during the treatment period with individual high dose animals showing a decrease compared to controls.

There was no evidence that darunavir was teratogenic in rabbits.

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.

Study no.: TMC 114-NC126

Conducting laboratory and location:

Date of study initiation: June 22, 2001

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: AA-5977-Batch-3-01, _____

Methods

Doses: Vehicle (1% Carboxy methylcellulose CMC/ 0.2% Tween 80 in water), 40, 200 or 1000 mg/kg/day

Species/strain: New Zealand white rabbits

Number/sex/group: 20 mated females per group

Route, volume: Oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: 3 animals dosed at 1000 mg/kg

Study design: darunavir, formulated in 1% CMC/ 0.2% Tween 80 in deionized water was administered once daily, by gavage, during gestation day (GD) 8 to GD 20. Four groups were dosed with, 0 (vehicle), 40, 200 and 1000 mg/kg/day in a dose volume of 10 ml/kg. Each group consisted of 20 presumed pregnant, female New Zealand white rabbits. Observations were made for clinical signs, body weight and food consumption. Necropsy was carried out after sacrificed on GD30. 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 viscera (including the brain) by fresh tissue examination. The fetuses were then eviscerated, preserved, stained with Alizarin Red S, and examined skeletally for abnormalities.

Results

Mortality (dams): there were two deaths where the cause was not identified, 1 at 40 and 1 at 1000 mg/kg/day.

Clinical signs (dams), Body weight (dams) and Food consumption: No effects

Toxicokinetics: Limited toxicokinetic evaluations were performed in this study. Three satellite animals were given the highest dose and used for evaluation on GD20. Maternal and fetal plasma levels were determined. Maternal and fetal plasma levels were determined. Maternal plasma concentrations ranged between 0.09 and 1.5 µg/ml and TMC 114 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 and these data which are shown in the following Table are considered representative of values expected in this present study. Exposures were only approximately 5% of that measured in the clinic.

TMC 114 (darunavir) C_{max} and AUC values after repeated p.o. administration in rabbits

TMC114 Dose (mg/kg/day)	Sampling Day	C _{max}	AUC ^a
		(µg/mL)	(µg.h/mL)
		F	F
40	Day 1 (GD8)	0.05	0.13
	Day 13 (GD20)	0.15	0.47
200	Day 1 (GD8)	0.15	0.52
	Day 13 (GD20)	0.60	1.81
1000	Day 1 (GD8)	1.92	6.26 ^b
	Day 13 (GD20)	1.74	6.00

^a AUC₀₋₂₄ after single dose (day 1) or AUC₀₋₂₄ after repeated dose, ^b AUC₀₋₂₄

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): 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.

Offspring (malformations, variations, etc.): There was one fetus at 40 mg/kg/day with external malformations. Visceral malformations were seen in five fetuses, two at 40 mg/kg/day and one each in the other groups. Skeletal malformations were seen in 21, 23, 23 and 19 fetuses in the four groups.

Study title: Oral (gavage) developmental toxicity study in the mouse

Initial studies carried out in New Zealand white rabbits demonstrated low systemic exposure in comparison with that in the human. The sponsor, therefore, carried out studies in mice in an effort to increase the animal exposure to a level closer to that seen in the clinic.

A non-GLP dose range-finding study was carried out in pregnant female CD-1 mice in which one control and three dosed animals per treatment group were administered darunavir at doses of 0 (vehicle, PEG400), 150, 450 or 1000 mg/kg/day through gestation day (GD) six to 15. The animals were sacrificed on GD18. The NOAEL was considered to be 1000 mg/kg/day based on the outcomes of the usual examinations made for a developmental toxicity test.

Key study findings: Pregnant CD-1 mice were administered darunavir formulated in PEG 400 once daily, by gavage, during gestation day (GD) 6 to 15. The following combinations of darunavir/ritonavir (mg/kg/day) were given: 0/0 (PEG400, vehicle), 150/0, 450/0, 1000/0, 1000/50 and 0/50. Each group consisted of 30 mated mice.

Seven animals were found dead or were sacrificed prematurely during the study. All but one were associated with dosing error. There was one animal in the 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 darunavir. With ritonavir alone, body weight gain and food consumption of the dams was reduced. In the group dosed with 1000 mg/kg/day darunavir and 50 mg/kg ritonavir, 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 considered to be primarily due to one litter in each group where a high number of abnormalities were observed. These were considered to be coincidental and unrelated to treatment with darunavir or ritonavir.

There was no evidence that darunavir was teratogenic in mice.

Based on the absence of relevant effects, the maternal NOAEL was considered to be 1000 mg/kg/day with darunavir alone. Ritonavir alone, or ritonavir given in combination with darunavir, reduced food consumption. There were no effects of darunavir treatment on embryo-fetal development at any of the doses administered. The embryofetal NOAEL was therefore considered to be 1000 mg/kg/day.

Study no.: TMC 114-NC172

Conducting laboratory and location:

Date of study initiation: 29 October, 2004

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Darunavir: 03P0468, — ritonavir: 21350VA, not given

Methods

Doses: darunavir /ritonavir (mg/kg/day) were given: 0/0 (PEG 400, vehicle), 150/0, 450/0, 1000/0, 1000/50 and 0/50.

Species/strain: — CD-1 (ICR) BR VAF/PLUS mice

Number/sex/group: See Table following:

The following table summarises the groups and dose levels used during the study:

Group number	Colour code	Number of mated females	Animal identification numbers	Dose level (mg eq/kg/day) TMC114 or TMC114 + RTV or RTV
1	White	30 ^a	1 - 30	Vehicle Control
2	Green	30 ^a	31 - 60	150
3	Yellow	30 ^a	61 - 90	450
4	Purple	30 ^a	91 - 120	1000
5	Red	30 ^a	121 - 150	1000 + 50
6	Pink	30 ^a	151 - 180	50
7	White #	8	181 - 188	Vehicle Control
8	Green #	10	189 - 198	150
9	Yellow#	10	199 - 208	450
10	Purple #	10	209 - 218	1000
11	Red #	10	219 - 228	1000 + 50
12	Pink #	10	229 - 238	50

a = expected to give 20 pregnant per group

= black corner added to label – Satellite groups

Route, formulation, volume, and infusion rate: Oral gavage, 10 ml/kg darunavir, 6.25 ml/kg ritonavir.

Groups 1-6 were designated main study groups. Groups 7-12 were designated satellite groups and were used for the assessment of exposure to the test article.

Study design: Pregnant CD-1 mice were administered darunavir formulated in PEG 400 once daily, by gavage, during gestation day (GD) 6 to 15. The following combinations of darunavir /ritonavir (mg/kg/day) were given: 0/0 (PEG 400, vehicle), 150/0, 450/0, 1000/0, 1000/50 and 0/50. Each group consisted of 30 mated mice. Observations were made for clinical signs, body weight and food consumption. On gestation day 18, the dams were sacrificed and necropsied. All animals sacrificed at the end of the study were subject to macroscopic examination. 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 for skeletal abnormalities.

