CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

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:	204-384
Supporting document number:	0
Applicant's letter date:	6/28/2012
CDER stamp date:	6/29/2012
Product:	SIRTURO (bedaquiline)
Indication:	SIRTURO is indicated in adults (≥ 18 years) as part of combination therapy of pulmonary multi-drug resistant tuberculosis.
Applicant:	Janssen Therapeutics, a Division of Janssen Products, LP 1125 Trenton-Harbourton Road Titusville, NJ 08560
Review Division:	Division of Anti-infective Products
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1 Executive Summary

1.1 Introduction

Bedaquiline is administered at 400 mg once a day for two weeks followed by 22 weeks of intermittent dosing (200 mg three times a week) for the treatment of tuberculosis infection in MDR-TB patients. The highest exposures observed during the intermittent dosing were measured after 8 weeks of treatment, where the mean $AUC_{(0\text{-}24h)}$ value was 22 µg*h/mL for bedaquiline and 6 µg*h/mL for the metabolite M2. Mean C_{max} levels were as high as 3.3 µg/mL during Week 2 (daily dosing phase) but steady state plasma levels were about 0.9 µg/mL at Week 8. These exposure data in man were derived from trial TMC207-C208. The safety implications of nonclinical studies were based on the exposure data achieved with the proposed dose.

Recommendations

1.1.1 Approvability

There are no nonclinical pharmacology or toxicology data that preclude the approval of SIRTURO.

1.1.2 Additional Non Clinical Recommendations

No additional nonclinical pharmacology or toxicology studies of SIRTURO are being recommended at this time.

1.1.3 Labeling

The following text is to be included in the prescribing information:

HIGHLIGHTS OF PRESCRIBING INFORMATION

Pregnancy: Use SIRTURO only if the potential benefit justifies the potential risk to the fetus.
Nursing Mothers: Discontinue nursing or discontinue SIRTURO taking into account the importance of the drug to the mother.

8.1 Pregnancy

Pregnancy Category B. Reproduction studies performed in rats and rabbits have revealed no evidence of harm to the fetus due to bedaquiline. In these studies, the corresponding plasma exposure (AUC) was 2-fold higher in rats compared to humans. There are, however, no adequate and well-controlled studies of SIRTURO in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

8.3 Nursing Mothers

It is not known whether SIRTURO or its metabolites are excreted in human milk, but rat studies have shown that drug is concentrated in breast milk.

In rats, treated with SIRTURO at doses 1-2 times the clinical dose (based on AUC comparisons), concentrations in milk were 6- to 12-fold higher than the maximum concentration observed in maternal plasma. Pups from these dams showed reduced body weights compared to control animals throughout the lactation period.

Because of the potential for adverse reactions in nursing infants, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

No mutagenic or clastogenic effects were detected in the *in vitro* non-mammalian reverse mutation (Ames) test, *in vitro* mammalian (mouse lymphoma) forward mutation assay and an *in vivo* mouse bone marrow micronucleus assay. Carcinogenicity testing of SIRTURO is ongoing and results are not available at this time.

SIRTURO had no effects on fertility when evaluated in male and female rats. No relevant drug-related effects on developmental toxicity parameters were observed in rats and rabbits at doses corresponding to plasma (AUC) exposures twice the clinical exposures.

There was no effect of maternal treatment with SIRTURO at any dose level on sexual maturation, behavioral development, mating performance, fertility or reproductive capacity of the F1 generation animals.

There was no effect of maternal treatment with bedaquiline at any dose level on sexual maturation, behavioral development, mating performance, fertility or reproductive capacity of the F1 generation animals. Body weight decreases in pups were noted in high dose groups during the lactation period after exposure to bedaquiline via milk and were not a consequence of in utero exposure. Concentrations of bedaquiline in milk were 6- to 12-fold higher that the maximum concentration observed in maternal plasma.

13.2 Animal Toxicology and/or Pharmacology

SIRTURO is a cationic, amphiphilic drugs (CAD) and induced phospholipidosis (at almost all doses, even after very short exposures) in drug-treated animals, mainly in cells of the monocytic phagocytic system (MPS). All species tested showed drug-related increases in pigment-laden and/or foamy macrophages, mostly in the lymph nodes, spleen, lungs, liver, stomach, skeletal muscle, pancreas and/or uterus. After treatment ended, these findings were slowly reversible. Phospholipidosis has been shown in numerous approved drugs and no specific adverse events have been ascribed to this finding.

Muscle degeneration was observed in several species at the highest doses tested. For example the diaphragm, oesophagus, quadriceps and tongue of rats were affected after 26 weeks of daily treatment at doses similar to clinical exposures based on AUC comparisons. These findings were not seen after a 12-week, treatment-free, recovery period and were not present in rats given the same dose biweekly. Degeneration of the fundic mucosa of the stomach, hepatocellular hypertrophy and pancreatitis were also seen in high dose animals.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical toxicology program was comprehensive, and included *in vitro* and *in vivo* studies using mice, rats, dogs, rabbits and guinea pig. The applicant evaluated the effects of bedaquiline administration for up to 3 months in mice, 6 months in rats and 9 months in dogs via daily and intermittent dosing (two or three times weekly). There were also evaluations of genotoxic potential, fertility, embryofetal toxicity, pre- and postnatal development, local tolerance, immunotoxicity and mechanistic studies. The studies conducted were adequate to characterize the toxicity of bedaquiline. Study designs, including species selection, dose selection and study durations were appropriate given the 24 week clinical regimen. Study conduct was also acceptable since most studies were conducted according to GLP.

In test species, bedaquiline bioavailability was between 36 and 79%, and drug achieved maximum plasma concentrations between 0.5 and 8 hours after dosing. Bedaquiline is metabolized mainly by cytochrome P450 (CYP 3A4) to its major metabolite, M2, via *N*-demethylation. Another metabolite, M3, is produced by the subsequent *N*-demethylation of M2. Bedaquiline is extensively bound to proteins, with plasma protein binding above 99.9% for all species, including humans. It is very slowly eliminated from the plasma, with extensive distribution to tissues (volume of distribution Vd_{ss} 60 times total body water). Tissues with the highest accumulation of drug were the adrenal gland, lung, spleen, liver, lymph nodes and thymus. Over time, in some tissues, the concentrations of metabolite M2 exceeded the levels of the parent compound. Elimination half life ranged from 2 days in mice to 50 days in dogs. Drug is excreted predominantly in the feces, with only 1-4% excreted via urine. In bedaquiline-treated dams which had recently given birth, bedaquiline and its metabolite M2 in milk were 4- to 12 times higher than plasma levels.

Adverse Events of Special Concern
Throughout the nonclinical development program certain toxicities were identified as being of special concern because of their seriousness and/or because of their observed incidence. These were QT prolongation, liver damage, pancreatitis, phospholipidosis and musculopathy.

Cardiotoxicity
Bedaquiline has shown some evidence of cardiotoxic potential in nonclinical studies, but the relevance of these findings to humans will be further investigated in upcoming clinical trials. Bedaquiline and its metabolite M2 were shown to inhibit IKr in hERG transfected kidney cells with IC_{50} values of 0.2 µg/mL for both compounds. This would not be considered a strong hERG blocker since, by comparison, the positive control astemizole, which is known to cause prolonged QT, inhibited IKr with an IC_{50} in the nanomolar range. Bedaquiline did not increase the QTc interval at plasma bedaquiline levels up to 3.6 µg/mL in the anesthetized guinea pig. QTc interval was also not increased in conscious telemetered dogs after single oral or intravenous dosing. In a mechanistic study (#1408-008), dogs dosed with 100 mg/kg bedaquiline for six days showed no

increases in QT interval. When dogs were then treated with 100 mg/kg moxifloxacin on Day 7, there was no real difference between the effect of moxifloxacin alone (+17 % compared to control) and the effect of moxifloxacin +bedaquiline, where the QT prolongation was +20 % compared to control. Exposures ($AUC_{(0\text{-}24h)}$) at 100 mg/kg were estimated to be much higher than clinical exposures.

In one six month dog study, dogs showed slight increases in the QTc interval (+12 to +16 %, (depending on the formula used to correct the QT) after two months of dosing at 40 mg/kg/day. At 20 mg/kg/day, ($AUC_{(0\text{-}24h)}$ about 130 mcg*h/mL), the QTc interval was only minimally increased. There were no QTc changes in dogs treated for six months at 10 mg/kg/day ($AUC_{(0\text{-}24h)}$ estimated at about 86 µg*h/mL) or in animals dosed twice weekly at 140 mg/kg ($AUC_{(0\text{-}24h)}$ estimated at about 170 mcg*h/mL). The observed QTc prolongation was consistent with the finding of hERG channel blockade and suggests that the QTc prolongation may be related to drug, albeit high doses.

Cardiac troponin (cTnI, a biomarker of cardiac toxicity) was increased at several time points in these 40/20 mg/kg/day dogs and those dosed twice weekly at 140 mg/kg. Cardiac lesions in these dogs consisted of minimal multifocal lymphohistiocytic infiltrates with degeneration of cardiomyocytes and/or minimal to slight endocardial fibrosis. These changes were also associated with elevated levels of total CK.

No similar EKG changes or cardiac lesions were detected in a 9 month dog study using a lower dose (18 mg/kg/day) despite substantial exposure ($AUC_{(0\text{-}24h)}$ about 154 µg*h/mL at week 13) and despite increases in cardiac troponins. In this instance, increases in cardiac troponins did not serve as a good biomarker of cardiac damage. No adverse cardiac effects were observed in dogs treated at 10 mg/kg/day, a dose which resulted in exposures 4 times the clinical exposure.

At this point in SIRTURO's development, only a limited number of subjects have been exposed to the SIRTURO, but some trial participants have experienced prolonged QT intervals. The prescribing information should describe the information available regarding the association between QT changes and SIRTURO administration. Also, future clinical use of this drug should include sufficient EKG monitoring to determine the degree of association between SIRTURO administration and QT prolongation (or torsades de pointes).

Phospholipidosis

Bedaquiline and its active metabolites are considered cationic, amphiphilic drugs (CAD) and, as such, induced phospholipidosis in drug-treated animals, mainly in cells of the monocytic phagocytic system (MPS). After bedaquiline administration, all species tested showed the accumulation of pigment-laden and/or foamy macrophages, mostly in the lymph nodes, spleen, lungs, liver, stomach, skeletal muscle, pancreas and/or uterus. Under electron microscopic examination, phospholipidosis was characterized by the prominent presence of intracytoplasmic lamellar inclusions in the affected tissues.

Phospholipidosis developed very quickly with bedaquiline and foamy vacuolization was seen even after single doses in mice. Findings are more frequent and severe with increasing dose and increasing dosing duration. A NOAEL was not determined for a number of studies as a result of

the presence of foamy macrophages and pigment-laden macrophages even at the lowest doses. These findings were slowly reversible upon treatment cessation and at least partial recovery was seen in dog and rat studies with recovery arms. The applicant also conducted *in vitro* studies which showed that M2 and M3 were stronger phospholipidosis-inducers than bedaquiline.

Phospholipidosis is reversible and has also been observed with several other drugs, including perhexiline, amiodarone, fluoxetine and gentamicin. This finding has not been shown to result in any functional changes.

Muscular degeneration
Degenerative changes in skeletal muscles were seen in mice, rats and dogs treated with high doses of bedaquiline. For example, minimal fibrohistiocytic infiltration and muscle fiber degeneration were observed in the tongue and, to a lesser extent, in the quadriceps in rats treated for 13 weeks with bedaquiline at 24 mg/kg. Myopathy was also observed in rats after six months of bedaquiline at 20 mg/kg/day ($AUC_{(0\text{-}24h)}$) of about 31 µg*h/mL) but this finding disappeared by the end of a 12 week, drug-free, reversibility period.

Stomach
In several studies, bedaquiline administration was associated with damage to the stomach lining. The degeneration and necrosis of the fundic mucosa of the stomach observed in one dog study of bedaquiline at 40 mg/kg/day after 13 weeks ($AUC_{(0to24h)}$) 94 µg*h/mL, about 4 times clinical exposure) was no longer seen in dogs after the dose was reduced to 20 mg/kg/day and dogs dosed for a total of 26 weeks. The absence of this finding at the end of 26 weeks at the reduced dose suggests that patients are also likely to recover from the adverse effects on the stomach upon cessation of dosing.

Pancreas
In several studies, focal to multifocal chronic pancreatitis with acinar cell atrophy was observed in mice and dogs. The pancreatic changes appear to be dose and duration related since pancreatitis was detected in dogs earlier (at 13 weeks) at the high dose, (40 mg/kg/day, ($AUC_{(0to24h)}$,94 µg*h/mL, about 4 times clinical exposure) but was also seen at lower doses after a longer duration of treatment (26 weeks at 10 mg/kg/day, $AUC_{(0to24h)}$,54 µg*h/mL, about 2 times the clinical exposure). Pancreatic inflammation was less common, but still present at the end of the 13-week recovery period in the 39-week dog study (18 mg/kg/day, $AUC_{(0to24h),}$ 173 µg*h/mL, about 8 times the clinical exposure). Pancreatitis and/or amylase increases have been observed in the completed clinical trials. Data is being collected in the Phase III clinical trial which will help to clarify the incidence of this finding in man.

Liver
In addition to the phospholipidosis noted above, hepatocellular centrilobular hypertrophy was seen in all species (for example in dogs treated at 40/20 mg/kg/day for 6 months, $AUC_{(0to24h),}$ 120 µg*h/mL, about 6 times the clinical exposure) and was often accompanied by increased liver weight and increases in liver enzymes. Mice may be more sensitive as they experienced increased AST levels in a 13 week study at doses one-half the human exposure. The applicant ascribes this increased sensitivity to increased M2 levels in the mice. Single cell necrosis was also observed in rats and mice. Although some changes, such as liver hypertrophy, were not detected at the end of

a reversibility period in the recovery studies, in some instances, signs of phospholipidosis persisted, although diminished.

Other Findings

Bedaquiline showed no evidence of genotoxicity, no adverse effects on mating, or fertility and it was not teratogenic. A two-year oral carcinogenicity study is ongoing and results will be available after the due date for this NDA.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number: 845533-86-0

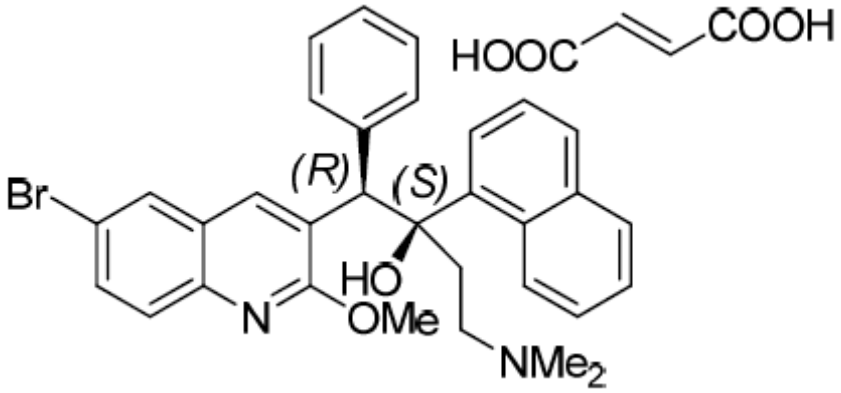
2.1.2 Generic Name: bedaquiline fumarate

2.1.3 Code Name: TMC207 or TMC207 fumarate or R403323 or JNJ-16175328-AEP.
During early development, [redacted] (b) (4) form of TMC207 was used and this was called R207910 or JNJ-16175328-AAA.

2.1.4 Chemical Name:
(1R,2S)-1-(6-bromo-2-methoxy-3-quinolinyl)-4-(dimethylamino)-2-(1-naphthalenyl)-1- phenyl-2-butanol compound with fumaric acid (1:1).

2.1.5 Molecular Formula: $C_{32}H_{31}BrN_2O_2 \cdot C_4H_4O_4$
Molecular Weight: 671.58

2.1.6 Structure



2.1.7 Pharmacologic class
Diarylquinoline antimyco-bacterial.

2.2 Relevant IND/s, NDA/s, and DMF/s
IND 69,600

2.3.1 Drug Formulation
[SIRTURO] is an uncoated, white, round biconvex tablet with a diameter of 11 mm an, containing 120.89 mg of bedaquiline fumarate drug substance, (equivalent to 100 mg bedaquiline [redacted] (b) (4)).This formulation was assigned the formulation number F001, and is referred to as bedaquiline 100-mg tablet (F001).

Table 1: Composition of the Bedaquiline 100-mg Tablet (F001)

Component	Function	Amount per tablet (mg)	(%)
Bedaquiline fumarate	Active ingredient	120.89	26.28
Lactose Monohydrate (b) (4) Starch Hypromellose 2910 15 mPa.s Polysorbate 20 Purified Water Microcrystalline Cellulose Croscarmellose Sodium (b) (4) Magnesium Stearate	(b) (4)		
Total tablet weight		460	100

Solubility

The drug substance is practically insoluble in aqueous media over a wide pH range and very slightly soluble in 0.01 N HCl. The drug substance is soluble in a variety of organic solvents.

