CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Antiviral Products
Center for Drug Evaluation and Research

Date:	March 2, 2011
Reviewer:	Hanan Ghantous, PhD, DABT Supervisory Interdisciplinary Scientist
NDA #/SS#/date:	202-022/000/7/23/2010
Sponsor:	Tibotec Inc.
Drug Product:	Rilpivirine
Indication:	HIV infection in treatment-naïve patients
Recommended Action:	Nonclinical data support approval

Rilpivirine, is a diarylpyrimidine derivative, a next generation non-nucleoside reverse transcriptase inhibitor (NNRTI) indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-naïve adult patients.

The safety of Rilpivrine was investigated in a number of toxicology studies including repeat-dose nonclinical toxicity studies (mice, rats, rabbits, dogs and cynomolgus monkeys), in genetic toxicity and carcinogenicity studies and in reproductive and developmental toxicity studies.

The primary toxicity findings in nonclinical studies were adrenal effects, generally characterized by increased serum progesterone and decreased cortisol levels observed in rats, dogs, and Cynomolgus monkeys. These effects are thought to be associated with an inhibition of steroidogenesis at the level of 21-hydroxylase (CYP21) and 17-hydroxylase (CYP17, in Cynomolgus monkeys only). In dogs, findings of premature activation and overstimulation of the ovaries may also be related to inhibition of steroidogenesis. Those effects on dog ovaries were noted at exposures 8 to 25 times higher than clinical exposures at the recommended dose of 25 mg q.d. Based on the nonclinical safety studies, adrenal function was carefully monitored in the clinical trials. However, Phase III and Phase IIb trials did not show any safety concerns with respect to adrenal function or endocrine events. Based on the nonclinical safety information, Rilpivrine was considered safe for use in humans in clinical trials and it was recommended to give particular attention to possible effects on adrenal and gonadal steroidogenesis.

On-going and planned trials in adolescents and pre-pubertal children should include endocrine safety monitoring, with monitoring of hormone levels and ovulation. Growth curves, pubertal status, breast development, menarche or evidence of either hyperandrogenism (hirsutism) or delayed adrenarche should be documented in these trials.

A Phase I clinical trial demonstrated a QT interval-prolonging effect of Rilpivirine at supratherapeutic doses. In follow-up nonclinical safety pharmacology studies, Rilpivirine demonstrated the potential to inhibit some potassium channels involved in cardiac action potential repolarization at concentrations approximately 10-fold greater than the clinical exposures. Given the clinical and nonclinical findings, adverse events that could be related to cardiac conduction abnormalities or to rate and rhythm disturbances were closely monitored in the Phase IIb and Phase III clinical trials. No clinically relevant QTc prolonging effect was observed with the recommended therapeutic dose of 25 mg q.d., however, patients with known risk for QT interval prolongation or Torsade de Pointes were excluded from the Phase III trials.

Rilpivirine was evaluated for carcinogenic potential by oral gavage administration to mice and rats for 2 years. Rilpivrine was positive in mice for hepatocellular neoplasms which is likely not relevant to humans. At the lowest tested doses in the carcinogenicity studies, the systemic exposures (based on AUC) to Rilpivirine were 21-fold (mice) and 3-fold (rats), relative to those observed in humans at the recommended clinical dose. Rilpivirine was not genotoxic.

The reproductive and developmental toxicity studies did not demonstrate any effects on fertility, fecundity, parturition, or maternal behavior at systemic exposures approximately 40-fold higher than the exposure in humans at the recommended clinical dose. In offspring from rats and rabbits treated with Rilpivirine during pregnancy and lactation, there were no toxicologically significant effects on developmental endpoints., at exposures 15 and 70 times higher than the exposure in humans at the recommended clinical dose.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Mark Seaton that the nonclinical data support an approval action for this Rilpivrine.

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/s/

HANAN N GHANTOUS
04/11/2011

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	202-022
Supporting document/s:	000
Applicant's letter date:	7/23/2010
CDER stamp date:	7/23/2010
Product:	Tradename (Rilpivirine)
Indication:	HIV infection in treatment-naïve patients
Applicant:	Tibotec Inc.
Review Division:	Division of Antiviral Products
Reviewer:	Mark Seaton, Ph.D.
Supervisor/Team Leader:	Hanan Ghantous, Ph.D., DABT
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Project Manager:	Robert Kosko, Pharm. D., M.P.H.

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

It is recommended that Rilpivrine be approved.

1.1.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.1.3 Labeling

The nonclinical Pharmacology/Toxicology portion of the sponsor's drug product label is modified in italics below:

8.1 Pregnancy

Pregnancy Category B

No adequate and well-controlled or pharmacokinetic studies of TRADE NAME™ use in pregnant women have been conducted. Studies in animals have shown no evidence of relevant embryonic or fetal toxicity or an effect on reproductive function [see *Nonclinical Toxicology (13)*]. In offspring from rat and rabbit dams treated with Rilpivirine during pregnancy and lactation, there were no toxicologically significant effects on developmental endpoints. The exposures at the embryo-fetal No Observed Adverse Effects Levels (NOAELs) in rats and rabbits were respectively 15 and 70 times higher than the exposure in humans at the recommended dose of 25 mg once daily ~~[see *Nonclinical Toxicology (13)*]~~. TRADE NAME™ should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

8.3 Nursing mothers

The Centers for Disease Control and Prevention recommend that HIV-infected mothers not breastfeed their infants to avoid risking postnatal transmission of HIV. *Studies in lactating rats and their offspring indicate that Rilpivirine was present in rat milk.* It is not known whether Rilpivirine is secreted in human milk. Because of both the potential for HIV transmission and the potential for adverse reactions in nursing infants, **mothers should be instructed not to breastfeed if they are receiving TRADE NAME™**.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis and Mutagenesis

Rilpivirine was evaluated for carcinogenic potential by oral gavage administration to mice and rats up to 104 weeks. Daily doses of 20, 60 and 160 mg/kg/day were administered to mice and doses of 40, 200, 500 and 1500 mg/kg/day were administered to rats. (b) (4)

- *In rats, there were no drug related neoplasms. In mice, Rilpivirine was positive for hepatocellular neoplasms in both males and females. The observed hepatocellular findings in mice may be rodent-specific.* At the lowest tested doses in the carcinogenicity studies, the systemic exposures (based on AUC) to Rilpivirine were 21-fold (mice) and 3-fold (rats), relative to those observed in humans at the recommended dose (25 mg q.d.).

Rilpivirine *in the absence and presence of a metabolic activation system* has tested negative in the *in vitro* Ames reverse mutation assay, (b) (4) and the *in vitro* clastogenicity mouse lymphoma assay. (b) (4) Rilpivirine did not induce chromosomal damage in the *in vivo* micronucleus test in mice [~~*see Nonclinical Toxicology (13.2).*~~]

Impairment of Fertility

No human data on the effect of Rilpivirine on fertility are available. In a study conducted in rats, there were no effects on mating or fertility with Rilpivirine up to 400 mg/kg/day, a dose of Rilpivirine that showed maternal toxicity. This dose is associated with an exposure that is approximately 40 times higher than the exposure in humans at the recommended dose of 25 mg once daily.

(b) (4)

1.2 Brief Discussion of Nonclinical Findings

Rilpivirine (Rilpivirine HCl), a diarylpyrimidine derivative, is a next generation non-nucleoside reverse transcriptase inhibitor (NNRTI). Rilpivirine is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-naïve adult patients.

Bioavailability of Rilpivirine in dogs was 80% at a dose of 5 mg/kg in PEG400 + citric acid, in rabbits was 54% at 5 mg/kg, in rats was ~30-40% at 5 mg/kg decreasing to 21% at 400 mg/kg, and in monkey was 24% at 5 mg/kg. In limited tissue distribution studies with rats and dogs, significant concentrations of Rilpivirine-associated radioactivity were found in adrenal glands and liver. The oral bioavailability of Rilpivirine has not been determined in humans.

In *in vitro* metabolism studies, Rilpivirine was primarily conjugated with glutathione, glucuronic acid or sulfuric acid. *In vivo* studies showed that the major metabolites, including the *N*-glucuronide of Rilpivirine and two glutathione conjugates (the cysteinyl glycine conjugate and the cysteine conjugate) identified as human metabolites, were present in one or more nonclinical species. Sufficient levels of the major human metabolites were present in nonclinical animal models such that the toxicological profile of Rilpivirine and its metabolites could be adequately assessed in nonclinical studies.

No adverse effects of Rilpivirine on the cardiovascular, respiratory, or central nervous systems were noted during initial safety pharmacology studies. Subsequently, a Phase I clinical trial demonstrated a QT interval-prolonging effect of Rilpivirine at supratherapeutic doses. In follow-up nonclinical safety pharmacology studies, Rilpivirine at concentrations approximately 10-fold greater than the clinical exposures demonstrated the potential to inhibit some potassium channels involved in cardiac action potential repolarization. Given the clinical and nonclinical findings, adverse events that could be related to cardiac conduction abnormalities or to rate and rhythm disturbances were closely monitored in the Phase IIb and Phase III clinical trials. No clinically relevant QTc prolonging effect was observed with the recommended therapeutic dose of TMC278 25 mg q.d., although it should be noted that patients with known risk for QT interval prolongation or Torsade de Pointes were excluded from the Phase III trials.

The primary toxicity findings in nonclinical studies were adrenal effects, generally characterized by increased serum progesterone and decreased cortisol levels, observed in rats, dogs, Cynomolgus monkeys, and possibly mice. These effects are thought to be associated with an inhibition of steroidogenesis at the level of cytochrome P450 21-hydroxylase (CYP21) and 17-hydroxylase (CYP17; inhibition of the latter was observed in Cynomolgus monkeys only). In dogs, findings of premature activation and overstimulation of the ovaries may also be related to inhibition of steroidogenesis. Those effects on dog ovaries were noted at exposures 8 to 25 times higher than clinical exposures at the recommended dose of 25 mg q.d. Premature ovulation, as was noted in immature dogs treated for four weeks, was not seen in immature Cynomolgus monkeys treated for eight weeks, although the lack of an early puberty effect in the monkeys may be related to the young age of the monkeys and the fact that the monkeys were still pre-pubertal at the end of the study.

Endocrine monitoring, including gonadal, adrenal and thyroid parameters, was included in clinical trials in order to assess adrenal and thyroid function. Additional safety assessments relating to adrenal function included ACTH stimulation testing, with measurements of basal and stimulated cortisol, 17-hydroxy (OH) progesterone, and aldosterone (the latter only in Phase III trials). Basal DHEA sulphate, progesterone, androstenedione, testosterone and LH were measured in Phase IIb and III clinical trials. There were no apparent increases in the incidence of endocrine events in Phase IIb or Phase III clinical trials. On-going and planned trials in adolescents and pre-pubertal children should include endocrine safety monitoring, with monitoring of hormone levels and ovulation. Growth curves, pubertal status, breast development, menarche or evidence of either hyperandrogenism (hirsutism) or delayed adrenarche should be documented in these trials.

Renal effects were observed in mice and dogs. In mice, findings in the kidney were limited to minimal to moderate nephropathy that was noted in half of the

female mice treated with the high dose, 320 mg/kg/day, which corresponded to systemic exposures more than 200-fold the human exposures at the recommended clinical dose. Findings of kidney toxicity in the dog were only noted at exposures more than 25-fold the exposure in humans at the recommended clinical dose. In dogs, renal effects were limited to acute interstitial nephritis in two males and minimal to slight corticomedullary mineralization in all females sacrificed at the end of the study. In the Phase III clinical trials, mean increases in serum creatinine and mean decreases in $eGFR_{creat}$ were seen over time with Rilpivirine. Using creatinine as a marker for estimated glomerular filtration rate (eGFR), there was a mean decrease from baseline in $eGFR_{creat}$ in the Rilpivirine group, which was stable over time. However, when GFR was estimated with a second biomarker, cystatin C, no decrease in GFR was seen. Rather, there was an increase in $eGFR_{cyst}$, indicating that there was no Rilpivirine-induced nephrotoxicity. Membranous glomerulonephritis was noted as an adverse drug reaction in one subject during clinical trials.

Effects on the thyroid gland were noted in rats. Dose related increases in diffuse follicular hypertrophy in the thyroid glands in male and female rats at all doses following six months of Rilpivirine administration correlated with increased thyroid gland weights. The thyroid changes were likely due to a rodent-specific mechanism related to altered metabolism of thyroid-related hormones. An increased incidence of swollen-vacuolated cells in the *pars distalis* of the pituitary in male rats from all treated groups was likely also related to altered thyroid hormone metabolism.

A slight to moderate increase in coagulation parameters, specifically activated partial thromboplastin time (APTT) and prothrombin time, was seen in male rats dosed for six months. The increases in APTT and prothrombin times were still present in high dose males after recovery. In clinical trials, there were no clinically relevant effects on coagulation.

The mechanism of action leading to a decrease in red blood cell parameters in rats, dogs and mice has not been determined. There were no indications of bone marrow suppression. Changes in hematology parameters were noted in high dose group animals only and appear to be reversible. In clinical trials, no significant hemoglobin abnormalities were noted in Phase 3 trials with Rilpivirine 25 mg q.d.

A series of in *vitro* and *in vivo* genotoxicity tests have shown Rilpivirine to be free of genotoxic potential. Rilpivirine did not show a potential for phototoxicity, skin irritation, or allergic or delayed sensitization reactions. Rilpivirine was a moderate eye irritant in an *in vitro* test.

The carcinogenic potential of Rilpivirine has been assessed in 2-year carcinogenicity studies in rats and in mice. At the lowest tested doses in the

carcinogenicity studies, the systemic exposures (based on AUC) to Rilpivirine were 21-fold (mice) and 3-fold (rats), relative to those observed in humans at the recommended dose (25 mg q.d.). In rats, Rilpivirine was negative for statistically significant drug related neoplasms. In mice, the tumor types that showed a statistically significant increase were hepatocellular adenomas and hepatocellular adenomas-carcinomas (combined). The findings are considered to be treatment related; however, the increased incidence of liver tumors is thought to result from a rodent-specific mechanism related to induction of hepatic enzymes, such that the tumors may not be relevant to humans.

The reproductive and developmental toxicity studies did not demonstrate any effects on fertility or fecundity, on parturition, or maternal behavior at systemic exposures approximately 40-fold higher than the exposure in humans at the recommended dose of 25 mg once daily. In offspring from rat and rabbit dams treated with Rilpivirine during pregnancy and lactation, minimal effects on bone ossification and other developmental endpoints were not considered toxicologically significant. Exposures in rats and rabbits were approximately 15- and 70-fold higher, respectively, than exposures at the recommended clinical dose.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number (Optional)

700361-47-3

2.1.2 Generic Name

Rilpivirine

2.1.3 Code Name

TMC278 (HCl salt); R 314585 or R278474 (free base)

2.1.4 Chemical Name

(E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile hydrochloride

2.1.5 Molecular Formula/Molecular Weight

$C_{22}H_{18}N_6$.HCl/ m.w. 402.88

2.1.6 Structure

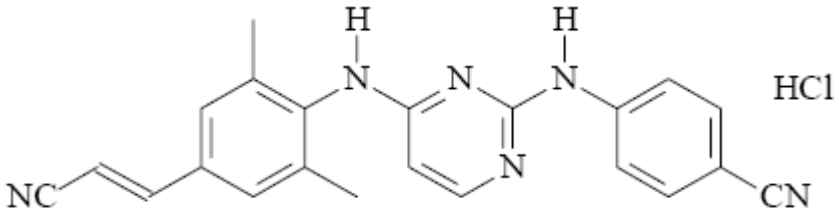

Figure 1: Structure of Rilpivirine

2.1.7 Pharmacologic class

Antiviral, non-nucleoside reverse transcript inhibitor

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 67,699

2.3 Clinical Formulation

2.3.1 Drug Formulation

The drug product (Rilpivirine (TMC278) (b) (4) is a white to off-white film-coated (b) (4) tablet containing 25 (b) (4) mg Rilpivirine as the free base.

Inactive Ingredients: Lactose monohydrate, NF; USP ;Polysorbate 20, USP ; Microcrystalline cellulose, NF ; Croscarmellose sodium, NF ; (b) (4) ; Magnesium stearate, NF ; (b) (4).

2.3.2 Comments on Novel Excipients

None

2.3.3 Comments on Impurities/Degradants of Concern

Three impurities, (b) (4) are present in drug substance at quantities that require qualification. (b) (4) have been qualified as spiked (b) (4) components of Rilpivirine test article in the Ames assay for mutagenicity, the mouse lymphoma assay for clastogenicity, and a one-month repeat dose study in rats. (b) (4) of TMC278 that was present in the test article during all pivotal nonclinical studies, and is therefore considered to be qualified.

(b) (4) is a synthesis intermediate and impurity of TMC278 HCL-salt. (b) (4) tested positive in a mutagenic assay and is considered to be clastogenic to human lymphocytes *in vitro*. (b) (4) tested negative in a dermal irritation study; however, an ocular irritancy test showed that (b) (4) produced eye irritation and is considered to have the potential to cause severe ocular irritancy *in vivo*. (b) (4) was a skin sensitizer following topical application of this drug to the dorsal surface of the ear (lymphocyte proliferation was observed). The levels of (b) (4) will be sufficiently controlled in the intermediate (free-base) drug substance, such that the impurity does not need to be specified in the final drug substance.

2.4 Proposed Clinical Population and Dosing Regimen

Rilpivirine is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-naïve adult patients.

The proposed dosing regimen is 25 mg (one 25 mg tablet) taken once daily with a meal.

2.5 Regulatory Background

IND 67,699 was opened in DAVP on September 29, 2004.

3 Studies Submitted

3.1 Studies Reviewed

Study Title	Study #
Safety Pharmacology	
Effects of R278474 on the membrane K+ current I_{Kr} in HERG transfected CHO cells compared to astemizole and terfenadine	TMC-278-CPF730
Effects of R278474 in the isolated, spontaneously beating right atrium of the guinea-pig.	TMC278-N168576
Effects of R278474 on cardio-hemodynamic and –electrophysiological parameters in combination with the determination of R278474 in plasma, heart and lung tissue in anaesthetized guinea-pigs: dose 0.16, 0.32, 0.64, 1.25, 2.5 and 5 mg/kg intravenously	TMC278-CPF643

Cardio-hemodynamic, cardio-electrophysiological and pulmonary effects of an intravenous infusion of 5 mg/kg in 1 hour of R278474 in anaesthetized dogs	TMC278-CPF648
Effects of R278474 on cardiovascular and behavior parameters in instrumented, awake dogs: single oral dose of 20 mg/kg	TMC278-CPF654
Electrophysiological evaluation of TMC278 (Rilpivirine: JNJ-16150108-AAA-23562791; TMC181560) in isolated, arterially-perfused rabbit ventricular wedge preparations	TMC278-NC341
Effects on cardiovascular and respiratory function in the telemetered dog.	TMC278-Exp5555
Single Dose Oral Safety Pharmacology Study in the Rat: The Modified Irwin's Test.	TMC278-Exp5560
Effects of Rilpivirine (JNJ-16150108-AAA-23562791, TMC278) on the membrane K+ current IKs in KvLQT1/minK-transfected CHO cells compared to HMR 1556	TMC278-NC342
HERG-Lite Assay: Effects of 10 Test Articles on Cloned hERG Channel Surface Expression in Mammalian Cells	TMC278-NC330
Effects of JNJ-16150108-AAA and JNJ-26892567-AAA on Cardiac Ion Channels Expressed in Mammalian Cells	TMC278-NC331
Effects of a daily oral dose of 10 mg/kg of TMC278 (JNJ-16150108-AAA) or 160 mg/kg p.o. of JNJ-26892567-AAA (efavirenz) during 16 days on ECG parameters, heart rate and body temperature in freely-moving telemetered guinea-pigs.	TMC278-CPF327
Pharmacology Data Report: Tibotec BVBA (Gastric acidity in rats)	TMC278-NC204
ADME/Pharmacokinetics	
Tissue distribution and placental transfer of 14C-TMC278, as studied by whole body autoradiography, in the pregnant Sprague-Dawley rat after single oral administration at 40 mg/kg	TMC278-NC109
A study of the effects of TMC278 hydrochloride on some hepatic enzyme activities after oral administration for three months at doses of 0, 20, 80, and 320 mg/kg/day to male and female Swiss albino CD1 mice.	TMC278-NC192
A study of the effects of TMC278 hydrochloride on some hepatic enzyme activities after oral administration for three months at doses of 0, 40, 120, and 400 mg/kg/day to male and female SD rats.	TMC278-NC193
A study on the pharmacokinetics and relative bioavailability of TMC278 in male beagle dogs after single oral administration of 3 different particle sizes of Drug Substance of TMC278.HCl (R314585) at 5 mg eq./kg	TMC278-NC257

General Toxicology	
Single Dose Oral Toxicity Study in the Rat	TMC278-Exp5559
Single dose escalation oral toxicity study followed by a 5-day repeated dose oral toxicity study in the beagle dog (tolerance study)	TMC278-EXP5461
2 week repeated dose oral toxicity study in the rat	TOX5535
2 week repeated dose oral toxicity study in the rat	TOX6813
4 week oral (gavage) immunotoxicity study in the rat	TOX5692
6 month repeat dose oral toxicity study with 1 month recovery in the rat	TOX6142
1 month repeated dose oral toxicity study in the beagle with 1 month recovery	TOX5650
6 month repeated dose oral toxicity study with a 3 month interim kill in the Beagle dog	TOX6110
52 week oral gavage toxicity study in the beagle dog	TOX6314
3 month repeated dose oral toxicity study in the Swiss mouse	TOX6739
4-week toxicity study by oral (gavage) in CB6F1-nonTgrasH2 mice	TMC278-NC121
5-Day Repeated Dose Oral Toxicity Study in the Female Rabbit.	TMC278-NC126
Carcinogenicity	
Carcinogenicity Study by Oral Gavage Administration to CD-1 Mice for 104 Weeks	TMC278-NC120
Carcinogenicity Study by Oral Gavage Administration to CD Rats for 104 Weeks	TMC278-NC123

Reproductive and Developmental Toxicology	
Oral developmental toxicity study in the rat	TOX6268
Oral developmental toxicity study in the rabbit	TOX6313
Oral (Gavage) Pre- and Postnatal Fertility Study in the Rat	TMC278-NC131
Oral (gavage) fertility study in the female rat	TOX6708
Oral (gavage) fertility study in the male rat	TOX6861
Genetic Toxicology	
In vitro bacterial reverse mutation test with Salmonella typhimurium	5693 and 5540
In vitro mammalian forward mutation test with L5178Y mouse lymphoma cells	5539
In vivo micronucleus test on bone marrow cells of mice	5538
In Vitro Bacterial Reverse Mutation Test of TMC278 in Salmonella typhimurium with human liver S9-mix	TMC278-NC279
(b) (4): Screening Chromosome Aberration Test in Human Lymphocytes *In Vitro*	TMC278-NC 184
Local Tolerance	
In Vitro Bovine Corneal Opacity-Permeability Eye Irritation Test	TMC278-NC202
In Vitro Bacterial Reverse Mutation Test with *Salmonella Typhimurium* (b) (4) (an intermediate)	TMC278-NC165
Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application)	TMC278 NC159
Acute Oral Toxicity in the Mouse or (b) (4)	TMC278 NC180

Rabbit Enucleated Eye Test on (b) (4)	TMC278-NC 181
Acute Dermal Irritation in the Rabbit on (b) (4)	TMC278-NC 182
Local Lymph Node Assay in the Mouse on (b) (4)	TMC278-NC 183
Nanosuspensions: Local lymph node assay (LLNA) (Topical application).	TMC278-NC199
Special Toxicology Studies	
1-month Repeated Dose Oral Toxicity Study in the Rat.	TMC278-NC117
1-Month Repeated Dose Oral Toxicity Study in the Beagle Dog.	TMC278-NC116
Endocrinological oral (gavage) 8-week study in the female sexually immature cynomolgus monkey	TMC278-NC248
In-Vitro Guinea-Pig Adrenal Cell Test for the evaluation of the cortisol biosynthesis.	Exp5653
A study on the effect of R278474 on the biosynthesis of cortisol in crude subcellular fractions of dog adrenal cortex.	FK4790
1-Month Repeated Dose Oral Impurity Qualification Study for TMC278.HCl in the Rat.	TMC278-NC314

3.2 Studies Not Reviewed

Study Title	Study #
2-Week Repeated Dose Oral Toxicity Study in the Swiss Mouse	TMC278-NC118
Five-day repeated dose oral toxicity study in the rat (investigative tolerance study)	TMC278-EXP5463
7-Day Repeated Dose Oral Toxicity Study in the Beagle Dog	TMC278-EXP5534
In Vitro Bacterial Reverse Mutation Test with R314585 (TMC278.HCl) in *Salmonella typhimurium*	TMC278-NC335

Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y Cells (MLA) using Microtitre® fluctuation technique	TMC278-NC336

3.3 Previous Reviews Referenced

Nonclinical studies, including safety pharmacology, ADME, repeat-dose toxicology, genetic toxicology, and reproductive toxicology studies to support the NDA have been reviewed previously. The reviews are attached to this document as appendices 1A – G and are summarized in the appropriate sections of this review.

4 Pharmacology

Rilpivirine (Rilpivirine HCl, TMC278), a diarylpyrimidine derivative, is a next generation non-nucleoside reverse transcriptase inhibitor (NNRTI). Rilpivirine is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-naïve adult patients.

4.1 Primary Pharmacology

Please see the Clinical Microbiology review by Lisa Naeger, Ph.D. for a complete review of the pharmacology of Rilpivirine.

4.2 Secondary Pharmacology

Rilpivirine did not cause *in vitro* inhibition of α- or β-adrenergic, dopaminergic, muscarinergic, serotonergic, opioid, interleukin, or chemokine receptors (up to 10 µM, 3.7 µg/mL), or human DNA polymerase α, β, or γ (up to 1000 µM, 366 µg/mL).

4.3 Safety Pharmacology

The safety pharmacology studies were previously reviewed (Appendix 1A). Findings are summarized below.

Test	Dose (mg/kg)	Results
Functional Observational Battery in Rats	40, 120 and 400 mg/kg	Motor affective and sensory-motor effects at high dose Slight autonomic impairment at high dose

Test	Dose (mg/kg)	Results
hERG Tail Current in CHO Cells	100 nM (37 ng/ml) 300 and 3000 nM	↓10, 33, and 80% tail current at studied concentrations
I_{ks} Current in CHO Cells	0.3 (0.11 µg/mL) – 10 µM	7.4 – 76.7% decrease; IC_{50} = 3.1 µM
CV: Isolated right guinea pig atrium		Not remarkable up to 1000 nM
CV Effects in Anesthetized Guinea Pigs	0.16 to 5 mg/kg	Not remarkable
CV and Respiratory Effects in Conscious, Telemetered Dogs	0, 20, 80 and 160 mg/kg	Not remarkable
CV and Respiratory Effects in Conscious, Telemetered Guinea Pigs	10 mg/kg for 16 days	No remarkable effects on heart rate, ECG parameters or temperature
Effect on Gastric Acid Release	10 mg/kg i.p.	Not remarkable

Neurological effects: Five male Sprague-Dawley rats per group were administered 40, 120 or 400 mg/kg TMC278 orally prior to being assessed using the modified Irwin test. No effects were seen at doses up to 120 mg/kg. At 400 mg/kg, motor affective (marked reaction in the finger withdrawal test) and sensory-motor (negative toe pinch test) responses were slightly affected (one rat affected per endpoint). There was a slight reduction in pupil size indicative of autonomic impairment at this dose. Most of the effects were seen 8 hrs post-dose and no effects were retained 24 hrs after dosing.

Cardiovascular effects: A concentration dependent reduction in the membrane K current was seen in CHO cells transfected with hERG. No significant effect was measured at 100 nM (37 ng/ml) but significant inhibition was measured at 300 and 3000 nM. Similarly, Rilpivirine reduced membrane current in an *in vitro* system that mimics the slowly activating delayed-rectifier K current in cardiac myocytes (IC_{50} = 3.1 µM). TMC278 had no effect in the isolated spontaneously beating right atrium of the guinea pig until drug concentrations reached 1000 nM. In isolated, arterially-perfused rabbit ventricular wedge preparations, Rilpivirine prolonged the QT interval at 1 uM (+6%) and 10 uM (+9%) compared to solvent. There were no effects on other measured parameters, including QRS duration and rate dependency, Tp-Te, rTP-Te, TdP score and contractile force, and no early after-depolarizations, TdP, ventricular tachycardia, ventricular fibrillation or in-excitability. When tested in anesthetized guinea pigs, TMC278 at intravenous bolus doses of 0.16 to 5 mg/kg had no significant effect on heart rate, mean arterial pressure and PQ and QRS intervals. QT interval was increased at the

high dose but the effect disappeared when corrected for heart rate using Bazett's formula (QT_cBazett). At 5 mg/kg, the median plasma level of TMC278 five minutes after injection was 9.15 µg/ml.

In conscious chronically instrumented dogs and guinea pigs, TMC278 given as a single oral dose of 20 mg/kg or 10 mg/kg, respectively, had no effect on heart rate, blood pressure, or other parameters of cardiac function. Likewise, no effects were noted on ECG parameters including QT and QT_c. In dogs, peak plasma levels were reached four hours after drug administration and amounted to 1.6 µg /ml and the average AUC_{0-4h} was 3.93 µg.hr/mL.

In male conscious telemetered dogs treated by gavage with 0, 20, 80 and 160 mg/kg of TMC278 in PEG400, cardiac hemodynamics and electrophysiological recordings were made at regular time intervals until 12 hrs after dosing. There were no significant effects on any measured parameters.

Pulmonary effects: In male conscious telemetered dogs treated by gavage with 0, 20, 80 and 160 mg/kg of TMC278 in PEG400, pulmonary parameters including respiratory rate, tidal volume, and minute volume were unchanged with drug treatment.