Results

Mortality (dams): Seven animals were found dead or were sacrificed prematurely during the study. All but one were associated with dosing error. There was one animal in the 1000/0 mg/kg/day where the cause was not identified.

Clinical signs (dams), body weights and food consumption: There were no relevant clinical signs or effects on body weight and food consumption with darunavir. With ritonavir alone, body weight gain and food consumption of the dams was reduced. In the group dosed with 1000 mg/kg/day darunavir and 50 mg/kg ritonavir, food consumption was also slightly reduced.

Toxicokinetics: Toxicokinetic analysis described non-linear kinetics of darunavir. Combining darunavir with ritonavir resulted in a higher exposure to the compound. In general, a decrease in exposure of ritonavir was seen when it was combined with darunavir. Systemic exposure values are given in the following two Tables.

Exposure (C_{max} and AUC) to darunavir

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

^a AUC_{0-∞} after single dose (day 1) or AUC_{0-24h} after repeated dose. ^b AUC_{0-24h}

Exposure (C_{max} and AUC) to ritonavir

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

^a AUC_{0-∞} after single dose (day 1) or AUC_{0-24h} after repeated dose, ^b AUC_{0-8h}

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): Offspring (malformations, variations, etc.):

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. Statistically significant increases in the number of major abnormalities were observed in groups given 450/0, 1000/0 and 1000/50 mg/kg/day. In each group, one litter was responsible for the unusually high number of abnormalities observed. The findings were considered coincidental and unrelated to treatment with darunavir or ritonavir. The incidence within each responsible litter is given in the following Table (one can see the Table in "Methods" to identify the group of each of the three litters).

Group number	animal number	Abnormality	Incidence within litter
3	90	Eye - uni or bilateral open,	4
		Excecephaly	7
		Frontal -uni-or bilateral malformed	7
		Parietal -uni-or bilateria absent	7
		Interparietal absent	7
		Occipital absent	7
4	91	Eye - uni or bilateral open,	1
		Palatine cleft	6
		Palate cleft	6
5	150	Eye - uni or bilateral open,	3
		Excecephaly	4
		Frontal -uni-or bilateral malformed	4
		Parietal -uni-or bilateria absent	4
		Interparietal absent	4
		Occipital absent	4

Following treatment with either darunavir or ritonavir, a small number of skeletal abnormalities related to the extent of ossification were seen. Although these reached statistical significance, they were not considered related to administration of darunavir or ritonavir (or a combination of

Number/sex/group: See Table below describing the group sizes, doses and identification numbers

Group number	Colour code	Number of animals	Animal identification numbers	Dose level (mg eq./kg/day) TMC114 (R319064)
1	White	6	1 – 6	Vehicle control
2	White #	6	7 – 12	Vehicle Control
3	Green	6	13 – 18	40
4	Yellow	6	19 – 24	200
5	Pink	6	25 - 30	1000

= black corner added to label

Route, formulation, volume, and infusion rate: Oral, gavage, darunavir in PEG 400, 10 ml/kg.

Satellite groups used for toxicokinetics: Day seven of lactation, four pups were bled at pre-dose, two and six hours postdose

At day 12 of lactation, four pups were bled at one, three and five hours postdose

Study design with parameters and endpoints examined: Darunavir, formulated in PEG 400 was administered once daily, by gavage, to mated females from day six of gestation through day seven of lactation. Two control groups and three treated groups were given, 0 (vehicle), 0 (vehicle), 40, 200 and 1000 mg/kg/day. Each group consisted of 6 presumed pregnant, mated female rats. Maternal clinical signs, body weight and food consumption were noted at regular intervals. Nesting and nursing behavior were observed after the dams were allowed to litter. Parturition, litter size and numbers of each sex were recorded. Clinical observations and pup body weight were also taken. On day six of lactation, eight male and eight 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. There were unexpected deaths and adverse findings after direct dosing of pups on day 12 of age, and the dose level of 1000 mg/kg/day (groups 2 and 5) was reduced to 500 mg/kg/day. Due to unexpected deaths and adverse findings after direct dosing of pups on day 13, these groups were terminated. Twelve male and 12 female pups from each group, which had not been selected for direct dosing, were used for blood sampling during the first six hours after dosing of the dams, for toxicokinetic analysis on day seven of lactation.

- 7. *Subacute 14-day oral toxicity study with TMC114 by daily gavage in the rat (Study TMC114-NC107; [REDACTED] Project 298248)*
- 8. *Three-month oral (gavage) repeat dose toxicity study in the rat (Study TMC114-NC130; [REDACTED] Project PHN1032)*
- 9. *Six-month oral (gavage) repeat dose toxicity study in the rat (Study TMC114-NC132; [REDACTED] Project PHN1034; Second draft 6 November 2002)*

Dog

- 10. *Subacute 14-day oral toxicity study with TMC114 by daily gavage in the dog (Study TMC114-NC106; [REDACTED] Project 298259)*
- 11. *Three-month oral (gavage) repeat dose toxicity study in the dog (Study TMC114-NC131; [REDACTED] Project PHN103)*
- 12. *Six-month oral (gavage) repeat dose toxicity study in the dog (Study TMC114-NC133; [REDACTED] Project PHN1035; Second draft 6 November 2002)*

Combination studies

Rat

- 13. *Subacute 14-day oral toxicity study with TMC114 and RTV by daily gavage in the rat (Study TMC114-NC141; [REDACTED] Project 343171; Second draft 7 November 2002)*
- 14. *Subacute 14-day oral toxicity study with TMC114 and RTV by daily gavage in the rat (Study TMC114-NC143; [REDACTED] Project 344936; Second draft 7 November 2002)*

Dog

- 15. *Subacute 14-day oral toxicity study with TMC114 and RTV by daily gavage in the dog (Study TMC114-NC140; [REDACTED] Project 343136; Second draft 7 November 2002)*

Genotoxicity

- 16. *Evaluation of the mutagenic activity of TMC114 in the Salmonella-typhymurium reverse mutation assay and the Escherichia coli reverse mutation assay ([REDACTED] Project 293063)*
- 17. *Evaluation of the ability of TMC114 to induce chromosome aberrations in cultured peripheral human lymphocytes ([REDACTED] Project 294288)*
- 18. *Micronucleus test in bone marrow cells of the mouse (Study TMC114-NC114, [REDACTED] Project 303874)*

Studies not reviewed within this submission:

Pharmacology

19. [REDACTED]

Pharmacokinetics

20. [REDACTED]
 21. [REDACTED]
 22. [REDACTED]
 23. [REDACTED]



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 § 552(b)(5) Deliberative Process

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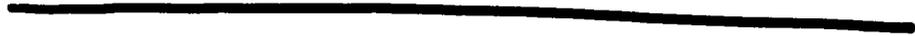


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TABLE OF CONTENTS –PHARMACOLOGY/TOXICOLOGY REVIEW**I. PHARMACOLOGY:**

Primary pharmacodynamics: TMC114 is a potent inhibitor of wild type HIV protease as well as clinically relevant mutants.