2.3.2 Comments on Novel Excipients

Many nonclinical studies were conducted using bedaquiline dissolved in (b) (4) which is known to cause phospholipidosis. Although drug-related phospholipidosis in these studies were typically increased compared to HPBCD controls, the effects are likely exaggerated due to the additive effects of two phospholipidogenic compounds.

2.3.3 Comments on Impurities/Degradants of Concern

2.4 Proposed Clinical Population and Dosing Regimen

Bedaquiline is indicated in adults (≥ 18 years) as part of combination therapy of pulmonary multi-drug resistant tuberculosis.

2.5 Regulatory Background

This is the first NDA submitted for this new molecular entity. It has never been approved for sale by any other agency.

3 Studies Submitted

3.1 Pivotal Studies Reviewed

Secondary Pharmacodynamics

870293,	*In vitro* Pharmacology: High throughput profile study of R207910

Safety Pharmacology

TMC207-CPF989	Effects of JNJ-16175328-AAA (R207910) and of astemizole (JNJ-120432-AAA, R043512) on the membrane K+ current IKr in HERG-transfected HEK293 cells [antituberculosis; DIP 178]
TMC207-NC197	Effects of JNJ-16175328-AAA-24248056 (TMC207) on the membrane K+ current IKr in HERG-transfected HEK293 cells compared to astemizole [ATP synthesis inhibitor]
TMC207-NC202	Effects of JNJ-28325583-AAA-31636185 on the membrane K+ current IKr in HERG-transfected HEK293 cells compared to astemizole [M2 metabolite; ATP synthesis inhibitor]
TMC207-NC227	Effects of JNJ-28325583-AAA-29630923 on the membrane K+ current IKr in HERG-transfected HEK293 cells compared to astemizole [M2 metabolite; ATP synthesis inhibitor]
TMC207-NC198	Effects of JNJ-16175328-AAA-24248056 (TMC207) on the membrane K+ current IKs in KvLQT1/minK-transfected CHO cells compared to HMR1556 [ATP synthesis inhibitor]
TMC207-NC203	Effects of JNJ-28325583-AAA-31636185 on the membrane K+ current IKs in KvLQT1/minK-transfected CHO cells compared to HMR1556 [M2 metabolite; ATP synthesis inhibitor]
TMC207-4133606	Effects of R207910 (JNJ-16175328-AAA) on the isolated, spontaneously beating right atrium of the guinea-pig [Anti-tuberculosis: Dip 1692].
TMC207-NC200	Effects of JNJ-16175328-AAA (TMC207) on the isolated, spontaneously beating right atrium of the guinea-pig [ATP synthase inhibitor; Dip 178]
TMC207-NC204	Effects of R207910 (JNJ-16175328-AAA) on the isolated, spontaneously beating right atrium of the guinea-pig (2001)
TMC207-1893592	Electrophysiological effect of JNJ-16175328-AAA (R207910) in isolated, Langendorff-perfused rabbit hearts (antituberculosis; Dip 178)
TMC207-NC201	Electrophysiological evaluation of JNJ-16175328-AAA-24248056 (TMC207) in isolated, arterially-perfused rabbit ventricular wedge preparations [ATP synthase inhibitor]
TMC207-NC205	Electrophysiological evaluation of JNJ-28325583-AAA-31636185 in isolated, arterially-perfused rabbit ventricular wedge preparations [ATP synthesis inhibitor]
TMC207-CPF732	Effects of JNJ-16175328-AAA on cardio-hemodynamic and –electrophysiological parameters in anaesthetized guinea pigs: dose 0.16, 0.32, 0.64, 1.25 and 2.5 mg/kg intravenously [Tuberculosis – Dip 178].
EXP6201	Single Dose Oral Safety Pharmacology Study in the Rat: the Modified Irwin's Test.
1692-03676	Moxifloxacin hydrochloride and TMC207 fumarate: cardiovascular evaluation following combined oral administration in the beagle dog.
TMC207-CPF761	Effects of JNJ-16175328-AAA (R207910) on cardiovascular and behavioral parameters in instrumented, awake dogs: dose 20 mg/kg orally [antituberculosis;

	Dip 178].
EXP6115	Effects on cardiovascular and respiratory function in the telemetered dog.

Single dose studies

TOX6202	Single Dose Oral Toxicity Study in the Swiss Mouse.
TOX6016	Single Dose Oral Toxicity Study in the Rat.

Repeat dose studies

TOX6022	15-Day Repeated Dose Oral Toxicity Study in the Rat.
TOX7419	R403323: 13-week Oral (Gavage) Administration Toxicity Study in the Mouse.
TOX6614	13-Week Toxicity Study by Oral Route (Gavage) in Rats.
TOX 7421	26 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 12 Week Treatment-Free Period.
TOX7305	Repeated Dose Oral Toxicity Study of TMC207 with 2-Month Interim Kill in the Beagle Dog (Mechanistic Study).
TOX9239	39 Week Oral (Gavage) Toxicity Study in Dogs followed by a 13 Week Recovery Period.

Genotoxicity

TOX6089	In Vitro Bacterial Reverse Mutation Test with *Salmonella typhimurium*.
TOX 6088	In Vitro Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique.
TOX 6090	Micronucleus Test on Bone Marrow Cells of Mice.

Reproductive and Developmental Toxicity

TOX7332	Oral Fertility Study of R403323 (TMC207) in the Male and Female Rat.
TOX 6657	Oral Developmental Toxicity Study in the Rat.
TOX 6656	Oral Developmental Toxicity Study in the Rabbit.
TOX9296	Pre- and Post-Natal Development Study in the CD Rat by Oral (Gavage) Administration.
TOX9405	TMC207-fumarate: Toxicity Study in the Juvenile Rat by Oral (Gavage) Administration.

Special Toxicology

TOX6926	Assessment of immunomodulating effects of TMC207 after repeated dose (4 weeks) oral administration in rats including toxicokinetics
TOX7484	Assessment of immunomodulating effects of R403323 (TMC207 fumarate) in a host resistance assay with Listeria monocytogenes after repeated dose (4 weeks) oral administration in rats.
TOX5731	1-Month Intermittent Dose Oral Toxicity Study in the Rat.
TOX5669	10-week Intermittent Dose Oral Toxicity Study in the Rat
TOX7539	5-day repeated dose oral toxicity study of TMC207-fumarate/M2 In The C57BL/6N Mouse
TOX9398	: *In Vitro* Bacterial Reverse Mutation Test with TMC207 fumarate spiked with 2% D007762 in *Salmonella typhimurium*

TOX 9394	Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique.
TOX7562	2-Week Repeated Dose Oral Toxicity Study in the Rat
TOX9483	*In Vitro* Bacterial Reverse Mutation Test with TMC207 fumarate spiked with 10% GA27 in *Salmonella typhimurium*
TOX6327	*In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium*
TOX8271	: Comparison of the cytotoxicity and phospholipidogenic potency of TMC207and R405786, the N-desmethyl metabolite of TMC207 in an *in vitro* phospholipidosis screening assay.

Studies not reviewed:

Dose range finding studies were not reviewed as well as studies which were duplicative. Since toxicities were generally similar within a species as duration of dosing increased and since bedaquiline is to be given for 24 weeks, some intermediate durations were not reviewed.

Previous Reviews Referenced: **None**

4 Pharmacology

4.1 Primary Pharmacology

Studies conducted to investigate the primary pharmacology of TMC207 are discussed in the Microbiologist's Review of this NDA. The mechanism of action of TMC207 appears to involve the inhibition of mycobacterial adenosine 5'-triphosphate (ATP) synthase, an enzyme essential for the generation of energy in mycobacteria.

Bedaquiline inhibits the proton pump of mycobacterial ATP-synthase 3 by binding to the subunit c of the enzyme. Subunit c of ATP synthase is responsible for the flow of protons (H^+ or Na^{++}) from the intercristae region in mitochondria and the periplasmic space in bacteria to the mitochondrial matrix and the bacterial cytoplasm, respectively. Bedaquiline thereby inhibits the production of energy in mycobacterial cells, resulting in cell death. This activity appears to be selective towards mycobacterial ATP synthase relative to eukaryotic mitochondria; bedaquiline IC_{50} for mycobacterial strains was 0.01 μM compared to an IC_{50} >200 μM for human cancer cells, demonstrating that bedaquiline has a > 20,000-fold lower affinity for human mitochondrial ATP synthase than it has for the mycobacterial ATP synthase.

4.2 Secondary Pharmacology

Study 870293: *In vitro* Pharmacology: High throughput profile study of R207910

TMC207 was screened *in vitro* for interactions with a standard panel of receptors, ion channels and transporters at a concentration of 10 μM (5.6 μg/mL) in buffer containing 0.1 μM dimethylsulfoxide (DMSO).

TMC207 exposure resulted in the inhibition of histamine2 receptor (87%), sodium channel (71%) and dopamine transporters (54%). No significant interaction of TMC207 (above 50 % inhibition) was observed with any of the other receptors or transporter binding sites tested, including adenosine, adrenergic, angiotensin, cholecystokinin, dopamine, endothelin, muscarinic, neurokinin, opioid, serotonin, vasopressin receptors or calcium, potassium, chloride channels or norepinephrine transporters).

4.3 Safety Pharmacology Studies

Study title:	Effects of JNJ-16175328-AAA (R207910) and of astemizole (JNJ-120432-AAA, R043512) on the membrane K^+ current I_{Kr} in HERG-transfected HEK293 cells
Study # :	TMC207-cpf989
Study report location:	EDR
Conducting laboratory and location:	Janssen Research & Development, Division of Janssen Pharmaceutica N.V Turnhoutseweg 30 2340 Beerse, Belgium.
Date of study initiation:	December 2001
GLP compliance:	No
QA statement:	Yes
Drug, lot #:	Not provided
Purity (%):	Not provided

Key Study Findings: TMC207 inhibited IKr with an IC_{50} that was above the micromolar range

Methods

In this study, whole-cell voltage clamp technique was used to determine the effects of TMC207 free base form (three concentrations ranging from 10^{-7} M to 3×10^{-6} M) on the IKr-like membrane potassium current in a human embryonic kidney cell line (HEK293) transfected with the human *ether-à-go-go*-related gene (HERG). Astemizole was used as the positive reference compound and was tested at three concentrations (3×10^{-9} M to 3×10^{-8} M). Time-matched solvent controls were also included.

Results

Data show that TMC207 resulted in a concentration-dependent decrease in the membrane K+ current (IKr) in HERG-transfected HEK293 cells compared to solvent, at concentrations 3×10^{-7} M and above (see Table S1, below). Under the same conditions, astemizole resulted in the inhibition of the IKr at nanomolar concentrations.

Table S1. Inhibition of HERG-mediated K+ current in HEK293 cells by increasing concentrations of cumulatively applied JNJ-16175328-AAA (R207910) compared to astemizole (JNJ-120432-AAA; R043512)

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
JNJ-16175328-AAA	1×10^{-7}	17.0 ± 1.5 (n = 3)	2.8 ± 2.1 (n = 4)
	3×10^{-7}	24.0 ± 4.6 (n = 3)	5.5 ± 1.4 (n = 4)
	3×10^{-6}	42.5 ± 5.4 (n = 4)	7.0 ± 0.9 (n = 4)
Astemizole	3×10^{-9}	27.3 ± 0.7 (n = 3)	
	1×10^{-8}	68.3 ± 1.9 (n = 3)	
	3×10^{-8}	92.7 ± 1.3 (n = 3)	

n = the number of cells tested

Conclusion

TMC207 inhibited IKr with an IC_{50} that was above the micromolar range while the IC_{50} of astemizole was in the nanomolar range.

Study title:	Effects of JNJ-16175328-AAA-24248056 (TMC207) on the membrane K_+ current I_{Kr} in HERG-transfected HEK293 cells compared to astemizole [ATP synthesis inhibitor]
Study # :	TMC207-nc197
Study report location:	EDR
Conducting laboratory and location:	Janssen Research & Development, Division of Janssen Pharmaceutica N.V Turnhoutseweg 30 2340 Beerse, Belgium.
Date of study initiation:	March 2008
GLP compliance:	No
QA statement:	Yes
Drug, lot #:	00420075
Purity (%):	98.4 %

Key Study Findings: TMC207 inhibited IKr with and IC_{50} of 3.68 x 10^{-7} M

Methods

In this study, whole-cell voltage clamp technique was used to determine the effects of TMC207 (six concentrations ranging from 10^{-8} M to 3 x 10^{-6} M) on the IKr-like membrane potassium current in a human embryonic kidney cell line (HEK293) transfected with the human *ether-à-go-go*-related gene (HERG). Astemizole was used as the positive reference compound and was tested at three concentrations ($3x10^{-9}$M, 10^{-8}M and $3x10^{-8}$M). Time-matched solvent controls were also included.

Results

Data show that TMC207 resulted in a concentration-dependent decrease in the membrane K+ current (IKr) in HERG-transfected HEK293 cells compared to solvent, at concentrations $3x10^{-8}$ M and above (see Table S2, below). The Hill equation was fitted to the data with an IC_{50} of 3.68 x 10^{-7} M and a Hill coefficient of 0.7.Under the same conditions, astemizole resulted in the inhibition of the IKr at nanomolar concentrations.

Table S2: Inhibition of HERG-mediated K+ current in HEK293 cells by increasing concentrations of cumulatively-applied JNJ-16175328-AAA-24248056 (TMC207) compared to astemizole

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
JNJ-16175328-AAA	1×10^{-8}	8.4 ± 3.7 (n = 5)	2.6 ± 1.3 (n = 5)
	3×10^{-8}	11.0 ± 2.9 (n = 5)#	0.4 ± 1.1 (n = 5)
	1×10^{-7}	30.0 ± 6.1 (n = 5)#	3.0 ± 1.7 (n = 5)
	3×10^{-7}	47.2 ± 7.1 (n = 5)#	0.2 ± 1.1 (n = 5)
	1×10^{-6}	68.0 ± 7.0 (n = 5)#	3.0 ± 3.0 (n = 5)
	3×10^{-6}*	79.0 ± 3.3 (n = 5)#	0.2 ± 2.6 (n = 5)
Astemizole	3×10^{-9}	41.0 ± 8.0 (n = 5)	
	1×10^{-8}	82.8 ± 7.1 (n = 5)	
	3×10^{-8}	96.6 ± 1.3 (n = 5)	

n = the number of cells tested
* NB: Analysis revealed a low recovery of the locally applied drug concentration (39%).
# $p < 0.05$
The drug-containing solution at 3×10^{-6} M precipitated over time; therefore all drug-containing solutions were prepared freshly and used within 110 min

Concentration analysis:

Analysis of the solution that was supposed to contain TMC207 at a concentration of 3×10^{-6} M revealed a low recovery of the locally-applied drug concentration. The actual concentration was 1.2×10^{-6} M (i.e. 39% of the target concentration).

Discussion

TMC207 inhibited IKr with and IC_{50} of 3.68×10^{-7} M. Although it seems evident that TMC207 inhibits IKr, the specific concentration is not clear as analysis revealed a low recovery of the locally-applied drug concentration (i.e. 39%).

Study title:	Effects of JNJ-28325583-AAA-31636185 on the membrane K_+ current I_{Kr} in HERG- transfected HEK293 cells compared to astemizole
Sponsor reference # :	TMC207-nc202nc
Study report location:	EDR
Conducting laboratory and location:	Janssen Research & Development, Division of Janssen Pharmaceutica N.V Turnhoutseweg 30 2340 Beerse, Belgium.
Date of study initiation:	April 2008
GLP compliance:	No
QA statement:	No
Drug, lot #:	31636185
Purity (%):	Not provided

Key Study Findings: TMC207 metabolite, M2 inhibited IKr with and IC_{50} of 9.2 x 10^{-7} M

Methods

In this study, whole-cell voltage clamp technique was used to determine the effects of JNJ-28325583-AAA-31636185 also known as the TMC207 metabolite M2 (six concentrations ranging from 10^{-8} M to 3 x 10^{-6} M) on the IKr-like membrane potassium current in a human embryonic kidney cell line (HEK293) transfected with the human *ether-à-go-go*-related gene (HERG). Astemizole was used as the positive reference compound and was tested at three concentrations ($3x10^{-9}$M, 10^{-8}M and $3x10^{-8}$M). Time-matched solvent controls were also included.

Results

Data show that M2 resulted in a concentration-dependent decrease in the membrane K+ current (IKr) in HERG-transfected HEK293 cells compared to solvent, at concentrations $3x10^{-8}$ M and above (see Table S3, below). The Hill equation was fitted to the data with an IC_{50} of 9.2 x 10^{-7} M and a Hill coefficient of 0.99.Under the same conditions, astemizole resulted in the inhibition of the IKr at nanomolar concentrations.

Concentration analysis:
Analysis of the solution that was supposed to contain M2 at a concentration of 3 x 10^{-6} M revealed a low recovery of the locally-applied drug concentration. The actual concentration was 1.8 x 10^{-8} M (i.e. 0.6 % of the target concentration).