Renal effects: Renal effects were not assessed in a Safety Pharmacology study. Renal histopathology was subsequently noted in repeat-dose studies in mice and dogs (see section 6.2 Repeat-dose Toxicity).

Gastrointestinal effects: Although not completed as part of the safety pharmacology battery *per se*, the ability of Rilpivirine (10 mg/kg i.p.) to inhibit pentagastrin-stimulated gastric acidity was assessed in fasted rats. No inhibition of gastric acidity was observed.

Abuse liability: Not examined

5 ADME/Pharmacokinetics/Toxicokinetics

5.1 ADME

The ADME/toxicokinetic studies were previously reviewed (Appendices 1A and 1F). Findings are summarized below.

Absorption

Oral absorption was assessed in several nonclinical species. Bioavailability of Rilpivirine in animal species can be ranked as dogs>rabbits>rats>monkeys. Absorption in dogs was 80% at 5 mg/kg in PEG400 + citric acid, in rabbit was

54% at 5 mg/kg, in rats was ~30-40% decreasing to 21% at 400 mg/kg, and in monkey was 24% at 5 mg/kg.

Distribution

Rilpivirine was extensively distributed throughout the body in rats, dogs and monkeys. In limited tissue distribution studies in rats and dogs, high concentrations were found in adrenal glands and liver (see table below, *excerpted from sponsor*).

Table 1: Tissue to Blood AUC0-4h Ratios of Total Radioactivity in Rats After a Single Oral Administration of ^{14}C-TMC278 base at 40 mg/kg.

Tissues/Organs	AUC_{0-4h} (µg.h/g)	Tissue/Blood AUC_{0-4h} ratio
Adrenal gland	18.5 (140 [a])	4.95 (6.76 [b])
Blood (LSC)	3.43 [c]	0.92
Blood (RLG)	3.74	1.00
Bone	NC [d]	-
Bone marrow	5.20	1.39
Brain	2.48	0.66
Brown fat	14.9	3.98
Eye ball (LSC)	9.55 (616 [e])	2.55 (20.5 [f])
Heart	6.98	1.87
Kidney	13.5 (58.9 [g])	3.61 (4.21 [h])
Liver	43.9 (265 [a])	11.7 (12.8 [b])
Lung	7.38	1.97
Additional Information		

[a] AUC_{0-96h}; [b] calculated with AUC_{0-96h}; [c] AUC_{0-24h} = 14.0 µg.h/g, AUC_{0-96h} = 20.7 µg.h/g and AUC_{0-336h} = 30.1 µg.h/g; [d] NC: not calculated, too limited data; [e] AUC_{0-336h}; [f] calculated with AUC_{0-336h}; [g] AUC_{0-24h}; [h] calculated with AUC_{0-24h}

-: not applicable; CA: citric acid; LSC: liquid scintillation counting; PEG400: polyethylene glycol 400; RLG: radioluminography

The tissue distribution of Rilpivirine-related radioactivity in pregnant Sprague-Dawley rats was highest in liver (approximately six times the levels measured in blood, as expressed by AUC_{0-8h}) followed by adrenal gland, lachrymal gland, kidney, brown fat (AUC_{0-8h} tissue/blood ratios=2-4), and lung, pancreas, salivary gland, heart, spleen (ratios=1.5-2).

After a single dose of radiolabeled Rilpivirine in dogs, there were no indications of tissue accumulation. The tissue to plasma ratio of radioactivity was high in liver and adrenal gland, indicating the affinity of radiolabeled Rilpivirine for these organs.

Metabolism

In vitro studies were conducted in mouse, rabbit, rat, dog and human hepatocytes. Rilpivirine was primarily conjugated with glutathione, glucuronic acid or sulfuric acid. Most of the metabolites found in human hepatocytes were also found in rodents; however, the *in vitro* metabolite profiles of dog and rabbit differed from those of human and rodent.

In vivo in rats, Rilpivirine is an inducer of hepatic microsomal CYP4A, CYP3A, CYP2B (not CYP2E) and microsomal UDP glucuronosyltransferase in males primarily. Conversely, in dogs Rilpivirine administered by gavage for six months had little effect on the parameters of hepatic xenobiotic metabolism. No evidence of induction on CYP1A, CYP2B, CYP2E, CYP4A forms, UDP glucuronosyltransferase activity and cytosolic GST activities were reported.

Table 2: Metabolism of Rilpivirine (% of dose) in Human and in Mice, Rats and Dogs after Oral Administration (*table excerpted from sponsor*)

Metabolites	Mice		Rats	Dogs	Human
	20 mg/kg	320 mg/kg	40 mg/kg	5 mg/kg	150 mg
5-Hydroxyl TMC278 at the pyrimidinyl moiety (M42)	18 – 26 [a]	9.2 – 13 [a]	2.8-3.6 [b]	5.3	16
Hydroxymethyl of TMC278 (M33)	0.5 – 0.7	1.3 – 1.0	0.54-0.54	8.7 (traces in plasma)	3.0 (seen in plasma)
Carboxylic acid metabolite of the cyanoethenyl moiety (M30)	1.6 – 3.1	1.5 – 1.2	0.47 - 0.05	3.1 [c]	2.7
Unknown (M35)	< 0.2	< 0.2	-	-	2.2
Tricyclic metabolite (M27) and carboxylic metabolite of M27 (M11)	0.3 - <0.2 [d]	<0.2 – 0.1 [d]	0.99–1.60 [f]	3.1 [g] (traces of M27 in plasma)	2.2 (M27 seen in plasma)
Glutathione derived conjugates (M13, M14, and M18)	9.6 – 7.9 [e]	8.7 – 7.3 [e]	0.03 - 0.46 [i]	< 0.08 [h]	1.2
N-glucuronide of TMC278 (M15)	-	-	-	traces in plasma	0.6 (seen in plasma)
Unchanged compound	8.8 – 7.9	33 – 34	47 - 43	45	26

[a] co-eluted with M41, M42 was estimated at 13.9-16.6% (20 mg/kg) and at 5.9-8.0% (320 mg/kg); [b] co-eluted with M41; [c] co-eluted with M48; [d] co-eluted with M28 and M29; [e] includes M17; [f] co-eluted with M24, M28 and M29; [g] including M23; [h] each of them; [i] M14 co-eluted with M12; In mice and rats, the first number in each box is male data and the second one female data

Excretion

In mice, rats and dogs, the predominant route of excretion of ^{14}C-Rilpivirine was via the feces. The majority of the total radioactivity was eliminated in feces as unchanged Rilpivirine in mice (33-34% at 320 mg/kg), in rats (43-47%) and in dogs (43%) at 48 hours after dosing. Only, in mice at 20 mg/kg, one metabolite M42 was the most abundant in feces. No radioactivity was detected in expired air. In human trials, approximately 85% of the administered dose was excreted in feces, and unchanged Rilpivirine represented about 26% of the administered dose in feces.

5.2 Pharmacokinetics/Toxicokinetics

Serum protein binding of Rilpivirine was above 99% for all species tested (including humans) over a wide range of concentrations. Additional toxicokinetic

parameters for Rilpivirine in nonclinical species and humans are provided in the tables below (*excerpted from sponsor*).

Table 3: Toxicokinetic Parameters from Repeat-dose Toxicology Studies with Rilpivirine.

Species	TMC278 formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	t_{max} (h)	AUC[a] (µg.h/mL)	$t_{1/2}$ (h)
Mouse	TMC278 in HPMC (0.5% w/v)	Day 1	M/15	20	9.9	2	60	2.0
				60	23	2	239	1.5
				160	41	6	440	1.7
			F/15	20	13	1	61	2.1
				60	24	2	182	1.7
				160	38	2	345	1.5
		Week 28	M/9	20	9.8	1	76	2.2
				60	22	1	230	2.8
				160	36	2	505	4.6
			F/9	20	9.9	1	51	2.7
				60	29	1	278	2.5
				160	58	1	766	3.7
Rat	TMC278 base in PEG400/CA (10%)	Day 1	M/6	40	2.9	1	19	2.3
				120	6.4	8	53	2.2
				400	9.1	8	92	3.5
			F/6	40	6.5	1	32	4.3
				120	8.5	0.5	83	2.6
				400	17	8	160	2.5
		Day 175[b]	M/3	40	1.7	2	12	3.9
				120	3.0	10	35	3.9
				400	6.2	10	73	4.4
			F/5	40	6.6	1	50	4.3
			F/5	120	8.8	0.5	116	5.6
			F/6	400	16	10	244	7.4

Table 3 (cont.): Toxicokinetic Parameters from Repeat-dose Toxicology Studies with Rilpivirine.

Species	TMC278 formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	t_{max} (h)	AUC[a] (µg.h/mL)	$t_{1/2}$ (h)
Rat	TMC278 in HPMC (0.5% w/v)	Day 1	M/9	40	1.6	2	19	6.6
				200	2.6	2	34	4.5
				500	4.5	4	45	4.5
				1500	6.1	8	58	7.8
			F/9	40	2.2	2	17	5.0
				200	4.2	4	40[c]	NC
				500	6.5	2	63	4.1
				1500	7.0	2	81	5.1
		Week 39	M/9	40	0.82	2	6.3	NC
				200	1.3	2	8.2	11
				500	1.8	0.5	14	8.0
				1500	2.2	2	18	NC
			F/9	40	2.1	0.5	14	NC
				200	4.7	2	41	7.6
			F/8	500	8.5	0.5	46	11
			F/9	1500	9.4	0.5	84	3.0
Pregnant rat	TMC278 base in PEG400/CA (10%)	Day 1 (GD 6)	F/6	40	4.9	1	33	3.3
				120	6.0	0.5	65	4.2
				400	14	8	182	5.5
		Day 11 (GD 16)	F/4	40	5.6	2	37	3.8
				120	7.2	1	63	4.0
			F/6	400	13	8	152	5.2
Juvenile rat (aged 25days)	TMC278 in HPMC (0.5% w/v)	Day 14	M/8	40	2.6	1	12	2.7
			M/7	120	3.7	4	34	4.4
			M/7	400	9.1	4	50	2.8
			F/8	40	5.8	1	18	2.3
			F/8	120	3.6	4	28	2.6
			F/7	400	7.3	4	53	2.0

Table 3 (cont.): Toxicokinetic Parameters from Repeat-dose Toxicology Studies with Rilpivirine.

Species	TMC278 formulation	Sampling Period	Sex/n	Dose (mg/kg/day)	C_{max} (µg/mL)	t_{max} (h)	AUC[a] (µg.h/mL)	$t_{1/2}$ (h)
Pregnant rabbit	TMC278 base in HPMC (0.5% w/v)	Day 1 (GD6)	F/3	5	6.4	11	95[b]	8.2
				10	9.7	11	162[b]	12
				20	13	9.3	219[b]	11
		Day 14 (GD19)	F/3	5	6.7	11	105	7.6
				10	10	8	170	8.4
				20	15	11	232	8.6
Dog	TMC278 base in PEG400/CA (10%)	Day 1	M/4	5	0.70	2	11[b]	ND
				10	0.90	1.5	15[b]	ND
				40	2.4	14	37[b]	ND
			F/4	5	0.75	2	9.7[b]	ND
			F/5	10	1.2	2.8	15[b]	ND
			F/4	40	2.5	8	41[b]	ND
		Day 363	M/4	5	1.1	9	17	ND
			M/2	10	1.3	4	24	ND
			M/4	40	4.1	3.7	65	ND
			F/4	5	1.5	1.5	19	ND
				10	2.2	7	36	ND
			F/3	40	5.5	2.7	61	ND
Monkey	TMC278 in HPMC (1%)/Tween 20	Day 55	F/8	100 b.i.d	0.14[c]	2.5[c]	2.7	7.8
			F/7	250 b.i.d	0.31[c]	4.6[c]	4.6	7.5

[a] : $AUC_{0-\infty}$ after single dosing and AUC_{0-24h} after repeated dosing, [b] : AUC_{0-24h}, [c] : after the first dosing, ND: not determined; CA : citric acid ; HPMC: hydroxypropyl-methylcellulose

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Table 4: Steady-state Pharmacokinetics of TMC278 After Administration of TMC278 25 mg q.d. in Healthy Subjects (Trial C130 and C152) and in HIV-1 Infected Subjects (Trials C209 and C215, Pharmacokinetic Substudies)

	Mean ± SD; t_{max}: Median (Range)			
	Healthy		HIV-1 Infected	
Parameter	C130	C152	C209	C215
Day 11 (Healthy) or any time point between Week 4 and 8 (HIV-1 Infected)				
N	16	57	12	32
t_{max}, h	5.0 (5.0 - 12.0)	5.0 (4.0 - 24.0)	4.01 (2.00 - 12.00)	4.00 (1.00 - 12.00)
C_{min}, ng/mL	66.48 ± 16.29	95.23 ± 29.07	61.79 ± 28.69	50.58 ± 27.94
C_{max}, ng/mL	145.5 ± 31.97	246.8 ± 74.36	138.6 ± 66.73	132.5 ± 74.79
AUC_{24h}, ng.h/mL	2235 ± 460.4	3324 ± 884.0	2133 ± 1016	1958 ± 964.5

N = maximum number of subjects with data.
Source: Module 2.7.2/Summary of Clinical Pharmacology Studies/Table 97.

6 General Toxicology

All studies in rats and dogs were conducted with Rilpivirine base dissolved in PEG400, usually with citric acid. Following the selection of Rilpivirine HCl salt as the chemical form to be marketed, one-month studies in rats and dogs compared the kinetics and toxicity of Rilpivirine base and Rilpivirine HCl. There were no toxicologically significant differences between the two drug forms. Summary tables from those bridging studies are included in section 6.2 Repeat-dose Toxicity, below. The repeat-dose studies in mice and Cynomolgus monkeys were conducted with Rilpivirine HCl suspended in aqueous hydroxypropyl methylcellulose (HPMC).

6.1 Single-dose Toxicity

Single dose studies were conducted in rats and dogs. In rats, there was no mortality following administration of 800 mg/kg Rilpivirine by oral gavage. Salivation was noted in all male rats and 2/6 female rats. $AUC_{0-\infty}$ values were 86 µg.h/ml in males and 233 µg.h/ml in females. In dogs, there were no effects noted following a single 40 mg/kg oral dose of Rilpivirine. Salivation, vomiting and softened feces were occasionally seen following a single 80 mg/kg dose.

6.2 Repeat-dose Toxicity

Systemic toxicity of Rilpivirine after repeat dosing was studied in mice, rats, rabbits, dogs and cynomolgus monkeys. Pivotal repeat dose studies were conducted in mice (3 months), rats (1 and 6 months), dogs (1, 3, 6, and 12 months), and Cynomolgus monkeys (8 weeks). The mouse study served as a dose range finding study for the carcinogenicity study in that species. A five day study in female rabbits served as a dose range finding study for an embryofetal development study in rabbits. The reversibility of toxic effects was investigated in rats and dogs.

Rats

In rats, the primary toxicological effects in one month and six month studies were increased thyroid hypertrophy, which was not entirely reversible. In the one month study, rats received 10, 40 or 160 mg/kg Rilpivirine by oral gavage. There were no early deaths, no significant clinical signs, no effects on body weight or food consumption, no ophthalmoscopic changes, and no changes in hematology or urinalysis parameters related to Rilpivirine treatment. Blood glucose levels were decreased in high dose females only. Macroscopically, increased liver weights and increased thyroid weights were noted in middle dose and high dose males and females. Microscopically, minimal follicular hypertrophy of the thyroid

was detected in middle dose and high dose males, and high dose females. There were no treatment related effects on the direct plaque forming cell (PFC; immunotoxicity) assay. Toxicokinetic parameters were evaluated at the end of the four week dosing period. Day 28 systemic exposures (AUC_{0-24}) were 7.2, 27.4 and 50.5 µg.h/mL and 14.0, 41.6 and 88.9 µg.h/mL for low, middle and high dose males and females, respectively. Based on histopathological findings in the thyroid at middle and high doses, the NOAEL was defined as the low dose (10 mg/kg), corresponding to systemic exposures of 7.2 and 14.0 µg.h/mL in males and females, respectively.

In the six month rat study, Rilpivirine (40, 120 or 400 mg/kg) formulated in PEG400 was administered by oral gavage. Early deaths (7/30, 6/20, 2/20, and 9/30 in control, low, middle and high dose group males, respectively and 4/30, 2/20, 1/20, and 2/30 in control, low, middle and high dose group females, respectively) were due to gavage errors related to the increased viscosity of the dosing formulation. From day 84 to the end of the dosing period, the test article was administered twice per day (b.i.d.) with 1.5 hours between doses. Clinical signs in moribund animals included rough haircoat, piloerection, wet urogenital region, crusty nose, labored breathing (dyspnea), nasal discharge, lameness, a small amount of feces and/or red tears (chromodacryorrhea). In surviving high dose group animals, there was an increase in salivation and wet urogenital region in males and females. All animals were normal at recovery. There were no test article related effects on body weights, food consumption or Ophthalmoscopic examinations. Clinical pathology assessments revealed changes in hematology and clinical chemistry parameters, all of which were reversible.

A slight to moderate increase in coagulation parameters, specifically activated partial thromboplastin time (APTT) and prothrombin time, was seen in all dosed males. Slight decreases in red blood cells (RBCs) and RBC parameters, including hemoglobin, hematocrit and eosinophils, were noted in high dose (400 mg/kg) males. In females, there was a slight decrease in eosinophils at all doses and a marginal decrease in mean cell volume and mean cell hemoglobin at the middle and high doses. The increases in APTT and prothrombin times were still present in high dose males after recovery. Hematology parameters had returned to normal.

At the middle dose (120 mg/kg), there were slight dose related increases in inorganic phosphate, albumin, urea nitrogen and alkaline phosphatase in males. In females, slight increases in albumin were detected. Additional clinical chemistry changes related to Rilpivirine administration at the high dose included slight decreases in triglycerides and calcium in males, and slight increases in total protein and albumin in females. All parameters were normal after recovery.

Macroscopically, findings were limited to swollen thyroid glands and more pronounced lobulation of the liver in middle dose and high dose animals, plus

swollen livers in high dose animals. Those changes correlated with increased thyroid weight at all dose levels and increases in liver weight at the middle and high dose. At recovery, increased thyroid weights remained, whereas liver weights were normal.

Microscopically, dose related increases in diffuse follicular hypertrophy in the thyroid glands in males and females at all doses correlated with increased thyroid gland weights. There was a dose related increase in hepatocellular hypertrophy in middle dose and high dose males and females, as well as increase in swollen-vacuolated cells in the *pars distalis* of the pituitary in males from all treated groups. In some high dose males, macrophages in the mesenteric lymph nodes had a swollen, vacuolated appearance. Only minimal changes in the thyroid were still present at recovery.

Calculated systemic exposures (AUC_{0-24}) are provided in the table below.

Table 5: AUC0-24 (µg.h/ml) of Rilpivirine during 6 Month Rat Toxicity Study

dose	40 mg/kg		120 mg/kg		400 mg/kg	
	AUC $_{0-24}$ (ug.h/ml)					
day	M	F	M	F	M	F
0	19.3	31.4	52.9	82.2	90.2	159.0
83	18.6	41.4	40.6	100.7	56.5	184.0
174	19.3	32.1	53.0	82.6	92.2	159.7

Due to effects on coagulation parameters and the thyroid and pituitary glands at the low dose (40 mg/kg), no NOAEL was defined in this study.

As noted above, repeat dose studies in rats were conducted with Rilpivirine base dissolved in PEG400, usually with citric acid. Following the selection of Rilpivirine HCl salt as the chemical form to be marketed, one-month studies in rats were conducted to compare the toxicity and toxicokinetics of Rilpivirine base and Rilpivirine HCl. The table below summarizes the one-month bridging study in rats. Briefly, male and female rats were administered test article, 10 or 400 mg/kg/day, by oral gavage. Rilpivirine base was administered in PEG400 with citric acid, whereas Rilpivirine HCl was formulated in 0.5% w/v HPMC. There were no significant differences in the toxicological effects of the two test articles. Assessment of toxicokinetic parameters indicated that at the high dose (400 mg/kg), systemic exposures were greater in animals dosed with Rilpivirine base (AUC = 90 – 149 µg.hr/mL) than in Rilpivirine HCl-dosed animals (33 – 86 µg.hr/mL).

Table 6: Summary of Rilpivirine Base and Rilpivirine HCl Bridging Study in Rats

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/Formulation)	Duration	Batch no.	Doses * (mg/kg)	NOAEL (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Rat/Sprague Dawley Main groups: 10M/10F Satellite groups:6M/6F	Oral/gavage (PEG400 + CA)	1-month	ZR27847 4PFA021	0 (vehicle), 10, 400 mg/kg/day TMC278 base	/	**10 mg/kg**: Thyroid: hypertrophy. C_{max} = 0.74-1.4 µg/mL (M-F); AUC_{0-24h} = 5.4-8.6 µg.h/mL (M-F) **400 mg/kg**: ↓ Hct (3%); potassium (13%); chloride (F: 2%); cholesterol F: (22%); urea nitrogen (F: 15%); ↑ albumin (M: 7%); protein (M: 5%); ↑ TSH (M: 51%, F: 15%); T_3 (M: 57%); ↓ T_4.(M: 61%, F: 52%). ↑ liver weight (M: 24%, F: 15%); ↑ thyroid weight (F: 60%). Liver: hypertrophy, Thyroid: hypertrophy. C_{max} = 7.7-13 µg/mL (M-F); AUC_{0-24h} = 90-149 µg.h/mL (M-F)	TMC278-NC117[b]/ 4.2.3.2.
	Oral/gavage (0.5% w/v HPMC)		ZR31458 5PFA011	0 (vehicle), 10, 400 mg/kg/day TMC278.HCl	/	**10 mg/kg**: Thyroid: hypertrophy C_{max} = 0.76-1.7 µg/mL (M-F); AUC_{0-24h} = 4.5-7.8 µg.h/mL (M-F) **400 mg/kg**: ↑ RBC (F: 4%); albumin (M: 5%); ↓ cholesterol (F: 10%); triglycerides (F: 35%); urea nitrogen (M: 18%, F: 11%); ↑ TSH (M: 49%, F: 38%), T_3 (M: 56%); ↓ T_4 (M: 37%, F: 44%); ↑ thyroid weight (F: 29%). Liver: hypertrophy, Thyroid: hypertrophy C_{max} = 4.8-9.0 µg/mL (M-F); AUC_{0-24h} = 33-86 µg.h/mL (M-F)	

*: dose of HCl salt is presented as base equivalents; [b]: GLP compliant study; AUC_{0-24h} : area under the concentration vs time curve till 24 h after dosing; CA: citric acid; C_{max}: maximum concentration; F: female; Hct: hematocrit; HPMC: hydroxypropyl methyl cellulose; M: male; PEG400: polyethylene glycol 400; RBC: red blood cells; T_3: triiodothyronine; T_4: tetraiodothyronine, thyroxine; TSH: thyroid stimulating hormone.

Dogs

There were no early deaths among dogs administered Rilpivirine for one month (5, 10, or 40 mg/kg/day). Clinical signs were limited to red vaginal discharge in middle and high dose dogs, seen during dosing but not at the end of the one month recovery period. High dose males had reduced food intake with associated weight loss. Changes to hematology (decreased RBCs, hematocrit and hemoglobin and increased WBCs in high dose animals) and clinical chemistry parameters (decreased albumin, total protein and triglycerides concentrations at middle and high doses, and increased concentrations of cholesterol and total bilirubin and activities of ALP and ALT at high dose) were normal at the end of the one month recovery period.

Progesterone concentrations tended to increased in a dose-dependent manner in all dose groups. The levels of ACTH were increased at the end of the dosing period, while cortisol levels tended to be decreased at 10 and 40 mg/kg/day.

Following one month of repeated dosing in dogs, target organs for the toxic effects of Rilpivirine included liver, adrenals, and the female genital tract. Ovary weights were increased across all doses in a dose-related manner. Histopathological findings in the liver included centrilobular perivascular inflammation with single cell necrosis, reticular endothelial system (RES) aggregates (i.e., monocytes and/or macrophages), and minimal bile duct

proliferation. At the end of recovery there was slight multifocal centrilobular perivascular inflammatory reaction with increased centrilobular clear appearance and increased RES aggregates. In the adrenals, dense, swollen *zona fasciculata* cells with reduced vacuolation and some leukocytic infiltration were noted. Changes to the female genital tract and mammary glands were seen in middle and high dose animals. Effects were described as cycle activation: Corpora lutea were noted, as were more prominent tertiary follicles. Changes in the adrenal gland and female genital tract were not present at the end of the recovery period.

As stated by the sponsor, Rilpivirine administration was associated with impaired adrenal glucocorticoid synthesis, as evidenced by histological changes in the adrenal fasciculata, a decrease in cortisol and an increase in ACTH levels. The increase in progesterone suggests a 21α-hydroxylase inhibition. Although there were histological changes in the adrenal reticularis as well, this was not reflected in any obvious mineralocorticoid effect. There was no evidence of electrolyte abnormalities or vascular collapse in the animals. There was evidence of stimulation in the mammary and genital tract tissues of the female dogs, but this was not reflected in serum estradiol levels.

As the sponsor suggests, Rilpivirine appears to be a partial inhibitor of 21α-hydroxylase activity. The increase in progesterone may be due to 21α-hydroxylase suppression in the adrenal gland and/or Cypc17 inhibition in the ovary. The stimulation of the female mammary tissue might be due to 1) direct stimulation from adrenal androgens, 2) stimulation by estradiol from the peripheral conversion of adrenal androgens, or 3) excess progesterone concentrations resulting from Cypc17 inhibition. The serum estradiol levels were not increased. The sponsor noted that the difficulty in interpreting the endocrine findings in this study due to the young age of the animals, but nonetheless reached the conclusion that Rilpivirine appeared to induce ovarian maturation and ovulation earlier than controls.

Calculated systemic exposures (AUC_{0-24}) are provided in the table below.

Table 7: AUC0-24 (µg*hr/ml) of Rilpivirine during 1 Month Dog Toxicity Study

Dose (mg/kg)	AUC_{0-24} (ug.h/ml)	
	Male	Female
5	27.1	36.8
10	102.6	46.6
40	204.2	160.2

Due to the increased ovary weights and endocrinology changes in low dose animals, a NOAEL was not defined in this study. At the low dose of 5 mg/kg, systemic exposure (AUC) values were 27 and 37 µg*hr/mL in males and females, respectively.

The six month repeat dose study in dogs included a three month interim sacrifice. Following three months of treatment of dogs with 0, 5, 10, or 40 mg/kg/day there were no significant test article-related changes among the parameters assessed at the interim sacrifice. There were no early deaths throughout the six month study, and no test article related effects on heart rate, ECG, ophthalmology, hematology and urinalysis. Clinical signs were limited to soft feces and occasional emesis in all treated groups, although effects were more pronounced at the high dose. High dose animals lost weight during the first week of dosing, presumably due to the noted decrease in food intake. Affected clinical chemistry parameters included increases in bilirubin and cholesterol, and increased activity of alkaline phosphatase in middle and high dose animals.

In high dose dogs, ovary weights were increased after three and six months of dosing. Macroscopically, after six months of treatment, in high dose females there was a slightly higher incidence of swollen ovaries, uterus and vagina. Histopathological effects on the female genital tract and adrenal glands related to altered steroidogenesis were similar to those noted in the one month study, and are discussed more below. There was an increase in the presence of brown pigmented macrophages in the liver in middle and high dose males and high dose females.

As was noted previously, Rilpivirine likely acts to inhibit 21α-hydroxylase enzyme in the adrenal gland, as evidenced by an increase in serum 17α-OH-progesterone (the proximal substrate for the enzyme), a decrease in serum cortisol, an increase in ACTH, and histological changes of hypertrophy in the adrenals. The sponsor has solicited an expert opinion on the endocrine findings from (b) (4) . In (b) (4) interpretation of the endocrine findings, the fact that serum ACTH was increased but values were in a lower range compared to the one month study is suggestive of adaptation in the adrenal gland, with stabilization of ACTH levels. (b) (4) suggests that the hypertrophy of the adrenals compensates for the initial reduction in circulating serum cortisol. The serum cortisol does not increase back to baseline, but rather stays constant between 3 and 6 months of treatment.

In addition, histological changes in the male and female gonads have been observed. In testes, minimal to slight Leydig cell hypertrophy was noted. According to the sponsor, the observed incidence and degree of atrophic seminiferous tubules with reduced spermiogenesis and cellular debris in the epididymides was within the normal background variation of this species at this age. Finally, ovarian follicles showed an increase in the number of tertiary and atretic follicles and regressive corpora lutea.

Calculated systemic exposures (AUC_{0-24}) are provided in the table below.

Table 8: AUC0-24 (µg*hr/ml) of Rilpivirine during 6 Month Dog Toxicity Study

Dose	5 mg/kg		10 mg/kg		40 mg/kg	
	AUC_{0-24} (ug.h/ml)					
day	M	F	M	F	M	F
0	10.6	9.2	21.8	20.0	22.6	10.2
85	21.3	17.6	27.8	27.2	41.3	51.5
176	21.1	17.4	25.8	31.9	68.3	43.1

Due to histopathological effects in adrenals and ovaries at the low dose of 5 mg/kg, a NOAEL was not defined in this study. Systemic exposures associated with the low dose were 21 and 17 µg*hr/mL in males and females, respectively.

No test article related mortalities resulted from oral administration of Rilpivirine (5, 10, or 40 mg/kg/day) to beagle dogs for one year. Clinical signs were limited to liquid feces and salivation in treated animals. Reduced body weight gain in males and females at all doses was noted, but the weight loss was not particularly dose related. Treated males had lower body weight gain at low dose (+21%), middle dose (+26%) and high dose (+16%) when compared to controls (+50%). There were no significant effects on food consumption over the one year dosing period. Heart rate and electrocardiogram parameters were not changed by treatment with Rilpivirine at the studied doses.