Mechanism of action: TMC114 is a potent inhibitor of wild type HIV protease as well as clinically relevant mutants.

Secondary pharmacodynamics: Not reviewed.

II. SAFETY PHARMACOLOGY:**Neurological effects:**

TMC114 did not cause CNS toxicities in rats at up to 2000 mg/kg (three dose levels: 20, 200 and 2000 mg/kg). No changes in measures of activity were seen in the functional observation battery assessment (autonomic, neuromuscular, sensorimotor, convulsive, excitability, general activity of behavior) and motor activity at 1, 6 and 24-h post-treatment.

Respiratory system effects:

TMC114 did not cause respiratory toxicities in rats. No treatment-related changes in ventilatory parameters (tidal volume, respiratory rate and minute volume) or airway resistance (total pulmonary resistance) were seen in rats at up to 2000mg/kg.

Cardiovascular effects:

TMC114 had no relevant cardiovascular effects in vitro. The hERG assay and sheep Purkinje fiber findings indicate that TMC114 did not block both cardiac potassium channels. TMC114, at up to 10 μ M, did affect **action potential duration (APD)**, **upstroke amplitude (UA)** or **resting membrane potential (RMP)**, and **maximum rate of depolarization (MRD)** in isolated Purkinje fibers in the sheep. Thus, TMC114 is unlikely to have an effect on the QT interval or QRS duration at 10 μ M, which is further supported by cardiovascular dog studies. TMC114 at a single dose of 120 mg/kg (estimated C_{max} : 16.6 μ g/ml; AUC: 73.7-81.6 μ g•h/ml) did not affect arterial pressures or ECG parameters (including QTc) in dogs. At this dose level, systemic exposure in dogs is approximately 2 fold higher than that predicted in humans at the proposed maximal clinical dose (300 mg TMC114/100 mg RTV, b.i.d. for 14 days, estimated C_{max} : 2.9 μ g/ml; AUC: 42.5 μ g•h/ml).

Neuro-behavior and motor activity in rats (Study No.: TMC114-NC116)**Method**

TMC114 was orally administered to Sprague-Dawley rats (n=6/sex/group) in three doses levels such that groups received a total dose of 0 (vehicle), 20, 200, or 2000mg/kg.

Results

TMC114 did not cause CNS toxicities in rats at up to 2000 mg/kg (three dose levels: 20, 200 and 2000 mg/kg). No changes in measures of activity were seen in the functional observation battery assessment (autonomic, neuromuscular, sensorimotor, convulsive, excitability, general activity of

behavior) and motor activity at 1, 6 and 24-h post-treatment.

Cardiovascular safety study (telemetry) in dogs (Study No.: TMC114-NC108)

Method and Result:

Cardiac function was evaluated in beagle dogs. **TMC114** (120 mg/kg/day) or the vehicle () was administered orally by gavage once daily. Mean arterial pressure, heart rate, systolic blood pressure, diastolic blood pressure, pulse pressure, electrocardiographic intervals (PR, QRS, QT, QTc), and ECG were recorded continuously from 2 hours prior to dosing until 24 hours after the ritonavir dose. TMC114 at a single dose of 120 mg/kg (estimated C_{max} : 16.6 μ g/mL; AUC: 73.7-81.6 μ g•h/ml) did not affect arterial pressures or ECG parameters (including QTc) in dogs. At this dose level, systemic exposure in dogs is approximately 2 fold higher than that predicted in humans at the proposed maximal clinical dose (300 mg TMC114/100 mg RTV, b.i.d. for 14 days, estimated C_{max} : 2.9 μ g/ml; AUC: 42.5 μ g•h/ml).

Cardiovascular safety study (hERG) (Study No.: TMC114-NC103)

Method and Result

TMC114, at up to 10 μ M, had no relevant effects on cardiac potassium channels in the hERG assay.

Cardiovascular safety in purkinje fibers (Study No.: TMC114-NC105)

Method and Result

TMC114, at up to 10 μ M, had no relevant cardiovascular effects on the sheep Purkinje fiber TMC114. No changes in **action potential duration (APD)**, **upstroke amplitude (UA)** or **resting membrane potential (RMP)**, and **maximum rate of depolarization (MRD)** were seen in isolated Purkinje fibers. Thus, TMC114 is unlikely to have an effect on the QT interval or QRS duration at 10 μ M.

Pulmonary safety study in rats (Study No.: TMC114-NC117)**Method**

Respiratory function was evaluated in Sprague-Dawley rats (n = 5 males) with at least 7 days between each treatment. On each treatment day the vehicle or TMC114 (2000mg/kg/day) was administered orally by gavage. Ventilatory and airway resistance measurements were recorded prior to dosing, and at approximately 2, 4, 24, and 48 hours after the first daily dose of GW640385X, with an additional recording approximately 2 hours after the second daily dose.

Results

TMC114 had no effect on ventilatory parameters (tidal volume, respiratory rate and minute volume) or airway resistance (total pulmonary resistance) in rats up to 2000mg/kg.

Gastrointestinal safety study in rats (Study No.: TMC114-NC120)

Renal effects: Not assessed

Abuse liability: Not determined

Other: None

III. PHARMACOKINETICS/TOXICOKINETICS**Summary**

ADME: Co-administration of 75mg/kg/day ritonavir (RTV) with 2000 mg/kg/day TMC114 significantly increased the TMC114 systemic exposure and C_{max} in rats. In the presence of 75 mg/kg/day RTV, the TMC114 AUC_{24h} increased 2.68- and 3.4-fold in Days 1 and 2, respectively. In the presence of RTV, TMC114 AUC_{24h} values in female rats were 4 times that of TMC114 AUC_{24h} values in male rats, although the reason for the difference in sex is not known at present. In dogs, co-administration of RTV with TMC114 did not increase the systemic TMC114 exposure. However, decreases in RTV AUC values were seen in rats and dogs co-administered with TMC114. TMC114 was highly protein-bound in rat, dog, and human plasma. TMC114 was rapidly metabolized in rat, dog, and human liver microsomes. The metabolic profiles of TMC114 generated from the rat, dog and human were qualitatively different. In rat liver microsomes, only one hydroxylated metabolite was found, while in dogs and humans, there were two different hydroxylated metabolites identified. Evaluation of hepatic microsomal cytochrome P450 (CYP) metabolism in dog, rat, and human liver microsomes indicated TMC114 is a good substrate for CYP3A (K_m : 3.5 μM for dog, 4.6 μM for human, and 21 μM for rat microsomes. Ketoconazole, a CYP3A inhibitor, significantly inhibit TMC metabolism in the dog and human liver microsomes.