Table S3, Inhibition of HERG-mediated K+ current in HEK293 cells by increasing concentrations of cumulatively applied (M2) JNJ-28325583-AAA-31636185 compared to astemizole.

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
JNJ-28325583-AAA	1×10^{-8}	9.4 ± 1.4 (n = 5)	3.0 ± 2.3 (n = 5)
	3×10^{-8}	16.4 ± 3.1 (n = 5)#	4.0 ± 2.4 (n = 5)
	1×10^{-7}	22.8 ± 2.0 (n = 5)#	5.4 ± 2.5 (n = 5)
	3×10^{-7}	34.4 ± 6.1 (n = 5)#	3.8 ± 1.9 (n = 5)
	1×10^{-6}*	63.2 ± 4.7 (n = 5)#	7.8 ± 2.6 (n = 5)
	3×10^{-6}*	88.0 ± 3.6 (n = 5)#	3.8 ± 2.9 (n = 5)
Astemizole	3×10^{-9}	43.8 ± 6.9 (n = 5)	
	1×10^{-8}	83.8 ± 7.3 (n = 5)	
	3×10^{-8}	97.2 ± 1.3 (n = 5)	

n = the number of cells tested
* NB: There might have been a possible issue in stability. Analysis of the solutions at 10^{-6} M and 3×10^{-6} M revealed a very low recovery (0.5% and 0.6%, respectively)
# $p < 0.05$
All drug-containing solutions were prepared freshly and used within 60 min

Discussion

M2 inhibited IKr with and IC_{50} of 9.2×10^{-7} M. Although it is clear that M2 inhibits IKr, the magnitude of the effect is not certain since concentration analysis revealed a low recovery of the locally-applied drug concentration (i.e. 0.6 %). These studies may in fact underestimate the effect of M2. *In vivo* studies in animals have not revealed any effect at nontoxic doses. A second study of M2, TMC207-TiDP13-NC227[1] showed a similar IC_{50} of 4.5×10^{-7} M (See Table S4, below. In that case also, concentration analysis showed that the actual concentration was 17 % of the intended concentration.

Patch clamp was also used to evaluate the effects of TMC207 and M2 on the membrane current IK_S. The maximum inhibition produced by TMC207[2] concentrations up to 10^{-5} M was 31 % (See Table S5, below) and the maximum inhibition produced by M2[3] concentrations up to 3×10^{-6} M was 42 % (See Table S6, below). In all cases, the concentrations determined from analyses were lower than the nominal concentrations. The sponsor has ascribed the low recovery of drug to high binding to glass. Regardless of the explanation for this phenomenon or the actual quantitative effect, *in vivo* and clinical studies have shown that bedaquiline appears to be associated with some QT prolongation.

Table S4: Inhibition of HERG-mediated K+ current in HEK293 cells by increasing concentrations of cumulatively applied M2 compared to astemizole. Data from Study # TMC207-TiDP13-NC227: Effects of JNJ-28325583-AAA-29630923 on the membrane K^+ current I_{Kr} in HERG-transfected HEK293 cells compared to astemizole

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
JNJ-28325583-AAA	1×10^{-8}	8.1 ± 1.8 (n = 7) #	0.0 ± 1.0 (n = 7)
	3×10^{-8}*	9.0 ± 1.7 (n = 6)#	0.0 ± 1.2 (n = 6)
	1×10^{-7}	22.6 ± 3.4 (n = 7)#	1.9 ± 1.7 (n = 7)
	3×10^{-7}*	32.2 ± 4.1 (n = 6)#	1.2 ± 2.0 (n = 6)
	1×10^{-6}	70.3 ± 5.3 (n = 7)#	2.1 ± 1.4 (n = 7)
	3×10^{-6}*	93.0 ± 4.3 (n = 5)#	2.8 ± 1.2 (n = 5)
Astemizole	3×10^{-9}	36.6 ± 6.2 (n = 5)	
	1×10^{-8}	77.6 ± 5.6 (n = 5)	
	3×10^{-8}	95.6 ± 1.3 (n = 5)	

n = the number of cells tested

* NB: Analysis revealed low recoveries of the locally-applied drug concentration (i.e. 5% at 3×10^{-8} M, 11% at 3×10^{-7} M and 17% at 3×10^{-6} M). The analyses were performed on the day of sampling.

# $p < 0.05$

All drug-containing solutions were freshly prepared and used within 90 min.

Table S5: Inhibition of Iks in CHO cells by increasing concentrations of (TMC207) compared to HMR1556. Data from Study # TMC207-NC198: *Effects of JNJ-16175328-AAA-24248056 (TMC207) on the membrane K^+ current I_{Ks} in KvLQT1/minK-transfected CHO cells compared to HMR1556*. Data show that TMC207 weakly inhibits IKs.

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
JNJ-16175328-AAA	3×10^{-9}	17.7 ± 2.2 (n = 6)#	4.8 ± 2.2 (n = 6)
	1×10^{-7}*	24.8 ± 4.9 (n = 6)#	4.7 ± 2.4 (n = 6)
	1×10^{-6}*	29.2 ± 5.0 (n = 5)#	2.4 ± 2.2 (n = 5)
	3×10^{-6}*	34.6 ± 8.0 (n = 5)#	2.4 ± 2.2 (n = 5)
	1×10^{-5}*	31.2 ± 3.9 (n = 5)#	5.6 ± 1.8 (n = 5)
HMR 1556	10^{-8}	20.4 ± 7.1 (n = 5)	
	10^{-7}	41.4 ± 4.7 (n = 5)	
	10^{-6}	91.4 ± 0.9 (n = 5)	

n = the number of cells tested
* NB: Analysis of the locally-applied drug concentration revealed a low recovery (range 22% to 51%)
10^{-5} M drug-containing solution precipitated in the Petri-dish
All drug-containing solutions were prepared freshly and used within 120 min
# $p < 0.05$

Table S6: Inhibition of KvLQT1/minK-mediated K+ current in CHO cells by increasing concentrations of M2 (JNJ-28325583-AAA-31636185) compared to HMR1556. Data are from Study # TMC207-NC203: *Effects of JNJ-28325583-AAA-31636185 on the membrane K^+ current I_{Ks} in KvLQT1/minK-transfected CHO cells compared to HMR1556*. Data show that M2 weakly inhibits IKs.

		Mean inhibition (%) ± SEM	
	Conc. (M)	Test drug	Solvent control
JNJ-28325583-AAA	3×10^{-7}	14.5 ± 2.9 (n = 6)#	4.2 ± 2.5 (n = 6)
	1×10^{-6}	23.0 ± 1.1 (n = 5)#	5.6 ± 1.8 (n = 5)
	3×10^{-6}*	41.8 ± 6.0 (n = 6)#	4.8 ± 2.2 (n = 6)
HMR1556	10^{-8}	20.4 ± 7.1 (n = 5)	
	10^{-7}	41.4 ± 4.7 (n = 5)	
	10^{-6}	91.4 ± 0.9 (n = 5)	

n = the number of cells tested
* NB: There might have been an issue in stability. Analysis of the locally-applied drug concentration revealed a very low recovery (0.6%).
All drug-containing solutions were prepared freshly and used within 95 min
# $p < 0.05$

Study PRD4133606: Effects of R207910 (JNJ-16175328-AAA) on the isolated, spontaneously beating right atrium of the guinea-pig
Study NC200: Effects of JNJ-16175328-AAA (TMC207) on the isolated, spontaneously beating right atrium of the guinea-pig
Study NC204: Effects of R207910 (JNJ-16175328-AAA) on the isolated, spontaneously beating right atrium of the guinea-pig (2001)

The drug's effect on rate and force of contraction and effective refractory period of the heart was evaluated using the isolated guinea pig atrium assay. At concentrations of 1, 3 and 10 μM, neither TMC207 (Study PRD4133606 and Study NC200) nor its metabolite (Study NC204) had any biologically significant adverse effect on the rate and force of contraction or refractory period. Concentration analysis showed that the actual exposure in the medium was only 19% of the intended concentration 10^{-5} M.

Electrophysiological effect of JNJ-16175328-AAA (R207910) in isolated, Langendorff- perfused rabbit hearts

Using **Langendorff** -perfused rabbit hearts, it was shown that TMC207 did not induce any harmful electrophysiological effects at concentrations between $1x10^{-7}$ and $3x10^{-6}$ M (1.7 μg/mL). Parameters evaluated included APD60 (the duration of the action potential at 60% repolarization), triangulation of the action potential (difference between APD30 and APD90), instability (the difference in the upper and lower quartile estimated APD60) reverse-use dependence of APD and intraventricular conduction time (IVC).

At the highest dose, 10^{-5} M, TM207 decreased coronary flow (-68 % of baseline), increased the instability (+7 ms of baseline vs. 0% of baseline with control) and caused ventricular fibrillation in one of 6 hearts. Ventricular tachycardia (VT; unlike TdPs) was increased in one of six hearts exposed to $3x10^{-7}$ M. At no point did the drug elicit did not elicit early afterdepolarizations (EADs) or torsade de pointes (TdPs). Since the ventricular tachycardia was not dose related, it was concluded that it was not drug-related. A marked slowing of IVC was observed in 3 of the 6 hearts at 10^{-5} M. TMC207 resulted in a number of changes at 10 μM (5.6 μg/mL). The NOAEL was 3 μM (1.7 μg/mL) although VT was observed in one heart at 0.3 μM (0.17 μg/mL). There were no similar findings in a study of awake, instrumented dogs.

TMC207-NC201: Electrophysiological evaluation of JNJ-16175328-AAA-24248056 (TMC207) in isolated, arterially-perfused rabbit ventricular wedge preparations.

TMC207-NC205: Electrophysiological evaluation of JNJ-28325583-AAA-31636185 in isolated, arterially-perfused rabbit ventricular wedge preparations

The isolated, arterially-perfused rabbit ventricular wedge preparation was used to investigate the potential of TMC207 and its metabolite M2 to produce electrophysiological effects in the heart at concentrations between 1×10^{-8} M to 1×10^{-5} M.

TMC207 or M2 did not did not significantly change the QT interval, QRS duration and QRS rate dependency, Tp-Te, rTp-Te, TdP score and contractile force (FC) of the ventricle slices, elicit early

afterdepolarizations (EADs), TdP, ventricular tachycardia (VT), ventricular fibrillation (VF) or in-excitability in any of the six isolated, arterially-perfused rabbit left ventricular wedge preparations.

TMC207-CPF732: Effects of JNJ-16175328-AAA on cardio-hemodynamic and electrophysiological parameters in anesthetized guinea pigs.

Intravenous administration of TMC207 to anesthetized guinea pigs at 0.16 to 2.5 mg/kg had no effect on the PQ or QRS interval or on the EKG morphology. All doses increased heart rate (up to + 10 %). Starting at 1.25 mg/kg, there was a transient increase in blood pressure (maximum change in mean arterial pressure was +29 %) and QT_C (Bazzett) was decreased (-6% compared to solvent). The median Cmax was 3.6 µg/mL after the i.v. injection of 2.5 mg/kg TMC207. The relevance of these findings after intravenous dosing is unclear since exposures will be greater than after oral dosing. Studies in awake, instrumented dogs and cardiovascular evaluations in the clinic trials have not revealed similar findings.

TOX6201: Single Dose Oral Safety Pharmacology Study in the Rat: The Modified Irwin's Test.

SPF Sprague-Dawley rats 5 males/dose were treated with single doses of 50, 200 or 800 mg/kg TMC207 in order to evaluate the neurofunctional integrity of the rat based on Irwin's method (Irwin, S. Psychopharmacologia (Berl.) 13, 222 - 257. 1968). A single oral administration of TMC207 to male Sprague-Dawley rats at 50 or 200 mg/kg body weight did not affect the neurofunctional integrity. There were no adverse clinical observations noted and body weight and weight gain increased at a normal rate during the 7-day observation period. At 800 mg/kg, effects were slight or isolated and included increased locomotor activity (slight) and aberrant motor-affective (increased alertness, slight) irritability (one animal), low defecation rate and sensor-motor responses (lack of startle response and lack of response to stimulus in one animal). The autonomic functions (eyes, secretions and excretions, piloerection) were slightly affected and in one male of the 800 mg/kg dosed group, a low body temperature and a decreased respiratory rate was also recorded. Very high doses appear to have some effect on sensory/motor function but a single dose of 200 mg/kg had no adverse effects.

Study 1692-03676: Moxifloxacin hydrochloride and TMC207 fumarate: cardiovascular evaluation following combined oral administration in the beagle dog.

TMC207 (100 mg/kg) or its vehicle [HP-β-CD], was administered to groups of dogs, 4/group, via oral gavage, once a day for 7 consecutive days. Moxifloxacin or its vehicle (0.5% sodium carboxymethylcellulose was administered to all groups via oral gavage, once on Day 7 approximately 15 minutes prior to the TMC207 dose. Body temperature, systolic, diastolic, and mean arterial blood pressures, heart rate, and ECG parameters (QRS duration and the RR, PR, QT intervals, and R Wave Amplitude) were monitored continuously for at least 24 hours prior to dosing (Day -1) for all animals and for at least 24 hours postdose on Days 1, 6, and 7 for animals at 0/0 and 100/100 mg/kg TMC207/ Moxifloxacin and for at least 24 hours postdose on Days 6 and 7 for animals at 0/100 mg/kg TMC207/ Moxifloxacin. On Day -5, untreated animals were continuously monitored for cardiovascular endpoints for at least 24 hours. These data were used in the calculation of the corrected QT interval throughout the study.

The study showed that adding 100 mg/kg moxifloxacin resulted in a 44 msec increase in QT interval and that the addition of TMC207 to the regimen did not cause any further increase in QT prolongation (See Table S7, below)

Table S7 Summary of QT interval values in instrumented dogs given moxifloxacin on Day 7 after 7 daily doses of bedaquiline compared to moxifloxacin alone or vehicle alone and/or TMC207.

Treatment	N	QT Interval Values, msec
Vehicle	**4**	**223 ± 7.1**
100 mg/kg Moxifloxacin	**4**	**267 ± 6.8**
100 mg/kg Moxifloxacin + 100 mg/kg TMC207	**4**	**282 ± 5.6**

Dosing with 100 mg/kg TMC207 did not increase the moxifloxicin-induced QT prolongation in instrumented dogs. Under the present experimental conditions, (short term dosing) TMC207 did not show the potential to increase the moxifloxicin-induced QT interval.

Study TOX6115: Effects on cardiovascular and respiratory function in the telemetered dog.

In this study 4 instrumented, telemetered male beagle dogs were given a single oral dose of 10, 40 and 160 mg/kg TMC207 to determine if there were any effects on EKG parameters (measured for 1 hour before and 12 hours after dosing) or respiratory parameters including tracheal air flow (5 minutes before dosing and 30 minutes, 2 hours and 6 hours after dosing)). There were no biologically significant effects on EKG or respiratory parameters.

Study CPF761: Effects of JNJ-16175328-AAA (R207910) on cardiovascular and behavioral parameters in instrumented, awake dogs: dose 20 mg/kg orally

A single oral dose of 20 mg/kg of the compound was administered to awake, chronically instrumented dogs (n = 4). Control values of the various parameters were recorded for at least 30 minutes predose and for 4 hours after dosing. Arterial blood samples were taken 30, 60 and 240 min after TMC207 administration. Control dogs received vehicle.

In awake instrumented dogs, TMC207 had no relevant or statistically significant effect on heart rate, blood pressure, pressure rate product, cardiac contractility (LV dp/dtmax/pd), cardiac relaxation (LV dP/dtmin min) and the duration of the PQ, QRS, QT, QTcB, QTcF and QTcVDW interval, QT dispersion and on ECG morphology.

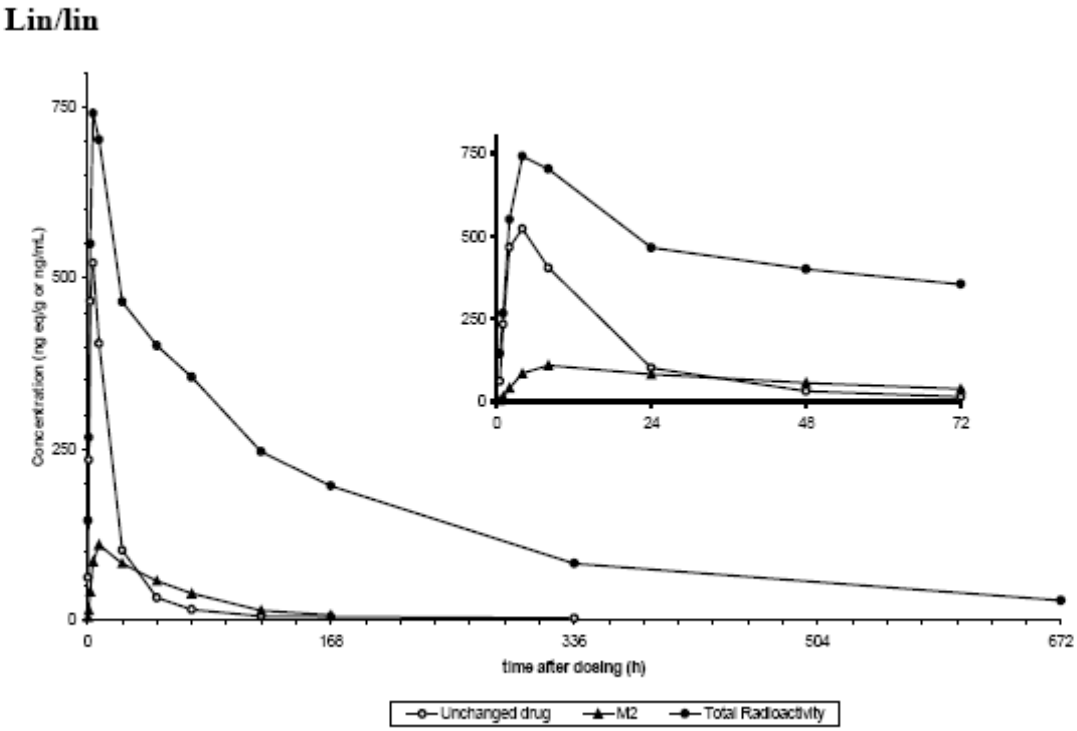
5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Absorption
After a single dose, the bioavailability of the TMC207 dose was 36%, 40% and 79% in the dog, monkey and rat, respectively. Drug achieved maximum plasma concentrations between 0.5 and 8 hours after dosing.