A significant ($p<0.05$) decrease was noted in total RBC's, hemoglobin, and packed cell volume in high dose males compared to controls. Increased activity of alkaline phosphatase in high dose animals (males and females) correlated with cholestasis seen in the histopathology assessment. A significant increased volume of urine in high dose males, associated with lower specific gravity, was also noted.

A dose related increase in serum progesterone concentrations in males was statistically significant up to weeks 35/39 [Note: due to early deaths unrelated to test article, replacement animals were brought on study four weeks from initial start of dosing] in middle dose and high dose animals, respectively. At weeks 48/52, the observed increase was significant only at the high dose. A dose related increase in 17-alphahydroxyprogesterone was significant in high dose males from wks 35/39 onward. No differences were noted in estrogen, cortisol, and ACTH.

In females up to weeks 35/39, the only statistically significant change was a decrease in the levels of serum cortisol in the two higher dose groups. At weeks 48/52, there was a significant decrease in cortisol levels for all treated groups and a significant lower mean cortisol in the middle and high dose groups when compared to controls.

Microscopically, moderate acute interstitial nephritis was seen in kidneys from two high dose males. This change was seen in the inner medulla and consisted of a large infiltration of the renal interstitium by neutrophils. In one male, this was associated with slight acute pelvic inflammation. Minimal to slight corticomedullary mineralization was seen in three high dose females. An increase in adrenal gland weight in high dose males and females (23% for both sexes) correlated with increased cellular density in zona fasciculata and reticularis and a reduction in neutral fat deposit. Ovary weights, increased in middle dose (40%) and high dose (119%) dogs, correlated with increased numbers of antral follicles and prominent early luteinized follicles in the high dose and prominent corpora lutea in the middle dose. There was minimal hypertrophy of the Leydig cells in high dose dogs without changes in the spermatogenic cycle. Thyroid weights were decreased in all treated males, and increased in all treated females. These changes ranged from 13% to 28% and were not particularly dose related. There was a decrease in prostate weight (39%) and spleen weight (56%) in high dose males. Changes in spleen, prostate and thyroid weights were without histological correlates.

According to (b) (4) the sponsor's expert endocrinologist, the adrenals and ovaries seemed to be of most concern as target organs for the toxicological effect of Rilpivirine. In male animals, evidence of a 21α-hydroxylase inhibition in the adrenals included elevated serum 17α-OH-progesterone and progesterone levels in a dose-response manner and histological changes within the adrenals. The enzymatic inhibition in this one year study seemed to be limited though, since hormone levels stayed within the physiologic range, did not result in an increase in ACTH secretion and were not associated with abnormal electrolytes or physical changes consistent with early puberty or hyperandrogenism. Although there were some histological changes in the female dog, these did not result in reduced sex hormone secretion from the ovary. Males showed evidence of some primary testicular dysfunction, based on the Leydig cell hypertrophy, lowered serum testosterone and elevated LH concentrations. As noted above, potential mechanisms to explain this elevation include inhibition of P450c17 enzyme in the testes, which could interfere with testosterone steroidogenesis, resulting in an increase in LH and Leydig cell hyperplasia. There was evidence of early puberty in the animals. Compared to no changes in the control animals, three treated females had ovulated and all other females had increased tertiary follicles. In males, there was slight Leydig cell hypertrophy, which may have resulted in the reduced serum testosterone levels.

Calculated systemic exposures (AUC_{0-24}) are provided in the table below.

Table 9: AUC0-24 (µg*hr/ml) of Rilpivirine during 12 Month Dog Toxicity Study

	AUC_{0-24h} (ug.h/ml)							
Day	0		89		272		362	
Dose	M	F	M	F	M	F	M	F
5	10.5	9.7	17.6	21.1	15.5	18.0	17.4	18.7
10	14.6	15.3	29.2	29.3	18.9	31.0	23.8	35.8
40	36.9	40.6	59.7	87.9	59.6	51.1	65.3	61.0

The principal target organs of toxicity seen in this study were the adrenals, ovaries and kidneys. Slight drug effects on body weight gain, serum calcium, cortisol and progestational steroids, as well as urine volume and adrenal histopathology occurred in dogs at the low dose (5 mg/kg); therefore, no NOAEL was defined in this study. The corresponding systemic exposures (AUC) were 17.4 and 18.7 µg*hr/ml in males and females, respectively.

The tables below summarize the one-month bridging study in dogs that compared the toxicokinetics and toxicity of Rilpivirine base and Rilpivirine HCl. There were no significant differences in the toxicological effects of the two test articles.

Table 10: Summary of Rilpivirine Bridging Study in Dogs: Rilpivirine Base

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/Formulation)	Duration	Batch no.	Doses (mg/kg)	NOAEL (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/beagle 3M/3F	Oral/gavage (PEG400 + CA)	1-month	ZR2784 74PFA0 21	0 (vehicle), 5, 40 mg/kg/day TMC278 base	/	**5 mg/kg**: Uterus: increased glandular development. C_{max} = 1.4-1.1 µg/mL (M-F); AUC_{0-24h} = 23-14 µg.h/mL (M-F) **40 mg/kg**: ↓ RBC (F: 7%, F), Hct (M: 12%, F: 5%), reticulocytes (M: 61%). ↓ IP (F: 11%), triglycerides (M: 53%), ↑ bilirubin (M: 39%, F: 62%), ALP (M: 1-fold, F: 2-fold), ALT (M: 5-fold, F: 2-fold), γGT (M: 3-fold); ↑ ACTH (M: 77%, F: 92%), progesterone (M: 10-fold), 17α-OH-progesterone (M: 34-fold), ↓ cortisol (M: 47%, F: 34%); Liver: Centrilobular and periportal inflammation, brown pigmented macrophages, single cell death and oval cell proliferation. Adrenal glands: Swollen cells with densely stained cytoplasm in zona reticularis and fasciculata and presence of foamy cells in the zona fasciculata. Ovaries: ↑ in cystic luteinized follicles or tertiary follicles. Uterus: ↑ in glandular development. Testes: Prominent Leydig cells. C_{max} = 3.8-2.5 µg/mL (M-F); AUC_{0-24h}= 73-43 µg.h/mL (M-F)	TMC278-NC116[a]/ 4.2.3.2.

[a]: GLP compliance; ACTH: adrenocorticotropic hormone; ALT: alanine aminotransferase, ALP: alkaline phosphatase; AUC_{0-24h} : area under the concentration vs time curve till 24 h after dosing; CA: citric acid; C_{max}: maximum concentration; F: female; γGT: gamma glutamyltransferase; Hct: hematocrit; IP: inorganic phosphate; M: male; PEG400: polyethylene glycol 400; RBC: red blood cell.

Table 11: Summary of Rilpivirine Bridging Study in Dogs: Rilpivirine HCl

Species/Strain No. and Gender/Group	Route/Method of Administration (Vehicle/Formulation)	Duration	Batch no.	Doses* (mg/kg)	NOAEL (mg/kg)	Noteworthy Findings	Study No./ Location in CTD
Dog/beagle 3M/3F	Oral/gavage (0.5% w/v HPMC)	1-month	ZR3145 85PFA0 11	0 (vehicle), 5, 40 mg/kg/day TMC278.HCl	/	**5 mg/kg:** ↓ IP (F: 10%). Uterus: ↑ in glandular development C_{max} = 0.89-0.70 µg/mL (M-F); AUC_{0-24h} = 13-7.5 µg.h/mL (M-F) **40 mg/kg:** Hct (M: 15%, F: 7%), reticulocytes (M: 43%). ↓ IP (F: 8%), ↑ bilirubin (M: 17%, F:100%), ALP (M: 3-fold, F: 3-fold), AST (F: 1-fold), ALT (M: 1-fold, F: 6-fold), γGT (F: 6-fold), ↑ ACTH (M: 59%, F: 54%), cortisol (M: 54%), progesterone (M: 10-fold), 17α-OH-progesterone (M: 29-fold). Liver: Centrilobular and periportal inflammation, brown pigmented macrophages, single cell death and oval cell proliferation. Adrenal glands: Swollen cells with densely stained cytoplasm in zona reticularis and fasciculata and presence of foamy cells in the zona fasciculata. Ovaries: ↑ in cystic luteinized follicles or tertiary follicles. Uterus: ↑ in glandular development. Testes: Prominent Leydig cells. C_{max} = 4.1-5.8 µg/mL (M-F); AUC_{0-24h} = 76-81 µg.h/mL (M-F)	TMC278-NC116[b]/ 4.2.3.2.

*: dose of HCl salt is presented as base equivalents; [b]: GLP compliant study; ACTH: adrenocorticotropic hormone; ALT: alanine aminotransferase, ALP: alkaline phosphatase; AST: aspartate aminotransferase; AUC_{0-24h} : area under the concentration vs time curve till 24 h after dosing; C_{max}: maximum concentration; F: female; γGT: gamma glutamyltransferase; Hct: hematocrit; HPMC: hydroxypropyl methyl cellulose; M: male.

Mice

Repeat-dose studies in mice were conducted to support dose selection for the two year carcinogenicity study. In a one-month study, CB6F1-nonTgrasH2 mice were administered 20, 80 or 320 mg/kg Rilpivirine. One high dose female was euthanized moribund on day 18 and a second high dose female was found dead on day 27. In both mice, liver and kidney lesions were noted at necropsy. Over the last two weeks of dosing, a slight (males) or marked (females) decrease in body weight was noted at the high dose. Food consumption was increased in high dose animals. Assessments of clinical pathology parameters revealed hematological and clinical chemistry changes, primarily at the high dose (see tables below, *excerpted from sponsor*).

Table 12: Changes in Hematology Parameters in Mice Administered Rilpivirine for One Month.

Sex	Male				Female			
Dose-level (mg eq./kg/day)	0	20	80	320	0	20	80	320
WBC (G/L)	4.94	4.08	4.31	3.01*	3.29	2.73	3.90	8.35*
% diff vs. controls	-	*-17*	*-13*	*-39*	-	*-17*	*+19*	*+154*
N (G/L)	1.21	0.94	0.91	0.43**	0.59	0.44	0.43	5.17*
% diff vs. controls	-	*-22*	*-25*	*-64*	-	*-25*	*-27*	*+776*
L (G/L)	3.52	3.01	3.11	2.45	2.59	2.21	3.34	2.99
% diff vs. controls	-	*-17*	*-12*	*-30*	-	*-15*	*+29*	*+15*
M (G/L)	0.09	0.06	0.08	0.04*	0.04	0.04	0.06	0.13*
% diff vs. controls	-	*-33*	*-11*	*-55*	-	*0*	*+50*	*+225*
Platelet (G/L)	1294	1249	1368	1478*	1122	1225	1146	1390*
% diff vs. controls	-	*-3*	*+6*	*+14*	-	*+9*	*+2*	*+24*

Statistically different from controls: *: $p<0.05$ and **: $p<0.01$,
WBC: white blood cell, N: neutrophils, L: lymphocytes, M: monocytes.

Table 13: Changes in Clinical Chemistry Parameters in Mice Administered Rilpivirine for One Month

Sex	Male				Female			
Dose-level (mg eq./kg/day)	0	20	80	320	0	20	80	320
Calcium (mmol/L)	2.33	2.22	2.38	2.54	2.25	2.2	2.23	2.85*
% diff vs. controls	-	*-5*	*+2*	*+9*	-	*-2*	*-1*	*+27*
Inorganic phosphorus (mmol/L)	1.61	1.45	1.63	1.69	1.78	1.77	1.77	2.53*
% diff vs. controls	-	*-10*	*+1*	*+5*	-	*-1*	*-1*	*+42*
Urea (mmol/L)	10.4	10.4	11.9	17.9**	11.3	13.3	15.6	17.9**
% diff vs. controls	-	*0*	*+14*	*+72*	-	*+18*	*+38*	*+58*
Tot. Bilirubin (µmol/L)	4	3	4	4	2	2	2	6
% diff vs. controls	-	*0.75X*	*0*	*0*	-	*0*	*0*	*3X*
Protein (g/L)	55	50	55	60	55	52	52	65
% diff vs. controls	-	*-9.1*	*0*	*9.1*	-	*-5.5*	*-5.5*	*18.2*
Albumin	30	29	30	35*	32	30	30	33
% diff vs. controls	-	*-3.3*	*0*	*16.7*	-	*-6.3*	*-6.3*	*3.1*
Cholesterol (mmol/L)	2.4	2.2	2.8	3.5**	2.0	1.8	2.4	4.2**
% diff vs. controls	-	*-8*	*+17*	*+46*		*-10*	*+20*	*+110*
ALP (IU/L)	210	222	238	631**	245	243	330	503**
% diff vs. controls	-	*1X*	*1X*	*3X*	-	*1X*	*1X*	*2X*

Statistically different from controls: *: $p<0.05$ and **: $p<0.01$.
ALP: alkaline phosphatase.

Changes in electrolytes are considered to be related to kidney histopathology, including minimal to moderate degenerative/necrotic nephropathy (see below).

Liver weights were increased in high dose males and females. Spleen and kidney weights were increased in high dose females only. Also in high dose animals, testes and ovary weights were decreased. Macroscopically, dark and/or swollen livers were present in all high dose males and 8/10 high dose females. Swollen spleens (6/10) and an irregular surface of the kidneys (7/10) was seen in high dose females, and to a lesser extent in males. Also in high dose females, thymus (7/10) and uterus (5/10) were reduced in size.

Microscopically, hepatocellular hypertrophy was noted in 3/10 low dose, 9/10 middle dose, and 10/10 high dose males, and 2/10 low dose, 9/10 middle dose and 9/10 high dose females. Areas of coagulative hepatocellular necrosis were noted in 2/10 high dose males. Moderate degenerative/necrotic nephropathy was noted in 1/10 middle dose and 3/10 high dose males, and 10/10 high dose females. In bone marrow, minimal to moderate myeloid cell hyperplasia was noted in both femur (5/10 males and 10/10 females) and sternum (3/10 males and 9/10 females) from high dose animals.

At the high dose, histopathological changes in ovary/uterus/vagina included absent hyperkeratosis in the vagina, mucification of the vaginal epithelium and uterine atrophy. In the adrenal gland, 4/10 high dose females did not have an obvious x-zone, which may be related to altered steroidogenesis.

Toxicokinetic parameters assessed after one month of dosing are presented in the table below (*excerpted from sponsor*).

Table 14: Toxicokinetic Parameters in CB6F1-nonTgrasH2 mice Administered Rilpivirine for One Month

Test Article: R314585

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Species/Strain: Mouse CB6F1-nonTgrasH2		Sample: Plasma		Study No.: 6740		
Feeding Condition: Fed		Analyte: TMC278		Location in CTD:		
Vehicle/Formulation: 0.5% Methocel aqueous solution		Assay: LC/MS/MS		GLP Compliance: GLP		
Route: Oral/Gavage						
Gender (M/F)/Number of Animals	M/3	F/3	M/3	F/3	M/3	F/3
Dose (mg TMC278 eq./kg/day)	20	20	80	80	320	320
Pharmacokinetic Parameters						
C_{max} (µg/ml)	13.5	15.3	30.0	37.1	65.5	69.1
t_{max} (h)	2.0	1.0	2.0	1.0	6.0	1.0
AUC (µg × h/ml)	63.7	54.0	250	240	1090	942
(Time for calculation –h)	0-24	0-24	0-24	0-24	0-24	0-24
$t_{1/2}$ (h)	3.1	5.1	1.4	2.1	28 [2]	- [1]
(Time for calculation –h)	12-24	12-24	12-24	12-24	12-24	- [1]

Additional Information

[1] no $t_{1/2}$ could be calculated since plasma levels increased between 12 and 24 h after dosing.

2] the estimated half-life does not only represent the elimination half-life (as probably seen at the lower dose levels), but possibly also a part of the absorption phase

In a three-month study in Swiss mice, administration of 20, 80 or 320 mg/kg Rilpivirine resulted in abdominal distension in high dose animals from 6 weeks on. A slight increase in body weight gain was noted at the middle dose, with a more pronounced effect on body weight (11%) and body weight gain (54%) in high dose males and females throughout the study. Food consumption was slightly to moderately increased in high dose animals. Assessments of clinical pathology parameters revealed changes in hematology and clinical chemistry, primarily at the high dose (320 mg/kg). Slight (less than 10%) decreases were seen in red blood cells (RBCs), hemoglobin, and hematocrit in high dose animals. There was a slight decrease in neutrophils (30%) and moderate decreases in lymphocytes and eosinophils (~50%) in high dose group males, but a slight decrease in thrombocytes (12%) and a slight increase in the number and % reticulocytes (50%) in high dose females. Middle dose (80 mg/kg) females had a slight increase in total protein, albumin and cholesterol, and a moderate decrease in triglycerides. High dose females had a slight increase in total protein and cholesterol, a moderate increase in urea nitrogen (25%; P<0.01) and a decrease in triglycerides. High dose males had a slight increase in inorganic phosphorus and a decrease in total bilirubin. High dose males and females had a slight increase in calcium, albumin, ALP (1.25-2X), and ALT (2-2.5X).

Macroscopically, the finding of dark liver was present in 4/10 middle dose females, with swollen spleens noted in two females. Dark and swollen livers were present in all high dose animals. Swollen spleens and an irregular surface of the kidneys were seen in three high dose females.

Microscopically, marginal histopathological changes in the adrenal, ovary/uterus, spleen and thymus from high dose animals had questionable toxicological significance. In the liver, there was an increased incidence of hepatocellular hypertrophy (centrilobular to diffuse) that was slight in middle dose males, moderate in middle dose females and prominent in high dose males and females. There was a moderate increase in single cell necrosis in high dose males and females, with minimal to slight focal necrosis in one female and one male at the middle dose and two females and three males at the high dose. Hepatocellular (small) vacuolation was minimal in two high dose males and minimal to slight in five high dose females. In the high dose only, there were minimal to slight increases in the incidence of Kupffer cell proliferation, brown pigmentation of Kupffer cells and extramedullary hematopoiesis.

There were no drug related effects on the kidneys in males, or low dose and middle dose group females. In 5/10 high dose females, there was minimal to moderate nephropathy, characterized by slight to marked multifocal tubular basophilia (with minimal presence of single cell death within the basophilic tubules), minimal to slight glomerulopathy (focal atrophic glomeruli with thickened Bowman's capsule among basophilic tubules), minimal to moderate mononuclear cell infiltration, minimal to slight interstitial fibrosis, minimal tubular dilation and slight cortical mineralization. In three of these animals, these changes were associated with minimal to slight inflammation within the capsule and adjacent tissue. One female had moderate exudative inflammation; two had minimal granulocytic infiltration and two had minimal focal single cell death not confined to the basophilic tubules.

In the bone marrow, there was an increase in the myeloid/erythroid ratio in high dose males and females.

Calculated systemic exposures ($AUC_{0-\infty}$) are provided in the table below (*excerpted from sponsor*).

Table 15: AUC0-∞ (µg.h/ml) of Rilpivirine during 3 Month Mouse Toxicity Study

Day	20 mg/kg		80 mg/kg		320 mg/kg	
	M	F	M	F	M	F
0	71	59	236	250	1010	707
30	*	74	263	313	860	1170
86	80	61	210	313	665	1360

* not available

Based on hepatocellular histopathology at the middle dose, the no observed adverse effect level (NOAEL) was defined as the low dose, 20 mg/kg. The corresponding systemic exposures ($AUC_{0-\infty}$) were 80 µg*hr/mL and 61 µg*hr/mL in males and females, respectively.

Rabbits

In a dose range finding study, female rabbits (n = 5/group) were dose with 100, 300, or 1000 mg/kg Rilpivirine for five days. Findings at the middle and high doses are summarized in the table below (*excerpted from sponsor*), and include clinical signs of decreased fecal output, body weight loss associated with decreased food consumption, decreased reticulocytes, and increased serum creatinine. There were no gross pathological findings. There was no histopathological assessment conducted in this pilot study.

Table 16: Summary of Findings in Female Rabbits.

	Doses (mg/kg body weight/day)			
	0	100	300	1000
Rabbit Nos.	1 - 5	11 - 15	21 - 25	31 - 35
Mortality [a]	0/5	0/5	0/5	0/5
Clinical observations - reduced faeceal output	N	P(+)(1/5)	P+++(5/5)	P+++ (5/5)
Body weight (BW) and weight gain	N	D+	D++ BW loss	D+++ BW loss
Food consumption	N	D+	D++	D+++
Haematology - reticulocytes	N	N	D+	D++
Serum analysis -creatinine	N	N	I+	I+
Gross pathology	N	N	N	N
Toxicokinetics	Available			

[a] Number of animals positive / total number of rabbits.
N = No treatment related effects; P = present; D = decrease; I = increase;
(+) marginal; + slight; ++ = moderate; +++ = pronounced.

Toxicokinetic parameters were assessed on Day 4 of dosing. Results are summarized in the table below (*excerpted from sponsor*).

Table 17: Toxicokinetic Parameters in Rabbits Administered Rilpivirine for Four Days.

Time (h) Dose group	Low 100 mg/kg		Medium 300 mg/kg		High 1000 mg/kg	
	Mean	SD	Mean	SD	Mean	SD
0 (predose)	30567	13588	130667	14224	131333	5686
0.5	23300	9350	109667	3512	109200	17542
1	32150[1)]	18880[1)]	104500	12379	108967	27104
2	31567	8819	105000	11136	120667	11930
4	43333	3955	98033	18205	117667	14572
8	57267	10124	115667	11372	122000	19053
24	42267	10391	115333	9238	131267	36036
C_{max} (ng/ml)	57267	10124	124000	2646	138333	23861
T_{max} (h)	8.0	0.0	13.3	9.2	18.7	9.2
C_{min} (ng/ml)	23167	9441	93733	14915	102500	23812
T_{min} (h)	0.7	0.3	1.8	1.9	0.7	0.3
AUC_{0-24h} (ng.h/ml)	1120241	198251 (17.7%[2)])	2695015	72819 (2.7 %[2)])	2970886	505969 (17.0%[2)])
C_{min}/C_{max}	0.40	0.12	0.73	0.18	0.72	0.14

1) n=2
2) % CV (coefficient of variation)

7 Genetic Toxicology

Genetic toxicology studies with Rilpivirine were reviewed previously (Appendix 1A). In summary, Rilpivirine was negative for genetic toxicology in the *in vitro* bacterial reverse mutation (Ames) assay, the *in vitro* mammalian forward mutation (chromosome aberration) assay, and the *in vivo* micronucleus test in mice.

Additional genetic toxicology studies were performed with (b) (4) a synthesis intermediate and impurity of TMC278 HCl salt. Those studies were reviewed previously (Appendix 1G). (b) (4) tested positive in a mutagenic assay and is considered to be clastogenic to human lymphocytes *in vitro*. The levels of (b) (4) will be sufficiently controlled in the intermediate (free-base) drug substance, such that the impurity does not need to be specified in the final drug substance.

8 Carcinogenicity

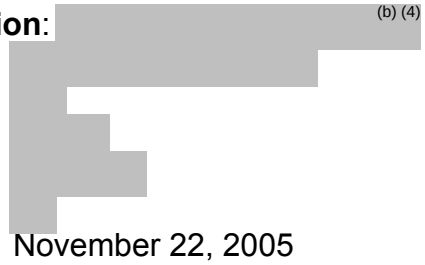
Study title: Carcinogenicity Study by Oral Gavage Administration to CD-1 mice for 104 Weeks

Key study findings: The CAC recommended doses for males and females of 0, 20, 60 and 160 mg/kg/day by oral gavage based on the AUC for the high dose in mice being >25-fold the human AUC, and minimal toxicity in previous studies. The sponsor accepted the recommended doses.

The doses used for this study were appropriately selected based on the AUC ratio. The steady state (week 28) $AUC_{0\text{-}24h}$ values for the high dose groups were 505 and 766 µg.h/mL for males and females, respectively, which were 210-fold and 319-fold the estimated mean steady-state human $AUC_{0\text{-}24h}$ of 2.4 µg.h/mL.

The tumor types that showed the greatest increase in treated mice, and for which the increase was statistically significant, were hepatocellular adenomas (alone) and hepatocellular adenomas-carcinomas (combined). In males, the incidence rates for those tumors reached statistical significance at the middle and high doses, whereas the incidence of hepatocellular carcinomas alone was increased at all doses but the increases did not reach statistical significance compared to controls. In females, the incidence of each tumor type alone, and the combined incidence of hepatocellular adenomas and carcinomas reached statistical significance at the middle and high dose. In a previous study, administration of Rilpivirine to rats for three months was associated with an induction of CYP enzymes and UDPGT. In a three month study in mice, hepatocyte hypertrophy, moderate increases in single cell necrosis, and accumulation of Kupffer cell pigmentation was noted. Since Rilpivirine is known to be associated with liver changes, an increase in adenomas and a slight increase in carcinomas in the two year study in mice might have been anticipated. The findings are considered to be treatment related; however, the increased incidence of liver tumors is thought to result from a rodent-specific mechanism related to induction of hepatic enzymes.

Study no.: TMC278--NC120
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)



Date of study initiation: November 22, 2005
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity:

Batch: ZR314585PUA	221	231	241	261
HPLC assay	99.7	98.8	98.6	100.4
Date received	25 Nov 2005	6 Feb 2006	6 Feb 2006	22 Sep 2006
Expiry date	28 Sep 2007	20 Dec 2007	20 Dec 2007	28 June 2008
Date of first use	13 Dec 2005	7 Mar 2006	1 Aug 2006	3 Oct 2006

CAC concurrence: The Exec. CAC recommended the doses used in the study (*see Appendix 2*). The Exec. CAC also concurred with the review conclusions (*see Appendix 3*).

Methods

Doses: 0, 20, 60, 160 mg eq/kg/day

Basis of dose selection: Dose selection was based on MTD due to kidney toxicity in females and on multiples of drug exposure in males.

Species/strain: Swiss CD1 mice, (b) (4)

Number/sex/group (main study): 60/sex/group

Route, formulation, volume: Oral gavage/suspension in 0.5% w/v Methocel in purified water/10 mL/kg body weight.

Frequency of dosing: Daily

Satellite groups used for toxicokinetics or special groups: 18 per sex per group for controls and 24 per sex per group for dosed groups.

Age: 41 to 48 days

Animal housing: Group housed in transparent boxes, food and water *ad libitum*, 12/12h light/dark cycle, vehicle groups were housed in separate racks from the treatment groups to further prevent exposure to test article

Restriction paradigm for dietary restriction studies: N/A

Drug stability/homogeneity: The concentration and homogeneity of TMC278 were checked in suspensions prepared during weeks 1, 13 (batch change), 26, 28, 36 (batch change), 39, 44 (batch change), 52, 65, 78, 91, 103 and were checked. Two week stability was assessed in the first preparation only. Concentrations, homogeneity and stability were acceptable.

Dual controls employed: No.

Interim sacrifices: N/A

Deviations from original study protocol: Minor deviations were noted in the study report. The deviations appeared to have no impact on the validity or integrity of the study.

Observation times

Mortality: Animals were checked at least twice daily for mortality or signs of morbidity.

Clinical signs: Animals were checked at least twice daily for signs of reactions to treatment. Detailed observations were made at and following the time of dosing according to the following schedule:

Week 1 - daily
Weeks 2 to 4 - twice weekly (middle and end of week)
Weeks 5 to 13 - once each week
Weeks 14 to 52 - once every two weeks
Week 53 onwards - once every four week.

A detailed weekly physical examination included palpation and a general health assessment.

Body weights: Body weights were recorded pre-study and day 0 of dosing, then daily for dosing volume adjustments for the first 16 weeks of treatment, followed by once every 4 weeks to coincide with food consumption measurements, and before necropsy.

Food consumption: Food consumption per cage (3 mice/cage) was recorded pre-study and day 0 of dosing, then daily for the first 16 weeks of treatment, followed by once every 4 weeks for the remainder of the study.

Histopathology: At necropsy.
Peer review: yes (x), no ( )

Toxicokinetics: Blood samples (0.5 mL on Day 1 and 0.4 mL in week 28) were drawn from the retro-orbital sinus into EDTA-containing tubes at 1, 2, 6, 12, and 24 hours post-dose.

Results

Mortality:

- Females receiving 160 mg eq/kg/day were terminated in Week 99 due to increasing mortality in the latter stages of the study, and the remaining females (low and middle dose groups) were terminated in Week 103. Males receiving 160 mg eq/kg/day had dosing stopped during Week 101 and, with the remaining groups, were sacrificed after 104 weeks as scheduled.

Table 18: Mortalities of the Toxicity Group Animals in the Mouse Carcinogenicity Study

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg eq./kg/day)	0	20	60	160	0	20	60	160
Group size	60	60	60	60	60	60	60	60
Week of treatment completed	104	104	104	100	102	102	102	98
Unscheduled Deaths total;	25	35	39	42*	40	37	30	43
Survival %	58	42	35	30	33	38	50	28

* kept off dose until the end Week 104, number of survivors at the end of Week 100 was 21.

- The trend test for mortality with dose was significant in males at 60 and 160 mg eq/kg/day. Compared to controls, only the 160 mg eq/kg/day dose group showed a significant increase in mortality. There was no treatment-related effect on mortality in female mice.
- The principal causes of death/moribundity are listed in the table below (*excerpted from sponsor*):

Table 19: Incidence Rates of Factors Contributing to Death in the Mouse Carcinogenicity Study

Group/sex Dose (mg eq./kg/day)	1M 0	2M 20	3M 60	4M 160	1F 0	2F 20	3F 60	4F 160
Hepatocellular carcinoma	1	1	1	4	0	0	0	3
Urogenital tract disease	10	13	1	17	3	0	1	3
Glomerulonephritis/ Chronic nephropathy	0	1	0	0	6	6	5	7
Malignant lymphoma	3	3	7	4	11	14	11	8
Ovarian cysts	-	-	-	-	3	1	3	1

Clinical signs:

There were no treatment-related effects.