IV. GENERAL TOXICOLOGY:**Acute toxicity studies****Single-dose studies**

Rat

44. Acute oral toxicity study with TMC114 in the mouse (Study TMC114-NC111; i [REDACTED] Project 303828)
45. Acute oral toxicity study with TMC114 in the rat (Study TMC114-NC101; [REDACTED] Project 293074)
46. Acute oral toxicity study with TMC114 in the rat (Study TMC114-NC104; [REDACTED] Project 299069)
47. Acute intravenous toxicity study with TMC114 in the rat (Study TMC114-NC110; [REDACTED] Project 303839)

Dog

48. Range finding oral toxicity study in male and female beagle dogs (Study TMC114-NC102; [REDACTED] Project 293502)
49. Single and repeated dose intravenous toxicity of TMC114 in male and female beagle dogs (Study TMC114-NC109; [REDACTED] Project 303841)

Multidose toxicity studies**Rats****TMC114 alone:**

- Toxicology findings noted in the 3-month and 6-month repeated dose toxicity study in rats included increased liver weights (centrilobular hepatocyte hypertrophy, dark liver), minimal hypertrophy/hyperplasia of bile duct changes in urinary parameters (\uparrow bilirubin in males, \uparrow protein in females, \uparrow urobilirubin and osmolality in males and females, and \uparrow relative kidney weights, dark kidneys in males) at 500 mg/kg/day, and alterations in hematology and blood chemistry (\downarrow RBC, \downarrow Hb, \downarrow Hct, \downarrow MCV, \uparrow reticulocyte count, \uparrow platelets, \uparrow APTT, \uparrow cholesterol, \uparrow total protein, \uparrow globulin, \uparrow K, \downarrow A/G ratio, \downarrow Triglycerides at week 6, 13, or 26) at 100 mg/kg/day, and 500 mg/kg/day. Effects at 20 mg/kg/day were limited to \uparrow reticulocyte count \uparrow APTT and \uparrow K in males. Thus, the NOAEL in the absence of RTV was considered to be <20 mg/kg/day. The human equivalent oral dose will be <3.2 mg/kg/day (conversion factors: 6.3 for rats).

TMC114+RTV:

- Toxicology findings noted in the 14-day repeated dose toxicity study in rats co-administered with TMC114 and RTV included increased liver weights (hepatocellular hypertrophy and vacuolation) and an increased incidence of follicular hypertrophy in the thyroid glands in female rats. In an additional 14-day repeated dose toxicity study in rats co-administered with TMC114 and RTV, two male rats and one male died at 0/50 mg/kg/day and 500/25 m/kg/day, respectively. At 0/50 mg/kg/day TMC114/RTV, decreases in Hct, increases in cholesterol, total protein and globulin were seen in females. Increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation in the liver) and increases in the incidence of hemopoiesis (primarily erythropoiesis) were also seen in the spleen in rats. At 150/25 mg/kg/day TMC114/RTV, an increased incidence of salivation was seen in females. Alterations in hematology and blood chemistry (\downarrow RBC, \downarrow Hb, \downarrow Hct, \downarrow MCV, \uparrow platelets, \uparrow APTT, \uparrow cholesterol and \uparrow Ca, \uparrow total globulin and protein, \downarrow A/G ratio) were also seen.

Accentuated lobular pattern of the liver (6/10 F) and liver enlargement (2/10 M) and 3/10 F), as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation) were also seen. Increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation in the liver), increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, and increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland were seen females only. At 500/25 mg/kg/day TMC114/RTV, an increased incidence of salivation was seen in females. Alterations in hematology and blood chemistry (↓RBC, ↓Hb, ↓Hct, ↓MCV, ↑platelets, ↑APTT, ↑cholesterol and ↑Ca, ↑total globulin and protein, ↑bilirubin, ↓A/G ratio) were also seen. Accentuated lobular pattern of the liver (1/10 M, 5/10 F) and liver enlargement (3/10 M, 7/10 F), as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation) were also seen. Increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, and increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland were seen in females. At 1000/50 mg/kg/day TMC114/RTV, an increased incidence of salivation was seen in females. Alterations in hematology and blood chemistry (↓RBC, ↓Hb, ↓Hct, ↓MCV, ↑platelets, ↑APTT, ↑cholesterol and ↑Ca, ↑total globulin and protein, ↑bilirubin, ↓A/G ratio) were also seen. Accentuated lobular pattern of the liver (1/10 M, 9/10 F) and liver enlargement (7/10 M, 9/10 F), as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation) were also seen. Increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, as well as increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland were also seen in males and females. Thus, while the RTV treatment-related liver effect (hepatocellular hypertrophy and vacuolation) was exacerbated by the TMC114 treatment, the RTV treatment-related hematological effect (RBC, ↓Hb, ↓Hct, ↓MCV, and ↑platelets) appeared not to be exacerbated by the TMC114 treatment. Additionally, the increased incidence of follicular hypertrophy/hyperplasia in the thyroid glands was confined to TMC114 treatment groups. The NOAEL in the presence of RTV could not be established. The human equivalent oral dose will be <24/4 mg/kg/day (conversion factors: 6.3 for rats).

Dogs:

TMC114 alone:

- Toxicological effects noted in the 3-month and 6-month repeated dose toxicity study in dogs (30, 60, 120 mg/kg/day TMC114) included an increased incidence of loose or liquid faces, vomiting and salivation at 30, 60, and 120 mg/kg/day, slightly increased relative liver weight in one female dog (absence of any histopathological correlates), a marginal increase in severity of thymic involution at 120 mg/kg/day. Thus, the NOAEL in the absence of RTV was considered to be 120 mg/kg/day. The human equivalent oral dose will be 65 mg/kg/day (conversion factors: 1.85 for dogs).