The mean C_{max} of M2 was about 2-fold lower than that of TMC207 in mice and was markedly lower in the other species, up to 6- to 8-fold lower in dogs and monkeys. By contrast, mean AUC was about 3-fold higher for M2 than for the parent drug in mice whereas in dogs and monkeys, mean M2 exposure was similar to parent drug exposure.

Figure P1: Mean plasma concentrations of total radioactivity, TMC207, and N-desmethyl TMC207 (M2) following a single oral administration of [^{14}C]-R403323 to the cynomolgus monkey at a nominal dose level of 10 mg/kg.



Distribution
Bedaquiline is extensively bound to proteins, with plasma protein binding above 99.9% for all species, including humans. After single IV administration, TMC207 was slowly eliminated from plasma and extensively distributed to tissues with a volume of distribution about 60-fold higher than the total body

water. Data from Study TMC207-NC159 showed that eight hours after a single oral dose of radioactive TMC207 in a cynomolgus monkey, the highest levels of parent drug and M2 were found in adrenals and liver (see Figure P2 and Table P1 and P2). Over time, the levels of M2 surpass the levels of parent compound (see Figure P3). After 17 weeks, levels of unchanged drug are highest in the pancreas and the liver, consistent with adverse findings and phospholipidosis in these tissues (see Figure P4)

Table P1. Pharmacokinetic parameters of TMC207 in tissues following a single oral administration of [^{14}C]-R403323 to the cynomolgus monkey at a nominal dose level of 10 mg/kg.

Tissue	t_{max}	C_{max}	$t_{½elim}$	$t_{½elim}$ lower	$t_{½elim}$ upper	t_{last}	$AUC_{(0-t)}$	$AUC_{(0-\infty)}$
Adrenals	8	29.20	261.9	168	1344	1344	2707	2749
Brain	8	0.329	ID	ID	ID	24	5.564	ID
Heart	8	2.710	230.7	168	1344	672	174.3	190.8
Kidney	8	5.090	246.1	168	1344	672	188.0	201.8
Liver	8	23.50	696.2	168	2856	2856	827.3	846.5
Lung (left lobe)	24	8.960	523.5	168	1344	1344	910.3	948.8
Lung (right lobe)*	8	3.300	45.9	8	168	168	242.1	260.6
BALC	24	5.500	19.3	8	1344	168	467.3	467.7
BALF	8	0.043	ID	ID	ID	24	0.858	ID
Ovaries	8	1.260	ID	ID	ID	24	17.87	ID
Pancreas	8	6.460	729.5	672	2856	2856	2005	2068
Skeletal Muscle	ID	ID	ID	ID	ID	ID	ID	ID
Spleen	8	3.940	31.3	8	168	168	114.4	117.5
Stomach	24	0.159	564.5	168	1344	1344	60.75	75.74
Thymus	24	4.220	184.6	168	672	1344	1382	1699

*: following bronchoalveolar lavage
BALC: bronchoalveolar lavage cells
BALF: bronchoalveolar lavage fluid
ID: Insufficient data to conduct extrapolation
t_{max} (hours): time of maximum concentration
C_{max} (µg TMC207 equivalents/g): maximum concentration
$t_{½elim}$ (hours): elimination half-life
$t_{½elim}$ lower (hours): lower time point used to determine elimination half-life
$t_{½elim}$ upper (hours): upper time point used to determine elimination half-life
t_{last} (hours): final measurable time point
$AUC_{(0-t)}$ (hr.µg TMC207 equivalents/g): area under concentration-time curve from time 0 to final measurable time-point
$AUC_{(0-\infty)}$ (hr.µg TMC207 equivalents/g): area under concentration-time curve from time 0 to infinity

Table P2: Pharmacokinetic parameters of M2 in tissues following a single oral administration of [^{14}C]-TMC207 to the cynomolgus monkey at a nominal dose level of 10 mg/kg.

Tissue	t_{max}	C_{max}	$t_{½elim}$	$t_{½elim}$ lower	$t_{½elim}$ upper	t_{last}	$AUC_{(0-t)}$	$AUC_{(0-\infty)}$
Adrenals	24	10.20	47.0	8	168	168	1014	1085
Brain	24	0.352	ID	ID	ID	24	5.012	ID
Heart	8	1.960	37.5	8	168	168	108.5	113.2
Kidney	8	6.880	35.7	8	168	168	408.7	422.8
Liver	8	15.20	589.4	168	2856	2856	1072	1092
Lung (left lobe)	24	31.90	224.8	168	1344	1344	3284	3301
Lung (right lobe)*	24	7.320	88.9	8	168	168	727.3	908.2
BALC	24	18.00	114.0	168	672	672	1513	1514
BALF	24	0.154	44.4	8	168	168	13.36	13.80
Ovaries	24	0.345	ID	ID	ID	24	6.564	ID
Pancreas	24	5.080	42.0	8	168	168	489.8	513.1
Skeletal Muscle	ID	ID	ID	ID	ID	ID	ID	ID
Spleen	8	4.400	35.8	8	168	168	333.7	344.2
Stomach	24	0.345	ID	ID	ID	24	4.428	ID
Thymus	24	2.890	2550	24	1344	1344	628.0	3671

*: following bronchoalveolar lavage
BALC: bronchoalveolar lavage cells
BALF: bronchoalveolar lavage fluid
ID: Insufficient data to conduct extrapolation
t_{max} (hours): time of maximum concentration
C_{max} (µg TMC207 equivalents/g): maximum concentration
$t_{½elim}$ (hours): elimination half-life
$t_{½elim}$ lower (hours): lower time point used to determine elimination half-life
$t_{½elim}$ upper (hours): upper time point used to determine elimination half-life
t_{last} (hours): final measurable time point
$AUC_{(0-t)}$ (hr.µg TMC207 equivalents/g): area under concentration-time curve from time 0 to final measurable time-point
$AUC_{(0-\infty)}$ (hr.µg TMC207 equivalents/g): area under concentration-time curve from time 0 to infinity

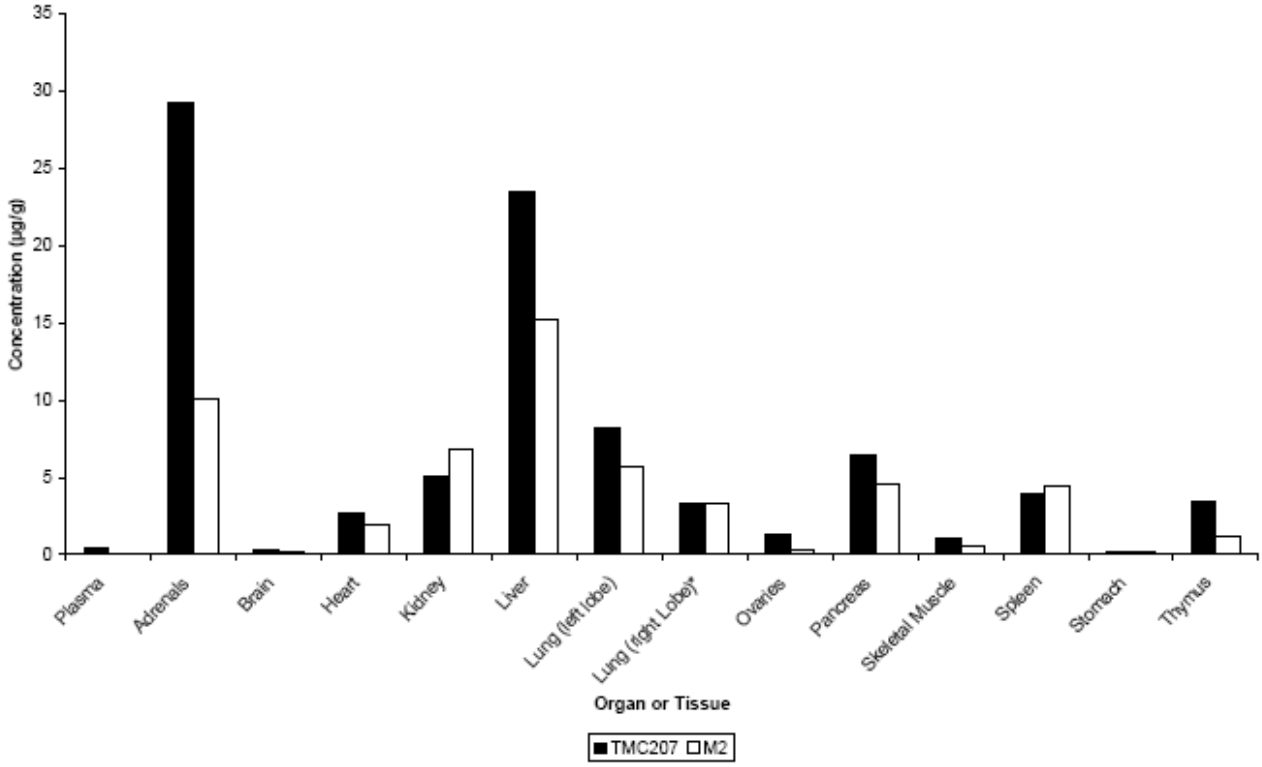
Metabolism

In all preclinical species and humans (see Figure P5), TMC207 was metabolized via Phase I reactions with the most important pathway being N-demethylation to M2, which was followed by a second N-demethylation to M3. Secondary metabolic pathways included oxidation at different parts of the molecule leading to minor metabolites. (See figure P5)

M2 was the major circulating metabolite in all preclinical species and humans. Its formation was extensive in mice, leading to a systemic exposure to M2 ($AUC_{(0-24h)}$) between 2- and 7-fold higher than that of the parent compound after 28-day and 13-week repeated dosing at 5 up to 30 mg/kg/day. M2 formation was less extensive in rats and dogs and even less in humans. M2- $AUC_{(0-24h}$ plasma levels were generally comparable to 2-fold lower than those of TMC207 in rats and dogs upon repeated administration of TMC207, and 3.5- to 4.5-fold lower in subjects with sputum smear positive pulmonary infection with MDR-TB.

Figure P2

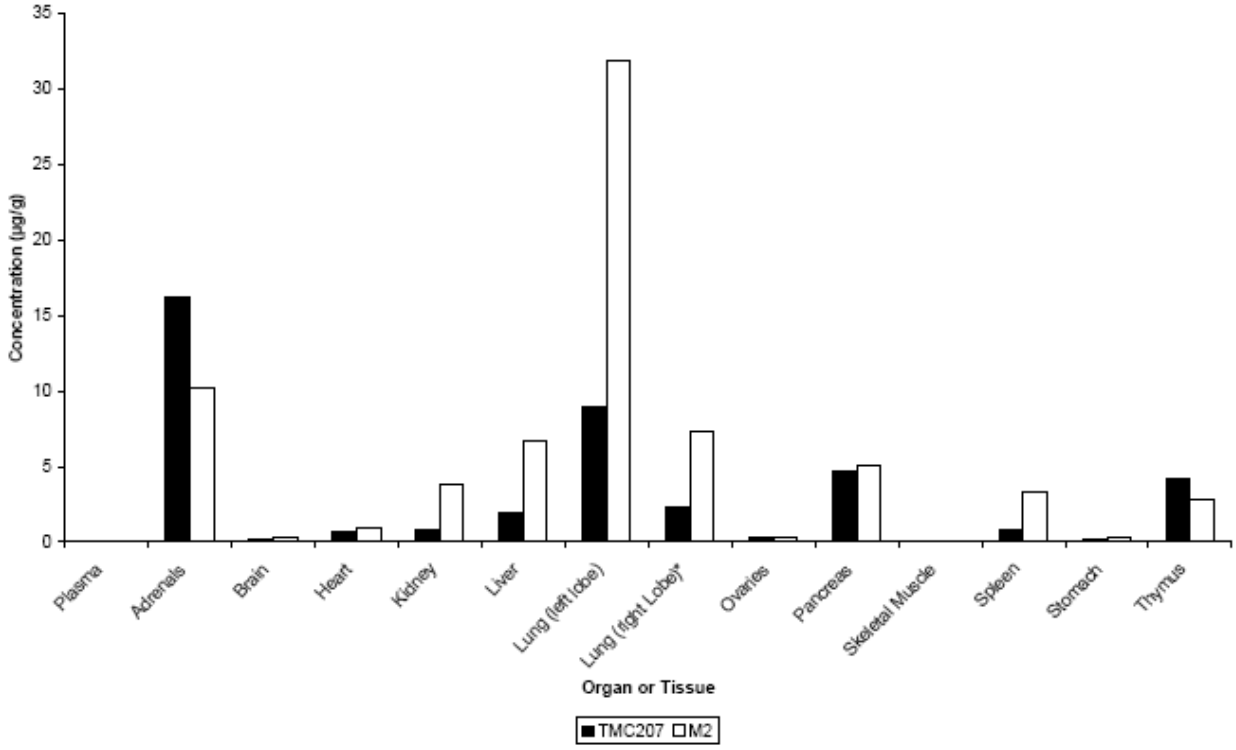
Mean concentrations of TMC207 and M2 in tissues 8 hours after a single oral administration of [^{14}C]-R403323 to the female cynomolgus monkey at a nominal dose level of 10 mg/kg (Group B)



* following bronchoalveolar lavage

Figure P3

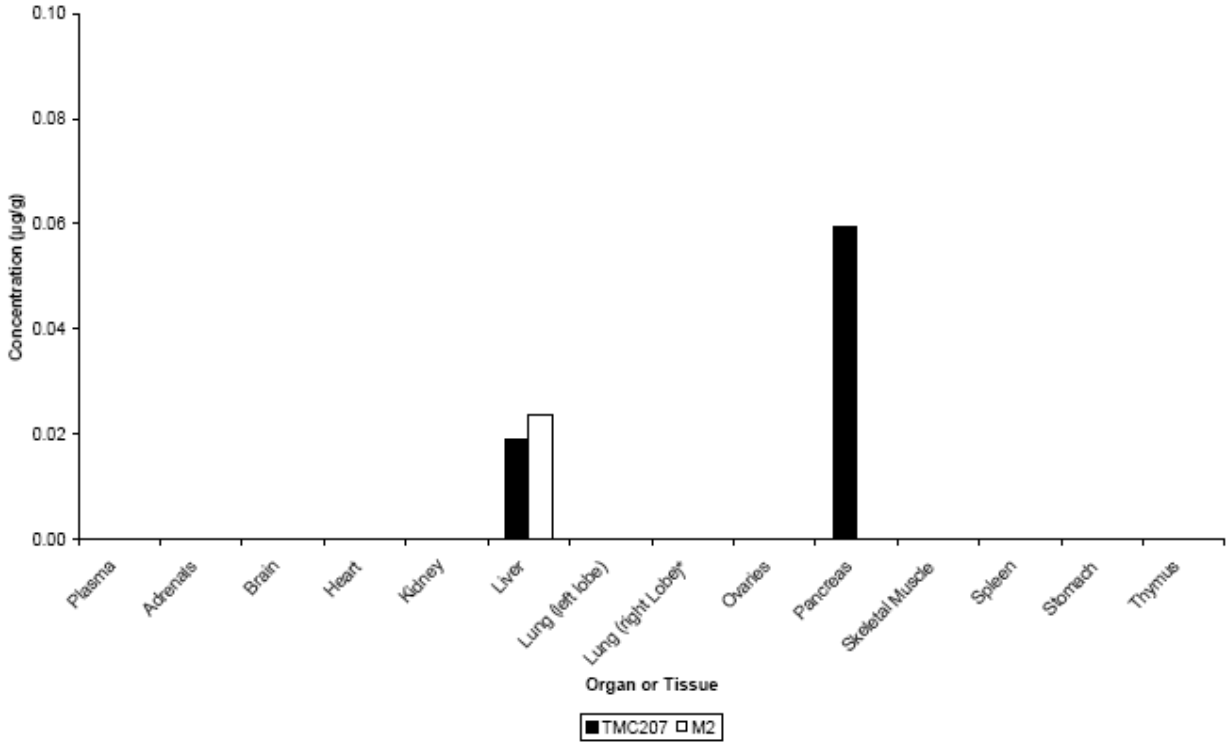
Mean concentrations of TMC207 and M2 in tissues 24 hours after a single oral administration of [^{14}C]-R403323 to the female cynomolgus monkey at a nominal dose level of 10 mg/kg (Group B)