Body weights: There were no toxicologically significant effects on body weight or body weight gain.

Food consumption:

There was no significant difference in food consumption in treated versus control animals.

Hematology:

Results of hematological assessments were highly variable. There were no toxicologically significant changes.

Organ weights:

Dose-related increases in liver weights were noted in all female dose groups compared to untreated controls (1.13-, 1.39-, and 2.23-fold higher in low, middle and high dose groups, respectively). Liver weights were increased in treated males compared to controls, but not in a dose-dependent manner (1.35-, 1.69-, and 1.18-fold higher in low, middle and high dose groups, respectively).

Gross pathology:

Liver: Macropathological findings in liver are summarized in the table below (*excerpted from sponsor*). The incidence of macroscopically observable liver masses was increased in mice from all groups. In animals (both sexes) sacrificed or dying during the study, there were dose-related increases in the following observations: dark, enlarged, lobular pattern accentuated, and pale areas. In males that survived to terminal necropsy, a slight increase in the incidence of pale livers was noted (0/35, 2/25, 4/21, and 4/18, in controls, low, middle, and high dose groups, respectively). In females that survived to terminal necropsy, an increase in the incidence of masses (1/20, 5/23, 8/30, and 15/17, in controls, low, middle, and high dose groups, respectively) and pale areas (2/20, 4/23, 4/30, and 10/17, in controls, low, middle, and high dose groups, respectively), was noted.

Table 20: Summary of Findings in the Liver for All Animals

Group/sex	1M	2M	3M	4M	1F	2F	3F	4F
Dose (mg eq./kg/day)	0	20	60	160	0	20	60	160
Liver								
Enlarged	2	3	2	11	7	7	10	30
Mass(es)	13	21	23	29	4	9	14	32
Pale area(s)	1	4	6	12	6	9	10	33
Number of tissues examined	60	60	60	60	60	60	60	60

Kidney: An increased incidence of granular kidneys was noted in high dose females (left kidney: 15, right kidney: 14) compared with controls (left kidney: 6, right kidney: 6).

Histopathology:
Neoplastic:

Lung:

- There was a marginally higher number of bronchioloalveolar adenocarcinomas in males given 160 mg eq/kg/day than in controls (6/60, 5/60, 0/60 and 10/60 in control, low, middle and high dose groups, respectively). The observation is considered to be incidental to TMC278 administration since the incidence rate fell among the historical control range and the same finding was not observed in high dose females.

Liver:

Males:

Evaluation of tumor findings: Oral administration of TMC278-HCl to male mice at doses of 20, 60 or 160 mg eq/kg/day produced a dose-related increase in hepatocellular adenomas in males at all doses. When compared with untreated controls, the increase was statistically significant at 60 and 160 mg/kg.

The combined incidence of hepatocellular adenomas and hepatocellular carcinomas was increased in a dose-related manner, and when compared to controls the increases were significant at all dose levels.

Also, in males the incidences of adenocarcinoma in cecum and hemangioma in spleen were considered to have statistically significant positive dose response relationships. The incidence of cecal adenocarcinomas were considered to be incidental (i.e., not related to administration) given the lack of cecal inflammation or hyperplasia and the lack of adenocarcinomas in other portions of the intestine at the high dose level. It should be noted, however, that an adenocarcinoma of the colon was noted in one control male, two low dose males, and one middle dose male. The rate of occurrence of cecal adenocarcinomas in the middle dose group fell within the background control range, and in the high dose group, just outside the background control range (5.3% versus 3.9%, respectively).

Females:

In female mice, the incidence of hepatocellular carcinomas, hepatocellular adenomas, and both tumors combined, increased with dose. The difference from controls was statistically significant at 60 and 160 mg eq/kg/day.

Non-neoplastic:

Liver:

- Increases in clear cell, eosinophilic, and basophilic foci in 160 mg eq/kg/day animals, were more notable in females.
- Hepatocyte eosinophilic hypertrophy was seen in livers of most high dose females, and to a lesser degree in middle dose females and high dose males.

- Increases in the incidence of brown pigmented Kupffer cells/macrophages and macrophages/mononuclear cells were seen in middle and high dose females, and to a lesser extent in high dose males. The increased pigmentation is thought to be due to lipofuscin accumulation, secondary to prolonged hepatocytes hypertrophy and increased hepatocytes turnover.

Kidney:

- Nephropathy/glomerulonephritis was increased in high dose females, and accounted for the granular appearance of kidney tissue from these animals. This is considered to be a treatment-related enhancement of a finding also seen in untreated controls.

Toxicokinetics:
Maximum blood concentrations were noted between one and two hours after gavage dosing. Exposure data is provided in the table below (*excerpted from sponsor*).

Table 21: Peak Plasma and AUC Values

	Dose (mg eq./kg)	Day 1 20	Day 1 60	Day 1 160	Week 28 20	Week 28 60	Week 28 160
				Males			
C_{max}	(µg/ml)	9.89	22.9	40.7	9.75	21.9	35.7
AUC	(µg.h/ml)	59.3	239	440	75.6	230	505
				Females			
C_{max}	(µg/ml)	13.3	24.0	38.4	9.89	28.5	57.5
AUC	(µg.h/ml)	61.2	182	345	51.0	278	766

Study title: Carcinogenicity Study by Oral Gavage Administration to CD Rats for 104 Weeks

Key study findings: Male and female Crl:CD rats were administered the equivalent of 40, 200, 500, or 1500 mg free base TMC278 HCl/kg/day for up to 104 weeks (males were treated for 104 weeks, females were treated for 98 weeks due to mortality). It should be noted that the high mortality rate in high dose females suggests that the maximum tolerated dose (MTD) was exceeded in that group. The doses used for this study were appropriately selected based on saturation of absorption. The steady state (week 39) $AUC_{0\text{-}24h}$ values for the high dose groups were 18.4 and 83.8 µg.h/mL for males and females, respectively, which were 8-fold and 35 fold the mean estimated steady-state human $AUC_{0\text{-}24h}$ of 2.4 µg.h/mL, respectively.

According to the test site, the need to terminate dosing of females at week 98 reflects a decline in longevity of the female CD rat. Assessment of the study was not apparently affected by study duration.

The tumor types that showed the greatest increase in rats were thyroid follicular cell adenomas (alone) and adenomas – carcinomas (combined). The tumor findings correlated with increased thyroid weights and increased serum thyroid stimulating hormone levels, among other effects. Rilpivirine slightly increased UDPGT activity in an *ex vivo* assay, and administration of Rilpivirine to rats for three months was associated with an induction of CYP enzymes and UDPGT. Therefore, the increased incidence of thyroid tumors at the high dose is thought to result from a rodent-specific mechanism related to altered metabolism of thyroid-related hormones.

The results indicate that while Rilpivirine is positive for thyroid carcinogenicity, the increased incidence does not reach statistical significance using the current statistical decision rules. Moreover, since thyroid adenomas and carcinomas are commonly seen in the rat at a low basal rate, and given the possible rodent-specific mechanism for tumor production, the relevance to humans is considered unlikely.

Study no.: TMC278-NC123
Volume #, and page #: electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 22 November 2005 (protocol signed)

GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: TMC278-HCl (alternative name R314585)
Batch/Lot number test article:

Batch: ZR314585PUA	221	231	241	261
HPLC assay	99.7	98.8	98.6	100.4
Date received	25 Nov 2005	6 Feb 2006	6 Feb 2006	22 Sep 2006
Expiry date	28 Sep 2007	20 Dec 2007	20 Dec 2007	28 June 2008
Date of first use	02 Dec 2005	23 Feb 2006	3 Aug 2006	28 Sep 2006
For first dosing in	Week 1	Week 13	Week 36	Week 44

CAC concurrence: The Exec. CAC concurred with the protocol (*see Appendix 2*). The Exec. CAC also concurred with the review conclusions (*see Appendix 3*).

Methods

Doses: 0, 40, 200, 500, 1500 mg eq/kg/day
Basis of dose selection: saturation of absorption and no dose limiting toxicity.
Species/strain: Sprague Dawley Rat
Number/sex/group (main study): 65/sex/group
Route, formulation, volume: Oral gavage; suspension; 10 mL/kg/day
Frequency of dosing: Once daily
Satellite groups used for toxicokinetics or special groups: 3/sex/group in control
6/sex/group in treatment groups
Age: ~ 42 days old on Day 0
Animal housing: Group housed with filtered and fresh air, positive pressure to the outside, with a controlled temperature range (19 to 23°C), and relative humidity range (40 to 70%), and artificial lighting on a 12/12 hr light/dark cycle.
Restriction paradigm for dietary restriction studies: Food and water *ad lib.*
Drug stability/homogeneity: Acceptable.
Dual controls employed: No
Interim sacrifices: No.
Deviations from original study protocol: None of significance that would alter interpretation of study results. NOTE: apparently high levels of test article in some locations of male and female

animals rooms based on swab samples collected during week 36.

Observation times

Mortality: Twice daily
Clinical signs: Detailed observations were recorded according to the following schedule:

Week 1: daily
Week 2 to 4: twice weekly
Week 5 to 13: once weekly
Week 14 to 52: once every two weeks
Week 53 onwards: once every four weeks

A weekly physical examination included palpation.

Body weights: Pre-study and day 0. Weekly for the first 16 weeks of dosing, then every four weeks.
Food consumption: Coinciding with recording of body weight measurements.
Histopathology: At necropsy
Peer review: Yes
Ophthalmic examinations: Before Day 0, weeks 26, 52, 98 (females) or 100 (males), and 103 (all surviving males).
Hematology/Blood chemistry: Weeks 52 and end of treatment.
Urinalysis: Weeks 51 and end of treatment
Tissue weights: The following tissues were weighed:

Adrenals	Ovaries with oviducts
Brain	Spleen
Heart	Testes L & R
Kidneys L & R	Thymus
Liver	Thyroid and parathyroids
Lungs with mainstream bronchi	

Toxicokinetics: Blood samples were taken from the satellite rats at pre-dose (before administration of the first dose) and at 0.5, 2, 4, 8, 12 and 24 h after dosing on Day 1, Weeks 27 and 39, and at necropsy (Week 60).

Results
Mortality:
There was no effect of treatment on mortality. The table below (*excerpted from sponsor*) summarizes the primary causes of death in the two year bioassay.

Table 22: Incidence Rates of Factors Contributing to Death in the Rat Carcinogenicity Study

Group/sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500	1F 0	2F 40	3F 200	4F 500	5F 1500
Adenoma of pars distalis										
Total	13	10	9	7	11	20	24	15	11	15
Mammary adenocarcinoma										
Total	0	1	0	0	0	4	4	8	2	5
Mammary fibroadenoma										
Total	0	0	0	0	0	12	8	4	7	7
Number of rats examined	40	41	38	37	44	45	46	35	33	34

Clinical signs:
There were no significant clinical observations. Head and dorsal brown staining, ventral yellow staining and hair loss (head and forelimbs) were increased in rats in the high dose groups. There was no dose-related increase in palpable swellings.

Body weights:
There was no treatment-related effect on body weight.

Food consumption:
There was no treatment-related effect on food consumption.

Ophthalmology:
Marked opacities in three high dose males were considered to be spontaneous age-related findings and not related to treatment with TMC278.

Hematology:
There was no treatment-related effect on hematologic parameters.

Clinical Chemistry:
Treatment-related effects on several clinical chemistry parameters were noted primarily in high-dose (1500 mg/kg) animals, and were more pronounced in males, although the changes do not appear to be toxicologically significant. In males, treatment-related increases noted in mean concentrations of hepatic enzymes alkaline phosphatase (ALP), alanine amino-transferase (ALT), aspartate amino-transferase (AST), gamma-glutamyl transferase were generally 2-fold or less at the high dose (ALT: 2.63-fold). Minimal increases in urea (1.14-fold) and creatinine (1.15-fold) were noted. Blood glucose levels increased slightly (1.19-fold.). Triglycerides and cholesterol concentrations tended to decrease with treatment (-24% and -15% at high dose, respectively).

Urinalysis:
Chloride excretion was increased more than 3-fold, and potassium excretion was decreased with treatment in both males and females (see tables below, *excerpted from sponsor*).

Table 23: Results of Urinalysis in Males-Week 51 and at the End of the Treatment Period

Parameter	Occasion	Group/sex				
		1M	2M	3M	4M	5M
		Dose (mg eq./kg/day)				
		Control	40	200	500	1500
pH	Term	7.9	8.1	8.1	7.9	7.1** (0.90X)
Potassium (mmol/L)	Term	130.7	127.5	106.5* (0.81X)	104.0* (0.80X)	111.3* (0.85X)
Chloride (mmol/L)	51	45.1	49.8	51.4	81.0** (1.80X)	163.8** (3.63X)
	Term	42.2	40.3	54.5 (1.29X)	61.9** (1.47X)	146.8** 3.48X)

Significant when compared with control: * $p<0.05$; ** $p<0.01$
Term = End of treatment period

Table 24: Results of Urinalysis in Females-Week 51 and at the End of the Treatment Period

Parameter	Occasion	Group/sex				
		1F	2F	3F	4F	5F
		Dose (mg eq./kg/day)				
		Control	40	200	500	1500
pH	51	6.9	6.5	6.6	7.0	6.0** (0.87X)
	Term	6.6	6.4	6.6	6.8	6.0** (0.91X)
Protein (g/L)	51	0.27	0.16	0.24	0.15	0.30** (1.11X)
Potassium (mmol/L)	51	160.4	150.1	137.2 (0.86X)	138.7 (0.86X)	129.9* (0.81X)
	Term	107.2	113.8	103.7	87.8** (0.82X)	90.9** (0.85X)
Chloride (mmol/L)	51	31.6	35.8	50.1** (1.59X)	87.0** (2.75X)	151.7** (4.80X)
	Term	34.9	35.3	51.3** (1.47X)	67.6** (1.94X)	120.7** (3.46X)

Significant when compared with control: * $p<0.05$; ** $p<0.01$
Term = End of treatment period

Gross pathology and histopathology correlates:
Mean thyroid and parathyroid weights were increased in high dose males (1.64-fold) and females (1.37-fold).
The following gross observations were recorded at necropsy (*tables excerpted from sponsor*):

Table 25: Gross Findings in Lungs and Bronchi

Group/Sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Pale area(s)	22	37	31	31	40
No. of animals examined	65	65	65	65	65

Table 26: Gross Findings in Liver

Group/Sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Dark area(s) No. of animals examined	12 65	12 65	20 65	11 65	17 65

Table 27: Gross Findings in Thyroids

Group/Sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Mass(es) No. of animals examined	2 65	6 65	6 65	3 65	4 65

Table 28: General Comments

Group/Sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Abnormal contents of GI tract (pale/white) No. of animals examined	6 65	9 65	18 65	15 65	32 65

Histopathology:

Neoplastic:

Liver: Increased incidence of hepatocellular adenomas in female rats at all doses and male rats at all but the low dose was not statistically significant in the trend analysis of pairwise comparisons.

Thyroid: A statistically significant increase in the incidence of follicular cell adenomas with carcinoma was seen in males and females from all dose groups (see table below, *excerpted from sponsor*).

Table 29: Summary of Treatment-related Findings in the Thyroid

Group/sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Follicular cell adenoma Total	1	3	4	5	5
Follicular cell carcinoma Total	0	0	2	1	2
Number of tissues examined	65	65	65	65	65
Total adenomas	1		17		
Percentage	1.5%		6.54%		
Total carcinomas	0		5		
Percentage	0.0%		1.92%		

Group/sex Dose (mg eq./kg/day)	1F 0	2F 40	3F 200	4F 500	5F 1500
Follicular cell adenoma Total	0	0	3	3	4
Follicular cell carcinoma Total	0	1	1	0	1
Number of tissues examined	65	65	65	65	65
Total adenomas	0		10		
Percentage	0.0%		3.85%		
Total carcinomas	0		3		
Percentage	0.0%		1.15%		

Skin: The increase in the incidence of skin lipoma in high dose males was statistically significant with respect to dose response, but was not considered to be related to treatment with TMC278, as the incidence was just above historical background levels, was not significantly increased in high dose males when compared to controls, and was not associated with pre-neoplastic findings in fat.

Mammary Gland: Mammary adenomas in female rats were found to have a statistically significant dose response. There were no dose-related histopathological findings that suggest this finding was related to treatment. Also, historical control data indicates that the incidence rate for mammary adenomas ranges from 1.14 to 32.00%.

Table 30: Results of Time-to-tumor Analysis for Benign Adenoma in Female Mammary Areas

Group	Dose level (mg eq./kg/day)	Initial group size	Number examined	Number of animals with tumours		Relative tumour rate (O/E)	Pairwise comparison *p*-value†	Trend test *p*-value#
				Observed (O)	Expected (E)			
1	0	65	65	0	0.99	0.00		
2	40	65	65	0	1.03	0.00	-	
3	200	65	65	0	1.02	0.00	-	
4	500	65	65	3	0.93	3.23	0.110	
5	1500	65	65	2	1.03	1.94	0.274	0.063

† One-tailed pairwise comparisons against control.
# One-tailed trend test using groups up to the respective row.

Non-neoplastic:
Non-neoplastic findings were noted in thyroid tissue from females at all dose levels and in males at 200, 500 and 1500 mg/kg (follicular cell hypertrophy and cystic follicular cell hyperplasia). Non-neoplastic findings were noted in nasal turbinates in high dose males and females (inflammatory exudate with hyperplasia and inflammatory cell infiltration of the respiratory epithelium with or without respiratory metaplasia of the olfactory epithelium).

Table 31: Non-neoplastic Findings in Thyroid Tissue from Males

Group/sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Follicular cell hypertrophy					
Minimal	1	0	2	1	1
Slight	0	1	2	3	3
Total	1	1	4	4	4
Cystic follicular cell hyperplasia					
Minimal	3	1	3	0	5
Slight	3	4	2	2	1
Moderate	0	0	0	0	1
Total	6	5	5	2	7
Number of tissues examined	65	65	65	65	65

Table 32: Non-neoplastic Findings in Nasal Turbinates from Males

Group/sex Dose (mg eq./kg/day)	1M 0	2M 40	3M 200	4M 500	5M 1500
Inflammatory exudate					
Total	1	2	2	3	39
Respiratory epithelium - hyperplasia					
Minimal	0	0	0	0	8
Slight	0	0	1	0	11
Moderate	0	0	0	0	5
Total	0	0	1	0	24
Respiratory epithelium - inflammation					
Minimal	2	0	0	0	5
Slight	0	0	0	1	17
Moderate	0	0	0	0	3
Total	2	0	0	1	25
Olfactory epithelium - respiratory metaplasia					
Minimal	0	0	0	0	0
Slight	0	0	0	0	5
Moderate	0	0	0	0	3
Total	0	0	0	0	8
Number of tissues examined	65	65	65	65	65

Table 33: Non-neoplastic Findings in Thyroid Tissue from Females

Group/sex Dose (mg eq./kg/day)	1F 0	2F 40	3F 200	4F 500	5F 1500
Follicular cell hypertrophy					
Minimal	0	1	3	1	6
Slight	0	1	1	1	2
Total	0	2	4	2	8
Cystic follicular cell hyperplasia					
Minimal	0	1	2	2	2
Slight	0	1	0	3	3
Moderate	0	0	0	0	0
Total	0	2	2	5	5
Number of tissues examined	65	65	65	65	65

Table 34: Non-neoplastic Findings in Nasal Turbinates from Females

Group/sex Dose (mg eq./kg/day)	1F 0	2F 40	3F 200	4F 500	5F 1500
Inflammatory exudate					
Total	0	0	0	1	26
Respiratory epithelium - hyperplasia					
Minimal	0	0	0	1	4
Slight	0	0	0	0	15
Moderate	0	0	0	0	2
Total	0	0	0	1	21
Respiratory epithelium - inflammation					
Minimal	0	0	0	1	2
Slight	0	0	0	0	11
Moderate	0	0	0	0	4
Total	0	0	0	1	17
Olfactory epithelium - respiratory metaplasia					
Minimal	0	0	0	0	2
Slight	0	0	0	0	7
Moderate	0	0	0	0	2
Total	0	0	0	0	11
Number of tissues examined	65	65	65	65	65

Toxicokinetics: Blood samples for toxicokinetic analysis were collected at 0.5, 2, 4, 8, 12 and 24 hours post-dose on Day 1, and during Week 27 and Week 39. Blood samples collected at necropsy (Week 60) were not analyzed. In male rats, systemic exposures to test article decreased with repeated exposures. Values for AUC were 2 to 4-fold lower at weeks 27 and 39 compared with Day 1. In females, systemic exposures were not significantly changed by repeated administration. As a result, sex-related differences in exposures were apparent at weeks 27 and 39 (see table below, *excerpted from sponsor*).

Table 35: Toxicokinetics of Rilpivirine Following Up to 39 Weeks of Oral Administration to Rats

	Day 1				Week 27				Week 39			
Dose (mg eq./kg)	40	200	500	1500	40	200	500	1500	40	200	500	1500
	Males											
C_{max} (µg/ml)	1.57	2.60	4.48	6.07	0.852	1.18	2.04	2.73	0.821	1.30	1.77	2.06
AUC (µg.h/ml)	19.1	33.5	44.5	57.7	4.54	11.3	15.5	20.1	6.26	8.15	13.8	18.4
	Females											
C_{max} (µg/ml)	2.16	4.23	6.47	6.97	2.23	5.23	5.69	10.0	2.11	4.72	8.47	9.38
AUC (µg.h/ml)	17.0	39.5	63.0	81.3	14.8	36.3	69.8	81.2	14.2	41.2	46.3	83.8

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9 Reproductive and Developmental Toxicology

The reproductive and developmental toxicology studies were previously reviewed (see Appendix 1D and 1E). Findings are summarized below.

9.1 Fertility and Early Embryonic Development

Three groups of 25 male Sprague-Dawley rats were dosed once daily with TMC278.HC1 (R314585) at dose levels of 100, 400 or 1600 mg/kg/day. A similar group of females received vehicle only and served as untreated controls. The males were dosed for 10 weeks prior to mating, during mating and for approximately 3-4 days after the mating period. Four groups of 25 females of the same strain were untreated and served as mates for the males. After the pre-mating dosing period, each male was paired with an untreated female, for up to ten days. The pregnant females were killed and subject to necropsy on Day 13 of gestation. The number of corpora lutea, implantations and live embryos were recorded. There was no effect upon any pregnancy parameter in any of the treated female groups. The number of corpora lutea, the extent of pre-and post-implantation loss and the number of live embryos in all groups were comparable

with the untreated controls. The NOAEL for male fertility was considered to be 1600 mg/kg for this study. Toxicokinetic parameters were not assessed.

Three groups of 24 female Sprague-Dawley rats were dosed once daily with TMC278 at dose levels of 40, 120, or 400 mg eq/kg/day. Test article was administered for 14 days prior to mating, during mating and up to day 7 of presumed pregnancy. There were no effects noted on estrus cycle or median pre-coital interval, copulation index, fertility rate, weight of gravid uterus, numbers of corpora lutea, implantations, live embryos or the extent of pre- or post-implantation loss. The NOAEL for female fertility was considered to be 400 mg/kg for this study. Toxicokinetic parameters were not assessed.

9.2 Embryonic Fetal Development

Studies on embryofetal development in rats and rabbits were reviewed previously (Appendix 1A). In rats, there was a slight increase in the incidence of dilated renal pelvis at the middle dose ($p<0.05$) and high dose ($p<0.01$). One high dose fetus exhibited absent skull bones, open vertebral arches and tongue protrusion. Another high dose fetus had deformed forelegs, tongue protrusion and local edema. All long bones of the fore and hindlimbs, pectoral and pelvic girdle bones and mandible of this fetus were bowed, ribs were wavy and ossification was generally reduced. Fetuses of the high dose group showed slightly increased incidences of reduced ossification of sternebrae and metacarpal bones ($p<0.05$). These findings were confined to fetuses of two litters, and were considered to be of minimal toxicological significance. In both pregnant females and fetuses the NOAEL was defined as the low dose (40 mg/kg) with an associated systemic exposure of 36.8 µg.h/ml (maternal AUC on Day 16). In pregnant females the NOAEL was based on slight dose related increases in the thyroid weight in the 120 and 400 mg/kg/day dose groups. In fetuses, the NOAEL was based on increased incidence of dilated renal pelvis.

In rabbits dosed with 5, 10, or 20 mg/kg/day, there were a number of major abnormalities in specific fetuses but no two the same and nothing significant on a per litter basis. Litters from six females were affected, three low dose, one middle dose, and two high dose animals. The incidence of branches of the left subclavian artery originating from the aorta was slightly increased in all treated groups compared to controls. This reached significance ($p<0.05$) in the high dose group and may be treatment related. The incidence of absent or hypoplastic interparietal bone was increased in all treated groups and reached significance in the high dose group. Those findings were considered to be of minimal toxicological significance. The NOAEL for pregnant females was considered to be at least 20 mg/kg/day (AUC = 232 µg.h/ml) based on a lack of findings at the high dose, and the NOAEL for fetuses was considered to be 10 mg/kg/day (maternal AUC = 170 µg.h/ml) based on the increased incidence of

branches of the left subclavian artery originating from the aorta, which reached statistical significance at the high dose.

9.3 Prenatal and Postnatal Development

No studies have been conducted to assess directly the excretion of TMC278 into milk. In a quantitative whole body autoradiography study in pregnant Sprague Dawley rats, some radioactivity was seen in the mammary glands (tissue/blood AUC0-8h ratio = 3), which indicates the potential for excretion of TMC278-related radioactivity via the milk. In a dose range finding study for a pre- and postnatal developmental study it was found that pups were exposed to TMC278 through the milk of the dams dosed with TMC278 (40, 120 and 400 mg/kg/day). On Day 7 of lactation, exposure (AUC0-24h) in pups was 0.62 and 0.74 µg.h/mL at 40 mg/kg, 0.94 and 0.91 µg.h/mL at 120 mg/kg and 1.9 and 1.8 µg.h/mL at 400 mg/kg in males and females, respectively. Exposure in pups dosed through milk on Day 7 of lactation was approximately 20- to 35-fold lower than in pups directly dosed by oral gavage on Day 25 of age.

A pre- and postnatal fertility study in rats was reviewed previously (Appendix 1G). In summary, F_0 females treated with 40, 120 or 400 mg/kg TMC278.HCl during gestation and lactation had no remarkable toxicological effects. The NOAEL for maternal toxicity was 400 mg/kg. Likewise, there was no effect of maternal treatment with TMC278.HCl on pup growth and no effect on pup survival or development during lactation or post-weaning. The NOAEL for pup development after maternal treatment was 400 mg/kg. Finally, there was no effect of maternal treatment with TMC278.HCl on fertility or mating performance of the F_1 males or females or on gestation of the F_1 females. The NOAEL was 400 mg/kg. Toxicokinetic parameters were not assessed in this study.

10. Special Toxicology Studies

10.1. Endocrine Studies

To clarify the effects of Rilpivirine on adrenal gland function and cortisol synthesis, the effects of Rilpivirine were assessed using isolated primary guinea-pig adrenal cells in culture. In summary, no decrease in cortisol levels was observed at low concentrations of Rilpivirine, but a moderate decrease in cortisol was observed at the high concentration. The median inhibition concentration (IC_{50}) value was 6.04×10^{-6}M.

In a separate study, adrenal gland toxicity and cortisol synthesis was assessed using crude, subcellular fractions of dog adrenal gland tissue. Specifically, the effects of Rilpivirine and a positive control on the metabolism of pregnenolone were studied. 75 ng/mL of Rilpivirine caused an overall 39% inhibition of the

conversion of pregnenolone when compared to the vehicle control. There was a dose-dependent increase in the progesterone : 11-deoxycorticosterone ratio and an increase in the 17-OH-progesterone : 11-deoxycortisol ratio in cell-free extracts. At 100 µM Rilpivirine, there was complete inhibition of corticosterone and 11-deoxycortisol synthesis. The study showed that the site of inhibition was at 21α-hydroxylase. The inhibition curve for steroid biosynthesis did not show saturation up to 100 µM.

A special endocrine study in dogs, previously reviewed by Alex Jordan, Ph.D. (Appendix 1A) is summarized here. A separate endocrine study in monkeys is reviewed below.

A single dose oral endocrinology study was performed in male beagles at doses of 20 and 80 mg/kg. TMC278 was formulated in PEG400 with 100 mg/ml citric acid. A vehicle control group was included. Plasma cortisol, ACTH and aldosterone levels were measured on the day of dosing and six days later. A CRF (Corticotropin Releasing Factor)-challenge test was done one day after dosing and six days later.

A single dose of TMC278 did not adversely affect plasma cortisol, aldosterone and ACTH levels. Results of the CRF challenge test were comparable between treated and vehicle control dogs indicating a normal functioning hypophysis and adrenal cortex.

Study Title Endocrinological oral (gavage) 8-week study in the female sexually immature cynomolgus monkey

Study no.:	TMC278-TiDP38-NC248-TOX8862
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11 August 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	TMC278 HCl, batch no. ZR314585PUA311,

Key Study Findings

This study assessed the effects of eight weeks of treatment with 200 and 500 mg/kg TMC278 HCl (100 mg/kg BID or 250 mg/kg BID) on adrenal gland physiology and sexual maturity of female cynomolgus monkeys. Hormonal changes are consistent with an inhibitory effect on steroid metabolism in the adrenal gland.

Methods

Doses:	100 and 250 mg/kg
Frequency of dosing:	Twice daily for 8 weeks
Route of administration:	Oral
Dose volume:	5 mL/kg/dose
Formulation/Vehicle:	1% w/v Methocel
Species/Strain:	Cynomolgus monkey/*Macaca fascicularis*
Number/Sex/Group:	8
Age:	1-2 years predose
Weight:	1.5-2.0 kg
Satellite groups:	none
Unique study design:	ACTH stimulation was used to diagnose effects on adrenal steroidogenesis.
Deviation from study protocol:	No deviations were noted.