TMC114+RTV:

- In the dog following repeated administration of TMC114/RTV for 2 weeks (0/10, 40/10, 120/10, 360/10 mg/kg/day TMC114/RTV), vomiting, salivation and shaking of the head were seen in all groups including the RTV control group. The frequency of these findings was exacerbated by treatment with TMC. Slight to moderate weight loss was seen in 2/3 RTV control females, in male (1/3) and females (3/3) at $\geq 40/10$ mg/kg/day, in males (2/3) and females (3/3) at 120/10 mg/kg/day, and in males (3/3) and females (3/3). Decreases in Cl levels were seen in males at 360/10 mg/kg/day. Feces with white particles were seen in one 120/10mg/kg/day male dog (1/3) at Week 2, as well as in males (2/3) and females (3/3). The significance of white particles in the feces of most animals at 360/10 mg/kg/day at Week 2 is uncertain, but it was considered to be non-absorbed particle of the test article. Based on the results, the NOAEL of TMC 114/RTV was considered to be 120/10 mg/kg/day. The human equivalent oral dose will be 64.9/5.4 mg/kg/day (conversion factors: 1.85 for dogs).
- **ADME:** Co-administration of 75 mg/kg/day ritonavir (RTV) with 2000 mg/kg/day TMC114 significantly increases the TMC114 systemic exposure and C_{max} in rats. In the presence of 75 mg/kg/day RTV, TMC114 AUC_{24h} increased 2.68-fold in Day 1 and 3.4-fold in Day 2. TMC114 AUC_{24h} decreased between Days 1 and 14 by approximately 46% and 59% in rats at 2000 mg/kg/day in the absence or presence of RTV. In the presence of RTV, TMC114 AUC_{24h} values in female rats were 3.7-fold higher than TMC114 AUC_{24h} values in male rats at 2000 mg/kg/day, although the reason for the difference in sex is not known at present. TMC114 was highly protein-bound in rat, dog, and human plasma. TMC114 was rapidly metabolized in rat, dog, and human liver microsomes. The metabolic profiles of TMC114 generated from the rat, dog and human were qualitatively different. In rat liver microsomes, only one hydroxylated metabolite was found, while in dogs and humans, there were two different hydroxylated metabolites identified. Evaluation of hepatic microsomal cytochrome P450 (CYP) metabolism in dog, rat, and human liver microsomes indicated TMC114 is a good substrate for CYP3A (Km: 3.5 μ M for dog, 4.6 μ M for human, and 21 μ M for rat microsomes. Ketoconazole, a CYP3A inhibitor, significantly inhibit TMC metabolism in the dog and human liver microsomes.
- **Issues of ingredient:** The oral formulation of TMC114 proposed for use in the clinical study (TMC-C133) contains an ingredient [REDACTED]. However, this component was not present in the TMC114 oral formulation of the animal studies (TMC114 oral formulation for the animal studies was prepared as a suspension in PEG400. Because of the lack of animal toxicological data, a justification should be made to address the safety of [REDACTED] in the clinical formulation.

V. GENETIC TOXICOLOGY:

Genotoxicity: TMC114 was non-mutagenic and non-clastogenic in a battery of genotoxicity evaluations. TMC114 was not genotoxic in vitro in the Ames test (up to 3300 μ g/plate) or a cytogenetics assay in human lymphocytes (up to 333 μ g/mL; \pm S9), or in vivo in a mouse micronucleus test (up to 2000mg/kg/day).

VI. CARCINOGENICITY:

No carcinogenicity studies have been conducted.

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Reproductive toxicity: A fertility study (Segment I) in rats and Segment II reproductive toxicity studies in rats and rabbits are ongoing. No reproductive toxicology studies in animals or humans have been reported. Therefore, the compound must not be administered to pregnant women, nursing mothers, or women of childbearing potential unless adequate birth control measures are in place.

VIII. SPECIAL TOXICOLOGY STUDIES: NONE

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

The IND package included 14 single and repeated dose toxicology and toxicokinetic studies up to 6 months in length in rats and dogs with TMC114 alone or with TMC114 in combination with RTV, three safety pharmacology studies, three genotoxicity studies, 8 non-clinical pharmacology studies. The IND package did not contain any relevant information pertaining to reproductive toxicology and carcinogenicity. Overall pre-clinical information submitted are considered adequate in support the repeated dose human Phase I trial proposed in this IND.

General Toxicology Issues:

Safety Pharmacology

- **CNS effects:** TMC114 did not cause CNS toxicities in rats at up to 2000 mg/kg (three dose levels: 20, 200 and 2000 mg/kg). No changes in measures of activity were seen in the functional observation battery assessment (autonomic, neuromuscular, sensorimotor, convulsive, excitability, general activity of behavior) and motor activity at 1, 6 and 24-h post-treatment.
- **Respiratory system effects:** TMC114 did not cause respiratory toxicities in rats. No treatment-related changes in ventilatory parameters (tidal volume, respiratory rate and minute volume) or airway resistance (total pulmonary resistance) were seen in rats at up to 2000mg/kg.
- **Cardiovascular effects:** TMC114 had no relevant cardiovascular effects in vitro. The hERG assay and sheep Purkinje fiber findings indicate that TMC114 did not block both cardiac potassium channels. TMC114, at up to 10 μ M, did affect **action potential duration (APD)**, **upstroke amplitude (UA)** or **resting membrane potential (RMP)**, and **maximum rate of depolarization (MRD)** in isolated Purkinje fibers in the sheep. Thus, TMC114 is unlikely to have an effect on the QT interval or QRS duration at 10 μ M, which is further supported by cardiovascular dog studies. TMC114 at a single dose of 120 mg/kg (estimated C_{max} : 16.6 μ g/mL; AUC: 73.7-81.6 μ g•h/mL) did not affect arterial pressures or ECG parameters (including QTc) in dogs. At this dose level, systemic exposure in dogs is approximately 2 fold higher than that predicted in humans at the proposed maximal clinical dose (300 mg TMC114/100 mg RTV, b.i.d. for 14 days, estimated C_{max} : 2.9 μ g/mL; AUC: 42.5 μ g•h/mL).

Multiple Dose Toxicology Study

Rats

TMC114 alone:

- **Toxicology findings noted in the 3-month and 6-month repeated dose toxicity study in rats included increased liver weights (centrilobular hepatocyte hypertrophy, dark liver),**

minimal hypertrophy/hyperplasia of bile duct changes in urinary parameters (↑bilirubin in males, ↑protein in females, ↑urobilirubin and osmolality in males and females, and ↑relative kidney weights, dark kidneys in males) at 500 mg/kg/day, and alterations in hematology and blood chemistry (↓RBC, ↓Hb, ↓Hct, ↓MCV, ↑reticulocyte count, ↑platelets, ↑APTT, ↑cholesterol, ↑total protein, ↑globulin, ↑K, ↓A/G ratio, ↓triglycerides at week 6, 13, or 26) at 100 mg/kg/day, and 500 mg/kg/day. Effects at 20 mg/kg/day were limited to ↑reticulocyte count, ↑APTT and ↑K in males. Thus, the NOAEL in the absence of RTV was considered to be <20 mg/kg/day. The human equivalent oral dose will be <3.2 mg/kg/day (conversion factors: 6.3 for rats).