* following bronchoalveolar lavage

Figure P4

Mean concentrations of TMC207 and M2 in tissues 17 weeks after a single oral administration of [^{14}C]-R403323 to the female cynomolgus monkey at a nominal dose level of 10 mg/kg (Group B)



* following bronchoalveolar lavage

Figure P5: Bedaquiline metabolism

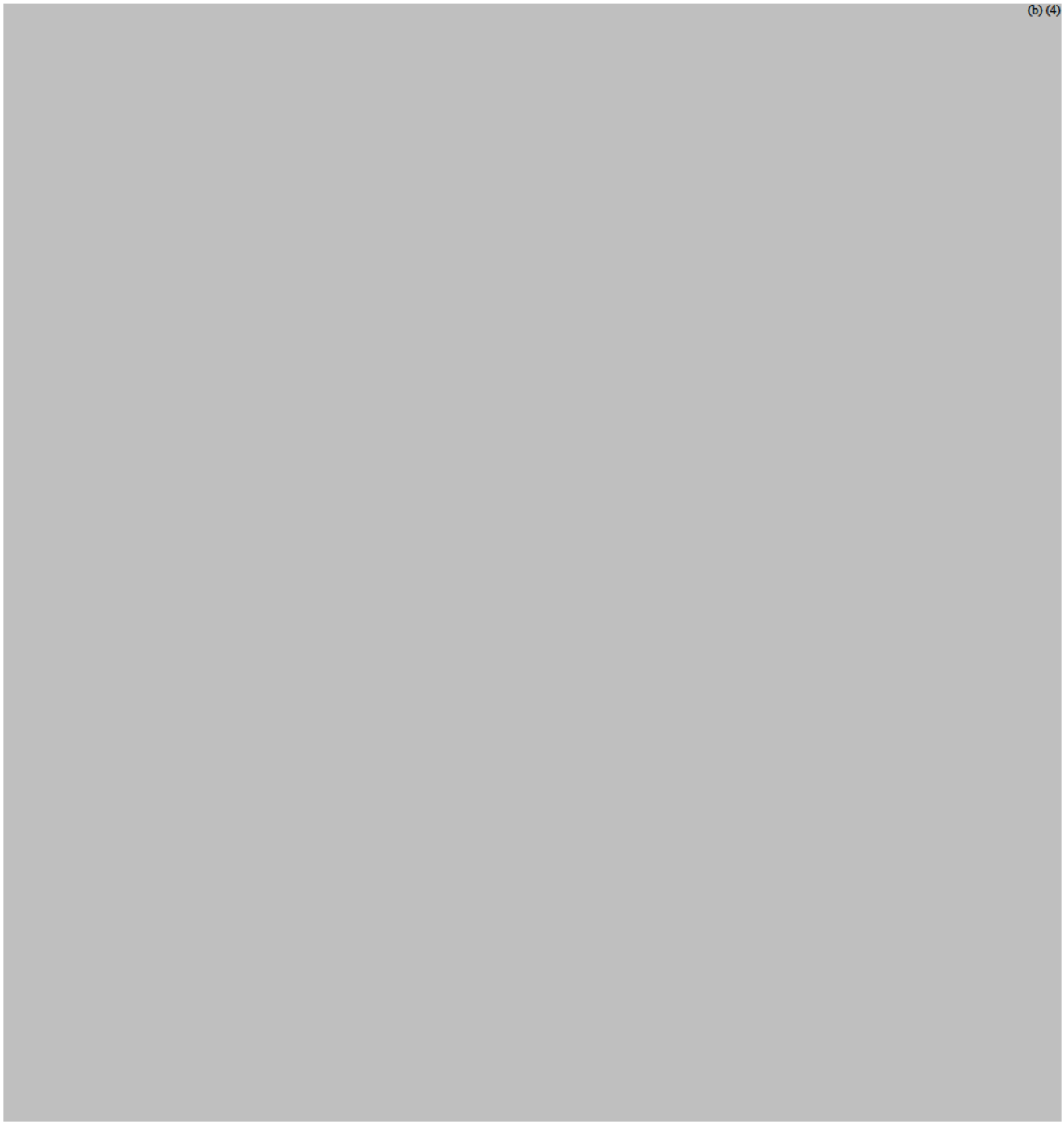


Table P3: Excretion of TMC207

Excretion balance

Sample	Time point (h)	Recovery (% of dose) (mean ± SD; n = 3)
Urine	0-336	2.522 ± 0.475
Faeces	0-336	58.00 ± 12.46
	336-1416*	5.78
	(0-∞)*	63.06
Cage wash	0-336	4.634 ± 3.131
Cage debris	0-336	3.279 ± 0.475
Swabs	0-336	2.868 ± 2.435
Total	0-336	71.30 ± 6.124
	0-∞*	78.20 ± 5.526

*: estimated from discontinuous 48-hour faeces collections

Bedaquiline was excreted very slowly and predominantly in the feces after oral dosing to rats, dogs and monkeys. As observed in patients, during the first 24 h after dosing, the main compound was unchanged (probably unabsorbed) drug. At later time-points the bulk of excreted material was mainly made up of M2 and M3 derivatives. In total, M2 and its derived metabolites accounted for 20-23% and 9-10% of the dose in rats and dogs, respectively.

6. General Toxicology-

6.1 Single-Dose Toxicity

Study title:	Single dose oral toxicity study in the mouse
Sponsor reference # :	TOX6202
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 18, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	00420075
Purity (%):	99.83 %
Frequency of dosing:	Single
Route of administration:	Oral gavage
Doses	0, 50, 200 or 800 mg/kg
Dose volume:	0.25 mL per 10 g body weight
Formulation/Vehicle:	20% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralised water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	SPF(CD1) mouse
Number/Sex/Group:	5
Age:	5 weeks old
Weight:	24 to 34 g

Key study findings: The maximum nonlethal single oral dose of TMC207 was 200 mg/kg. Mortality, liver toxicity and phospholipidosis occurred in high dose animals at a dose equivalent to 65 mg/kg or 4 g for a 60 kg patient.

Mortality

Four males and 2 females died or were sacrificed moribund at 800 mg/kg (see Table S3, below). Cause of death was not determined for decedents.

Table S1. Mortality in mice treated with a single dose of TMC207.

Day	Number	Circumstances
5	1	1F found dead
7	3	1F, 1M found dead 1 M moribund sacrifice
8	1	1M moribund sacrifice
10	1	1M moribund sacrifice

Clinical signs:
There were no clinical signs at 50 mg/kg. At 200 mg/kg, animals showed soft feces. Prior to death, 800 mg/kg mice showed bad condition, cyanosis, hypothermia, piloerection, ptosis and/or deep set eyes, while surviving animals showed soft feces.

Body weights:
At 800 mg/kg, mice showed body weight loss (males -30% and females -17 %), 7 days after dosing. On Day 14 after dosing, body weight remained reduced by 10 % in 800 mg/kg males compared to controls.

Gross pathology:
Findings in animals that died included wasting, pale swollen liver, dark content in small intestines, congested lungs. There was no clear relationship between these findings and the deaths.

Histopathology:
Histopathology was conducted in vehicle and high dose animals. There were no significant findings in females at 800 mg/kg. The one surviving high dose male that was evaluated showed hepatocellular single cell necrosis and hepatocellular hypertrophy with phospholipidosis (foamy vacuolization, and infiltration of the cells of the mononuclear phagocyte system).

Discussion:
The maximum nonlethal single oral dose of TMC207 was 200 mg/kg, which was associated with soft feces. Above this dose findings included mortality, phospholipidosis, weight loss, hepatocellular hypertrophy and single cell hepatocellular necrosis. Cause of death was not determined for decedents.

This study shows that liver findings and phospholipidosis can occur even after single doses, in high enough doses.

Study title:	Single dose oral toxicity study in the rat
Sponsor reference	TOX6016
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	June 5, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	00420075
Purity (%):	99.83 %
Frequency of dosing:	Single dose
Doses	0, 50, 200, 800 mg/kg
Route of administration:	Oral gavage
Dose volume:	2.5 mL/100g body weight
Formulation/Vehicle:	40% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralised water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	Sprague Dawley rat
Number/Sex/Group:	5/sex/dose group
Age:	5 weeks old
Weight:	144-209 g
Satellite groups:	Toxicokinetics animals: 4/sex/dose group

Key study findings:
No adverse effects were observed at 50 mg/kg. The maximum nonlethal single oral dose of TMC207 was 200 mg/kg, which was associated with soft feces and salivation and a mean $AUC_{(0-\infty)}$ values of 179 µg*h/mL.

Results
Mortality and clinical signs
Clinical signs and mortality were recorded daily. One 800 mg/kg male rat died 7 days after dose administration. Clinical signs included soft feces (all groups including controls), salivation, (transient in one animal at 200 mg/kg, but more common at 800 mg/kg) and bad condition in one (800 mg/kg) rat with ptosis, wet urogenital area, chromodacryorrhea, rough hair coat, crusty nose, salivation and few feces.

Body weights:
There was no biologically significant weight change in rats treated with single doses of TMC207 up to 800 mg/kg.

Gross pathology:
There were no gross pathology findings that were clearly related to drug although small thymus, which has been recorded in other studies, was observed in a single animal.

Table S2. Pharmacokinetics of TMC207 in male rats after a single oral dose.

Dosage group	Low: 50 mg/kg		Medium: 200 mg/kg		High: 800 mg/kg	
Sex	Males		Males		Males	
Time (h)	Mean	SD	Mean	SD	Mean	SD
0.5	1306	579	1435	641	1962	775
1	1149	394	1628	463	2037	616
2	1295	462	1913	561	2379	917
4	1218	406	1983	898	2869	1273
8	1331	426	2379	947	3494	1747
24	449	104	2989	395	7232	1999
96	29	9	93	26	1519	1197
168	15	4	55	13	279[1)]	147
336	<10		20	3	76[1)]	43
C_{max} ng/ml	1498	407	3286	330	7232	1999
T_{max} h	3	4	20	8	24	0
$t_{1/2,\ 24\text{-}96\ h}$ h	18.7	3.5	14.3	1.2	43.6[4)]	40.4
$t_{1/2,\ 96\text{-}336\ h}$ h	80.4[3)]	17.0	116.2	28.7	68.2[1)5)]	8.5
$AUC_{0\text{-}336\ h}$ ng.h/ml	NC		129044	13732	416286[1)]	118062
$AUC_{0\text{-}inf}$ ng.h/ml	36733	4094	132478	12478	424076[1)6)]	123441

[1)] n = 3
[2)] NC: Not calculated
[3)] The half-lifes were calculated from 96 h till 168 h post-dose.
[4)] Excluding satellite rat FK62 due to high inter- and intra-individual variability, a mean $t_{1/2,\ 24\text{-}96\ h}$ value of 23.4 ± 1.0 h (n = 3) was calculated.
[5)] Rat FK62 is excluded for the calculation of the mean $t_{1/2,\ 96\text{-}336\ h}$ value due to the lack of blood samples from 168 h post-dose onward.
[6)] Rat FK62 is excluded for the calculation of the mean value due to large extrapolation (56.6 %) in the calculation of $AUC_{0\text{-}inf}$ and the lack of blood samples from 168 h post-dose onward.

Table S3: Pharmacokinetics of TMC207 in female rats after a single oral dose.

Single Dose Oral Toxicity Study — Mean data

R207910 – OR/GAV – RAT — Females

Dosage group		Low: 50 mg/kg		Medium: 200 mg/kg		High: 800 mg/kg	
Sex		Females		Females		Females	
Time (h)		Mean	SD	Mean	SD	Mean	SD
0.5		657	390	737	191	1665	446
1		1143	498	1618	559	2244	326
2		1278	358	1929	219	2360	391
4		1265	180	2437	267	3193	533
8		1542	93	2761	431	4508	306
24		975	174	3778	1756	6540	459
96		151	28	312	86	574	139
168		98	12	178	44	302	60
336		<25		115	54	181	32
C_{max}	ng/ml	1696	143	4082	1396	6540	459
T_{max}	h	5	4	15	11	24	0
$t_{1/2,\ 24\text{-}96\ h}$	h	27.4	4.5	21.4	5.7	20.4	1.5
$t_{1/2,\ 96\text{-}336\ h}$	h	140.9	(n = 2)	209.7	122.9	163.8	42.4
$AUC_{0\text{-}336\ h}$	ng.h/ml	81240	(n = 2)	209121	59424	358913	19500
$AUC_{0\text{-}inf}$	ng.h/ml	89304	6478	250092	91129	402860	18245

In rats, single oral doses of TMC207 resulted in plasma and AUC values increased less than dose proportionally. Mean $AUC_{(0\text{-}336h)}$ values were 179 and 388 µg*h/mL at 200 and 800 mg/kg. Tmax increased with increasing dose (3 to 24 hours) and terminal half lives ranged from 80 to 209 hours.

Summary of individual study findings:

No adverse effects were observed at 50 mg/kg. The maximum nonlethal single oral dose of TMC207 was 200 mg/kg, which was associated with soft feces and salivation and an estimated mean $AUC_{(0\text{-}336h)\ value}$ was 179 µg*h/mL. One satellite (800 mg/kg) animal died 7 days after dosing and cause of death was not determined. Above 200 mg/kg, findings included mortality, bad condition and small thymus.

Repeat-Dose Toxicity

Study title:	15-Day repeated dose oral toxicity study in the rat
Sponsor reference # :	TOX6022
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	June 3, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	00420075
Purity (%):	98.4 %

Key Study Findings:
The NOAEL for 15-day, daily dosing in rats was 6 mg/kg, which resulted in an exposure ($AUC_{(0\text{-}24h)}$ value of 13 µg*h/mL. Liver effects (single cell necrosis and hepatocellular hypertrophy) and phospholipidosis developed within 14 days, at clinical exposures.

Methods

Frequency of dosing:	Daily for 15 days
Route of administration:	Oral gavage
Dose volume:	0.5 mL/ 100g body weight
Formulation/Vehicle:	10% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralized water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	Rat/Sprague Dawley
Doses:	0, 6, 12 and 24 mg/kg
Number/Sex/Group:	10
Age:	5 weeks old
Weight:	158-200 g

Results

Mortality and Clinical signs:
Clinical observations were recorded daily. There were no drug-related clinical signs or deaths at any dose during the study.

Body Weights:
Body weights were recorded on Days -4, 0, 7, 14 and 15. There were no effects on body weight during the study.

Food Consumption:
Food consumption was recorded on Days 7 and 14. There were no drug-related effects on feed consumption at any dose during the study.

Hematology and Clinical Chemistry
Blood was drawn for hematology and clinical chemistry, prior to terminal kill. Findings observed at 12 mg/kg included increased cholesterol (+22 %, females only) and decreased alkaline phosphatase (-17 %, females only). At 24 mg/kg, changes included increased cholesterol (+22 %), decreased glucose (-14 %, males), alkaline phosphatase (- 18 %), and total bilirubin (-32 %) and decreased AST (-16 %, males).

Gross Pathology
Liver weights were 10 % higher than controls at 24 mg/kg. There was a dose-related increase in several abdominal/liver findings in dosed rats. See Table R1, below.

Table R1: Gross pathology findings in rats treated with TMC207 for 15 days.

Finding	**12 mg/kg**	**24 mg/kg**
Pronounced lobulation of liver	3/20	8/20
Pale liver	1/20	3/20
Swollen liver	7/20	9/20
Increased peritoneal fluid	1/20	5/20

Histopathology
At 12 and 24 mg/kg findings in males included centrilobular single cell necrosis as well as hepatocellular hypertrophy, leukocyte infiltration and evidence of phospholipidosis (hepatocellular vacuolation). At the same doses, females showed signs of phospholipidosis (perilobular hepatocellular vacuolation).

Toxicokinetics

Table R2: Mean maximum plasma concentrations and pharmacokinetics parameters on Day 14

Dosage Group	**6 mg/kg/day**	**12 mg/kg/day**	**24 mg/kg/day**
C_{max} (ng/ml	0.5	1.1	1.8
$AUC_{(0\text{-}24h)}$ (µg.h/ml)	6.1	13.1	23.3

Discussion and conclusion

Data from clinical Trial C208[+] showed that doses of 400 mg q.d. for 14 days resulted in ($AUC_{(0\text{-}24h)}$ values of about 33 µg*h/mL in MDR-TB patients (see Table 20**, below). These data from this 15-day study suggest that liver findings occur at exposures about half those obtained in the clinic and that adverse liver effects could develop in the liver of patients in as little as 14 days, during the initial daily dosing phase of the regimen. Monitoring for adverse liver findings should be initiated early in the course of treatment with TMC207.