Observations and Results

Mortality

There were no early deaths.

Clinical Signs

Respiratory signs (panting or raspy breathing) were present in 1/8 animals in each treated group but not in untreated controls.

Body Weights

There was no effect of treatment on body weight.

Menses

There were no effects noted.

Hematology

No remarkable findings.

Clinical Chemistry

No remarkable findings.

Urinalysis

No remarkable findings.

Gross Pathology

Ovarian cysts noted in high dose monkeys were regarded by the sponsor as background findings.

Organ Weights

Adrenal glands, brain, heart, kidney, liver, pituitary, spleen, ovaries, and thyroid/parathyroid were weighed. There were no remarkable findings.

Histopathology

Adequate Battery

Yes.

Peer Review

None noted.

Histological Findings

Slight, diffuse thyroid follicular cell hypertrophy was noted in 1/8 group 1 (untreated controls) animals, 3/8 group 2 (100 mg/kg BID) animals and 4/8 group 3 (250 mg/kg BID) animals. The sponsor considered ovarian cysts (severe, follicular, focal) seen in two group 3 animals to be normal background findings in Cynomolgus monkeys.

Special Evaluation

ACTH Stimulation Test

Cortisol: Treatment with TMC278 HCl resulted in a decrease in baseline cortisol levels in all groups, from approximately 1.600 nmol/L at pre-dose to approximately 1.100 nmol/L on dosing day 43. On stimulation with ACTH, there was no apparent effect of treatment on cortisol response. Cortisol levels increased similarly in all groups including controls.

17-hydroxyprogesterone (17-HP): Baseline levels of 17-HP were not affected by treatment with TMC278; however, stimulated levels were higher in treated animals. Levels of 17-HP rose 3.8-fold over baseline in group 2 animals, and 3.2-fold in group 3 animals following ACTH stimulation, compared to a 2.2-fold increase in untreated controls post-stimulation.

Progesterone: Progesterone levels following ACTH challenge were markedly increased in treated animals, with the greatest effect noted on day 15 of dosing. Progesterone levels increased 103% in group 2 animals and

113% in group 3 animals, compared to a 70% increase in group 1 (untreated controls).

Androstenedione: Baseline levels for androstenedione in treated animals were lower than untreated controls, but without a clear dose response.

5-Dehydroepiandrosterone (DHEA): DHEA levels tended to be lower in high dose animals.

In summarizing the study results, the sponsor-selected expert in endocrinology concluded that the hormonal changes seen in this study are considered to be related to treatment with Rilpivirine and demonstrate that Rilpivirine has an influence on the adrenal steroidogenesis via inhibition of one or more enzymes. An inhibitory effect on 21α-hydroxylase is suggested by the increase in the proximal substrate of 17-OH-progesterone and progesterone, thus impairing synthesis of both the glucocorticoid and mineralocorticoid pathway, respectively. This was seen in a dose-response manner. The decline in DHEA is considered to be highly suggestive of a 17α-hydroxylase inhibition as well. Also, it was concluded that Rilpivirine likely has an inhibitory influence on 17,20 lyase, as evidenced by the increased baseline and stimulated levels of 17-OH-progesterone and the decreased levels of the androgens, DHEA and androstenedione. However, this inhibition cannot explain the increased baseline and stimulated levels of progesterone.

Regarding gonadal function, there was neither any histological abnormality observed in the ovaries, nor any reduction in circulating estrogen, nor any elevation in pituitary luteinizing hormone (LH) secretion. As expected, histological analysis of these immature cynomolgus monkey ovaries at termination of the study revealed immature ovaries. No effect on ovulation could be detected. As concluded by the endocrinology expert's analysis, this data suggests that the enzyme inhibitory effect is limited to the adrenal gland, since no effects on LH plasma levels and no histopathological effects on the ovaries could be observed. The treatment-related changes on the basal progesterone and estradiol levels are considered to be due to the effects on the adrenal steroidogenesis. Adrenal steroidogenesis was clearly affected, but it is likely only a partial enzymatic blockade. This conclusion was based on the observations that the drug did not result in adrenal crisis (severe vascular collapse), hypotension or electrolyte abnormalities. These immature female monkeys showed no evidence of ovarian pathology, but this might be a function of their pre-pubertal status.

Toxicokinetics

Exposure to TMC278 increased less than dose-proportionally between 100 to 250 mg/kg BID. Systemic exposures following repeated dosing were similar to Day 1 exposures in a previously performed study (b) (4) study 2041-011; see table below *excerpted from sponsor*).

Table 36: Toxicokinetic Parameters of TMC278 in Monkeys following Repeated BID Doses

	Female	
Group	2 (Intermediate)	3 (High)[1)]
Dose (mg eq./kg/day)	2 x 100[2)]	2 x 250[2)]
	Day 55	
C_{max1} (ng/ml)	144	313
C_{max2} (ng/ml)	181	307
AUC_{0-24h} (ng.h/ml)	2684	4620

[1)] n = 7

2) Bid (bis in die = twice a day) dosing; 8 hours apart

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Stability and Homogeneity

According to the study report, ten day stability and homogeneity assessments of TMC278 HCl in 1% Methocel with 0.5% Tween 20 at +5°C ±3°C were performed by (b) (4) before the first day of dosing of the test item. Results were found to be acceptable.

10.2. Local Tolerance

Several local tolerance studies were previously reviewed (Appendix 1G). In summary, TMC278.HCl [20% (m/m) suspension] induced an increase in corneal opacity (no increase in permeability.) and the drug is now classified as a moderate eye irritant (*in vitro* score=32.5). In a study of dermal toxicity, TMC278 did not induce significant or irreversible damage to the skin and is considered to be "not irritating" to rabbit skin. Likewise, TMC278 (HCl) and nanosuspension formulations of TMC278 (base) failed to elicit proliferative lymph nodal responses higher than those observed for control groups with stimulation index values less than 3 fold for all test concentrations and routes of exposure. This conclusion holds true even when the skin barrier is breeched via subcutaneous injection. Based on this finding the sponsor suggested that TMC278 (base) is unlikely to posses sensitizing properties.

10.3. Juvenile Study

While no studies in juvenile animals were conducted *per se*, the effects of direct administration of Rilpivirine for two weeks on postnatal development were evaluated in rat pups. Pups were taken from vehicle-treated dams that were part

of a dose range finding peri- and postnatal development study. Selected pups were dosed with 400 mg/kg/day by oral gavage from LD 12 up to and including LD 25. They were observed regularly for clinical signs, morbidity, and mortality and their body weight was recorded. Blood samples for toxicokinetics were taken on LD 25. Necropsy was performed after the last sampling.

Two pups from the group treated with 400 mg/kg/day showed decreased activity and decreased body weight and were sacrificed moribund on day 20 and day 24 of age, respectively. Other clinical signs included cold body surface and extremities, rapid breathing, piloerection and unsteady gait. At necropsy, one of the sacrificed pups had incompletely collapsed lungs and adhesions in the thoracic cavity, likely due to gavage trauma. There were no necropsy findings in the second euthanized pup. There were no other findings.

Systemic exposures in dosed pups are presented below (*table excerpted from sponsor*).

Table 37: Systemic Exposures to Rilpivirine in Rat Pups by Lactation and After Two Weeks of Oral Dosing with Rilpivirine

Dose (mg/kg/day)	Sampling Day	C_{max} (µg/mL)		AUC_{0-24h} (µg.h/mL)	
		M	F	M	F
40	LD7 [a]	0.05	0.07	0.62	0.74
	LD25 [b]	2.6	5.8	12	18
120	LD7	0.08	0.08	0.94	0.91
	LD25	3.7	3.6	34	28
400	LD7	0.16	0.14	1.9	1.8
	LD25	9.1	7.3	50	53
400*	LD25	6.4	4.9	43	39

M: males, F: females, LD: lactation day, [a]: pups from dams dosed as indicated exposed in utero and via lactation (n = 12/sex), [b]: pups from dams as indicated exposed in utero and via lactation and dosed by gavage from LD12 – LD25 (n = 8/sex), *: pups from control dams dosed by gavage from LD12 – LD25 (n = 8/sex)

11. Integrated Summary and Safety Evaluation

Rilpivirine (TMC278 HCl), a diarylpyrimidine derivative, is a non-nucleoside reverse transcriptase inhibitor (NNRTI). Rilpivirine is indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-naïve adult patients.

Absorption of an oral dose in nonclinical species ranged from 24% in monkeys to 80% in dogs. Rilpivirine is extensively (>99%) bound to serum proteins and rapidly and widely distributed among tissues, including the placenta and fetus, following an intravenous dose. Plasma half-lives varied greatly among nonclinical species, from 4.4 hours in the rat to 31 hours in the dog. In humans, the median terminal half-life of Rilpivirine was approximately 45 to 50 hours. Metabolism in mice and dogs (and humans) is predominantly via oxidation, whereas conjugation accounts for the primary metabolism pathways in rats. In

nonclinical species and humans, plasma concentrations of parent drug were greater than all metabolites combined. Rilpivirine in the parent form was primarily eliminated by fecal excretion.

Repeat dose studies were performed in mice (up to 3 months), rats (up to 6 months), non-pregnant rabbits (up to 5 days), dogs (up to 12 months), and immature female cynomolgus monkeys (up to 8 weeks). *In vitro* and *in vivo* genotoxicity studies were conducted. Two-year oral carcinogenicity studies were conducted in mice and rats. The reproductive and developmental toxicity studies included fertility studies in male and female rats, embryo-fetal developmental studies in rats and rabbits, and a peri- and postnatal developmental study in rats.

Early nonclinical toxicology studies were performed using Rilpivirine base. Later studies used Rilpivirine HCl, the current clinical formulation. One month repeat dose bridging studies in rats and dogs were conducted to compare the toxicity of the two formulations and there were no significant differences.

No adverse effects of Rilpivirine on the cardiovascular, respiratory, or central nervous systems were noted during initial safety pharmacology studies. Subsequently, a Phase I clinical trial demonstrated a QT interval-prolonging effect of Rilpivirine at supratherapeutic doses. In follow-up safety pharmacology studies, Rilpivirine demonstrated the potential to inhibit some potassium channels involved in cardiac action potential repolarization. Adverse events that could be related to cardiac conduction abnormalities or to rate and rhythm disturbances were monitored in the Phase IIb and Phase III clinical trials. No clinically relevant QTc prolonging effect was observed with the recommended therapeutic dose of TMC278 25 mg q.d., although it should be noted that patients with risk for QT interval prolongation or Torsade de Pointes were excluded from the Phase III trials.

A summary of systemic exposures, related NOAELs, and margins of exposure (ratio of nonclinical : clinical exposures) is presented in tabular form below.

Table 38: Systemic Exposure Margins for Rilpivirine in Nonclinical Repeat Dose Toxicity Studies.

Species	Study Type	Dose (mg/kg/day)	AUC $_{0\text{-}24hr}$ (µg.hr/mL)		Margin (Ratio of Animal to Human Exposure)[a]	
			Male	Female	Male	Female
Mouse	3-month	20 (NOAEL)	80	61	33	25
	Carcinogenicity	20 (LOAEL)	76	51	32	21
Rat	1-month	10 (NOAEL)	7.2	14.0	3	6
	6-month	40 (LOAEL)	19.3	32.1	8	13

Species	Study Type	Dose (mg/kg/day)	AUC $_{0\text{-}24hr}$ (µg.hr/mL)		Margin (Ratio of Animal to Human Exposure)[a]	
			Male	Female	Male	Female
Rat	Teratogenicity	40 (Female NOAEL)		37		15
	Carcinogenicity	40 (LOAEL)	6.3	14	3	6
Rabbit	Teratogenicity	10 (Fetal NOAEL)		170		70
Dog	1-month	5 (LOAEL)	27.1	36.8	11	15
	6-month	5 (LOAEL)	21.1	17.4	9	7
	12-month	5 (LOAEL)	17.4	18.7	7	8
Cynomolgus monkey	8-week	200 (LOAEL)		2.7		1.1
Human		25 mg q.d.	2.4			

[a]AUC 2.4 µg.hr/mL in clinical Phase III study (25 mg q.d.)

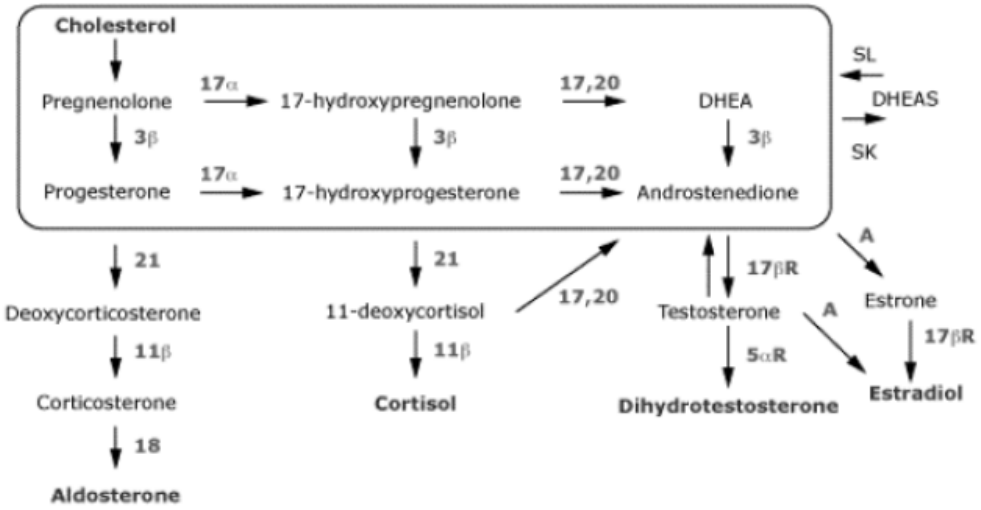
The following target organs were identified in repeat dose studies:

Adrenal gland

The primary toxicity findings in nonclinical studies were adrenal effects observed in rats, dogs, Cynomolgus monkeys, and possibly mice. These effects are thought to be associated with an inhibition of the steroidogenesis at the level of 21-hydroxylase (dogs and Cynomolgus monkeys) and 17-hydroxylase (Cynomolgus monkeys).

In repeat dose studies in dogs, the main toxicological effect was on steroidogenesis, with swollen ovaries, uterus and vagina in females and reduced spermiogenesis, Leydig cell hyperplasia and debris in epididymis in males. There were changes in endocrine parameters including higher progesterone and 17αOH-progesterone concentrations in males but not in females. Lower cortisol concentrations were seen in treated females throughout the treatment period whereas there were no effects or only slight effects in males. There was a dose related increase in the weight of the adrenals with increased cellular density in zona fasciculata and reticularis and a reduction in neutral fat deposit in all dosed females and high dose males. There was an increase in ovarian weight and numbers of antral follicles and prominent early luteinized follicles at the high dose. Prominent corpora lutea were seen at the middle

dose. In males, there was minimal hypertrophy of the Leydig cells in high dose dogs but without changes in the spermatogenic cycle.



The first step in adrenal steroid synthesis is the combination of acetyl CoA and squalene to form cholesterol, which is then converted into pregnenolone. The enclosed area contains the core steroidogenic pathway utilized by the adrenal glands and gonads.

17α = 17α-hydrolylase (CYP17, P450c17); 17,20 = 17,20 lyase (also mediated by CYP17); 3β = 3β-hydroxysteroid dehydrogenase; 21 = 21-hydroxylase (CYP21A2, P450c21); 11β = 11β-hydroxylase (CYP11B1, P450c11); 18 refers to the 2-step process of aldosterone synthase (CYP11B2, P450c11as), resulting in the addition of an hydroxyl group that is then oxidized to an aldehyde group at the 18-carbon position; 17βR = 17β-reductase; 5αR = 5α-reductase; DHEA = dehydroepiandrostenedione; DHEAS = DHEA sulfate; A = aromatase (CYP19)

Figure 2: Synthetic Pathways for Adrenal Steroid Synthesis in Non-Human Primates and Humans

A study in immature (approximately 18 months of age) female Cynomolgus monkeys assessed the effects of eight weeks of treatment with 200 and 500 mg/kg TMC278 HCl (100 mg/kg BID or 250 mg/kg BID) on adrenal gland physiology and sexual maturity. Observed hormonal changes were consistent with an inhibitory effect on steroid metabolism in the adrenal gland. The premature ovulation noted in immature dogs treated for four weeks was not found in immature Cynomolgus monkeys, although the lack of an early puberty effect in the monkeys may be related to the young age of the monkeys and the fact that the monkeys were still pre-pubertal at the end of the study. Female Cynomolgus monkeys reach sexual maturity at approximately four years of age.

Endocrine monitoring, including gonadal, adrenal and thyroid parameters, was included in clinical trials in order to assess adrenal and thyroid function. Additional safety assessments relating to adrenal function included ACTH stimulation testing, with measurements of basal and stimulated cortisol, 17-hydroxy (OH)

progesterone, and aldosterone (the latter only in Phase III trials). Basal DHEA sulphate, progesterone, androstenedione, testosterone and LH were measured in Phase IIb and III trials. There were no apparent increases in the incidence of endocrine events in Phase IIb or Phase III clinical trials.

As noted above, in dogs adrenal effects were characterized by early puberty, i.e., premature activation and overstimulation of the ovaries. The potential for Rilpivirine to cause early puberty in humans has not been assessed in clinical trials. On-going and planned trials in adolescents and pre-pubertal children should include endocrine safety monitoring, with monitoring of hormone levels and ovulation. Growth curves, pubertal status, breast development, menarche or evidence of either hyperandrogenism (hirsutism) or delayed adrenarche should be documented in these trials.

Liver

The hepatic effects observed following repeated administration of Rilpivirine to mice include increased incidence of hepatocellular hypertrophy (centrilobular to diffuse), and a moderate increase in single cell necrosis in high dose males and females, with minimal to slight focal necrosis in one female and one male at the middle dose and two females and three males at the high dose. Findings in rats included increased liver weights. In mice and rats, hepatic effects are likely due to a rodent-specific mechanism related to induction of metabolism enzymes. In dogs, pigmentation of liver and gall bladder cells, indicative of suppression of bile flow, was noted after three months or more of Rilpivirine administration. In dogs, hepatic findings were present at exposures approximately 10-fold the exposure in humans at the recommended clinical dose. No effect on bile flow has been noted in clinical trials.

Thyroid

In rats, dose related increases in diffuse follicular hypertrophy in the thyroid glands in males and females at all doses following six months of Rilpivirine administration correlated with increased thyroid gland weights. The thyroid changes were likely due to a rodent-specific mechanism related to altered metabolism of thyroid-related hormones. An increased incidence of swollen-vacuolated cells in the *pars distalis* of the pituitary in male rats from all treated groups was likely also related to altered thyroid hormone metabolism.

Kidney

In the nonclinical studies, histopathological effects in kidney were only observed in mouse and dog, although an increase in serum creatinine was noted after five days of dosing in rabbits (no histopathology assessment was conducted). For mice, in a one month study, changes in blood electrolytes are considered to be related to kidney histopathology findings, primarily seen in high dose mice, including minimal to moderate degenerative/necrotic nephropathy. In the 3-month study, renal findings were limited to mild to moderate nephropathy at the high dose, 320 mg/kg/day, which corresponded to systemic exposures more than 200-fold the human exposures at the recommended clinical dose.

Findings in the dog were only noted in a 12-month study at the highest dose of 40 mg/kg/day, corresponding to more than 25-fold the exposure in man at the recommended clinical dose. In the Phase III clinical trials, mean increases in serum creatinine and mean decreases in $eGFR_{creat}$ were seen over time with Rilpivirine. Using creatinine as a marker for estimated glomerular filtration rate (eGFR), there was a mean decrease from baseline in $eGFR_{creat}$ in the Rilpivirine group, which was stable over time. However, when GFR was estimated with a second biomarker, cystatin C, no decrease was noted; rather, there was an increase in $eGFR_{cyst}$. The cystatin C results indicate that there was no Rilpivirine-induced nephrotoxicity.

Coagulation

A slight to moderate increase in coagulation parameters, specifically activated partial thromboplastin time (APTT) and prothrombin time, was seen in male rats dosed for six months. The increases in APTT and prothrombin times were still present in high dose males after recovery. In clinical trials, there were no clinically relevant effects on coagulation.

Hematology

Slight decreases in RBC's, hemoglobin, hematocrit and eosinophils were noted in high dose (400 mg/kg) male rats. In female rats, there was a slight decrease in eosinophils at all doses and a marginal decrease in mean cell volume and mean cell hemoglobin at the middle and high doses. Hematology parameters had returned to normal at the end of the recovery period.

In dogs, changes to hematology parameters (decreased RBCs, hematocrit and hemoglobin and increased WBCs in high dose animals) were normal at the end of the one month recovery period.

> In mice, slight (less than 10%) decreases were seen in red blood cells (RBCs), hemoglobin, and hematocrit in high dose animals. There was a slight decrease in neutrophils (30%) and moderate decreases in lymphocytes and eosinophils (~50%) in high dose group males, but a slight decrease in thrombocytes (12%) and a slight increase in the number and % reticulocytes (50%) in high dose females.
>
> The mechanism of action leading to a decrease in red blood cell parameters in rats, dogs and mice has not been determined. There were no indications of bone marrow suppression. Changes in hematology parameters were noted in high dose group animals only and appear to be reversible. In clinical trials, no significant hemoglobin abnormalities were noted in Phase 3 trials with Rilpivirine 25 mg q.d.

There were no significant findings in *in vitro* and *in vivo* genotoxicity assays.

Due to the chronic nature of the indication (HIV infection), two carcinogenicity studies (a 2 year study in mice and a 2 year study in rats) were conducted. At the lowest tested doses in the carcinogenicity studies, the systemic exposures (based on AUC) to Rilpivirine were 21-fold (mice) and 3-fold (rats), relative to those observed in humans at the recommended dose (25 mg q.d.).

The tumor types that showed the greatest increase in treated mice, and for which the increase was statistically significant, were hepatocellular adenomas and hepatocellular adenomas-carcinomas (combined). In males, the incidence rates for those tumors reached statistical significance at the middle and high doses. The incidence of hepatocellular carcinomas was increased at all doses but the increases did not reach statistical significance compared to controls. In females, the incidence of each tumor type alone, and the combined incidence of hepatocellular adenomas and carcinomas reached statistical significance at the middle and high dose. In a three month study in mice, hepatocyte hypertrophy, moderate increases in single cell necrosis, and accumulation of Kupffer cell pigmentation was noted. Since Rilpivirine is known to be associated with liver changes, an increase in adenomas and a slight increase in carcinomas in the two year study in mice might have been anticipated. The findings are considered to be treatment related; however, the increased incidence of liver tumors may result from a rodent-specific, non-genotoxic mechanism related to hepatic CYP form induction.

The tumor types that showed the greatest increase in rats were thyroid follicular cell adenomas and adenomas – carcinomas (combined), although the increased incidence does not reach statistical significance. The tumor findings correlated with increased thyroid weights and increased serum thyroid stimulating hormone

levels, among other effects. Rilpivirine slightly increased UDPGT activity in an *ex vivo* assay, and administration of Rilpivirine to rats for three months was associated with an induction of CYP enzymes and UDPGT. Therefore, the increased incidence of thyroid tumors at the high dose may result from a rodent-specific mechanism related to altered metabolism of thyroid-related hormones.

There were no significant findings related to fertility, and no clear indication of effects on the developing fetus. In rats, an increased incidence of dilated renal pelvis in fetuses at middle and high doses, and in rabbits an increased incidence of branching of the left subclavian artery originating from the aorta and hypoplastic intraparietal bones in fetuses at the high dose are considered to be of minor toxicological or biological significance. Exposures in rats and rabbits were approximately 15- and 70-fold higher, respectively, than exposures at the recommended clinical dose.

Regarding studies in neonatal and juvenile animals, in an embryo-fetal development toxicity study, rat pups delivered by treated dams were dosed for two weeks with Rilpivirine. There were no test article-related effects on the pups. No specific juvenile studies were conducted in dogs; however, in all dog studies the animals were considered to be sexually immature at the start of dosing.

In local tolerance tests, Rilpivirine was negative for phototoxicity (*in vitro*), skin irritation (rabbits) and delayed-type hypersensitivity (mouse). Rilpivirine was a moderate eye irritant *in vitro*.

12. References

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Appendix 1A

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 67,699
Review number: 1
Sequence number/date/type of submission: 000/9/29/04/original IND
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Tibotec, Inc
Manufacturer for drug substance: J&J Pharm Res and Dev, or Janssen Pharm, Belgium

Reviewer name: Alex Jordan
Division name: DAVDP
HFD #: 530
Review completion date: 12/9/04

Drug:

Trade name:
Generic name:
Code name: R 314585 or TMC278 (HCl salt) or R278474 (free base)
Chemical name: (E) 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylpheny]amino]-2-pyrimidinyl]amino] benzonitrile
CAS registry number: 700361-47-3
Molecular formula/molecular weight: 366.42
Structure:

Relevant INDs/NDAs/DMFs: original IND

Drug class: non-nucleoside reverse transcript inhibitor

Intended clinical population: HIV patients

Clinical formulation: 25 mg (b) (4) l TMC278 (b) (4)

Route of administration: oral

Proposed clinical protocol: open label, randomized trial in 16 healthy subjects to investigate the Pk interaction between ddI and TMC278 at steady state. During session I, all subjects will receive 150 mg TMC278 from day 1 to day 7. In session II, after a two wk washout, subjects in gp 1 will receive 400 mg ddI for 14 days with additional 150 mg TMC278 from day 1 to 7. Subjects in gp 2 will receive 400 mg ddI for 14 days with additional 150 mg TMC278 from day 1 to 7. Plasma concentrations of TMC278 and ddI will be determined. Safety, tolerability, lab and CV parameters will be assessed.

Previous clinical experience: Phase I trials in healthy and HIV-1 infected subjects. Maximum dose 150 mg. Maximum duration 14 days.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: safety pharm, pharmacokinetics, 2 wk, 1 mo and 6 mo tox studies in rats, 1 and 6 mo tox studies in dogs, genotox, teratology in rats and rabbits.
Studies not reviewed within this submission: various minor studies

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Mechanism of action: inhibition of reverse transcriptase

Drug activity related to proposed indication:

TMC278 is an NNRTI, with IC_{50} values ranging from 0.1 to 7.9 nM for a panel of NNRTI resistant mutants.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects: 5 male Sprague-Dawley rats per gp were administered 40, 120 and 400 mg/kg TMC278 orally for the modified Irwin test. No effects were seen at doses up to 120 mg/kg. At 400 mg/kg, motor affective (marked reaction in the finger withdrawal test) and sensori-motor (negative toe pinch test) responses were slightly affected (1 rat affected per endpt). There was a slight reduction in pupil size indicative of autonomic impairment at this dose. Most of the effects were seen 8 hrs post-dose and no effects were retained 24 hrs after dosing.

Cardiovascular effects: A concentration dependent reduction in the membrane K current was seen in CHO cells transfected with hERG. No effect at 100 nM (37 ng/ml) but effects seen at 300 and 3000 nM. TMC278 had no effect in the isolated spontaneously beating right atrium of the guinea pig until drug concentrations reached 1000 nM. When tested in 9 anesthetized guinea pigs, TMC278 at iv bolus doses of 0.16 to 5 mg/kg had no significant effect on heart rate, mean arterial pressure and PQ and QRS intervals. QT interval was increased at the high dose but the effect disappeared when corrected for heart rate using Bazett's formula (QT_cBazett). At 5 mg/kg, the median plasma level of TMC278 5 min after inj was 9.15 ug/ml.

In 4 anesthetized dogs, TMC278 was infused iv over a 1 hr period at a rate of 2 ml/kg/h. In this experiment, the vehicle PEG400 had significant effects on many cardiovascular parameters and drug effects could not be determined.

In 7 conscious chronically instrumented dogs, TMC278 given as a single oral dose of 20 mg/kg, had no effect on heart rate, blood pressure, or any other parameters of cardiac function or ECG parameters including QT and QT_c. Peak plasma levels were reached 4 hrs after drug admin and amounted to 1.6 ug/ml and the average AUC_{0-4h} was 3.93 ug.h/ml.

In 4 male conscious telemetered dogs treated by gavage with 0, 20, 80 and 160 mg/kg of TMC278 in PEG400. Cardiac hemodynamics and electrophysiological recordings were made at regular time intervals until 12 hrs after dosing. There were no significant effects on any parameters.

Pulmonary effects:

In the male dog study mentioned above, pulmonary parameters respiratory rate, tidal vol, and minute vol were unchanged with drug treatment.

Renal effects: not examined

Gastrointestinal effects: not examined

Abuse liability: not examined

Other:

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.3 Absorption

Oral absorption in rats was ~30-40% decreasing to 21% at 400 mg/kg. Dog was 80% at 5 mg/kg in PEG400 + citric acid. Rabbit was 54% at 5 mg/kg. Monkey was 24% at 5 mg/kg.

2.6.4.4 Distribution

R278474 was extensively distributed throughout the body in rats, dogs and monkeys. In limited tissue distribution studies in rats and dogs, high concentrations were found in adrenal glands and liver.

2.6.4.5 Metabolism

In vitro studies were conducted in mouse, rabbit, rat, dog and human hepatocytes. R278474 was primarily conjugated with glutathione, glucuronic acid or sulfuric acid. Most of the metabolites found in human hepatocytes were also found in rodents. The metabolite profiles of dog and rabbit differed from those of human and rodent.

2.6.4.6 Excretion
Following a radioactive (^{14}C) dose of 10 mg/kg to rats, ~1% of the dose was recovered in urine and 28% of radioactivity was recovered in feces. No radioactivity was detected in expired air.