TMC114+RTV:

- Toxicology findings noted in the 14-day repeated dose toxicity study in rats co-administered with TMC114 and RTV included increased liver weights (hepatocellular hypertrophy and vacuolation) and an increased incidence of follicular hypertrophy in the thyroid glands in female rats. In an additional 14-day repeated dose toxicity study in rats co-administered with TMC114 and RTV, two male rats and one male died at 0/50 mg/kg/day and 500/25 m/kg/day, respectively. At 0/50 mg/kg/day TMC114/RTV, decreases in Hct, increases in cholesterol, total protein and globulin were seen in females. Increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation in the liver) and increases in the incidence of hemopoiesis (primarily erythropoiesis) were also seen in the spleen in rats. At 150/25 mg/kg/day TMC114/RTV, an increased incidence of salivation was seen in females. Alterations in hematology and blood chemistry (↓RBC, ↓Hb, ↓Hct, ↓MCV, ↑platelets, ↑APTT, ↑cholesterol and ↑Ca, ↑total globulin and protein, ↓A/G ratio) were also seen. Accentuated lobular pattern of the liver (6/10 F) and liver enlargement (2/10 M) and 3/10 F), as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation) were also seen. Increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation in the liver), increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, and increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland were seen females only. At 500/25 mg/kg/day TMC114/RTV, an increased incidence of salivation was seen in females. Alterations in hematology and blood chemistry (↓RBC, ↓Hb, ↓Hct, ↓MCV, ↑platelets, ↑APTT, ↑cholesterol and ↑Ca, ↑total globulin and protein, ↑bilirubin, ↓A/G ratio) were also seen. Accentuated lobular pattern of the liver (1/10 M, 5/10 F) and liver enlargement (3/10 M, 7/10 F), as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation) were also seen. Increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, and increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland were seen in females. At 1000/50 mg/kg/day TMC114/RTV, an increased incidence of salivation was seen in females. Alterations in hematology and blood chemistry (↓RBC, ↓Hb, ↓Hct, ↓MCV, ↑platelets, ↑APTT, ↑cholesterol and ↑Ca, ↑total globulin and protein, ↑bilirubin, ↓A/G ratio) were also seen. Accentuated lobular pattern of the liver (1/10 M, 9/10 F) and liver enlargement (7/10 M, 9/10 F), as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation) were also seen. Increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, as well as increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland

were also seen in males and females. Thus, while the RTV treatment-related liver effect (hepatocellular hypertrophy and vacuolation) was exacerbated by the TMC114 treatment, the RTV treatment-related hematological effect (RBC, ↓Hb, ↓Hct, ↓MCV, and ↑platelets) appeared not to be exacerbated by the TMC114 treatment. Additionally, the increased incidence of follicular hypertrophy/hyperplasia in the thyroid glands was confined to TMC114 treatment groups. The NOAEL in the presence of RTV could not be established. The human equivalent oral dose will be $24/4$ mg/kg/day (conversion factors: 6.3 for rats).

Dogs:

TMC114 alone:

- Toxicological effects noted in the 3-month and 6-month repeated dose toxicity study in dogs (30, 60, 120 mg/kg/day TMC114) included an increased incidence of loose or liquid faces, vomiting and salivation at 30, 60, and 120 mg/kg/day, slightly increased relative liver weight in one female dog (absence of any histopathological correlates), a marginal increase in severity of thymic involution at 120 mg/kg/day. Thus, the NOAEL in the absence of RTV was considered to be 120 mg/kg/day. The human equivalent oral dose will be 65 mg/kg/day (conversion factors: 1.85 for dogs).

TMC114+RTV:

- In the dog following repeated administration of TMC114/RTV for 2 weeks (0/10, 40/10, 120/10, 360/10 mg/kg/day TMC114/RTV), vomiting, salivation and shaking of the head were seen in all groups including the RTV control group. The frequency of these findings was exacerbated by treatment with TMC. Slight to moderate weight loss was seen in 2/3 RTV control females, in male (1/3) and females (3/3) at $\geq 40/10$ mg/kg/day, in males (2/3) and females (3/3) at 120/10 mg/kg/day, and in males (3/3) and females (3/3). Decreases in Cl levels were seen in males at 360/10 mg/kg/day. Feces with white particles were seen in one 120/10 mg/kg/day male dog (1/3) at Week 2, as well as in males (2/3) and females (3/3). The significance of white particles in the feces of most animals at 360/10 mg/kg/day at Week 2 is uncertain, but it was considered to be non-absorbed particle of the test article. Based on the results, the NOAEL of TMC 114/RTV was considered to be 120/10 mg/kg/day. The human equivalent oral dose will be 64.9/5.4 mg/kg/day (conversion factors: 1.85 for dogs).
- **Genotoxicity:** TMC114 was non-mutagenic and non-clastogenic in a battery of genotoxicity evaluations. TMC114 was not genotoxic in vitro in the Ames test (up to 3300 $\mu\text{g}/\text{plate}$) or a cytogenetics assay in human lymphocytes (up to 333 $\mu\text{g}/\text{mL}$; $\pm\text{S9}$), or in vivo in a mouse micronucleus test (up to 2000 mg/kg/day). Animal studies evaluating the carcinogenicity of GW640385X have not yet been performed.
- **Reproductive toxicity:** A fertility study (Segment I) in rats and Segment II reproductive toxicity studies in rats and rabbits are ongoing. No reproductive toxicology studies in animals or humans have been reported. Therefore, the compound must not be administered to pregnant women, nursing mothers, or women of childbearing potential unless adequate birth control measures are in place.
- **ADME:** Co-administration of 75 mg/kg/day ritonavir (RTV) with 2000 mg/kg/day TMC114 significantly increases the TMC114 systemic exposure and C_{max} in rats. In the presence of 75

mg/kg/day RTV, TMC114 AUC_{24h} increased 2.68-fold in Day 1 and 3.4-fold in Day 2. TMC114 AUC_{24h} decreased between Days 1 and 14 by approximately 46% and 59% in rats at 2000 mg/kg/day in the absence or presence of RTV. In the presence of RTV, TMC114 AUC_{24h} values in female rats were 3.7-fold higher than TMC114 AUC_{24h} values in male rats at 2000 mg/kg/day, although the reason for the difference in sex is not known at present. TMC114 was highly protein-bound in rat, dog, and human plasma. TMC114 was rapidly metabolized in rat, dog, and human liver microsomes. The metabolic profiles of TMC114 generated from the rat, dog and human were qualitatively different. In rat liver microsomes, only one hydroxylated metabolite was found, while in dogs and humans, there were two different hydroxylated metabolites identified. Evaluation of hepatic microsomal cytochrome P450 (CYP) metabolism in dog, rat, and human liver microsomes indicated TMC114 is a good substrate for CYP3A.

- **Issues of ingredient:** The oral formulation of TMC114 proposed for use in the clinical study (TMC-C133) contains an ingredient [REDACTED]. However, this component was not present in the TMC114 oral formulation of the animal studies (TMC114 oral formulation for the animal studies was prepared as a suspension in PEG400. Because of the lack of animal toxicological data, a justification should be made to address the safety of [REDACTED] in the clinical formulation.