** The following Table 20 was taken from the applicant's Summary of Clinical Pharmacology Studies.

Table 20: Comparison of Plasma Exposures Obtained From the Population Pharmacokinetic Model for TMC207 in Plasma After Administration of TMC207 400 mg q.d. (Weeks 1 and 2) and 200 mg t.i.w. (Weeks 3 to 24) in MDR-TB Infected Subjects (Trial C208, Stage 2)

Parameter	Mean ± SD	
	TMC207 400 mg q.d. + BR Week 2	TMC207 200 mg t.i.w. + BR Week 24
N	76	51
C_{max}, ng/mL	2485 ± 676	1321 ± 422
C_{24h}, ng/mL	888 ± 329	-
AUC_{24h}, ng.h/mL [a]	32942 ± 8910	-
C_{48h}, ng/mL	-	516 ± 244
AUC_{48h}, ng.h/mL [a]	-	32621 ± 12569
C_{72h}, ng/mL	-	477 ± 239
AUC_{72h}, ng.h/mL [a]	-	44475 ± 18366

N = maximum number of subjects with data.
[a] AUC was measured within the dosing interval, which was 24 hours at Week 2 (q.d. regimen) and either 48 or 72 hours at Week 24 (t.i.w. regimen).
Source: Module 5.3.5.1/TMC207-C208-CRR-Stage-2-Interim-Analysis/Section 5.2

Study title: R403323: 13 week oral gavage administration study in the mouse

Study no.:	TMC207-NC142 (Tox7419)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	17 October 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	ZR403323PFA021
Purity:	99.3 %

Key Study Findings: This study was designed to serve as a dose range finding study for the carcinogenicity study. Mortality, moribundity and widespread histological changes indicate that the 30 mg/kg dose was above the MTD. There were no drug-related deaths at 20 mg/kg where the ($AUC_{(0\text{-}24h)}$ value was about 7 µg*h/mL on Day 90). The NOAEL was 5 mg/kg. The applicant eventually decided not to conduct a two year carcinogenicity study in mice due excessive toxicity.

Methods

Doses:	0, 5, 10, 20 and 30 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral, gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	20 % w/v HP-β-CD
Species/Strain:	Mouse/ Crl:CD1(ICR)
Number/Sex/Group:	10/sex/dose group
Age:	46-49 days
Weight:	Males: 31-42 g Females: 21-33 g
Satellite groups:	Pharmacokinetics (12/sex/dose)

Results

Mortality

Six animals from the 30 mg/kg dose group were sacrificed due to moribund condition. Postmortem examination revealed prominent skeletal muscular degeneration/necrosis (rhabdomyolysis) at this dose. One 10 mg/kg/kg female died as a result of a gavage accident.

Clinical Signs

Clinical observations were recorded daily. Clinical signs in 30 mg/kg animals included hunched posture, thinness, tremors, sluggish movement, staining of fur, raised hair, pale/cold body, semi-closed eyes/ tears/colored tears and impaired mobility. Closed eyes/tearing was also observed in 20 mg/kg animals.

Body Weights
Body weights were recorded twice predose (in the acclimatization period) and weekly during dosing. Body weight was reduced by 17 % in 30 mg/kg animals compared to controls.

Feed Consumption
Feed consumption was recorded weekly. Feed consumption was reduced by 26 % in 30 mg/kg animals.

Ophthalmoscopy
Ophthalmological examinations were conducted on all main study animals pretreatment and on control and high dose animals in Week 12. There were no adverse ophthalmological findings.

Hematology and Clinical Chemistry
Blood was collected in Week 13 for hematology and clinical chemistry evaluations. There were no significant findings at the 5 or 10 mg/kg doses. At 20 mg/kg, changes were restricted to increases in HDW (hemoglobin concentration distribution width, +16 %) and total amylase (+27 %, males only) as well as decreases in phospholipids. At 30 mg/kg, decreases were observed in HGB (-17 %), HCT (-15 %), MCH (-12%), lymphocytes (-48%, males only) albumin (-16 %, females only) and phospholipids (-30 %, males only), and increases were seen in HDW (+35 %), RDW (+26%), reticulocytes (+33%), neutrophils (+300 %), AST (+126 %), ALT(+156 %, females only), CK (+340 %, males only), globulin (+27 %, males only), amylase (+ 70 %, males only).

Gross Pathology
Gross pathology was evaluated at necropsy. Findings at 30 mg/kg included mottled, pale livers, with large spleens (also at 20 mg/kg) and large mesenteric lymph nodes. Other changes were isolated and sporadic.

Organ Weights
Liver weights were increased by 12 and 33 % respectively at 20 and 30 mg/kg and spleen weights were increased by 48 % in 30 mg/kg females only.

Histopathology
The battery of tissues evaluated was adequate. At 10 mg/kg, histopathology findings were limited to increased diffuse intra alveolar macrophages in the lung. At 20 mg/kg, changes included chronic musculopathy in the sternum, hepatocellular hypertrophy, and single cell necrosis in the liver, pigmented macrophages in the liver, increased diffuse intra alveolar macrophages in the lung, histiocytosis in the mesenteric lymph nodes, chronic inflammation and degeneration of the quadriceps muscle.
The mortality/moribundity findings indicated that the 30 mg/kg dose was above the MTD. At this dose, additional findings were observed in the adrenal gland (reduced numbers of large vacuoles in the X-zone, females only), coagulating glands (inflammation and single cell necrosis), esophagus (vacuolar degeneration of the tunica muscularis), liver (granulocytic infiltration, necrotizing inflammation, abscesses, extramedullary haematopoiesis), lung (purulent inflammation), mesenteric lymph node (granulocytic infiltration, abscesses), pancreas (interstitial fibrohistiocytosis with acinar atrophy), mandibular salivary glands (atrophy, megakaryocytes, single cell necrosis, granulocytic infiltration, increased mitoses), skeletal muscle (mineralization), spleen (hyperplasia, extramedullary haematopoiesis, granulocytic infiltration, lymphoid atrophy, inflammation, atrophy), stomach (atrophy/degeneration of the glandular stomach) thymus (atrophy) and uterus (inflammation and granulocytes).

Toxicokinetics

Table R3: Mean plasma concentrations and pharmacokinetics parameters on Day 28

Dosage Group	(5 mg/kg/day)	(10 mg/kg/day)	(20 mg/kg/day)	(30 mg/kg/day)
C_{max} (µg/ml	0.5	0.9	1.1	1.1
$AUC_{(0-24h)}$ µg.h/ml	4.2	7.2	9.3	14

Table R4: Mean plasma concentrations and pharmacokinetics parameters on Day 90

Dosage Group	(5 mg/kg/day)	(10 mg/kg/day)	(20 mg/kg/day)	(30 mg/kg/day)
C_{max} (µg/ml	0.4	0.8	0.7	0.8
$AUC_{(0-24h)}$ µg.h/ml	3.7	6.8	6.6	9.6

Stability and Homogeneity
Stability testing revealed that test article was present at levels between 90 to 107 % of the nominal concentrations.

Discussion
This study was designed to serve as a dose range finding study for the carcinogenicity study. The poor condition/moribundity (hunched posture, thinness, tremors, sluggish movement, staining of fur, raised hair, pale/cold body, semi-closed eyes/ tears/colored tears and impaired mobility) observed at the 30 mg/kg dose indicated that this dose was above the MTD.

The NOAEL was 5 mg/kg/day although findings at the next higher dose (10 mg/kg/day) were mild and restricted to phospholipidosis, as indicated by diffuse intra alveolar macrophages in the lung. Target organs at 20 mg/kg included skeletal muscle (sternum and quadriceps muscle), liver, lung and lymph nodes and possibly the pancreas as indicated by increased amylase and decreased phospholipids. Multiple mortalities at 30 mg/kg indicated this dose was above the MTD. At the high dose, findings were widespread and in addition to tissues targeted at 20 mg/kg, included spleen, adrenals, coagulating glands pancreas, mandibular salivary glands, stomach and uterus. Mortality at 30 mg/kg indicated that this dose was above the maximum tolerated dose and the NOAEL was 5 mg/kg/day.

The applicant eventually decided not to conduct a two year carcinogenicity study in mice due excessive toxicity and because the pharmacokinetics profile in mice (excessive levels of M2) was not reflective of the clinical profile.

Study title:	13 Week Toxicity Study by Oral Gavage) Rats
Sponsor reference # :	TOX6614
Study report location:	EDR
Conducting laboratory:	(b) (4) [redacted]
Date of study initiation:	July 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	ZR207910PFA011
Purity (%):	99.5 %
Dose volume:	5 mL/kg/day
Formulation/Vehicle:	20% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralized water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	20
Age:	7 weeks old
Weight:	228 to 336 g
Satellite groups:	Toxicokinetics

Key Study Findings:
The NOAEL for 13 week, daily dosing was 1.5 mg/kg, a dose with an $AUC_{(0\text{-}24h)}$ value of 2.9 µg*h/mL. Liver and thymus effects were observed at 6 mg/kg doses, where $AUC_{(0\text{-}24h)}$ values were about 8 µg*h/mL. At 24 mg/kg, where exposures were 1-2 time clinical exposures and lymph nodes, spleen and lungs, liver, tongue, skeletal muscle, kidneys, thyroid and spleen were affected. Intermittent dosing (at 10 mg/kg/day alternate days) virtually eliminated toxicity

Methods
Groups of 20 rats/sex were dosed, by oral gavage, with TMC207 fumarate), for 13 weeks. Groups I-IV received TMC207 at 0 (vehicle), 1.5, 6 or 24 mg/kg/day, while group V received 10 mg/kg TMC207 fumarate every other day. A satellite group of 6 rats/sex/dose group which received the same doses was evaluated for toxicokinetics.

Observations and Results

Mortality
Animals were checked for morbidity/mortality twice daily. There were three deaths during the study, but none were considered to be drug-related. One control female and one satellite male were found dead after blood sampling and these deaths were attributed to the blood sampling procedure (from the orbital sinus). One low dose animal was sacrificed with an abscess/ulcerated mass on Day 59, but since this was only seen in the low dose, it was concluded that this finding was not drug-related.

Clinical Signs
No drug-related findings were observed in any animals. Findings observed were sporadic or transient and not dose-related.

Body Weights
Body weight was measured weekly. High dose animals showed body weights that were 21 % lower than controls at Week 14.

Feed consumption
Feed consumption was measured once per week. Feed consumption was not significantly affected by TMC207 at any dose.

Ophthalmoscopic examinations
Ophthalmoscopic examinations were conducted predose and at the end of dosing. There were no treatment-related adverse findings in any rats.

Hematology and Clinical Chemistry
Blood was drawn for hematology and clinical chemistry evaluations at the end of dosing. Changes at 24 mg/kg affected WBC (+17%), neutrophils (+153 %), monocytes (+173%), lymphocytes (-24%, females), platelets (+31 %, females), fibrinogen (+19 %). triglycerides (+23%) and cholesterol (+23 %) and ALP (-45%). AST was increased at 6 mg/kg (+77 %, females) and 24 mg/kg (+155%). ALT was increased at 6 mg/kg (+60 %), and 24 mg/kg (+51 %, males).

Gross Pathology
At 24 mg/kg, there were increases in relative organ weights in the adrenals (+28%), liver (+19 %) and spleen (+19 %). There was also a decrease in the relative thymus weight at 24 mg/kg and an increase in the number of rats with small thymus at 6 and 24 mg/kg/day.

Histopathology

At 24 mg/kg/day, histopathology findings included increased incidences of foamy, macrophages in the popliteal lymph nodes, spleen and lungs, hepatocellular hypertrophy, fibrohistiocytic inflammation in the tongue and skeletal muscle, tubular basophilia in the kidneys, follicular cell hypertrophy in the thyroid, small marginal zone in the spleen and muscle fiber degeneration in the tongue and skeletal muscle.

Toxicokinetics
Mean AUC values increased approximately proportionately with dose on Day 85. Exposures at 10 mg/kg every other day was similar to exposures seen with 6 mg/kg/day. At 6 mg/kg/day, effects on the liver were present, as indicated by the increases in AST/ALT levels.

Table R5: Mean C_{max} and AUC values for TMC207 in rats on Days 29 and 85 of dosing

	Dose (mg/kg/day)	1.5		6		24		10 (eod)	
	Day	29	85	29	85	29	85	29	85
C_{max} (µg/mL)	Male	0.150	0.277	0.705	1.02	2.12	2.27	0.661	1.03
	Female	0.294	0.443	0.994	1.52	2.92	4.00	1.11	1.21
AUC (µg.h/mL)	Male	1.04	1.36	6.88	9.34	25.0	27.5	9.90	12.2
	Female	2.77	4.35	12.4	20.4	46.7	59.7	24.8	26.6

eod: every other days

Discussion:

Repeated dosing of TMC207 in rats for 13 weeks at 24 mg/kg targeted the organs expected based on prior studies, including lymph nodes, thymus, spleen and lungs, liver, tongue, skeletal muscle, kidneys, thyroid and spleen. The liver and thymus appeared to be most sensitive, since small thymus and liver enzyme changes were observed at 6mg/kg. Effects were widespread at clinical exposures.

Dosing on alternate days (10 mg/kg every other day) resulted in similar exposures to animals dosed at 6 mg/kg/day, but no significant toxicity. Intermittent dosing is being used in patients and these data confirm that toxic effects can be reduced by this approach.

The NOAEL was 1.5 mg/kg, a dose with an $AUC_{(0\text{-}24h)}$ value of 2.9 µg*h/mL, a value 0.13 times the clinical exposure.

Study title:	R403323: 26 Week Oral (Gavage) Administration Toxicity Study in the Rat with a 12 Week Treatment-Free period
Sponsor reference #	TMC207-NC109
Covance study number	1073-097
Study report location:	DARRTS
Conducting laboratory :	(b) (4)
Date of study initiation:	October 18, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	ZR403323PFA021
Purity (%):	99.3 %

Key Study Findings:
No NOAEL could be determined because prostatitis, thymic atrophy and phospholipidosis were present at the lowest dose (5 mg/kg/day, a dose with an AUC $_{(0\text{-}24h)}$ of 11.7 µg*h/mL, about half the clinical exposure. For biweekly dosing, the NOAEL could not be determined since adverse effects including large liver and prostatitis were observed at the only dose studied (20 mg/kg, equivalent to about 1.4 times the clinical exposure based on AUC comparisons).

Methods
Groups of 20 rats/sex were dosed, with R403323, (R207910, TMC207 fumarate), for 26 weeks. Groups I-IV received TMC207 fumarate at 0 (vehicle), 5, 10 or 20 mg/kg/day, while group V received 20 mg/kg TMC207 biweekly. Recovery groups of 10 rats/sex/dose group which received 0, 20 mg/kg/day and 20 mg/k biweekly groups were evaluated over a 12-week treatment-free period to assess the reversibility of adverse effects.

Table R6: Experimental design

Group	TMC207 (mg/kg)	Number of animals					
		Main study		Recovery		Toxicokinetics	
		Male	Female	Male	Female	Male	Female
1	0	20	20	10	10	4	4
2	5	20	20			6	6
3	10	20	20			6	6
4	20	20	20	10	10	6+	6+
5	20*	20	20	10	10	9	9

* animals were dosed biweekly.
+ The last 3 males and 3 females from this group were retained for the 12 week recovery period
Body weights and clinical observations were recorded for toxicokinetics animals but not reported.

Frequency of dosing:	Daily (Groups 1-4) or biweekly
Route of administration:	Oral gavage
Dose volume:	5 mL/kg/day
Formulation/Vehicle:	20% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralised water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	20
Age:	22-43 days old
Weight:	0.2-0.3 kg
Satellite groups:	Recovery and Toxicokinetics
Unique study design:	Blood was collected for pK analysis on Days 1 and 13

Results

Mortality
There were four deaths during the study, but none were considered to be drug-related. One control male (week 18) and one 20 mg/kg/day female (week 30) died as a result of dosing accidents. One 20 mg/kg/day male died due to a hemolymphoreticular tumor (week 9). The cause of death was not established in one male from the 10 mg/kg/day group (week 23).

Clinical Signs
The most common clinical signs were protruding eyes and pale teeth, which were seen in 1/19 females at 10 mg/kg and 8/30 females at 20 mg/kg/day and 1/30 males at 20 mg/kg biweekly. Pale teeth were observed at 20 mg/kg/day in 4/30 females.

Body Weights
There was no significant difference between control and drug-treated animals at the end of dosing, although, in the 20 mg/kg/day group, body weight gain was lower than controls between weeks 13-26 (-27 for males and -53 % for females).

Hematology
Hematology changes were restricted to animals receiving 20 mg/kg/day and at week 26, included increases in absolute and % reticulocytes (+22 to 55 %), WBC (+21 %, males), increased neutrophils (+ 63 to 108 %), decreased lymphocytes (-13 to 17 %) and increased plateletcrit (+20%, females).