2.6.4.7 Pharmacokinetic drug interactions

2.6.4.8 Other Pharmacokinetic Studies: Protein binding was above 99% for all species tested (including humans) over a wide range of concentrations.

2.6.4.9 Discussion and Conclusions No unusual Pk data.

2.6.4.10 Tables and figures to include comparative TK summary

Human data: When given to healthy subjects at a single oral dose of 50 mg, the $AUC_{0\text{-}144h}$ of R278474 is 6.7 ug.h/ml and the C_{max} is 0.25 ug/ml.

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Plasma concentrations of R278474 following oral dosing in males for 14 days

	Day 1			Day 14		
Dose	25 mg	75 mg	150 mg	25 mg	75 mg	150 mg
$AUC_{0\text{-}t}$ ug.h/ml	1.3	4.4	5.7	3.6	11.2	17.7
C_{max}	0.10	0.33	0.46	0.22	0.66	1.07

Only approximately 0.01-0.03% of unchanged R278474 was excreted in the urine.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

Animal toxicokinetic data are given in the specific tox study reviews

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: In rats, the primary effects were increased thyroid hypertrophy, which was not entirely reversible. Slight increase in APTT and prothrombin time and slight decrease in eosinophils and RBC parameters all of which were reversible. In dogs the main effect was on steroidogenesis with swollen ovaries, uterus and vagina in females and reduced spermiogenesis, leydig cell hyperplasia and debris in epididymis in males.

Genetic toxicology: negative Ames, in vitro chrom ab and micronucleus tests

Carcinogenicity: not done

Reproductive toxicology: No study on effects on fertility was performed. Developmental studies in rats and rabbits demonstrated that TMC278 had no effects on

pregnancy parameters but did have some minor effects on various aspects of development that were statistically significant but of dubious physiological significance.

Special toxicology: Special endocrine study did not demonstrate any adverse drug effects following a single dose of 20 or 80 mg/kg R278474 on plasma cortisol, ACTH or aldosterone levels or any adverse effects to a CRH stimulation test in 6 male beagles.

2.6.6.2 Single-dose toxicity
Two male and two female rats (plus 4/sex for toxicokinetics) were given 800 mg/kg TCM278 by oral gavage and followed for 1 day. There was no mortality and all males and 2/6 females showed salivation during the day of dosing. $AUC_{0-\infty}$ values were 86 ug.h/ml in males and 233 ug.h/ml in females.

2.6.6.3 Repeat-dose toxicity

Study title: 2 week repeated dose oral toxicity study in the rat

Key study findings: effects on thyroid

Study no.: 5535
Volume #, and page #: 9, pg 001
Conducting laboratory and location: Global Preclinical Development (GPD), Beerse, Belgium
Date of study initiation: 3/02
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: R278474, batch no. ZR278474PLA011, 96.2%

Methods

Doses: 0, vehicle, 40, 120, 400 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 5/sex/gp
Route, formulation, volume, and infusion rate: oral gavage; water or soln of PEG400 and 100 mg/ml citric acid/ vol 10 ml/kg.
Satellite groups used for toxicokinetics or recovery: 0
Age: 6 wks
Weight: 148-239 g

Results:

Mortality: none

Clinical signs: dose related increased salivation in males and in females, HD only.

Body weights: no effect

Food consumption: no effect

Ophthalmoscopy: no changes

EKG: not done

Hematology: no toxicology signif changes

Clinical chemistry: decrease in sodium and chloride in females, decreased chloride in males, and increased albumin and total protein in males.

Urinalysis: increased urine vol in MD and HD males only.

Gross pathology: no effects

Organ weights: no tox signif effects except or increased thyroid wt in females

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

Dose related hypertrophy of thyroid follicular epithelium in all dosed males and MD and HD females. No other signif tox changes.

Toxicokinetics: not reported

Study title: 4 week oral (gavage) immunotoxicity study in the rat

Key study findings: No negative effects on the direct plaque-forming cell assay

Study no.: TOX5692
Volume #, and page #: 10, pg 001
Conducting laboratory and location: (b) (4)

Date of study initiation: 4/03
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: R278474, batch ZR278474PFA021,

Methods

Doses: 0, vehicle, 10, 40, 160 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 10/sex/gp
Route, formulation, volume, and infusion rate: oral gavage; water or soln of PEG400 and 100 mg/ml citric acid; vol 10 ml/kg
Satellite groups used for toxicokinetics or recovery: 8/sex/gp for toxicokinetics or immunoassays

Age: 6 wks
Weight: 134-205 g

Results:

Mortality: none

Clinical signs: none drug related

Body weights: no effects

Food consumption: no effects

Ophthalmoscopy: no changes

EKG: not done

Hematology: no relevant changes

Clinical chemistry: decreased glucose in HD females

Urinalysis: no effects

Gross pathology: no significant changes

Organ weights: increased liver wt in MD and HD males and females, increased thyroid wt in MD and HD males and females.

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes ( ), no ( )
minimal follicular hypertrophy of the thyroid in MD and HD males and HD females

Immunology; No treatment related effects on the direct plaque forming cell (PFC) assay.

Toxicokinetics: On day 28, AUC_{0-24} male values were 7.2, 27.4 and 50.5 ug.h/ml for LD, MD and HD respectively. Females were 14.0, 41.6 and 88.9.

Study title: 6 month repeat dose oral toxicity study with 1 month recovery in the rat.

Key study findings: thyroid hypertrophy that was not reversible in HD. Slight decrease in RBC and clotting parameters that was reversible.

Study no.: TOX6142
Volume #, and page #: 17 pg 116
Conducting laboratory and location: Global Preclinical Development, Beerse, Belgium

Date of study initiation: 8/03
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: R278474, batch ZR278474PFA021, purity not stated

Methods

Doses: 0, 40, 120, 400 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 20/sex/gp
Route, formulation, volume, and infusion rate: oral gavage; soln of PEG400 and 100 mg/ml citric acid; vol 10 ml/kg.
Satellite groups used for toxicokinetics or recovery: 10/sex in control and HD and 6/sex/dosed gp for toxicokinetics
Age: 6 wks
Weight: 136-226 g

Results:

Mortality: several deaths due to gavage accidents due to increased vol and viscosity of the formulation (especially the high dose gp). From day 84 forward, all animals were dosed bid with 1.5 hr between doses. During the next 4 wks, 7 males and 2 females died or were sacrificed, of which 6 deaths were considered possibly due to gavage accidents, 2 to trauma and 1 unknown. No deaths were considered drug related.

Clinical signs: In rats that died or were sacrificed, there was rough haircoat, piloerection, wet urogenital region, crusty nose, dyspnea, nasal discharge, lameness, a small amount of feces and/or chromodacryorrhea. In the HD gp, there was an increase in salivation and wet urogenital region in males and females. All animals were normal at recovery.

Body weights: no drug related changes in body wt or body wt gain

Food consumption: no toxicological significant changes

Ophthalmoscopy: no drug related changes

EKG: not done

Hematology: Slight to moderate somewhat dose related increase in APTT and prothrombin time in all dosed males. Slight decrease in RBC's, hemoglobin, hematocrit and eosinophils in HD males. In females, there was a slight decrease in eosinophils at all doses and a marginal decrease in mean cell vol and mean cell Hb in MD and HD. The increase in APTT and prothrombin times were still present in HD males after recovery. All other parameters had returned to normal

Clinical chemistry: At the MD, slight dose related increase in inorganic phosphate, albumin, urea nitrogen and Alk Phos in males. In females, slight increase in albumin. At

the HD, additional changes included slight decrease in triglycerides and Ca in males and a slight increase in total protein and albumin in females. All was normal at recovery.

Urinalysis: no drug related changes

Gross pathology: Swollen thyroid glands and more pronounced lobulation of the liver in MD and HD plus swollen liver at HD.

Organ weights: Increase in thyroid wt at all dose levels. Increase in liver wt at MD and HD. At recovery, increased thyroid wt remained, liver wt was normal.

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

Dose related increase in diffuse follicular hypertrophy in the thyroid glands in males and females at all doses. Dose related increase in hepatocellular hypertrophy in MD and HD males and females. Increase in swollen-vacuolated cells in the pars distalis of the pituitary in males from all treated gps. Swollen-vacuolated appearance of macrophages in the mesenteric lymph nodes of some HD males. Only minimal changes in the thyroid were still present at recovery.

Toxicokinetics:

dose	40 mg/kg		120 mg/kg		400 mg/kg	
	AUC $_{0\text{-}24}$ (ug.h/ml)					
day	M	F	M	F	M	F
0	19.3	31.4	52.9	82.2	90.2	159.0
83	18.6	41.4	40.6	100.7	56.5	184.0
174	19.3	32.1	53.0	82.6	92.2	159.7

A large gender discrepancy not seen in dogs. Not sure about humans.

Study title: 1 month repeated dose oral toxicity study in the beagle with 1 month recovery

Key study findings: Effects on steroidogenesis in females, some liver effects.

Study no.: 5650
Volume #, and page #: 11, pg 119
Conducting laboratory and location: GPD
Date of study initiation: 10/02
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: R278474, batch no. ZR27847PFA011, 98.5% pure

Methods

Doses: 0, vehicle, 5, 10, 40 mg/kg/day
Species/strain: beagle dogs
Number/sex/group or time point (main study): control and HD had 5/sex/gp. MD and LD had 3/sex/gp. Extra controls and HD used for recovery.
Route, formulation, volume, and infusion rate: oral gavage; soln in PEG400 and 100 mg/ml citric acid; 1 ml/kg vol.
Satellite groups used for toxicokinetics or recovery: 2/sex in control and HD for recovery
Age: 5-8 mo
Weight: 8.4-14.3 kg

Results:

Mortality: none

Clinical signs: red vaginal discharge, MD and HD females. Normal at recovery.

Body weights: HD males lost wt during the study. They gained more wt than controls during recovery but were still lighter than controls at end.

Food consumption: same as body wt. normal at recovery

Ophthalmoscopy: no effects

EKG: no effects on ECG or heart rate.

Hematology: decreased RBC's, hematocrit and hemoglobin in HD males and females. Normal at recovery.

Clinical chemistry: HD dogs had marginal increases in bilirubin, AP, and ALT. all normal at recovery except for AP.

Urinalysis: no relevant changes

Gross pathology: In MD and HD, there was swollen cervix, ovaries, uterus and vagina in 2/3 females at each dosage gp. Normal at recovery.

Organ weights: increased ovarian wts at MD and HD. normal at recovery

Histopathology: Adequate Battery: yes (X), no ( )—explain
Peer review: yes (X), no ( )
Changes in MD and HD
Liver: centrilobular perivascular inflammation with single cell necrosis, RES aggregates, and minimal bile duct proliferation.
Adrenal: Dense zona fasciculata cells with reduced vacuolation in MD and HD dogs. Cells swollen and some leukocytic infiltration.

Female genital tract: activation, including mammary glands at MD and HD. Changes in adrenal and female genital tract were normal after recovery. Still slight multifocal centrilobular perivascular inflammatory reaction with increased centrilobular clear appearance and increased RES aggregates.

Toxicokinetics:

Plasma TMC278 levels after 27 days of oral dosing in Beagles

Dose (mg/kg)	AUC_{0-24} (ug.h/ml)	
	Male	Female
5	27.1	36.8
10	102.6	46.6
40	204.2	160.2

Other:

Study title: 6 month repeated dose oral toxicity study with a 3 month interim kill in the Beagle dog

Key study findings: Effects on steroidogenesis

Study no.: TOX6110
Volume #, and page #: 20, pg 252
Conducting laboratory and location: Global Preclinical Development, Beerse, Belgium.
Date of study initiation: 7/03
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: R278474, batch ZR278474PFA021,

Methods

Doses: 0, 5, 10, 40 mg/kg/day
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 3/sex/gp
Route, formulation, volume, and infusion rate: oral gavage; soln of PEG400 and 100 mg/ml citric acid; vol 1 ml/kg.
Satellite groups used for toxicokinetics or recovery: 3/sex/gp
Age: 8-8.5 mo.
Weight: 8.3-14.8 kg

Results:

Mortality: none

Clinical signs: soft feces and occasional emesis LD to HD. At HD, more pronounced soft, mucous and pale feces, salivation (females only) and vomiting.

Body weights: At HD slight decrease in BW during first wk of dosing. No changes subsequently.

Food consumption: Same as BW.

Ophthalmoscopy: No changes

EKG: No changes in ECG or other cardiac parameters.

Hematology: No drug induced changes

Clinical chemistry: Slight increase in ALP at all dose levels in females and at MD and HD in males. In females there was a slight increase in bilirubin at all dose levels and at HD for males.

Urinalysis: No relevant changes.

Gross pathology: No changes in males or females up to 3 months and up to 10 mg/kg for 6 months. At 6 months in HD females, there was a slightly higher incidence of swollen ovaries, uterus and vagina.

Organ weights: No relevant organ wt changes in males. In females, there was a dose related increase in ovarian, uterine and vaginal wt seen at all dose levels.

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

Several changes related to altered steroidogenesis in the adrenal, ovaries and testes/epididymides. Adrenal mainly changes in staining droplets. In ovaries an increase in tertiary and atretic follicles and corpora lutea. In testes there was leydig cell hyperplasia and hypertrophy in MD and HD and multifocal atrophic tubules at the HD. Marginally reduced spermiogenesis with decrease spermatozoa in the epidydimides in 1 MD and1 HD male. Presence of cellular debris in the epididymides was seen in MD and HD. There was an increase in presence of brown pigmented macrophages in the liver in MD and HD males and HD females.

Toxicokinetics:

Dose	5 mg/kg		10 mg/kg		40 mg/kg	
	AUC_{0-24} (ug.h/ml)					
day	M	F	M	F	M	F
0	10.6	9.2	21.8	20.0	22.6	10.2
85	21.3	17.6	27.8	27.2	41.3	51.5
176	21.1	17.4	25.8	31.9	68.3	43.1

The plasma levels measured in this study are significantly lower than those seen in the 1 month dog study. The reason for the discrepancy is unclear but the sponsor seems to think that these data are closer to the true levels.

Special Endocrine Study; A single dose oral endocrinology study was performed in male beagles at doses of 20 and 80 mg/kg. R278474 was formulated in PEG400 with 100 mg/ml citric acid. A vehicle control gp was included.

Plasma cortisol, ACTH and aldosterone levels were measured on the day of dosing and 6 days later. A CRF (Corticotropin Releasing Factor)-challenge test was done one day after dosing and 6 days later.

A single dose of R278474 did not adversely affect plasma cortisol, aldosterone and ACTH levels. Results of the CRF challenge test were comparable between treated and vehicle control dogs indicating a normal functioning hypophysis and adrenal cortex.

Toxicokinetics: $AUC_{0\text{-}inf}$ averaged 87.3 ug.h/ml for the LD and 104.6 ug.h/ml for the HD.

2.6.6.4 Genetic toxicology

Study title: In vitro bacterial reverse mutation test with Salmonella typhimurium

Key findings: negative study

Study no.: 5693 and 5540 (two identical tests with identical results one of which is reported here)

Volume #, and page #: 23, pg 164
Conducting laboratory and location: GPD, Beerse, Belgium
Date of study initiation: 2/03
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R278474, batch ZR278474PFA011, 98.9% pure

Methods

Strains/species/cell line: Salmonella typhimurium TA1535, TA1537, TA102, TA98 and TA100

Doses used in definitive study: 8-500 ug/plate

Basis of dose selection: significant precip at high dose

Negative controls: DMSO

Positive controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, 2-aminoanthracene, 4-nitroquinoline-N-oxide

Incubation and sampling times: incubated for 2 days

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): study was valid

Study outcome: negative with and without metabolic activation

Study title: In vitro mammalian forward mutation test with L5178Y mouse lymphoma cells

Key findings: negative study

Study no.: 5539

Volume #, and page #: 23, pg 229
Conducting laboratory and location: GPD, Beerse, Belgium
Date of study initiation: 3/02
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R278474; batch ZR278474PLA011; 96.2% pure.

Methods

Strains/species/cell line: mouse lymphoma L5178Y cells

Doses used in definitive study: to 500 ug/ml for 3h w/o S9; to 35 ug/ml for 3 h w S9; to 100 ug/ml for 24h w/o S9.

Basis of dose selection: top doses limited by precipitation and cell death

Negative controls: DMSO

Positive controls: Methyl methanesulfonate for the non-activation portion and N-nitrosodimethylamine for the activation portion.

Incubation and sampling times: 3 hr w and wo S9 followed by 24 hrs exposure w/o S9 followed by 2 days growth without drug before being plated and counted.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): studies were valid

Study outcome: negative for small and large colonies

Study title: In vivo micronucleus test on bone marrow cells of mice

Key findings: study negative

Study no.: 5538

Volume #, and page #: 23, pg 274
Conducting laboratory and location: GPD, Beerse, Belgium
Date of study initiation: 4/02
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R278474; batch ZR278474PLA011; 96.2% pure.

Methods

Strains/species/cell line: male and female Albino Swiss (CD1) mice.

Doses used in definitive study: 100, 400 and 1600 mg/kg

Basis of dose selection: top dose was maximum feasible dose due to solubility/vol.

Negative controls: PEG 400 and 100 mg/ml citric acid

Positive controls: cyclophosphamide

Incubation and sampling times: 24 and 48hrs after drug administration

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): study was valid

Study outcome: no increase in structural or numerical chromosomal aberrations.

Toxicokinetics

dose	100 mg/kg		400 mg/kg		1600 mg/kg	
sex	males	females	males	females	males	females
$AUC_{0\text{-}6h}$ (ug.h/ml	58	130	62	258	307	287

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Studies on impairment of fertility and peri and postnatal development have not been conducted.

Embryofetal development

Study title: Oral developmental toxicity study in the rat

A pilot study was first conducted using doses of 40, 120 and 400 mg/kg/day. These doses produced no toxicological changes. In order to demonstrate drug related toxicity, the sponsor weighed the thyroid and noted an increase in thyroid wt and hypertrophy of the thyroid follicular epithelium. These results were considered evidence of a MTD.

Key study findings: no significant teratology findings

Study no.: TOX6268
Volume #, and page #: 24, pg. 068
Conducting laboratory and location: GPD, Beerse, Belgium
Date of study initiation: 11/03
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R278474; batch ZR278474PFA021, 99.5% pure

Methods

Doses: 0, 40, 120, 400 mg/kg
Species/strain: Female Sprague-Dawley rats
Number/sex/group: 24/gp
Route, formulation, volume, and infusion rate: oral gavage, soln in PEG 400 with 100 mg/ml citric acid; 1 ml/100 g BW
Satellite groups used for toxicokinetics: 18/gp
Study design: drug given pregnancy days 6 to 17 inclusive
Parameters and endpoints evaluated:

Results

Mortality (dams): none treatment related

Clinical signs (dams): none

Body weight (dams): slight decrease in BW gain in MD and HD. Significant between MD and control on days 10-13.

Food consumption (dams): slight decrease MD and HD

Adrenal weight: Slight, insignificant increase in thyroid wt at MD and HD.

Toxicokinetics: $AUC_{0\text{-}24h}$ at day 6 was 33, 65 and 128 ug.h/ml for LD, MD, HD, respectively, and on day 16, the values were 37, 63 and 152.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): no treatment related effects

Offspring (malformations, variations, etc.): slight increase in the incidence of dilated renal pelvis in MD ($p<0.05$) and HD ($p<0.01$). One HD fetus exhibited absent skull bones, open vertebral arches and tongue protrusion. Another HD fetus had deformed forelegs, tongue protrusion and local edema. All long bones of the fore and hindlimbs, pectoral and pelvic girdle bones and mandible of this fetus were bowed, ribs were wavy and ossification was generally reduced.

Fetuses of the HD gp showed slightly increased incidences of reduced ossification of sternebrae and metacarpal bones ($p<0.05$). These findings were confined to fetuses of two litters.

Study title: Oral developmental toxicity study in the rabbit

A pilot study was conducted using doses of 25, 75 and 150 mg/kg. One LD and 1 HD dam were sacrificed during the study because of abortion of their litters. At the end of the study, no viable fetuses existed in dams of the MD and HD due to early resorptions. The dams also exhibited reduced fecal output, reduced body wt gain and decreased food consumption.

A second pilot study was conducted at doses of 20 or 60 mg/kg/day. In the HD gp consisting of 6 females, 2 dams aborted prior to term, 2 exhibited total early resorptions and 2 had increased post-implantation losses of 33 and 43%. Sponsor considered 20 mg/kg to be the MTD.

Key study findings: no significant teratology findings

Study no.: TOX6313
Volume #, and page #: 25, pg 060
Conducting laboratory and location: GPD, Beerse, Belgium
Date of study initiation: 3/04
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity:

Methods

Doses: 0, 5, 10, 20 mg/kg
Species/strain: albino New Zealand White rabbits
Number/sex/group: 20/gp
Route, formulation, volume, and infusion rate: oral gavage; aqueous suspension in 0.5% hydroxypropylmethylcellulose (Methocel)
Satellite groups used for toxicokinetics: none
Study design: drug given on pregnancy days 6 to 19 inclusive.
Parameters and endpoints evaluated:

Results

Mortality (dams): none

Clinical signs (dams): no drug related effects

Body weight (dams): no effects

Food consumption (dams): no effects

Toxicokinetics: $AUC_{0\text{-}24h}$ were 95ug.h/ml on day 6 and 105 on day 19 at the LD, 162/170 at MD and 219/232 at HD.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): no effects

Offspring (malformations, variations, etc.): There were a number of major abnormalities in specific fetuses but no two the same and nothing significant on a per litter bases (6 females, 3 LD, 1 MD 2 HD).

The incidence of branches of the left subclavian artery originating from the aorta was slightly increased in all treated gps compared to controls. This reached significance ($p<0.05$) in the HD gp and may be treatment related. The incidence of absent or hypoplastic interparietal bone was increased in all treated gps and reached significance in the HD gp.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: Sponsor completed 6 month toxicity studies in rats and dogs. The primary effect seems to be on the endocrine system and steroidogenesis in both rats and dogs. The exact nature of these effects is unknown but there were effects on the thyroid (effect on thyroid may be secondary to effects on CYP_{450} enzymes in the liver), adrenal, possibly pituitary and the sex organs of both males and females. Other findings include effects on the liver (rat and dog) and increased eosinophils and clotting times (rat only). In rats, the effects on thyroid and blood clotting were not completely reversible after 1 month.

A special endocrine study in male beagles showed no obvious effects on the adrenal/pituitary axis.

In humans given the proposed dose of 150 mg for 14 days, the AUC of parent drug was 17.7 ug.h/ml. In rats, AUC values were significantly higher with the low dose of 40 mg/kg in the 6 month study giving plasma AUC values of ~20-35 ug.h/ml and the high dose giving values of ~75-170 ug.h/ml for males and females, respectively. In beagles, the values for males and females were similar. For the low, medium and high doses, the AUC values were ~20, 28 and 50 ug.h/ml. There were no effects on the pituitary/adrenal axis following a single oral dose in dogs that gave AUC values of 87 and 107 ug.h/ml for the low and high doses.

TMC278 was not genotoxic in the standard battery of genotox tests.

Fertility studies were not performed. Some mild effects on the fetuses of rats and rabbits but no common effects and no clear teratology signal.

Internal comments: Parameters to monitor in clinical trials should include steroidogenesis and blood clotting. These concerns have been communicated to the sponsor.

External comments (to sponsor): none

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

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/s/

Alexander W. Jordan
12/16/04 01:06:21 PM
PHARMACOLOGIST

James Farrelly
12/21/04 08:42:45 AM
PHARMACOLOGIST

Appendix 1B

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 67,699
Review number: 2
Sequence number/date/type of submission: 020; 5/9/05; IT
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Tibotec, Inc
Manufacturer for drug substance: J&J Pharm Res and Dev, Janssen Pharm, Belgium

Reviewer name: Alex Jordan
Division name: DAVDP
HFD #: 530
Review completion date: 6/13/05

Drug:

Trade name:
Generic name:
Code name: R 314585 or TMC278 (HCl salt) or R278474 (free base)
Chemical name: (E) 4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylpheny]amino]-2-pyrimidinyl]amino] benzonitrile
CAS registry number: 700361-47-3
Molecular formula/molecular weight: 366.42
Structure:

Relevant INDs/NDAs/DMFs:

Drug class: non-nucleoside reverse transcript inhibitor

Intended clinical population: HIV patients

Clinical formulation: 25 mg (b) (4) l TMC278 (b) (4)

Route of administration: oral

Proposed clinical protocol: Ongoing or completed open label, randomized trial in 16 healthy subjects to investigate the Pk interaction between ddI and TMC278 at steady state. During session I, all subjects will receive 150 mg TMC278 from day 1 to day 7. In session II, after a two wk washout, subjects in gp 1 will receive 400 mg ddI for 14 days with additional 150 mg TMC278 from day 8 to 14. Subjects in gp 2 will receive 400 mg ddI for 14 days with additional 150 mg TMC278 from day 1 to 7. Plasma concentrations of TMC278 and ddI will be determined. Safety, tolerability, lab and CV parameters will be assessed.

Previous clinical experience: Phase I trials in healthy and HIV-1 infected subjects. Maximum dose 150 mg. Maximum duration 14 days.

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: 2 week rat tox, 3 month mouse tox; correction on pK from original IND review.
Studies not reviewed within this submission: none

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Inhibition of reverse transcriptase

Drug activity related to proposed indication:

TMC278 is an NNRTI, with IC_{50} values ranging from 0.1 to 7.9 nM for a panel of NNRTI resistant mutants.

2.6.6 TOXICOLOGY

2.6.6.3 Repeat-dose toxicity

Sponsor conducted a 1 month study in rats (TOX6385) comparing the toxicity of TMC278 acid form vs free base at doses of 10 and 400 mg/kg. There were no toxicologically significant differences between the two drug forms.

Study title: 3 month repeated dose oral toxicity study in the Swiss mouse

Key study findings:

Study no.: TOX6739
Volume #, and page #: vol 3 pg 1
Conducting laboratory and location: J&J pharm GPD Beerse, Belgium
Date of study initiation: 8/04
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: TMC278; batch ZR314585PFA011; 100.3 % pure

Methods

Doses: 20, 80, 320 mg/kg
Species/strain: Swiss mice
Number/sex/group or time point (main study): 10/sex/gp + 12/sex extra in vehicle control gp
Route, formulation, volume, and infusion rate: oral gavage; 0.5% methocel aqueous suspension
Satellite groups used for toxicokinetics or recovery: 15/sex/dosed gp
Age: 6 wks
Weight: 19.6-31.8 g
Sampling times:
Unique study design or methodology (if any):

Results:

Mortality: no test article related mortality

Clinical signs: Abdominal distension in HD only animals from 6 wks on.

Body weights: Slight increase in BW gain in MD. Moderate increase in BW (11%) and BW gain (54%) in HD males and females throughout study duration

Food consumption: Slight to moderate increase in MD and HD animals

Ophthalmoscopy: not done

EKG: not done

Hematology: Slight decrease in RBC's, HB, HCT in HD animals (less than 10%). Slight decrease in neutrophils (30%) and moderate decrease in lymphocytes and eosinophils (~50%) in HD males. Slight decrease in thrombocytes (12%) and slight increase in number and % reticulocytes (50%) in HD females.

Clinical chemistry: MD females had a slight increase in total protein, albumin and cholesterol and a moderate decrease in triglycerides. HD females had a slight increase in total protein and cholesterol, a moderate increase in urea nitrogen (25%; $P<0.01$) and a decrease in triglycerides. HD males had a slight increase in inorganic phosphorus and a decrease in total bilirubin. Both HD males and females had a slight increase in calcium, albumin, ALP (1.25-2X), and ALT (2-2.5X).

Urinalysis: not done

Gross pathology: Dark liver in 4/10 MD females, swollen spleen in two females. Dark and swollen liver in all HD animals. Swollen spleen and an irregular surface of the kidneys was seen, each in three HD females.

Organ weights (specify organs weighed if not in histopath table):

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

Drug induced toxicity occurred in higher dosed males and females in the adrenals, bone marrow, liver, ovary/uterus, spleen and thymus. Changes in the adrenal, ovary/uterus, spleen and thymus occurred only in the HD and were marginal and of dubious toxicological significance.

In the liver, there was an increased incidence of hepatocellular hypertrophy (centrilobular to diffuse) that was slight in MD males, moderate in MD females and prominent in HD males and females. Moderate increase in single cell necrosis in HD males and females. Minimal to slight focal necrosis in 1 female and 1 male at the MD and 2 females and 3 males at the HD. Hepatocellular (small) vacuolation was minimal in 2 HD males and minimal to slight in 5 HD females. In the HD only, there was an increased incidence of Kupffer cell proliferation, brown pigmentation of Kupffer cells and extramedullary hematopoesis. There changes were seen in males and females and were minimal to slight.

There were no drug related effects on the kidneys in males or LD and MD females. In 5/10 HD females, there was minimal to moderate nephropathy, characterized by slight to marked multifocal tubular basophilia (with minimal presence of single cell death within the basophilic tubules), minimal to slight glomerulopathy (focal atrophic glomeruli with thickened Bowman's capsule among basophilic tubules), minimal to moderate mononuclear cell infiltration, minimal to slight interstitial fibrosis, minimal tubular dilation and slight cortical mineralization. In 3 of these animals, these changes were associated with minimal to slight inflammation within the capsule and adjacent tissue. One female had moderate exudative inflammation; 2 had minimal granulocytic infiltration and 2 had minimal focal single cell death not confined to the basophilic tubules.

In the bone marrow, there was an increase in the myeloid/erythroid ratio in HD males and females.