Risk assessment

Risk benefit assessment is based on the toxicology data obtained from the 3-month and 6-month repeated oral dose toxicity and toxicokinetics studies in dogs and rats with TMC114 alone and from the 14-day repeated dose toxicity and toxicokinetics studies in dogs and rats co-administered with TMC114/RTV. Safety margins are calculated based on the maximum daily doses of TMC114 in the phase I study proposed by the sponsor.

Toxicology findings observed in the 3-month and 6-month repeated dose toxicity study in rats included increased liver weights, minimal hypertrophy/hyperplasia of bile duct changes in urinary parameters at 500 mg/kg/day, and alterations in hematology and blood chemistry at 100 mg/kg/day and 500 mg/kg/day. Effects at 20 mg/kg/day were limited to ↑reticulocyte count, ↑APTT and ↑K in males. Thus, the NOAEL in the absence of RTV was considered to be <20 mg/kg/day. The HED will be <3.2 mg/kg/day (conversion factors: 6.3 for rats).

Toxicology findings seen in the 14-day repeated dose toxicity study in rats co-administered with TMC114 and RTV included alterations in hematology and blood chemistry, accentuated lobular pattern of the liver and liver enlargement and, as well as increases in absolute liver weights and relative liver weights, increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, and increased incidence of follicular

hypertrophy/hyperplasia in the thyroid gland. The NOAEL in the presence of RTV could not be established. The HED will be <24/4 mg/kg/day (**conversion factors: 6.3 for rats**).

Toxicological effects noted in the 3-month and 6-month repeated dose toxicity study in dogs (30, 60, 120 mg/kg/day TMC114) included an increased incidence of loose or liquid feces, vomiting and salivation at 30, 60 and 120 mg/kg/day, slightly increased relative liver weight in one female dog (absence of any histopathological correlates), a marginal increase in severity of thymic involution at 120 mg/kg/day, **Thus, the NOAEL in the absence of RTV was considered to be 120 mg/kg/day. The human equivalent oral dose will be 65 mg/kg/day** (conversion factors: 1.85 for dogs; safety margins: 3.2 for the 1200 mg/day maximum daily dose for a 60 kg human).

In the dog following repeated administration of TMC114/RTV for 2 weeks, vomiting, salivation and shaking of the head were seen; the frequency of these findings was exacerbated by treatment with TMC. Slight to moderate weight loss was seen in all dose groups. Decreases in CI levels were seen in males at 360/10 mg/kg/day. Feces with white particles were seen in $\geq 120/10$ mg/kg/day dogs.

Based on the results, **the NOAEL of TMC 114/RTV was considered to be 120/10 mg/kg/day. The HED will be 65/5 mg/kg/day** (conversion factors: 1.85 for dogs). The margins of safety for the clinical dose (300/100 mg TMC114/RTV b.i.d., or 5/1.7 mg/kg/day TMC114/RTV b.i.d) are calculated to be 6.5 and 1.5 for TMC114 and RTV, respectively. Safety margins over the proposed clinical daily dosage do not exist for rats.

Recommendations (internal):

The results of the nonclinical toxicity studies in dogs (TMC114 alone for 6 months and TMC114/RTV for two weeks) and the data from previous human clinical studies support the daily TMC114/RTV doses up to 300/100 mg for 9 days, by the oral route, proposed in the clinical protocol. The margins of safety are calculated to be 6.5 and 1.5 for TMC114 and RTV, respectively.

- Toxicology findings noted in the 14-day repeated dose toxicity study in rats co-administered with TMC114 and RTV included alterations in hematology and blood chemistry, increases in the incidence of hemopoiesis (primarily erythropoiesis) in the spleen, accentuated lobular pattern of the liver and liver enlargement and, as well as increases in absolute liver weights and relative liver weights (with hepatocellular hypertrophy and vacuolation). Thus, liver function tests and hematological endpoints should be monitored in subjects receiving TMC114/RTV in clinical trials.
- Co-administration of TMC114/RTV in rats for 14 days demonstrated an increased incidence of follicular hypertrophy/hyperplasia in the thyroid gland in animals. Thus, thyroid functions (plasma TSH, plasma free T4, and T3RU) should be monitored in subjects receiving GW640385X in clinical trials.
- The sponsor should conduct reproductive toxicity studies in rats (Segment I and II) and

rabbits (Segment II) with TMC114 alone or in combination with RTV as soon as possible. Because no reproductive toxicology studies in animals or humans have been performed, TMC114 must not be administered to pregnant women, nursing mothers, or women of childbearing potential unless adequate birth control measures are in place.

Recommendations (to the sponsor):

- The starting daily dose of 300/100 mg/kg TMC114 is considered reasonably safe to proceed in the perspective of pharmacology and toxicology.
- Please conduct further studies, such as a T-cell dependent antibody response assay (the Plaque assay), or a host resistant assay, to evaluate the potential for TMC114 to suppress immune function. For more information, please see the CDER Guidance for Industry, Immunotoxicology Evaluation of Investigational New Drugs (October 2002).

Labeling with basis for findings: N/A

Reviewer signature: Hao Zhang, M.D.

Supervisor signature: James Farrelly, Ph.D.

cc:

HFD-530/IND 62477

HFD-530/JFarrelly

HFD-530/CSO

HFD-530/MO

HFD-530/GLunn

HFD-530/MICRO

X. APPENDIX/ATTACHMENTS:

Appendix table 1 Exposure Ratio of TMC114 in Rats, Dogs Following Repeat Dose Administration of TMC114 With or Without RTV and in Human Following Administration of TMC114

Study Type Report No.	Dose of TMC114 mg/kg/day	Sex	Mean C _{max} ^a µg/mL	Mean AUC _{0-24h} ^b µg·h/mL	Ratio of Animals to Human AUC Following TMC114±RTV administration	
TMC114 Rat 3-month (Week 13 TK) (TMC114-NC132)	<u>20 (NOAEL)</u>	M	TMC114 0.78	TMC114 2.10	0.04	
		F	1.66	3.66	0.07	
	100	M	5.51	27.35	0.54	
		F	8.16	28.16	0.55	
	500	M	9.00	67.62	1.32	
		F	9.68	71.84	1.41	
	Rat 6-month (Week 26 TK) (TMC114-NC132)	<u>20 (NOAEL)</u>	M	1.03	2.92	0.06
			F	1.77	4.38	0.09
		100	M	7.11	31.60	0.62
			F	12.8	35.79	0.70
		500	M	10.30	63.83	1.24
			F	24.50	121.02	2.37
TMC114+RTV Rat 14-day (Day 14 TK) ^c (TMC114-NC141)		0/50	M	TMC114/RTV 0/4.72	TMC114/RTV 0/35.35	--
			F	0/3.62	0/38.73	--
	150/25	M	9.09/0.34	68.26/2.25	1.34	
		F	7.95	98.95/1.50	1.94	