Clinical Chemistry
Clinical chemistry changes in the 20 mg/kg dose group included changes in AST (+50%), CPK (+56%), triglycerides (-43%), urea (-15%), albumin (-11%) and AG ratio (-14%). Cholesterol levels increases were seen in all groups except the 5 mg/kg animals [(18-24 %) by week 26], but these changes were not dose-related. Urea (-18%) and triglycerides (-42%) were also reduced in 20 mg/kg intermittent dose animals.

Gross Pathology
Kidney weights were 11 and 16 % higher than controls in females dosed at 10 and 20 mg/kg/day respectively. Thymus weights were 20 lower than controls in 20 mg/kg/day females. There was an increase in the number of animals with large mesenteric lymph nodes and pale focus in the lungs at 20 mg/kg/day. Pale focus in the lungs was also increased at 10 mg/kg/day.

Histopathology
At 20 mg/kg/day, histopathology findings included increased incidences of foamy, eosinophilic and/or brown pigmented macrophages and/or granulomatous inflammation/abscesses and these were found in various combinations in the mesenteric lymph nodes, mandibular lymph nodes, popliteal lymph nodes, uterus, lung spleen and thymus. Foamy macrophages were also observed in the popliteal lymph nodes at the intermediate dose (10 mg/kg/day). No NOAEL could be established for this study since prostatitis, thymic atrophy, phospholipidosis (foamy/eosinophilic macrophages in the mesenteric lymph nodes), and corticomedullary mineralization were observed at all doses.

Reversibility
At the end of the recovery period, high dose animals still showed decreased plateletcrit % (-35 %), and PDW (-15 %), decreased thymus weight (-36%), foamy or brown pigmented macrophages (in the popliteal lymph nodes, thymus and lungs).

Intermittent dosing
Dosing at 20 mg/kg twice weekly resulted in adverse effects which were similar but less severe than those seen with daily dosing. Adverse effects included increased incidence of protruding eyes (1/30 males), large liver (12/40), pale focus on lungs (7/40 animals), foamy/eosinophilic macrophages in mesenteric lymph nodes (7/40 animals) and brown pigmented macrophages in the thymus (21/40 animals), prostatitis (12/20) and myopathy of the tongue (1/40 animals). Reduced urea (-18 %) and triglycerides (-42%) were of questionable toxicological significance.

Reversibility
At the end of the recovery period, intermittently dosed animals showed reduced mean lymphocyte count (-27 %), but similar individual values were seen in control animals. There were also slightly higher incidences of prostatitis and brown pigmented macrophages in the thymus and compared to controls.

Table R7: Toxicokinetics

R207910								
	Male				Female			
	Day 1							
Treatment group	2	3	4	5	2	3	4	5
Dose (mg eq./kg)	5	10	20	20[1)]	5	10	20	20[1)]
C_{max} (ng/ml)	380	555	1390	1140	399	817	1610	1710
AUC_{0-inf} (µg.h/ml)	3.42	5.37	13.5	11.7	4.94	10.5	29.2	22.9
$AUC_{0-168\ h}$ (µg.h/ml)[2)]	-	-	-	30.0	-	-	-	67.9
	1 Month							
Dose (mg eq./kg)	5	10	20	20[1)]	5	10	20	20[1)]
C_{max} (ng/ml)	627	615	1300	1290	750	1480	2230	1960
$AUC_{0-24\ h}$ (µg.h/ml)	5.54	7.58	16.1	18.3	9.44	20.2	35.0	32.1
$AUC_{0-168\ h}$ (µg.h/ml)[2)]	38.8	53.1	113	43.7	66.1	141	245	106
	6 Months							
Dose (mg eq./kg)	5	10	20	20[1)]	5	10	20	20[1)]
C_{max} (ng/ml)	691	843	1480	1560	1040	2360	3280	2380
$AUC_{0-24\ h}$ (µg.h/ml)	6.62	10.6	17.4	24.7	16.8	36.1	44.6	37.6
$AUC_{0-168\ h}$ (µg.h/ml)[2)]	46.3	74.2	122	69.5	118	253	312	163

Discussion and conclusion

Dosing was adequate, based on the toxicities observed at the high dose. Daily TMC207 dosing for 26 weeks at 20 mg/kg/day resulted in hematology and serum chemistry changes and phospholipidosis and affected the predicted tissues (lungs, liver, thymus, prostate and skeletal muscle). The vehicle, HP-β-CD, is known to be associated with kidney changes and phospholipidosis and these were seen at all doses in this study. After a 12 week reversibility period, some findings had disappeared but some (decreased thymus weight [-36%], and phospholipidosis) persisted. Findings after intermittent dosing were less severe, but these animals were not free from adverse effects at the end of the reversibility period (prostatitis and phospholipidosis persisted).

These data show that TMC207 effects are dose and duration dependent and clinical exposures are expected to affect various tissues after 6 months of daily dosing. While many of these findings are reversible upon cessation of dosing, the process appears to be slow since phospholipidosis persisted even after a 12 week reversibility period. Intermittent dosing results in somewhat fewer adverse effects but these also were only slowly reversible as phospholipidosis was evident at the end of the reversibility period. The other signs that persisted after the end of the reversibility period were prostatitis and reduced thymus weights which were of questionable toxicological importance.

Study title:	Six-month repeated dose oral toxicity study of TMC207 with 2-month interim kill in the beagle dog (mechanistic study).
Study no.:	TMC207-NC165
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	August 9, 2005
GLP compliance:	Yes but exceptions did not affect the interpretability of the data
QA statement:	Yes
Drug, batch #:	ZR403323PFA021
Purity	99.3 %
Species	Dog
Strains:	beagle
#/sex/group	3
Satellite groups:	Toxicokinetics
Age:	5-6 months old
Weight:	8 kg (males); 5 kg (females)
Doses	0, 10 or 40/20* mg/kg/day and 140 mg/kg biweekly. *Due to pronounced body weight loss and severe clinical signs, the dose level for the high dose was lowered to 20 mg eq./kg/day from day 57 onwards.
Route	Oral gavage
Formulation	aqueous solution containing 40% w/v Hydroxypropyl-ß-cyclodextrin (HP-ß-CD)
Dose volume	5 mL/kg

Key Study Findings

No NOAEL could be determined for this study because at the lowest dose, 10 mg/kg/day (about three times the clinical exposure), findings included discolored lungs, thymus atrophy, degeneration of the stomach wall, pancreatitis, inflammation in the testes and phospholipidosis. Findings at higher doses included dose related QT prolongation, degeneration of cardiac myocytes and skeletal muscle degeneration. With the exception of the degeneration of cardiac myocytes, these findings were consistent with those from other animal species.

Background

This study was conducted to determine the effects of TMC207 in dogs after 6 month of daily and intermittent dosing. In addition, biomarkers for cardiac and muscle injury (canine cardiac Troponin I, creatine kinase and myoglobin) and pancreatic pathology (amylase, lipase and Trypsin-like Immunoreactivity (cTLI)) and gastric pathology (Gastrin-17) were measured.

Results:

Mortality: There was no unscheduled mortality during this study.

Clinical signs: Clinical signs were recorded daily. No clinical signs were recorded at 10 mg/kg/day. During the first 2 months, high dose (40 mg/kg) and biweekly dosed animals showed thinness, excessive salivation and vomiting. In addition 40 mg/kg/day animals showed dehydration, conjunctivitis and decreased activity.

Body weights: On day 35 and 56 animals dosed at 40 mg eq./kg/day had a lower mean body weight of -19% and -30%, respectively, when compared with control.

Despite of the initiation of the supplementary feeding (Nutri-Plus gel®) in the 40 mg eq./kg/day dose group from day 36 onwards, body weight progressively decreased, resulting in up to 2 kg body weight loss in one male (No. 45) and one female dog (No. 145) after a 2-month treatment period. Because of this pronounced body weight loss, the dose level was decreased to 20 mg eq./kg/day (daily dosing) on day 57 and the interim sacrifice was performed after 2 months of dosing instead of after the planned 3 months of dosing.

Food consumption: Initially, dogs were group housed except for a 6 hour period in the morning where they were housed individually and had access to feed (250 to 400g depending on weight). However, as a result of the observed weight loss, a system of continuously individual housing was applied (Week 10 onwards) and dogs had continuous access to their food. Also, all male dogs weighing less than 12 kg received 350 g food per day and all female dogs weighing less than 8 kg received 300 g/day. Additionally all dogs of group B, dosed daily with R403323 at 40 mg eq/kg b.w./day, were given a daily supplement of Nutrigel-plus® at the daily dose of 4 cm/kg b.w./day from October 5, 2005 up to the end of the dosing period, in addition to their normal food supply.

At 10 mg/kg, drug administration had not significant impact on feed consumption. At 40 mg/kg, feed consumption was markedly reduced (-61 %) compared to controls at Week 8. After lowering the dose to 20 mg/kg/day and supplementing the feed with Nutri-Plus gel®) from day 36 onwards, feed consumption was comparable to controls from Week 13 onwards.

Intermittently dosed animals consumed about 18% less feed than control animals at Week 8, but were comparable to controls by Week 26.

Ophthalmoscopy: Ophthalmoscopy exams were performed on Days 56, with additional evaluations conducted a various times over the dosing period. Congested conjunctiva was observed in 40/20 mg/kg animals and intermittently dosed animals on Day 56. A number of other transient findings appeared and resolved during the course of the study including intolerance to light, small corneal opacities and mottling of the tapetal fundus.

Electrocardiography: Electrocardiography evaluations were conducted pre trial, after one month, prior to interim kill and at the end of the dosing period. QT intervals (corrected by Bazett, Fridericia and van de Water) were increased by 12-16 % at the 8 week timepoint. After dose reduction to 20 mg/kg/day, no QT prolongation was detectable.

Table R8. Mean ECG values in control and 40 mg/kg dogs recorded in Week 8

	Control Males/Females	40 mg/kg/day Males/Females	Mean % increase Compared to control
QT interval	173/174	216/217	
QT_c interval (msec) Bazett	247/246	276/273	+ 11 %
QT_c interval (msec) Fridericia	219/219	254/253	+ 16 %
QT_c interval (msec) Van de Water	217/217	250/249	+ 15 %
Overall Mean (msec)	228	259	+ 14 %

Table R9. Mean ECG values in control and 40 mg/kg dogs recorded in Week 26

	Control Males/Females	40 mg/kg/day Males/Females	Mean % increase Compared to control
QT interval	186/166	207/207	
QT_c interval (msec) Bazett	266/244	259/249	-1 %
QT_c interval (msec) Fridericia	236/214	240/234	+ 5%
QT_c interval (msec) Van de Water	230/213	236/231	+ 6 %
Overall Mean (msec)	233	241	+ 3 %

Hematology and Clinical Chemistry
Hematology evaluations were conducted predose, during Week 5, prior to interim kill (2 months), Week 14 and Week 27. Hematology changes were moderate at 40 mg/kg at Week 8. At Week 8, Values, expressed as (proportion compared to control) are shown on Table R10, below.

Table R10: Effects of TMC207 on hematology parameters compared to vehicle controls.

	40/20 mg/kg		140 mg/kg twice weekly	
	Males	Females	Males	Females
Fibrinogen (mg/dl)	2.6	4.1		
WBC (103/µl)	2.0	2.3	1.4	1.7
RBC (106/µl)	0.89		0.88	
HGB (g/dl)	0.87		0.85	
HCT (%)	0.86	0.88	0.84	
Reticulocytes (10^3/µl)	0.29	0.16	0.52	
Thrombocytes (10^3/µl)	1.5	1.8		
Neutrophils (10^3/µl)	2.5	3.2	1.5	1.4
Lymphocytes (10^3/µl)	0.84	0.83		
Monocytes (10^3/µl)	2.5	2.0	1.8	2.3
Total protein	0.83	0.76		
Albumin	0.69	0.61		
Cholesterol	1.3	1.4	1.2	1.4
ALP		1.6		1.5
AST	4.8	5.3		
ALT	5.8	8.1	1.6	
GGT	1.5			
CK	13	22		

After dose reduction, at the Week 27 timepoint, the only change detected in the 40/20 dose group was an almost 3-fold increase in reticulocytes in females only. At Week 27, intermittently dosed animals showed increases in fibrinogen (+47 %), WBC (+76 %), thrombocytes (+62 %), neutrophils (+80 %) and monocytes (157 %) and reduced albumin (-10%).

Biomarkers

At week 9, increased cardiac troponin I was observed at 40 mg eq./kg/day (4 times control levels), and in intermittently dosed animals (3 times control levels, females only) suggesting the presence of cardiac damage in male and female dogs. At week 13, after dose reduction to 20 mg e.q./kg/day, cTnI levels remained only slightly increased in males only, (+ 62 % while levels in females were comparable to controls. At Week 26, cardiac troponin I remained increased by 2 to 4-fold in intermittently dosed dogs. These increased troponin I levels correlated with increases in lymphohistiocytic infiltrate and single cell degeneration in the hearts of high dose (40/20 mg/kg) and intermittently treated dogs at the two month and at the 26 week kills. Treatment at a dose of 10 mg/kg/day for 6 months had no impact on cardiac troponin I levels at any time.

After two and three months of dosing, myoglobin was increased in the 40/20 mg eq./kg/day dose group in animals of both sexes. This finding was not observed in intermittently dosed animals at any time point and was not seen in any dogs at the end of dosing. This finding correlated well with the histopathological findings of skeletal muscle damage which was observed in the interim sacrificed dogs (after 2 months), but not present in any animals sacrificed at 26 weeks.

Serum gastrin-17 levels, known to be specific for the antrum of the stomach in humans, showed no relevant changes in all dose groups during the entire course of the study despite degeneration of cells in the stomach at all doses.

No marked changes in Canine Trypsin-Like Immunoreactivity (TLI) levels, indicative for pancreas effects, were observed in male and female dogs treated with test article.

Organ weights:
At the interim kill, 40/20 mg/kg animals showed decreases in weight of the popliteal lymph nodes (-30 %) and spleen (-40 %), compared to control animals, but these differences were not present at the end of the 26 week dosing period.

Gross pathology:
At the lowest dose, the only findings were white/tan/discolored focus in the lungs which was not dose related, found in all bedaquiline-dose groups, and observed at the 2 month kill and at the terminal kill. The only additional finding at the 40/20 mg/kg dose was pale liver in males only. The following findings were reversible, since they were seen at the interim kill in high dose animals, but not at the terminal kill: discoloration of the popliteal lymph nodes and spleen, small thymus, and congestion of the lacrimal glands. At the end of dosing, intermittently dosed animals showed effects in the lung (discoloration), thymus (discoloration) and pancreas (small/firm/discolored with minor pancreatic duct area swollen).

Histopathology:

Two month interim kill
At the interim kill, in addition to widespread phospholipidosis, findings included degeneration of cardiac myocytes (6/6 high dose and 4/6 intermittent dose animals), degeneration of the bile duct with cell necrosis and swollen appearance (2/6 high dose dogs), skeletal muscle degeneration (quadriceps, diaphragm, tongue, 6/6 high dose dogs), degeneration of glandular epithelial cells in the stomach (all dose groups) and thymus atrophy (all dose groups).

At the end of dosing, phospholipidosis was widespread and present at all doses as evidenced by pigmented/foamy /prominent macrophages/histiocytes, in the gall bladder, lacrimal glands, large intestines, larynx, lung, mesenteric and politeal lymph nodes, Peyer's patches, duodenum. Other organs affected at all doses included the stomach degeneration of fundic glands (all animals), testes, (inflammation in all daily-dosed animals, and pancreas (dose-related chronic inflammation, 1/6 animals at 10 mg/kg).

Six month terminal kill
At the end of dosing, histopathological changes in the heart were restricted to high and intermittently dosed animals. Single cell degeneration in the heart was seen in (3/6) high dose and (4/6) intermittently dosed dogs. Hepatocellular hypertrophy was observed in 4/6 high dose dogs and 6/6 intermittently dosed animals. Chronic inflammation in the pancreas was observed in the high dose (4/6 animals) and intermittent dose (4/6 animals) groups.

Table R11: Mean plasma concentrations and pharmacokinetics parameters in Day 175

Dosage Group	10 mg/kg/day	40/20 mg/kg/day	140 mg/kg/2x weekly
C_{max} (ng/ml	3.8	6.2	8.9
AUC $_{(0\text{-}24h)}$ µg.h/ml	70	120	963

Administration of TMC207 to dogs at 10 mg/kg/day for 6 months resulted in mean AUC $_{(0\text{-}24h)}$ values that were about 70 µg.h/ml or about three times the level seen in the clinic.

Discussion
The doses were adequate as indicated by the frank toxicity at the high dose and the reduced toxicity at the lowest dose. No NOAEL could be determined for this study because at the lowest dose, 10 mg/kg/day, findings included discolored lungs, thymus atrophy, degeneration of the stomach wall, pancreatitis, inflammation in the testes and phospholipidosis. Exposures at this dose were about three times the clinical exposure.

Within the first two months of dosing, 40 mg/kg TMC207 resulted in a 16 % increase in QTc interval. When the dose was reduced to 20 mg/kg on Day 57 due to excessive toxicity, this finding disappeared.