Toxicokinetics:

$AUC_{0-\infty}$ (ug.h/ml) of TMC138 during 3 month mouse toxicity study

Day	20 mg/kg		80 mg/kg		320 mg/kg	
	M	F	M	F	M	F
0	71	59	236	250	1010	707
30	*	74	263	313	860	1170
86	80	61	210	313	665	1360

* not available

Study title: 2 week repeated dose oral toxicity study in the rat

Key study findings: minimal toxic effects

Study no.: TOX6813
Volume #, and page #: 3, pg1
Conducting laboratory and location: J&J; Global Preclinical Development (GPD), Beerse, Belgium
Date of study initiation: 9/04
GLP compliance: yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: R278474, batch no. ZR314585PFA011, 96.2%

Methods

Doses: 0, vehicle, 400, 1500, 2000 mg/kg/day
Species/strain: Sprague-Dawley rats
Number/sex/group or time point (main study): 5/sex/gp
Route, formulation, volume, and infusion rate: oral gavage; aqueous suspension containing 0.5% Methocel (hydroxypropyl methcellulose)
Satellite groups used for toxicokinetics or recovery: 3/sex/gp
Age: 6 wks
Weight: 156-211 g

Results:

Mortality: none

Clinical signs: no effects

Body weights: no effect

Food consumption: no effect

Ophthalmoscopy: not done

EKG: not done

Hematology: no changes at LD. At 1500 mg/kg, there was a slight increase in APTT (19%) and prothrombin time (20%) in males, no changes in females. At 2000 mg/kg, slight increase in APTT (14%) and PT (15%) in males, no changes in females.

Clinical chemistry: MD females had a marginal decrease in Cl, and a slight decrease in glucose, urea N and creatinine, no changes in males. HD females had a decrease in chloride, glucose, urea N and creatinine, decrease in total bilirubin in males.

Urinalysis: Slight decrease in sp gravity and triple phosphate crystals and a slight increase in urinary vol in all dosed males. Slight decrease in specific gravity and triple phosphate crystals and moderate increase in urine vol in MD and HD females.

Gross pathology: no effects

Organ weights: no tox signif effects except for slight to moderate increase in thyroid wt of females at all doses (42% LD, 62% MD, 55% HD).

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

Pituitary gland: minimal multifocal vacuolation in two HD males, no effects in females.

Thyroid gland: Minimal follicular epithelium hypertrophy in one HD male. In females, minimal hypertrophy of follicular cells was observed in 1 control, 2 LD, 4 MD and 3 HD animals.

Toxicokinetics:

$AUC_{0-\infty}$ (ug.h/ml) of parent drug

dose	400 mg/kg		1500 mg/kg		2000 mg/kg	
day	M	F	M	F	M	F
0	50	78	153	152	103	206
13	42	96	86	115	77	147

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: The 3 month mouse toxicity study is a dose ranging study for the mouse carcinogenicity study. Based on liver or kidney toxicity, the MTD may be used or sponsor could use exposure multiples as well. TMC278 is not genotoxic and the plasma blood levels of parent drug at the high dose of 320 mg/kg are 665 and 1360 ug.h/ml for males and females, respectively. The maximum human dose currently being used is 150 mg which produced an AUC in males after 14 days dosing of 17.7 ug.h/ml.

The 2 week rat study used doses up to the MFD of 2000 mg/kg without significant toxicity. In the original 2 wk rat tox study, doses up to 400 mg/kg produced increased thyroid wt in females and thyroid follicular hypertrophy in males and females. In this study of doses up to 2000 mg/kg, thyroid wts increased by a maximum of 55% in females. There was minimal thyroid hypertrophy, mainly in females. The effects on the thyroid don't seem to get any worse with higher doses. There was no adverse effect of TMC278 on the thyroid in mice.

Internal comments: Sponsor will submit carcinogenicity protocols for both rat and mouse in the near future. The sponsor states that the submissions will contain the dose range

finding data as well as the proposed doses. The protocols and doses will be reviewed when received.

In the original review, the plasma blood levels from the high dose 6 month rat study were incorrect. Below are the data from the original review and below that the correct data.

Toxicokinetics:

from original review

dose	40 mg/kg		120 mg/kg		400 mg/kg	
	AUC_{0-24} (ug.h/ml)					
day	M	F	M	F	M	F
0	19.3	31.4	52.9	82.2	90.2	159.0
83	18.6	41.4	40.6	100.7	56.5	184.0
174	19.3	32.1	53.0	82.6	92.2	159.7

corrected data

dose	40 mg/kg		120 mg/kg		400 mg/kg	
	AUC_{0-24} (ug.h/ml)					
day	M	F	M	F	M	F
0	19.2	31.4	52.9	82.2	90.2	159.0
83	18.6	41.4	40.6	100.7	56.5	184.0
174	11.7	49.8	35.0	116.0	72.8	243.7

The plasma blood levels at the 400 mg/kg dose level were higher in the 6 month rat study than in the 2 wk study. Sponsor did not comment on the difference but it may be due to the different vehicles used in the studies.

External comments (to sponsor): none

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/s/

Alexander W. Jordan
7/5/05 11:12:18 AM
PHARMACOLOGIST

James Farrelly
7/5/05 03:09:00 PM
PHARMACOLOGIST

Appendix 1C

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 67,699
Review number:
Sequence number/date/type of submission: No. 028/8/12/05/IT
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Tibotec, Inc
Manufacturer for drug substance:

Reviewer name: Alex Jordan
Division name: DAVDP
HFD #: 530
Review completion date: 10/28/05

Drug:

Trade name:
Generic name:
Code name: TMC278; R278474
Chemical name:
CAS registry number:
Molecular formula/molecular weight:
Structure:

Relevant INDs/NDAs/DMFs:

Drug class:

Intended clinical population:

Clinical formulation:

Route of administration: oral

Proposed clinical protocol:

Previous clinical experience:

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission: 52 wk dog tox study
Studies not reviewed within this submission: none

2.6.6.3 Repeat-dose toxicity:

Study title: 52 week oral gavage toxicity study in the beagle dog

Key study findings:

Study no.: TOX6314
Volume #, and page #: vol 1 pg 1
Conducting laboratory and location: (b) (4)
Date of study initiation: may, 2004
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: R278474, Batch no. ZR278474PFA011, purity 98.3%

Methods

Doses: 5, 10, 40 mg/kg/day
Species/strain: beagle dogs
Number/sex/group or time point (main study): 4/sex/gp
Route, formulation, volume, and infusion rate: oral gavage, solution of citric acid in PEG400.
Satellite groups used for toxicokinetics or recovery: none
Age: 5 or 6 months
Weight: 6.3 – 9.3 kg
Sampling times:
Unique study design or methodology (if any):

Results:

Mortality: Five deaths in all gps due to gavage accidents. No drug related deaths

Clinical signs: increase in liquid feces and salivation

Body weights: Treated males had lower body wt gain when compared to controls. controls +27%, 50%; LD +21, 34%; MD +26%, 48%; HD +16%, 25%, males, females, respectively. Changes in LD and HD were statistically significant.

Food consumption: Over the 1 yr period, there were no significant differences between treated and controls.

Ophthalmoscopy: Not done

EKG: No effects of heart rate, cardiac conduction or cardiac rhythm and wave forms.

Hematology: Significant ($p<0.05$) decrease in total RBC's, Hb, and PCV in high dose males compared to controls.

Clinical chemistry: Scattered significant differences throughout the study. At study end there was significantly lower serum Alb, Ca and P and increased ALP in HD males compared to controls. In HD females, there was significantly lower K, Ca, prot and increased Cl when compared to control. All these changes were minor and of dubious toxicological significance.

Urinalysis: Significant increased volume of urine in HD males associated with a lower sp gravity. No changes in females.

Hormone Analysis: Dose related increase in serum progesterone concentrations in males. Significant up to weeks 35/39 in MD and HD. At wks 48/52 increase was significant only for HD. Dose related increase in 17 alpha OH progesterone that was significant in HD males from wks 35/39 onward.
No differences in estrogen, cortisol, ACTH

In females up to wks 35/39, the only statistically significant change was a decrease in the AUC of serum cortisol in the two higher dose gps. At wks 48/52 there was a significant decrease in AUC of cortisol for all treated gps and a significant lower mean cortisol in the MD and HD when compared to controls.

Gross pathology: Changes restricted to the 5 animals that died or were sacrificed moribund from gavage accidents. In addition, in one HD female, there was enlarged nodular thymus, enlarged adrenals and kidneys. The liver was mottled and the pancreas was edematous.

Organ weights (specify organs weighed if not in histopath table):
Increase in adrenal wt in HD males and females (23% for both sexes). Ovary wts were increased in MD (40%) and HD (119%). There was decrease thyroid wt in all treated males and increased thyroid wt in all treated females. These changes ranged from 13% to 28% and were not particularly dose related. There was a decrease in prostate wt (39%) and spleen wt (56%) in HD males.
Changes in spleen, prostate and thyroid wts were without histological correlates.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no ( )
Adrenal: Minimal to moderate increase in cytoplasmic density in the zona reticularis and zona fasciculata of the adrenals in 3 males and all HD females, in 1 male and all MD females and in all LD females. Most of the animals had the lowest grade of severity with the oil red O stain indicating a reduced neutral fat deposit in the adrenal cortex cells. Also, there was an increase in the size of the zona fasciculata in 1 male and 3 HD females and 1 MD female. Two males and all HD females had pigment deposits in the cortex.

Liver: Minimal to moderate yellow pigmentation in liver of 1 male and HD females and 1 MD female. This change consisted of aggregates of yellow pigment in the hepatocytes close to the canaliculi and in the canaliculi themselves which appeared slightly distended. Kupffer cells also had pigment deposits. In 3 HD males there was also minimal to slight

prominent brown pigment in the epithelium of the gall bladder. These changes suggest a trend to cholestasis.

Kidneys: Moderate acute interstitial nephritis was seen in 2 HD males. This change was seen in the inner medulla and consisted of a large infiltration of the renal interstitium by neutrophils. In 1 male this was associated with slight acute pelvic inflammation. Minimal to slight corticomedullary mineralization was seen in 3 HD females.

Testes: Minimal hypertrophy of the Leydig cells was seen in 2 HD males without any histological changes in the seminiferous tubules.

Ovaries: There was an increase in the number of antral follicles in all treated females. This was associated in 2 HD females with prominent early luteinized follicles and in 2 MD females with prominent corpora lutea.

Toxicokinetics:

AUC of parent compound

	$AUC_{0\text{-}24h}$ (ug.h/ml)							
Day	0		89		272		362	
Dose	M	F	M	F	M	F	M	F
5	10.5	9.7	17.6	21.1	15.5	18.0	17.4	18.7
10	14.6	15.3	29.2	29.3	18.9	31.0	23.8	35.8
40	36.9	40.6	59.7	87.9	59.6	51.1	65.3	61.0

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: Administration of TMC278 to beagles for 1 year was accompanied by reduced body weight gain in males and females at all doses but not particularly dose related. The main serum changes were in liver enzymes that correlated with the cholestasis seen in the histopath exam. There were also changes in calcium, sodium, chloride, potassium and creatinine concentrations that may be correlated with changes seen in the kidneys at the HD. There were changes in endocrine parameters including higher progesterone and 17αOH progesterone concentrations in males but not in females. Lower cortisol concentrations were seen in treated females throughout the treatment period whereas there was no effect or only slight effect in males. There was a dose-related increase in the weight of the adrenals with increased cellular density in zona fasciculata and reticularis and a reduction in neutral fat deposit in all dosed females and HD males. There was an increase in ovarian wt and numbers of antral follicles and prominent early luteinized follicles in the high dose. Prominent corpora lutea were seen in the MD. There was minimal hypertrophy of the leydig cells in HD but without changes in the spermatogenic cycle.

Internal comments: The principal target organs of toxicity seen in this study were the adrenals, ovaries and kidneys. Slight drug effects on body wt gain, serum calcium, cortisol and progestational steroids and urine volume and adrenal histopath occurred in low dose dogs so there was no NOAEL in this study. The AUC's at the 5 mg/kg dose were around 15-20 ug.h/ml. At the current high human dose of 150 mg, the AUC after 14 days dosing in males is 17.7 ug.h/ml. The sponsor currently is monitoring possible toxicities to the endocrine system in the clinical trials.

External comments (to sponsor): none

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/s/

Alexander W. Jordan
10/27/2005 01:44:06 PM
PHARMACOLOGIST

James Farrelly
11/1/2005 10:38:36 AM
PHARMACOLOGIST

Appendix 1D

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

IND number: 67,699
Review number:
Sequence number/date/type of submission: 019;4/13/05;GC
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Tibotec
Manufacturer for drug substance:

Reviewer name: Alex Jordan
Division name: DAVP
HFD #: 530
Review completion date: 11/11/05

Drug:

Trade name:
Generic name:
Code name: TMC278
Chemical name:
CAS registry number:
Molecular formula/molecular weight:
Structure:

Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Oral (gavage) fertility study in the female rat

Key study findings: no effects

Study no.: TOX6708
Volume #, and page #: vol 1, pg 1
Conducting laboratory and location: Global preclinical development, J&J; Cork, Ireland
Date of study initiation: Aug, 2004
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: TMC278; batch ZR314585PFA011; 99.5% pure

Methods

Doses: 40, 120, 400 mg.eq./kg/day

Species/strain: Sprague-Dawley rat
Number/sex/group: 24 females/gp
Route, formulation, volume, and infusion rate: gavage, 0.5% aqueous hydroxypropyl methylcellulose (Methocel) suspension
Satellite groups used for toxicokinetics: none
Study design: drug given for 14 days prior to mating, during mating and up to day 7 of presumed pregnancy
Parameters and endpoints evaluated:

Results

Mortality: none

Clinical signs: none drug related

Body weight: no toxicologically significant effects

Food consumption: slight increase in HD

Toxicokinetics: not done

Necropsy: no changes related to treatment

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): no effects on estrus cycle or median pre-coital interval, copulation index, fertility rate, weight of gravid uterus, numbers of corpora lutea, implantations, live embryos or the extent of pre- or post-implantation loss.

Study title: Oral (gavage) fertility study in the male rat
This was an interim report on a male fertility study that is short on specifics
Study no. was TOX6861
Twenty-five male SD rats were given TMC278 in 0.5% Methocel by oral gavage 100, 400 or 1600 mg/kg/day for 10 wks prior to mating, during mating and for approximately 2 wks after the end of the mating period. They were mated to untreated SD females.

One MD male died, cause unknown
no significant clinical signs
no body wt changes
no FC changes
Copulation and fertility indices in treated gps were comparable to controls
Females necropsied on day 13 of pregnancy had no differences in number of corpora lutea, the extent of pre- and post-implantation loss and the number of live embryos.
There was no effect on sperm motility (average path velocity or straight line velocity of sperm) or sperm concentration.
No differences were observed between control and HD males in amount of spermatozoa, or the histopathology of the testes or epididymides.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary: Because of the effects of TMC278 on the endocrine system in rats, the Division asked the sponsor to submit the results from male and female fertility studies prior to exposing normal volunteers to the drug. The female fertility study was ongoing and was submitted on 2/10/05 serial no. 009. The male study was submitted as a separate, unaudited study on 3/11/05 serial no 013 and the testes and epididymides histopath on 4/13/05 serial no 018. A submission submitted 4/13/05 serial no. 019 referred to the negative results of all three studies and requested the Divisions permission to initiate multiple dosing in healthy volunteers. That request was approved.

There were no effects in the female fertility study. In the justification for doses, sponsor referred to a one and 6 month rat studies using the same doses. There was little toxicity but some changes in hormone levels including decreased glucocorticoid levels. There was no maternal toxicity in this study and no toxicokinetics was performed.

TMC278 had no adverse effects on male or female fertility.

Internal comments: no action indicated

External comments (to sponsor): none

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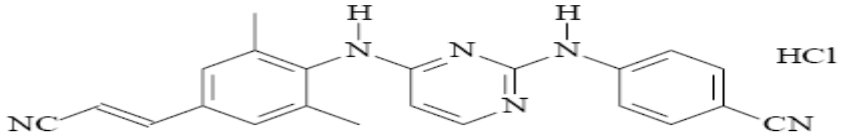
/s/

Alexander W. Jordan
11/9/2005 08:24:41 AM
PHARMACOLOGIST

James Farrelly
11/9/2005 09:50:40 AM
PHARMACOLOGIST

Appendix 1E

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND NUMBER:	67,699
VOLUME/DOCUMENT TYPE:	IT
SEQUENCE NUMBER/DATE:	111/2-07
SPONSOR:	Tibotec, Inc; 1020 Stony Hill Road, Suite 300, Yardley, PA 19067
MANUFACTURER:	J&J Pharm Res and Dev, or Janssen Pharm, Belgium
INFORMATION TO SPONSOR:	Yes () No (x)
REVIEWER NAME:	Kuei-Meng Wu
DIVISION NAME:	DAVDP
HFD NO.:	HFD-530
DRUG:	TMC278 (HCl salt); R 314585 or R278474 (free base)
CAS No.	700361-47-3
CHEMICAL NAME:	(E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile hydrochloride
FORMULA/MW:	402.88; $C_{22}H_{18}N_6$.HCl
STRUCTURE:	
DRUG CLASS:	Antiviral, on-nucleoside reverse transcript inhibitor
INDICATION:	Treatment of HIV Infection
CLINICAL FORMULATION:	25 mg (b) (4) TMC278 (b) (4); white to off-white film-coated (b) (4) tablets containing 25 (b) (4) TMC 278 as the free base. 27.5, (b) (4) of the hydrochloride are equivalent to 25, (b) (4) of the free base, respectively. Inactive Ingredients: Lactose monohydrate, NF; USP ;Polysorbate 20, USP ; Microcrystalline cellulose, NF ; Croscarmellose sodium, NF (b) (4) Magnesium stearate, NF ; (b) (4)
ROUTE OF ADMINISTRATION	Oral

DISCLAIMER: TABULAR AND GRAPHICAL INFORMATION IS FROM SPONSOR'S SUBMISSION UNLESS STATED OTHERWISE.

This amendment contains a final study report on male fertility in rats. The study did not product remarkable toxicity findings on TMC278 at the doses investigated. The study is reviewed as follows:

Study title: **Oral (Gavage) Male Fertility Study in the Rat**
Study no.: NC124
Laboratory: (b) (4)
Study initiation: 11/2004
GLP: yes (x) no ()
QA report: yes (x) no ()
Lot #, % purity: ZR31458PFA011 and ZR31458PFA021
Formulation/ vehicle: Aqueous suspension containing vehicle [0.5 % (w/v) F4M Premium Methocel]

METHODS: Three groups of 25 male Sprague-Dawley rats were dosed once daily with TMC278.HC1 (R314585) at dose levels of 100, 400 or 1600 mg/kg/day. A similar group of females received vehicle only and served as Controls. The males were dosed for 10 weeks prior to mating, during mating and for approximately 3-4 days after the mating period. Clinical signs and bodyweights were recorded. Food consumption was recorded during the pre-pairing period. Four groups of 25 females of the same strain were untreated and served as mates for the males. Clinical and mortality checks were carried out daily.

After the pre-mating dosing period, each male was paired with an untreated female, for up to ten days. The pregnant females were killed and subject to necropsy on Day 13 of gestation. The number of corpora lutea, implantations and live embryos were recorded.

The males were subject to macroscopic necropsy approximately three to four weeks after the end of the mating period. The testes and epididymides were removed, weighed and examined histopathologically. In addition, the liver and thyroids were removed, fixed, weighed and retained. Immediately after removal, one of the cauda epididymides from all males was sampled for assessment of motility and concentration using CASA (computer assisted sperm motility analysis) technology, Hobson Tracker, U.K. For sperm morphology a smear was prepared and examined by light microscopy.

Tissue	Weigh	Fix	Slide Preparation	Microscopic Examination
Liver	✓	✓		
Thyroids	✓	✓		
Testes	✓	✓	✓	✓
Epididymides	✓	✓	✓	✓

Dosing: 0 (control), 100, 400 or 1600 mg/kg TMC278.HC1 oral gavage

Group Number	Colour code	Number of animals Males	Females	Animal identification numbers Males	Females	Dose level TMC278.HCL (R314585) (mg/base eq./kg/day)
1	White	25	25	1-25	121-145	0
2	Green	25	25	26-50	146-170	100
3	Yellow	25	25	51-75	172-195	400
4	Pink	25	25	76-100	196-220	1600
5	White #	10	10	101-110*	221-230*	0
6	Pink #	10	10	111-120*	231-240*	1600

= Black corner added to label
* = Not required following outcome of pregnancy status of main study animals

Species/strain: Crl:CD (SD) IGS BR VAF PLUS strain rats

#/sex/group: 20

TK group: 9 animals per sex and per dose group

Route, volume: Oral gavage, 1 ml/100g BW

Toxico-kinetics: Not done.

RESULTS:

Mortality None.

Clinical signs: Unremarkable

Body weights: Unremarkable

Food consumption: Unremarkable

Mating & Fertility With the exception of one pair in the 100 mpk who mated after 6 days, all others mated within 4 days of the pairing period. Copulation and fertility indices in all groups were similar to those of the Controls.

Organ weights: There were no drug related findings at post mortem examination. Bodyweight-related liver and thyroid weights were elevated in all groups receiving TMC278.HC1 but there was no effect on the weights of the testes or epididymides.

Sperm Motility & Morphology: There was no reduction in average path velocity (VAP) or straight line velocity (VSL) of sperm in any of the groups receiving TMC278.HCl, values were similar to the Controls indicating no adverse effect on sperm motility. There was no adverse effect of treatment on sperm concentration or sperm morphology in any group receiving TMC278.HCl, values were similar to those of the Controls.

Testicular Histopathology: No remarkable histopathological changes in the epididymides or testes (1600 mg/kg) are reported.

Implantation in Females At mid-term necropsy (Day 13 of gestation) there were 25, 25, 24 and 25 females with implantations in the Control, 100, 400 or 1600 mpk, respectively.

Other Pregnancy Parameters There was no effect upon any pregnancy parameter in any of the treated female groups. The number of corpora lutea, the extent of pre-and post-implantation loss and the number of live embryos in all groups were comparable with the Controls.

Conclusions: The NOAEL for male fertility was considered to be 1600 mg/kg for this study. No toxicokinetics was performed to support the drug exposure. No regulatory comments are needed for this submission.

Kuei-Meng Wu, Ph.D.
Reviewing Pharmacologist
DAVDP

Concurrences:
HFD-530/Dep Dir/PTL/JFarrelly
Wu/Pharm/4/22/07

Disk: HFD-530/JFarrelly

cc:
HFD-530 IND 67,699(111)
HFD-530/Division File
HFN-340
HFD-530/CSO/
HFD-530/MO/
HFD-530/Chem/
HFD-530/Micro/
HFD-530/Pharm/
HFD-345

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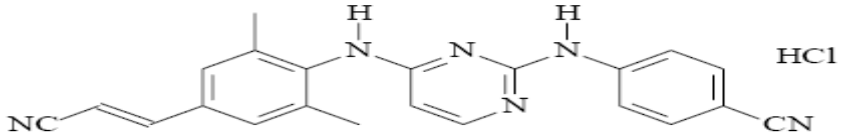
/s/

Kuei Meng Wu
4/27/2007 03:02:44 PM
PHARMACOLOGIST

James Farrelly
5/2/2007 07:59:09 AM
PHARMACOLOGIST

Appendix 1F

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND NUMBER:	67,699
VOLUME/DOCUMENT TYPE:	IT
SEQUENCE NUMBER/DATE:	127/4-07
SPONSOR:	Tibotec, Inc; 1020 Stony Hill Road, Suite 300, Yardley, PA 19067
MANUFACTURER:	J&J Pharm Res and Dev, or Janssen Pharm, Belgium
INFORMATION TO SPONSOR:	Yes () No (x)
REVIEWER NAME:	Kuei-Meng Wu
DIVISION NAME:	DAVDP
HFD NO.:	HFD-530
DRUG:	TMC278 (HCl salt); R 314585 or R278474 (free base)
CAS No.	700361-47-3
CHEMICAL NAME:	(E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile hydrochloride
FORMULA/MW:	402.88; $C_{22}H_{18}N_6$.HCl
STRUCTURE:	
DRUG CLASS:	Antiviral, on-nucleoside reverse transcript inhibitor
INDICATION:	Treatment of HIV Infection
CLINICAL FORMULATION:	25 mg(b) (4) TMC278 (b) (4); white to off-white film-coated (b) (4) tablets containing 25 (b) (4) TMC 278 as the free base. 27.5, (b) (4) of the hydrochloride are equivalent to 25, (b) (4) of the free base, respectively. Inactive Ingredients: Lactose monohydrate, NF; USP ;Polysorbate 20, USP ; Microcrystalline cellulose, NF ; Croscarmellose sodium, NF ; (b) (4) ; Magnesium stearate, NF ; (b) (4).
ROUTE OF ADMINISTRATION	Oral

DISCLAIMER: TABULAR AND GRAPHICAL INFORMATION IS FROM SPONSOR'S SUBMISSION UNLESS STATED OTHERWISE.

COMMENTS

This amendment contains an AMES and a series of animal PK reports on TMC278. They are summarized as follows:

(1) Tissue distribution and placental transfer of 14C-TMC278, as studied by whole-body autoradiography, in the pregnant Sprague-Dawley rat after single oral administration at 40 mg/kg. (TMC278-NC109)(Test facility: Global Preclinical Development, Beerse site Department of Toxicology/Pathology, Janssen Pharmaceutica N.V. Turnhoutseweg, 30 B-2340 Beerse, Belgium)

This study showed that the tissue distribution of TMC278 related total radioactivity (TR) in the female pregnant Sprague-Dawley rat, as expressed by the AUC0-8h, measured in the liver were about 6 times that in blood, followed by adrenal gland, lachrymal gland, kidney, fat (AUC0-8h tissue/blood ratios=2-4), and lung, pancreas, salivary gland, heart, spleen (ratios=1.5-2).

(2) A study of the effects of TMC278 hydrochloride on some hepatic enzyme activities after oral administration for 6 months at doses of 0, 5, 10, 40 mg/kg/day to male and female beagle dogs. (TMC278-NC140) (b) (4)

This animal pk study showed that TMC278 by gavage for six months had little effect on the parameters of hepatic xenobiotic metabolism in dogs. No evidence of induction on CYP1A, CYP2B, CYP2E, CYP4A forms, UDPglucuronosyltransferase activity and cytosolic GST activities were reported by this study.

(3) A study of the effects of TMC278 hydrochloride on some hepatic enzyme activities after oral administration for three months at doses of 0, 40, 120, and 400 mg/kg/day to male and female SD rats. (TMC278-NC193) (b) (4)

This rat pk study showed that TMC278 is an inducer of hepatic microsomal CYP4A, CYP3A, CYP2b (not CYP2E) and microsomal UDP glucuronosyltransferase in males primarily.

(4) A study on the pharmacokinetics and relative bioavailability of TMC278 in male beagle dogs after single oral administration of 3 different particle sizes of Drug Substance of TMC278.HCl (R314585) at 5 mg eq./kg (TMC278-NC257)(Test facility: Global Preclinical Development, Beerse site Department of Toxicology/Pathology, Janssen Pharmaceutica N.V. Turnhoutseweg, 30 B-2340 Beerse, Belgium)

This single-dose pk study compared relative bioavailability of the drug with different particle sizes in dogs:

Mean C_{max} = 926, 316 and 121 ng/ml after 15 µm, 85 µm, 216 µm, respectively. $AUC_{0-\infty}$ =20500, 10600 and 4600 ng.h/ml, respectively.

(b) (4)

[redacted]

(5) In Vitro Bacterial Reverse Mutation Test of TMC278 in Salmonella typhimurium with human liver S9-mix (TMC278-NC279)(Test facility: Global Preclinical Development, Beerse site Department of Toxicology/Pathology, Janssen Pharmaceutica N.V. Turnhoutseweg, 30 B-2340 Beerse, Belgium)

This study showed a negative AMES test result on TMC 278. TMC278 (R278474) in the presence of pooled human liver S9-mix has no mutagenic properties towards the various *S. typhimurium* strains (strains TA98, TA1537, TA100 and TA1535) under the test conditions described in this report up to the maximum test concentration of 500 µg/plate.

Conclusions: No regulatory comments are needed for this submission.

Kuei-Meng Wu, Ph.D.
Reviewing Pharmacologist
DAVDP

Concurrences:
HFD-530/Dep Dir/PTL/JFarrelly
Wu/Pharm/4/22/07

Disk: HFD-530/JFarrelly

cc:
HFD-530 IND 67,699(127)
HFD-530/Division File
HFN-340
HFD-530/CSO/
HFD-530/MO/
HFD-530/Chem/
HFD-530/Micro/
HFD-530/Pharm/
HFD-345

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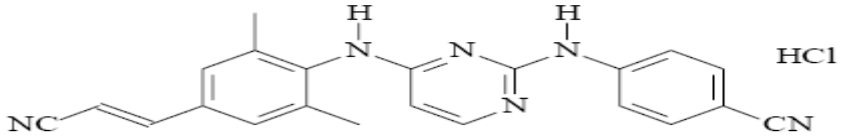
/s/

Kuei Meng Wu
5/8/2007 12:50:21 PM
PHARMACOLOGIST

James Farrelly
5/8/2007 01:00:54 PM
PHARMACOLOGIST

Appendix 1G

PHARMACOLOGY/TOXICOLOGY COVER SHEET

IND NUMBER:	67,699
VOLUME/DOCUMENT TYPE:	IT
SEQUENCE NUMBER/DATE:	113/2-07
SPONSOR:	Tibotec, Inc; 1020 Stony Hill Road, Suite 300, Yardley, PA 19067
MANUFACTURER:	J&J Pharm Res and Dev, or Janssen Pharm, Belgium
INFORMATION TO SPONSOR:	Yes (x) No ()
REVIEWER NAME:	Kuei-Meng Wu
DIVISION NAME:	DAVDP
HFD NO.:	HFD-530
DRUG:	TMC278 (HCl salt); R 314585 or R278474 (free base)
CAS No.	700361-47-3
CHEMICAL NAME:	(E)-4-[[4-[[4-(2-cyanoethenyl)-2,6-dimethylphenyl]amino]-2-pyrimidinyl]amino]benzonitrile hydrochloride
FORMULA/MW:	402.88; $C_{22}H_{18}N_6$.HCl
STRUCTURE:	
DRUG CLASS:	Antiviral, on-nucleoside reverse transcript inhibitor
INDICATION:	Treatment of HIV Infection
CLINICAL FORMULATION:	25 mg(b) (4) TMC278 (b) (4) white to off-white film-coated (b) (4) tablets containing 25 (b) (4) TMC 278 as the free base. 27.5, (b) (4) of the hydrochloride are equivalent to 25, (b) (4) of the free base, respectively. Inactive Ingredients: Lactose monohydrate, NF; USP ;Polysorbate 20, USP ; Microcrystalline cellulose, NF ; Croscarmellose sodium, NF ; (b) (4) ; Magnesium stearate, NF ; (b) (4).
ROUTE OF ADMINISTRATION	Oral

DISCLAIMER: TABULAR AND GRAPHICAL INFORMATION IS FROM SPONSOR'S SUBMISSION UNLESS STATED OTHERWISE.