Rat 14-day (Day 14 TK) ^a (TMC114-NC143)	500/25	M	12.90/0.27	102.40/0.79	2.00	
		F	10.98	117.39/0.57	2.30	
	1000/50	M	14.93/0.29	111.20/1.45	2.18	
		F	13.55	126.24/1.38	2.47	
	2000/0	M	8.52	73.98	1.45	
		F	8.98	21.50	0.42	
	2000/75	M	16.83/1.16	192.80/6.75	3.77	
		F	21.5/1.17	356.86/14.90	6.98	
	0/75	M	0/7.71	0/63.75	--	
		F	0/3.25	0/62.34	--	
TMC114 Dog 3 month (Day 86 TK) (TMC114-NC131-NCTK)	30	M	TMC114 4.34	TMC114 10.84	0.21	
		F	3.59	8.43	0.16	
		60	M	9.71	33.28	0.65
			F	8.09	24.26	0.47
		<u>120 (NOAEL)</u>	M	<u>20.90</u>	<u>108.84</u>	<u>2.13</u>
			F	16.90	96.17	1.88
	Dog 6month (Week 26 TK) (TMC114-NC133-NCTK)	30	M	7.91	22.37	0.44
			F	11.36	21.77	0.43
		60	M	15.00	44.00	0.86
			F	16.50	53.11	1.04
		<u>120 (NOAEL)</u>	M	<u>19.83</u>	<u>70.28</u>	<u>1.38</u>
			F	<u>25.95</u>	<u>97.30</u>	<u>1.90</u>
TMC114+RTV Dog 14-day (Day 12 TK) (TMC114-NC140)	TMC114/RTV 0/10	M	TMC114/RTV 0/7.00	TMC114/RTV 0/25.04	--	
		F	0/9.13	0/30.83	--	
	40/10	M	12.50/9.95	48.44/41.81	0.95	
		F	14.37/5.03	46.15/15.32	0.90	
	<u>120/10 (NOAEL)</u>	M	<u>23.93/3.01</u>	<u>103.26/9.12</u>	<u>2.02</u>	
		F	<u>22.3/3.89</u>	<u>105.24/13.10</u>	<u>2.06</u>	
	360/10	M	19.43/2.62	84.43/7.90	1.65	
		F	20.33/1.68	63.96/3.28	1.25	
	TMC114+RTV HIV patient 14-day (Day 14 TK) (TMC114-C207)	<u>300/100 (b.i.d.)</u> <u>600/100 (b.i.d.)</u> <u>900/100 (qd.)</u>	M/F (n)	TMC114/RTV	TMC114/RTV	
			12	4.4	51.1 (27.5-81.2)	--
12			5.7	70.0 (25.1-15.4)	--	
9			6.6	63.6 (44.0-72.4)	--	

a.: Note that TMC114 caused dose dependent decreases in the RTV AUC and C_{max} in both rats and dogs.

Attachment #2

Attachment #2 is the FAX to the sponsor from the Executive CAC relating to the dosages proposed in the protocols for the two year carcinogenicity studies in rats and mice.

Executive CAC

Date of Meeting: January 11, 2005

Committee: Joseph Contrera, Ph.D., HFD-901, Acting Chair

C. Joseph Sun, Ph.D., HFD-570, Alternate Member

Lois Freed, Ph.D., HFD-120, Alternate Member

Jim Farrelly, Ph.D., HFD-530, Pharmacology Supervisor and Presenting Reviewer

Author of Draft: Jim Farrelly

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be

submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 62,477

Drug Name: TMC114

Sponsor: Tibotec, Inc.

Background: TMC114 is an HIV protease inhibitor currently being developed, in combination with a low dose of ritonavir, for treatment of highly drug experienced HIV infected patients. The rationale for combination with a low dose of ritonavir is to improve the oral bioavailability of TMC114.

Since the drug will be used chronically, carcinogenicity studies are expected to be carried out. The sponsor has submitted two protocols for review, one for a two-year carcinogenicity study in mice and another for a two-year carcinogenicity study in rats.

Mouse Carcinogenicity Study Protocol and Dose Selection

The mouse 2-year carcinogenicity study will use CD1 mice with 70 males and females per dose. The doses proposed by the sponsor were 150, 450 or 1000 mg/kg/day in both males and female mice. The doses were based on the outcome of a three-month gavage study in CD1 mice. Dosing in the 2-year study will be by gavage at 10 ml/kg of drug in vehicle, PEG400. Additional groups of males and females will be dosed by gavage with vehicle alone. At the end of the study, the sponsor will examine an appropriate battery of tissues and organs. All tissues and organs at all doses (including control) will be examined histologically. Animals will be housed one per cage.

Dose selection was based on maximum feasible dose via gavage. No dose-limiting toxicity was observed at the high-dose in the 3-month dose range-finding study. Greater systemic exposure was elicited in mice when the animals were administered drug by gavage rather than in the feed. The maximum concentration of drug in the vehicle was 100 mg/ml. At higher doses, precipitate formed in the solution. Dosing by gavage at volumes greater than 10 ml/kg was lethal due to viscosity of the solution. The maximum feasible dose, therefore, was 1000 mg/kg/day.

Rat Carcinogenicity Study Protocol and Dose Selection

The rat 2-year carcinogenesis study will use Sprague Dawley rats with 70 males and females per dose. The doses proposed by the sponsor were 20, 100 or 500 mg/kg/day in both male and female rats. The doses were based on the outcomes of three-month and six month gavage studies in rats. Dosing in the 2-year study will be by gavage at 5 ml/kg of drug in vehicle, PEG400. Additional groups of males and females will be dosed by gavage with vehicle alone. At the end of the study, the sponsor will examine an appropriate battery of tissues and organs. All tissues and organs at all doses (including control) will be examined histologically. Animals will be housed two of the same sex per cage.

Dose selection, in rats (similar to mice) was based on maximum feasible dose via gavage.

No dose-limiting toxicity was observed in either the three- or six-month studies. Greater systemic exposure was elicited in rats when the animals were administered drug by gavage rather than in the feed. The maximum concentration of drug in the vehicle was 100 mg/ml.

At higher doses, precipitate formed in the solution. Dosing by gavage at volumes greater than 5 ml/kg was lethal due to viscosity of the solution. The maximum feasible dose, therefore, was 500 mg/kg/day.

Executive CAC Recommendations and Conclusions:

Mouse:

The Committee concurred with the sponsor's proposed doses of 150, 450, and 1000 mg/kg/day by oral gavage based on MFD (viscosity of solution).

Since TMC114 is to be administered in combination with a low dose of ritonavir, the Committee recommended that an additional group be administered TMC114 (at the high dose) in combination with a dose of 50 mg/kg/day of ritonavir; this combination is expected to increase systemic exposure to TMC114.

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

James Farrelly
6/22/2006 08:18:56 AM
PHARMACOLOGIST