COMMENTS

This amendment contains a series of animal PK, secondary pharmacology and toxicity study reports on TMC278 and (b) (4) is a synthesis intermediate and impurity of TMC278 HCL-salt or R314585. (The studies conducted with (b) (4) were addressed in the Annual Report; p.102-103, submitted on December 13, 2006, SN 117). The results of this submission showed:

1. TMC278 is a hepatic CYP enzymes inducer in mice.
2. TMC278 is an eye irritant.
3. TMC278 did not produce remarkable reproductive toxicities (maternal, pup, F0, F1) in a fertility study conducted in rats (up to 400 mg/kg).
4 (b) (4) tested positive in AMES and human lymphocytes (in vitro) assays and is a positively mutagenic and clastogenic substance.
5. (b) (4) is both an eye irritant and a skin sensitizer.

Summaries of the studies are provided below:

(1) *In Vitro* Bovine Corneal Opacity-Permeability Eye Irritation Test (TMC278-NC202)(Test facility: Global Preclinical Development, Beerse site Department of Toxicology/Pathology, Janssen Pharmaceutica N.V. Turnhoutseweg, 30 B-2340 Beerse, Belgium)

This study showed that TMC278.HCl [20% (m/m) suspension] induced an increase in corneal opacity (no increase in permeability.) and the drug is now classified as a moderate eye irritant (*in vitro* score=32.5).

(2) A study of the effects of TMC278 hydrochloride on some hepatic enzyme activities after oral administration for three months at doses of 0, 20, 80, and 320 mg/kg/day to male and female Swiss albino CDl mice. (TMC278-NC192)

(b) (4)

This animal pk study showed that (a). TMC278 is an inducer of hepatic microsomal CYP4A CYP3A and possibly other CYP subfamily forms in both male and female mice (from oral gavage study for three months), (b). TMC278 is an inducer of microsomal UDP glucuronosyltransferase (male mice with 80, 320 mg/kg/day female mice with 20, 80, 320 mg/kg/day).

(3). Secondary enzyme/receptor assay battery conducted by (b) (4) showed unremarkable results on all tests including possible inhibition of the pentagastrin-stimulated gastric acidity in fasted rats.

(4). *In Vitro* Bacterial Reverse Mutation Test with *Salmonella Typhimurium* (b) (4) (an intermediate) (NC165, Test facility: Global Preclinical Development, Beerse site Turnhoutseweg 30B-2340 Beerse, Belgium)

(b) (4) tested positive in mutagenic assay using *S. typhimurium* strains TA1537 in the absence of S9-mix and TA98 in the presence of S9-mix.

The test was carried out using standard operating procedures which are based on the most recent guidelines for performing this test [in triplicate, using
five strains of *Salmonella typhimurium*, TA1535, TA1537, TA102, TA98 and TA100,
in the absence and in the presence of a rat liver metabolic activation system (S9-mix)].
(b) (4) concentration used: 78.13, 156.25, 312.5, 625, 1250, 2500 and 5000 µg/plate (in DMSO). With the strain TA1537 in the absence of S9-mix and TA98 in the presence of S9-mix, a biologically significant increase in the reversion rate was observed.

(5). TMC278: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application). (NC159; Test Facility (b) (4)

This study showed that TMC278 did not induce significant or irreversible damage to the skin and is considered to be "not irritating" to rabbit skin.

(6). ACUTE ORAL TOXICITY IN THE MOUSE on (b) (4) **(NC180, November 2004 by** (b) (4)

This study was performed to assess the acute oral toxicity of the test material following a single oral administration in the female outbred albino mouse. The method followed the OECD Guidelines for the Testing of Chemicals No. 423 “Acute Oral Toxicity – Acute Toxic Class Method” (adopted 17 December 2001).
All animals at 2000 mg/kg were found dead one day after dosing. Signs of systemic toxicity noted during the study were hunched posture, lethargy, ataxia, ptosis, decreased respiratory rate, labored respiration, occasional body tremors, splayed gait, pilo-erection, increased salivation, emaciation and dehydration.
One animal treated at 300 mg/kg was killed in extremis three days after dosing. LD50 of (b) (4) in the female outbred albino mouse was estimated to be in the range of 300 – 500 mg/kg.

(7). RABBIT ENUCLEATED EYE TEST on (b) (4) **(NC 181, October 2004 by** (b) (4)

This ocular irritancy test showed that (b) (4) produced eye irritation and is considered to have the potential to cause severe ocular irritancy in vivo.

(8). ACUTE DERMAL IRRITATION IN THE RABBIT on (b) (4) **(NC 182, 11/2004 by** (b) (4)

(b) tested negative in a dermal irritation study and is thus label as such: Primary Irritation Index: 0.0; Classification: Non-Irritant.

(9). LOCAL LYMPH NODE ASSAY IN THE MOUSE on (b) (4) (NC 183, 11/2004 by (b) (4)

This skin sensitizing test using the Local Lymph Node Assay(delayed type hypersensitivity) in the CBA/Ca strain of mouse showed that (b) (4) was a skin sensitizer following topical application of this drug to the dorsal surface of the ear (lymphocyte proliferation was observed).

(10). EVALUATION OF THE SKIN SENSITIZATION POTENTIAL OF TMC278 AND VARIOUS NANOSUSPENSIONS OF TMC278 (BASK) IN THE LOCAL LYMPH NODE ASSAY (NON-GLP STUDY by (b) (4) August 2006)

This study demonstrated that TMC278 (HCl) and nanosuspension formulations of TMC278 (base) fail to elicit proliferative lymph nodal responses higher than those observed for control groups with stimulation index values less than 3 fold for all test concentrations and routes of exposure. This conclusion holds true even when the skin barrier is breeched via subcutaneous injection [the positive control test substance, streptozotocin, induced an SI of approximately 22 fold, indicative of a strong sensitizer, using the subcutaneous method of exposure.] Based on this finding the sponsor suggested that TMC278 (base) is unlikely to posses sensitizing properties.

(11). (b) (4): SCREENING CHROMOSOME ABERRATION TEST IN HUMAN LYMPHOCYTES *IN VITRO* (NC 184, 1/2004 by (b) (4)

This study showed (b) (4) clastogenic to human lymphocytes (b) (4)
Precipitation occurred at and above 1043.5 µg/ml in the without-metabolic activation exposure groups and at and above 521.75 µg/ml in the with-metabolic activation group.

The dose range for the Preliminary Toxicity test was 8.15 to 2087 µg/ml. Based on the mitotic index data (MI), test material induced toxicity in all three of the exposure groups. Microscopic assessment of the slides prepared from the cultures showed that metaphase cells were present at up to 1043.5 µg/ml in the pulse exposure groups and 260.88 µg/ml in the continuous exposure group. Therefore, the selection of the dose range for the chromosome aberration test was limited by toxicity for all exposure groups. The dose levels of the controls and the test material used in the chromosome aberration test are presented in the table below:

Concentration range of (b) (4) (µg/ml)
4(20)-hour without S9 0*, 65.22, 130.44*, 260.88*, 521.75*, 782.63, 1043.5, MMC 0.4*
4(20)-hour with S9 0*, 65.22, 130.44*, 260.88*, 521.75*, 782.63, 1043.5, CP 7.5*
24-hour without S9 0*, 16.3, 32.61, 65.22*, 130.44*, 260.88*, 391.32, MMC 0.2*
[MMC = Mitomycin C CP = Cyclophosphamide]

Based on MI, the test material was more toxic in the Chromosome Aberration Test than was observed in the Preliminary Toxicity Test.

In the 4(20) hours exposure groups there were scorable metaphase cells at up to 521.75 µg/ml. In the 4(20) hours exposure in the absence and presence of metabolic activation the MI was 37% and 32% respectively at this dose level.

In the 24 hours exposure group without S9 there were scorable metaphases up to 260.88 µg/ml and there was toxicity induced cell synchronization with an increase in MI at 65.22 and 130.44 µg/ml but complete mitotic inhibition at 391.32 µg/ml. Therefore, acceptable levels of toxicity were achieved in all cases.

All of the vehicle control cultures had frequencies of cells with chromosome aberrations within the expected range. The positive control materials [MMC = Mitomycin C CP = Cyclophosphamide] induced statistically significant increases in the frequency of cells with aberrations. It was therefore considered that the metabolic activation system was shown to be functional and the test method itself was operating as expected.

The test material induced a statistically significant increase in the frequency of cells with aberrations in the absence of metabolic activation (S9) after 4 hours exposure at the maximum dose level (521.75 µg/ml). An additional 100 metaphase cells were scored from the B culture to confirm the response. Similar numbers of cells with aberrations were observed in cells from both cultures. Furthermore, the frequency of cells with aberrations exceeded historical maxima for vehicle controls and the aberrations that were seen included an excess number of chromatid exchanges, which are rarely seen in cells from vehicle control cultures.

There was no evidence of a response in the 4(20) hours exposure with-S9 at this dose level or in the 24 hours exposure without metabolic activation at the maximum scorable dose level, 260.88 µg/ml.

The test material did not induce a statistically significant increase in the numbers of polyploid cells in any of the exposure groups.

The test material induced a statistically significant increase in the frequency of cells with chromosome aberrations in the absence of a liver enzyme metabolizing system after a 4(20)-hour exposure at the maximum scorable dose level only. The test material was therefore considered to be clastogenic to human lymphocytes in vitro.

(12)

Study title: **Oral (Gavage) Pre- and Postnatal Fertility Study in the Rat**

Study no.: NC131

Laboratory: (b) (4)

Study initiation: 11/2004

GLP: yes (x) no ()
QA report: yes (x) no ()
Lot #, % purity: ZR314585PUA031, 98.8%
Formulation/ vehicle: Aqueous suspension containing vehicle [0.5 % (w/v) F4M Premium Methocel] (hydroxypropylmethylcellulose)
METHODS: Three groups of twenty-five, time-mated, female Sprague-Dawley rats were dosed, once daily from Day 6 of gestation to Day 20 of lactation, inclusive, with suspensions of the TMC278, TMC278.HC1. The dose levels used (expressed as base) were 40, 120 and 400 mg/kg (Groups 2 to 4, respectively). A group of twenty-five similar rats, following the same dosing regime, served as Controls (Group 1) and received the vehicle.

Group number	Colour code	Number of mated F0 females	Animal identification numbers	Concentration (mg/mL) TMC278.HCl	Dose level (mg base eq./kg/day) TMC278.HCl
1	White	25	1-25	0	Vehicle Control
2	Green	25	26-50	4	40
3	Yellow	25	51-75	12	120
4	Pink	23	76-100	40	400

Maternal clinical signs, bodyweights and food consumption were recorded.

The females were allowed to litter and the total litter size and numbers of each sex were recorded. Pups were examined for abnormalities and weighed individually on Days 1, 4, 7, 14 and 21 of lactation. The number exhibiting ears open, the static righting reflex, eyes open, startle response and papillary light reflex were recorded on Days 3, 5, 15 and 21 of lactation, respectively.

The F0 females were sacrificed at weaning of their litters on Day 21 of lactation. All females were examined macroscopically and the numbers of implantation scars were counted.

Group number	Colour code	Number of F1 animals		Animal identification numbers	
		Males	Females	Males	Females
1	White	20	20	101-120	181-200
2	Green	20	20	121-140	201-220
3	Yellow	20	20	141-160	221-240
4	Pink	20	20	161-180	241-260

Approximately one week after the start of weaning the Fl generation, 20/sex/offspring_group were randomly selected (at least one from each of the weaned litters). The selected offspring were allowed to mature, untreated and the effects on growth, development, behavior and reproductive performance were assessed.

Dosing: 0 (control), 40, 120 and 400 mg/kg TMC278.HC1 oral gavage
Species/strain: Crl:CD (SD) IGS BR VAF PLUS strain rats
#/sex/group: See above
TK group: None
Route, volume: Oral gavage, 1 ml/100g BW
RESULTS: F0 Generation:

There were no mortalities or clinical observations recorded that were considered to be related to treatment with the TMC278. There was no effect of treatment on maternal bodyweight performance or food consumption or on the duration of gestation. There was no effect of treatment on the number of live litters or the mean number of pups per litter. There were no findings recorded at maternal necropsy that were related to treatment.

F1 Generation pre-weaning:
There was considered to be no effect of treatment on pup clinical condition or upon pup survival in any group treated with TMC278.HC1, compared with the Controls. [At 120 mg/kg, the survival rate to Day 4 of lactation (97.6 %) was slightly lower than that of the Controls although this comprised only 8 pup losses. Due to the high survival rate in the Control group (99.8 %) statistical significance was achieved, but this was considered to be coincidental and unrelated to treatment. At 400 mg/kg the mean number of pups surviving to Day 4 of lactation was also slightly but statistically significantly reduced (93.5 %) when compared with the Controls. This lower viability, however, was mainly attributable to one litter, Female 93, where seven of the nine pups born were found dead or missing, presumed cannibalized, between completion of parturition and Day 1 of lactation. This finding should be considered not to be of toxicological significance. At 40 and 120 mg/kg the mean lactation index showed that pup survival between Day 4 and Day 21 of lactation was slightly reduced but did not achieve statistical significance when compared with the Controls and was reflected in a decreased lactation index. The absence of a similar effect at 400 mg/kg confirmed that there was no relation to maternal treatment with the test article. As a consequence of these slight reductions in the viability and lactation indices the overall mean cumulative survival of pups to weaning was slightly lower for groups receiving the test article than for the Controls. This finding was considered to be associated with the pup deaths recorded for the sentinel animals during this period.]

Pup absolute bodyweights and bodyweight gain to weaning for all TMC278 treated groups were similar to or marginally greater than the Controls. Pup development during lactation was considered to be unaffected by maternal treatment with the TMC278.

There were no macroscopic findings at necropsy of Fl pups pre- or post-weaning that were related to maternal treatment with the TMC278.

Fl Generation post-weaning:
There were no early decedents in the Fl generation and there were no clinical observations recorded that were considered to be related to maternal treatment. There was no effect of maternal treatment with the TMC278 on bodyweight performance.

There was considered to be no effect of treatment on locomotor activity, hearing acuity or learning ability and memory retention of the Fl generation following maternal treatment with TMC278.HC1. Where parameters showed differences form the Controls these were considered to be of no or of little toxicological significance.

Sexual development, fertility and mating performance were unaffected by maternal treatment with the TMC278 and there was no effect on pregnancy parameters for Fl females.

Conclusion:

F0 females: Maternal treatment with TMC278.HC1 at 40, 120 and 400 mg/kg during gestation and lactation did not elicit toxicity. NOAEL for maternal toxicity=400 mg/kg.

Pup growth and pup/Fl development: There was no effect of maternal treatment with TMC278.HC1 on pup growth and no effect on pup survival or development during lactation or post-weaning. NOAEL for pup development after maternal treatment=400 mg/kg.

Fl reproductive performance: There was no effect of maternal treatment with TMC278.HC1 on fertility or mating performance of the Fl males or females or on gestation of the Fl females. NOAEL=400 mg/kg. No toxicokinetics was performed to support the drug exposures in this study.

No regulatory comments are needed for this submission.

Kuei-Meng Wu, Ph.D.
Reviewing Pharmacologist
DAVDP

Concurrences:
HFD-530/Dep Dir/PTL/JFarrelly
Wu/Pharm/5/2/07

Disk: HFD-530/JFarrelly

cc:
HFD-530 IND 67,699(113)
HFD-530/Division File
HFN-340
HFD-530/CSO/
HFD-530/MO/
HFD-530/Chem/
HFD-530/Micro/
HFD-530/Pharm/
HFD-345

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/s/

Kuei Meng Wu
5/14/2007 11:54:09 AM
PHARMACOLOGIST

James Farrelly
5/15/2007 02:18:43 PM
PHARMACOLOGIST

Appendix 2

Food and Drug Administration
Center for Drug Evaluation and Research
Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: July 25, 2005

To: Dr. Hua Zheng	**From:** Adele Seifried
Company: Tibotec, Inc.	HFD-024
Fax number: (609) 730-7501	**Fax number:** 301-480-8329
Phone number: (609) 730-7509	**Phone number:** 301-443-5344

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report - IND 67,699

Total no. of pages including cover: 4

Comments:

Document to be mailed: " YES ☑ NO

Executive CAC
Date of Meeting: July 19, 2005
Mouse/Rat Carcinogenicity Dose-Selection Protocol

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Abby Jacobs, Ph.D., HFD-024, Member
Terry Peters, D.V.M., HFD-520, Substitute Alternate Member
Jim Farrelly, Ph.D., HFD-530, Team Leader
Alex Jordan, Ph.D., HFD-530, Presenting Reviewer

Author of Draft: Alex Jordan

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-year 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 #: 67,699
Drug Name: TMC278
Sponsor: Tibotec, Inc.

Background: TMC278 is a nonnucleoside reverse transcript inhibitor for the treatment of HIV. Sponsor submitted protocols for rat and mouse carcinogenicity studies in amendments 023 and 022, respectively. Dose proposals were based on a two week study with doses up to 2000 mg/kg and a 6 month study with doses up to 400 mg/kg in rats and a three month study with doses up to 320 mg/kg in mice.

Mouse Carcinogenicity Study Protocol and Dose Selection

The Sponsor proposed a two-year carcinogenicity study in Swiss mice with doses based on an MTD due to kidney toxicity in females and on multiples of drug exposure in males. Doses recommended were 0, 20, 80, 160 mg/kg/day for males and 0, 5, 20, 80 mg/kg/day for females. The dosages are free base equivalents of the administered HCl salt (R314585) given in 0.5% Methocel (hydroxypropyl methylcellulose) by gavage.

Rat Carcinogenicity Study Protocol and Dose Selection

The Sponsor proposed a two-year carcinogenicity study in Sprague-Dawley rats with doses based on saturation of absorption. Between 400 and 2000 mg/kg exposure increased by only 1.8 and 1.5 fold in males and females, respectively. Doses recommended were 0, 40, 120, 400 mg/kg/day for males and females. The dosages are free base equivalents of the administered HCl

salt (R314585) given in 0.5% Methocel (hydroxypropyl methylcellulose) by gavage.

Executive CAC Recommendations and Conclusions:

For the mouse carcinogenicity study the Committee recommended doses for males and females of 0, 20, 60 and 160 mg/kg/day by oral gavage based the AUC for the high dose in mice being > 25-fold the human AUC and minimal toxicity.

For the rat carcinogenicity study the Committee recommended doses of 0, 200, 500 and 1500 mg/kg by oral gavage based on saturation of absorption and no dose limiting toxicity.

_____
David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\
/Division File, HFD-530
/JFarrelly, HFD-530
/AJordan, HFD-530
/DAraojo, HFD-530
/ASeifried, HFD-024

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/s/

David Jacobson-Kram
7/25/05 11:38:22 AM

Appendix 3

Executive CAC
Date of Meeting: January 11, 2011

Committee: Paul Brown, Ph.D., OND IO, Acting Chair
Todd Bourcier, Ph.D., DMEP, Rotating Member
Linda Fossom, Ph.D., DPP, Rotating Member
Hanan Ghantous, Ph.D., DABT, DAVP, Team leader
Mark Seaton, Ph.D., DAVP, Presenting Reviewer

Author of Draft: Mark Seaton, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations.

NDA #202-022
Drug Name: Rilpivirine
Sponsor: Tibotec, Inc.

Rat Carcinogenicity Study
For the rat carcinogenicity study, the CAC recommended doses of 0, 200, 500 and 1500 mg/kg by oral gavage based on saturation of absorption and the absence of dose limiting toxicity in previous studies. The sponsor accepted the Agency's recommendation but chose to add an additional low dose of 40 mg/kg. The doses used for this study were appropriately selected based on saturation of absorption. The steady state (week 39) $AUC_{0\text{-}24h}$ values for the high dose groups were 18.4 and 83.8 µg.h/mL for males and females, respectively, which were 8-fold and 35 fold the mean estimated steady-state human $AUC_{0\text{-}24h}$ of 2.4 µg.h/mL, respectively.

The tumor types that showed the greatest increase in rats were thyroid follicular cell adenomas (alone) and adenomas – carcinomas (combined), although the increased incidence does not reach statistical significance using the current statistical decision rules. The tumor findings correlated with increased thyroid weights and increased serum thyroid stimulating hormone levels, among other effects. Rilpivirine slightly increased UDPGT activity in an *ex vivo* assay, and administration of rilpivirine to rats for three months was associated with an induction of CYP enzymes and UDPGT. Therefore, the increased incidence of thyroid tumors at the high dose is thought to result from a rodent-specific mechanism related to altered metabolism of thyroid-related hormones.

Mouse Carcinogenicity Study
The CAC recommended doses for males and females of 0, 20, 60 and 160 mg/kg/day by oral gavage based on the AUC for the high dose in mice being >25-fold the human AUC, and minimal toxicity in previous studies. The sponsor accepted the recommended doses. The doses used for this study were appropriately selected based on the AUC ratio. The steady state (week 28) $AUC_{0\text{-}24h}$ values for the high dose groups were 505 and 766 µg.h/mL for males and females, respectively, which were 210-fold and 319-fold the estimated mean steady-state human $AUC_{0\text{-}24h}$ of 2.4 µg.h/mL.

The tumor types that showed the greatest increase in treated mice, and for which the increase was statistically significant, were hepatocellular adenomas (alone) and hepatocellular adenomas-carcinomas (combined). In males, the incidence rates for those tumors reached statistical significance at the middle and high doses, whereas the incidence of hepatocellular carcinomas alone was increased at all doses but the increases did not reach statistical significance compared to controls. In females, the incidence of each tumor type alone, and the combined incidence of hepatocellular adenomas and carcinomas reached statistical significance at the middle and high dose. In a previous study, administration of rilpivirine to rats for three months was associated with an induction of CYP enzymes and UDPGT. In a three month study in mice, hepatocyte hypertrophy, moderate increases in single cell necrosis, and accumulation of Kupffer cell pigmentation was noted. Since rilpivirine is known to be associated with liver changes, an increase in adenomas and a slight increase in carcinomas in the two year study in mice might have been anticipated. The findings are considered to be treatment related; however, the increased incidence of liver tumors is thought to result from a rodent-specific mechanism related to induction of hepatic enzymes.

Males

	Cont	Low	Med	High	Dose	P_Low	P_Med	P_High
HEPATOCELLULAR ADENOMA	8	14	21	19	0.0022*	0.0694	0.0020*	0.0015*
HEPATOCELLULAR-ADENOMA+CARCINOMA	11	19	24	27	<0.001*	0.0371	0.0029*	<0.001*

Females

	Cont	Low	Med	High	Dose	P_Low	P_Med	P_High
HEPATOCELLULAR ADENOMA	1	0	8	16	<0.001*	1.0000	0.0194	<0.001*
HEPATOCELLULAR CARCINOMA	0	0	2	7	<0.001*	.	0.2646	0.0032*
HEPATOCELLULAR-ADENOMA+CARCINOMA	1	0	9	20	<0.001*	1.0000	0.0101	<0.001*

Rilpivirine is considered positive for liver carcinogenicity. Since liver adenomas and carcinomas are commonly seen in the mouse at a low basal rate, and given the possible rodent-specific mechanism for tumor production, the relevance to humans is considered unlikely.

Executive CAC Recommendations and Conclusions:

Rat:

- The Committee agreed that the study was adequate, noting prior Exec CAC concurrence with the protocol.
- The Committee concluded that the study was negative for statistically significant drug related neoplasms.

Mouse:

- The Committee agreed that the study was adequate, noting prior Exec CAC concurrence with the protocol and modifications to the protocol during the study.
- The Committee concluded that the study was positive for hepatocellular adenomas (alone) in males and females, hepatocellular carcinomas (alone) in females and hepatocellular adenomas-carcinomas (combined) in both males and females. These tumors may not be relevant to humans.

Paul Brown, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DAVP
/Hanan Ghantous, Ph.D., DABT, Team leader, DAVP
/Mark Seaton, Ph.D., Reviewer, DAVP
/ Robert G. Kosko. Jr., Pharm.D., M.P.H., DAVP
/ASeifried, OND IO

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/s/

ADELE S SEIFRIED
01/12/2011

PAUL C BROWN
01/12/2011

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/s/

MARK J SEATON
03/23/2011

HANAN N GHANTOUS
03/23/2011

Comments on N202022 Rilpivirine
From: A Jacobs, AD
Date: 3/10/11

1. I concur with the pregnancy category
2. There are no outstanding pharm/tox issues for this NDA

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/s/

ABIGAIL ABBY C C JACOBS
03/10/2011

April 11, 2011

In this review's opening table, "BLA" should have been "NDA."

This is corrected in the 4/11/2011 REV-NONCLINICAL-03 (General Review). That review replaces this one.

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Antiviral Products
Center for Drug Evaluation and Research

Date:	March 2, 2011
Reviewer:	Hanan Ghantous, PhD, DABT Supervisory Interdisciplinary Scientist
BLA #/SS#/date:	202-022/000/7/23/2010
Sponsor:	Tibotec Inc.
Drug Product:	Rilpivirine
Indication:	HIV infection in treatment-naïve patients
Recommended Action:	Nonclinical data support approval

Rilpivirine, is a diarylpyrimidine derivative, a next generation non-nucleoside reverse transcriptase inhibitor (NNRTI) indicated in combination with other antiretroviral agents for the treatment of HIV-1 infection in treatment-naïve adult patients.

The safety of Rilpivrine was investigated in a number of toxicology studies including repeat-dose nonclinical toxicity studies (mice, rats, rabbits, dogs and cynomolgus monkeys), in genetic toxicity and carcinogenicity studies and in reproductive and developmental toxicity studies.

The primary toxicity findings in nonclinical studies were adrenal effects, generally characterized by increased serum progesterone and decreased cortisol levels observed in rats, dogs, and Cynomolgus monkeys. These effects are thought to be associated with an inhibition of steroidogenesis at the level of 21-hydroxylase (CYP21) and 17-hydroxylase (CYP17, in Cynomolgus monkeys only). In dogs, findings of premature activation and overstimulation of the ovaries may also be related to inhibition of steroidogenesis. Those effects on dog ovaries were noted at exposures 8 to 25 times higher than clinical exposures at the recommended dose of 25 mg q.d. Based on the nonclinical safety studies, adrenal function was carefully monitored in the clinical trials. However, Phase III and Phase IIb trials did not show any safety concerns with respect to adrenal function or endocrine events. Based on the nonclinical safety information, Rilpivrine was considered safe for use in humans in clinical trials and it was recommended to give particular attention to possible effects on adrenal and gonadal steroidogenesis.

On-going and planned trials in adolescents and pre-pubertal children should include endocrine safety monitoring, with monitoring of hormone levels and ovulation. Growth curves, pubertal status, breast development, menarche or evidence of either hyperandrogenism (hirsutism) or delayed adrenarche should be documented in these trials.

A Phase I clinical trial demonstrated a QT interval-prolonging effect of Rilpivirine at supratherapeutic doses. In follow-up nonclinical safety pharmacology studies, Rilpivirine demonstrated the potential to inhibit some potassium channels involved in cardiac action potential repolarization at concentrations approximately 10-fold greater than the clinical exposures. Given the clinical and nonclinical findings, adverse events that could be related to cardiac conduction abnormalities or to rate and rhythm disturbances were closely monitored in the Phase IIb and Phase III clinical trials. No clinically relevant QTc prolonging effect was observed with the recommended therapeutic dose of 25 mg q.d., however, patients with known risk for QT interval prolongation or Torsade de Pointes were excluded from the Phase III trials.

Rilpivirine was evaluated for carcinogenic potential by oral gavage administration to mice and rats for 2 years. Rilpivrine was positive in mice for hepatocellular neoplasms which is likely not relevant to humans. At the lowest tested doses in the carcinogenicity studies, the systemic exposures (based on AUC) to Rilpivirine were 21-fold (mice) and 3-fold (rats), relative to those observed in humans at the recommended clinical dose. Rilpivirine was not genotoxic.

The reproductive and developmental toxicity studies did not demonstrate any effects on fertility, fecundity, parturition, or maternal behavior at systemic exposures approximately 40-fold higher than the exposure in humans at the recommended clinical dose. In offspring from rats and rabbits treated with Rilpivirine during pregnancy and lactation, there were no toxicologically significant effects on developmental endpoints., at exposures 15 and 70 times higher than the exposure in humans at the recommended clinical dose.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Mark Seaton that the nonclinical data support an approval action for this Rilpivrine.

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/s/

HANAN N GHANTOUS
03/23/2011

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202022 **Applicant:** Tibotec **Stamp Date:** July 23, 2010

Drug Name: TMC278 (rilpivirine) **NDA/BLA Type:** NME

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			Not applicable.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-202022	ORIG-1	TIBOTEC INC	TMC278

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/s/

MARK J SEATON
09/01/2010

HANAN N GHANTOUS
09/01/2010