An interim kill at the two month timepoint revealed findings consistent with other repeat dose rat and mouse studies, including phospholipidosis, degeneration of the stomach wall and thymus atrophy which were already present at all doses. High dose animals showed effects in the liver, heart, and skeletal muscle.

For intermittently dosed animals, findings at the terminal sacrifice were qualitatively similar, but generally more severe than those seen at the interim sacrifice. For the 40/20 mg/kg animals, findings at the terminal sacrifice were occasionally less severe than those at the interim sacrifice because the earlier sacrifice reflected exposure to the higher 40 mg/kg dose, while terminally sacrificed animals reflected more of the effect of the reduced dose (20 mg/kg).

Despite obvious changes in the stomach, no increases in serum Gastrin-17 (biomarker for stomach insult) were observed. Also, despite pancreatitis being present in all dose groups, Canine Trypsin-Like Immunoreactivity (TLI), a biomarker for pancreas insult) was not increased at any time during the study. However, increases in creatine kinase were consistent with the muscle damage observed.

Summary:
No NOAEL could be determined for this study because at the lowest dose, 10 mg/kg/day, findings included discolored lungs, thymus atrophy, degeneration of the stomach wall, pancreatitis, inflammation in the testes and phospholipidosis. Exposures at this dose were about three times the clinical exposure. Findings at higher doses included dose related QT prolongation, degeneration of cardiac myocytes and skeletal muscle degeneration. With the exception of the degeneration of cardiac myocytes, these findings were consistent with those from other animal studies.

Study title:	TMC207: 39 Week Oral (Gavage) Toxicity Study in Dogs followed by a 13 Week Recovery Period
Sponsor referenc	TMC207-TiDP13-NC111
J&JPRD Test Site Ref. No:	TOX9239
Study report location:	DARRTS
Conducting laboratory:	(b) (4)
Date of study initiation:	16 January 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	ZR403323PUA051
Purity (%):	99.8 %

Key Study Findings:
No significant adverse effects were observed at 2 mg/kg/day for 39 weeks besides phospholipidosis, which is known to be reversible. The 2 mg/kg dose resulted in exposure similar to clinical exposures. Intermittent dosing resulted in findings consistent with previous TMC207 studies, and these findings were reversible at the end of a 13 week recovery period.

Methods

Table R12: Experimental design

Group #	TMC207 Dose mg/kg	Main study	Recovery Study
1	0	4/sex	2/sex
2	2	4/sex	
3	6	4/sex	
4	18	4/sex	2/sex
5	14*	4/sex	2/sex

*dogs were dosed three times weekly on Monday, Wednesday and Friday

Frequency of dosing:	Daily (Groups 1-4) or biweekly
Route of administration:	Oral gavage
Dose volume:	5 mL/kg/day
Formulation/Vehicle:	20% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralised water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	Beagle dog
Number/Sex/Group:	2
Age:	7-8 months old
Weight:	Females, 6-8 kg; Males 7-10 kg
Satellite groups:	2 dogs/sex from the vehicle, high and intermittent dose group were evaluated for reversibility after a 13-week drug free period

Results

Mortality:
Three 18 mg/kg animals were sacrificed in moribund condition. Although the factors contributing to the morbidity could not be determined in two cases, demise was attributed to peritonitis and pancreatitis in one animal. All three showed thinness, yellow/brown/green colored pigment laden macrophages in various tissues and either dark or small or gelatinous pancreas.

Clinical signs:
Clinical signs were recorded daily and drug-related clinical signs at the highest dose included thin appearance, loose or liquid feces and vomiting.

Body weights:
Body weights were recorded twice weekly and were reduced by about 13 % in high dose animals and 10 % at 6 mg/kg/day.

Food consumption:
Food consumption was recorded daily. At the end of dosing, consumption was reduced by about 22 % at the 6 mg/kg and 18 mg/kg doses.

Ophthalmoscopy:
Ophthalmoscopy exams were conducted during Weeks 13, 26, 38 and Week 13 of recovery. There were no abnormal findings in the ophthalmology examinations.

EKG:
Electrocardiography evaluations were conducted pretrial and during Weeks 13, 26, 38 and Week 13 of recovery. There were no remarkable electrocardiographic findings at any time points.

Hematology:
At the low dose, findings were generally mild, and consisted of very slight decreases in total protein and albumin (10-13 %).

At 6 mg/kg, Week 39 changes included reduced HGB (-11 %, males only), decreased RBC (-11 %, males only), increased neutrophils (+29 %, males only) and decreased phosphate (-22 %).

The highest (18 mg/kg/day) dose, resulted in decreased hemoglobin (-14 %), decreased hematocrit (-14 %), increased WBC's (+24 %), increased neutrophils (+40%) decreased total protein (-13%), decreased albumin (-15%), decreased phosphate (-22 %).

Doses of TMC207 (3x weekly) resulted in decreased reticulocytes (-29 %), decreased phosphate (-20 %).

Gross pathology:
At the high dose findings included pale focus in the lungs, dark coloration in the thymus, tonsils, lymph nodes (mandibular, mesenteric, inguinal, lumbar, mediastinal, bronchial) and gelatinous pancreas.

Organ weights:
At 6 mg/kg, thymus weights were reduced by 55 %, females only, At 18 mg/kg, thymus weights were reduced by a mean of 62 %, (both sexes). Intermittently dosed animals showed thymus weights which were reduced by 59%, females only. Changes in pancreas weights were sporadic (males, +29 % and females -15 %) with no discernible relationship to dose.

Histopathology:

Daily dosing
At the low dose, phospholipidosis was evident as vacuolation of the zona fasciculate of the adrenal gland, foamy macrophages in the lung and pigmented macrophages in lung, mandibular lymph nodes, mammary gland, spleen, stomach, thymus and uterus.

At 6 mg/kg, animals showed foamy and/or pigmented macrophages in the gall bladder, larynx, lymph nodes, mammary glands, ovary, stomach, thymus as well as inflammatory cell infiltration in the stomach and chronic inflammation of the pancreas.

At the high dose findings included vacuolation of the zona fasciculate of the adrenal gland, foamy macrophages and interstitial inflammation in the lung and pigmented macrophages in the adrenals, caecum, gall bladder, heart, ileum, lung, larynx, liver, spleen, stomach, thymus, uterus, mammary gland and mandibular and mesenteric lymph nodes.

Recovery-Daily Dosing
After a 13 week recovery period, 18 mg/kg animals still showed discolored lungs, dark thymus, dark lymph nodes and pale pancreas, atrophy (mucosal) and inflammatory cell infiltration (lamina propria) of the stomach. Evidence of residual phospholipidosis could be observed as pigmented macrophages in the adrenals, caecum, gall bladder, heart, lung, bronchial, lumbar, mandibular mediastinal and mesenteric lymph nodes, liver, mammary gland, ovary, pancreas, spleen, stomach, thymus and uterus.

Intermittent dosing
Intermittent doses of TMC207 (3x weekly) resulted in inflammatory cell infiltration of the stomach and phospholipidosis (vacuolation of the zona fasciculate of the adrenal gland, foamy macrophages in the

lung and pigmented macrophages in gall bladder, lung, larynx, liver, spleen, stomach, thymus, uterus, mammary gland and mandibular and mesenteric lymph nodes).

Recovery-Intermittently dosed animals
After a 13-week recovery period, the only remaining findings were pigmented macrophages and moderate inflammation of the pancreas.

Table R13: Mean plasma concentrations and pharmacokinetics parameters in Week 39

Dosage Group	2 mg/kg/day	6 mg/kg/day	18 mg/kg/day	14 mg/kg/ 3x weekly
C_{max} (µg/ml	1.4	3.4	8.9	5.0
$AUC_{(0-24h)}$ µg.h/ml	25	60	173	119

Summary of individual study findings:

Dosing was adequate, based on the toxicities seen in the low and high doses. No significant adverse effects were observed at 2 mg/kg/day for 39 weeks besides phospholipidosis, which is known to be reversible. The 2mg/kg dose resulted in exposure similar to clinical exposures (Table R13). Intermittent dosing resulted in findings consistent with previous TMC207 studies, and these findings were reversible at the end of a 13 week recovery period.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title:	*In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium*
Study no.:	TOX6089
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	September 15, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #,:	00420075
Purity:	99.83 %

Key Study Findings: TMC207 showed no evidence of genotoxicity at doses up to 1000 µg/plate, with or without metabolic activation.

Methods	
Strains:	*Salmonella typhimurium*, TA1535, TA1537, TA102, TA98 and TA100.
Concentrations in definitive study:	15.63, 31.25, 62.50, 125, 250, 500,
Basis of concentration selection:	In a non-GLP Study 5618, precipitation was observed at concentration of 250 µg/plate and above. Bacteriotoxicity was detected by evaluation of the decreased numbers of revertants at 500 µg/plate and above.
Negative control:	DMSO
Positive control:	2-Nitrofluorene, 5 µg/plate Sodium azide, 1 µg/plate 9-amino-acridine, 50 µg/plate 4-nitroquinoline-N-oxide, 5 µg/plate
Formulation/Vehicle:	Drug was dissolved in DMSO
Incubation time:	Plates were counted after 2 days of incubation in the dark at 37°C.

Study Validity

Bacterial tester strains were selected based on ICH guidelines. Study was valid because positive controls showed expected response and dose selection was adequate based on the presence of precipitate. S9 preparation was from (b) (4) and prepared according to Ames (1983).

Results

Test article was evaluated at doses up to 1000 µg/plate, and although precipitate was evident at concentrations of 125 µg/plate and above, these plates were readable. Although some mild toxicity was observed, as indicated by slight reductions in revertants at 1000 µg/plate, compared to controls, there were no increases in revertants at any dose, in any strain, in the presence or absence of S9 activation.

Discussion

TMC207 showed no genotoxic potential in this study (see Table G1, below). The evaluation was adequate in that it was conducted according to GLP with a signed quality assurance document. The solvent was appropriate and the test compound stable within the test period (97-98 % of nominal concentration). The strain selection was adequate and consistent with ICH guidelines. Concentrations were appropriate, based on precipitation detected in this and previous studies. Historical control revertant counts were within the range of historical controls and the positive controls resulted in increased revertants as expected, with and without S9.

Conclusion

TMC207 did not show any evidence of genotoxic potential in the presence or absence of metabolic activation.

Table G1: Revertant counts in plate incorporation test in the presence and absence of S9 activation

		Tester strain				
		TA98	TA100	TA1535	TA1537	TA102
Test Article	**Conc (µg/plate)**					
		Number of revertant colonies				
Without metabolic activation						
TMC207	0	14	149	5	8	209
TMC207	15.63	11	148	8	6	213
TMC207	31.25	15	131	11	5	215
TMC207	62.5	11	131	8	5	195
TMC207	125	13	138	6	6	200
TMC207	250	14	145	4	3	207
TMC207	500	17	153	12	6	192
TMC207	1000	14	144	9	5	183
2-NF[1]	5	779				
Na azide[2]			437	169		
9-AA[3]					168	
4-NNO[4]						1845
With metabolic activation						
TMC207	0	19	131	9	8	263
TMC207	15.63	19	134	11	9	292
TMC207	31.25	19	134	8	5	321
TMC207	62.5	20	131	10	10	287
TMC207	125	20	124	9	5	274
TMC207	250	18	141	6	7	259
TMC207	500	17	137	10	8	280
TMC207	1000	11	129	5	6	231
[5]2-AA		395	536	127		
[5]2-AA					106	688

[1]2-Nitrofluorene, [2] Sodium azide, [3]9-Amino-Acridine, [4]4-Nitroquinoline-N-Oxide
[5]2-amino-anthracine

7.2 *In Vitro* Mouse Lymphoma Assay

Study title:	*In Vitro* Mammalian Forward Mutation Test with L5178Y Mouse Lymphoma Cells (TK-locus) using the Microtiter® Fluctuation Technique
Study no.:	TOX6088
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	11 August 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	00400275
Purity (%)	99.83 %

Key Study Findings: TMC207 showed no evidence of mutagenic potential.

Methods

Cell line:	Mouse lymphoma L5178Y cells
Concentrations in definitive study:	3-hour incubation: 5, 10, 25, 50, 75, 100, 125, 150 and 200 (µg/plate) 24- hour incubation: 1, 2.5, 5, 10, 20, 30, 40, 50 and 60 µg/plate
Basis of concentration selection:	In a nonGLP study Exp5619, TMC207 was tested up to 200 µg/plate. Precipitation was observed at concentrations ≥ 75 µg/plate. Relative suspension growth was never below 36 % after 3 hours, but was 28 % at 40 µg/plate for the 24 hour incubation.
Negative control:	DMSO
Positive control:	Methyl methanesulfonate MMS
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Cells were exposed for 3 h (with and without S9) and for 24 h (without S9) to TMC207 in HEPES-buffered culture medium. Following the exposure period, cells were washed and cultured over an expression period of 48 h. Cell concentrations were then adjusted to 1 x 10^4 cells/ml. From at least six selected cultures, approximately 2000 cells from each culture were plated into each well of six 96-well plates, in selective cloning medium supplemented with TFT for selection of TK$^{-/-}$ cells. All plates were incubated for 9-15 days to allow the formation of colonies. Then, wells containing colonies were identified by eye and counted. The percentage of small colonies was also evaluated.

Results

Study Validity

The study was deemed valid because (1) the vehicle controls showed effects that were consistent with historical controls, (2) positive controls exhibited appropriate responses, (3) concentrations were appropriate as indicated by the precipitates seen at concentrations above 75 µg/plate (4) the ability to recover small colonies was demonstrated by the sizing of the positive controls.

Criteria for positive

Test item was considered mutagenic if the mutant frequency was above 2 times the negative (vehicle) control mutant frequency, a concentration-related increase in mutant frequency was observed and this effect was reproducible in an independent repeat experiment.

Study Outcome

Mutant frequency in TMC207 –treated cells was comparable to MF in vehicle control animals. In the same assays, the positive control substances methyl methanesulfonate (MMS) and N-nitrosodimethylamine (DMN) induced significant increases (> 2-fold) in mutant frequency compared to the vehicle control. These data show the sensitivity of the test and the metabolizing activity of the S9-mix.

Bedaquiline showed no evidence of mutagenic properties when evaluated in L5178Y Mouse Lymphoma Cells in the presence or absence of metabolic activation after 3 hour or 24 hour incubation. In no instance did the mean MF achieve a level double that of control cultures. See Table G2, below. TMC207 was negative in this mutagenicity assay.

Table G2: Suspension growth (SG), plating efficiency (PE), relative total growth (RTG) and mutation frequency (MF) after 3 hour treatment without metabolic activation.

3h treatment without metabolic activation

Test item	Concentr. (µg/ml)	SG		PE		RTG [5]	MF	
		Total [1]	Relative [2]	Total [3]	Relative [4]		Total [6]	Corrected [7]
Vehicle DMSO	0	38		118			85	68
R207910	5	34	91	108	92	83	69	59
	10	33	86	-	-	-	-	-
	25	33	86	110	93	81	78	66
	50	29	77	116	98	75	90	73
*	75	22	58	98	83	48	85	81
*	100	21	55	94	79	43	85	85
**	125	20	52	108	92	47	94	82
**	150	18	47	112	95	45	89	75
***	200	15	41	94	79	32	80	80
Positive Control (MMS)	15	29	75	58	49	37	345	789

* : precipitation (dosis-effect)

[1] : Total SG = (0 hr cells/ml) / 10E5 x ((24 hr cells/ml) / 2x10E5) x ((48 hr cells/ml) / 2x10E5)

[2] : Relative SG = SG (test) / SG (vehicle) x 100%

[3] : Total PE = (-ln(number of empty wells/total number of wells))/number of cells per well x 100%

[4] : Relative PE = PE (test) / PE (vehicle) x 100%

[5] : RTG = Relative SG x Relative PE/100

[6] : Total MF = sum of mutant colonies in all six 96-well plates

[7] : Corrected MF = (-ln(number of empty wells/total number of wells))/number of cells per well x 100/PE

Table G3: Suspension growth (SG), plating efficiency (PE), relative total growth (RTG) and mutation frequency (MF) after 3 hour treatment with metabolic activation.

3h treatment with metabolic activation

Test item	Concentr. (µg/ml)	SG		PE		RTG [5]	MF	
		Total [1]	Relative [2]	Total [3]	Relative [4]		Total [6]	Corrected [7]
Vehicle DMSO	0	46		100			76	71
R207910	5	43	94	103	103	97	85	78
	10	40	87	95	95	83	89	88
	25	35	77	103	103	79	80	73
	50	32	69	105	105	73	88	79
*	75	12	27	88	88	24	104	113
*	100	8	16	87	87	14	116	130
**	125	4	8	82	82	7	89	103
**	150	2	4	-	-	-	-	-
***	200	2	4	-	-	-	-	-
Positive Control (DMN)	400	35	76	69	69	52	260	437

* : precipitation (dosis-effect)

.

[1] : Total SG = (0 hr cells/ml) / 10E5 x ((24 hr cells/ml) / 2x10E5) x ((48 hr cells/ml) / 2x10E5)

[2] : Relative SG = SG (test) / SG (vehicle) x 100%

[3] : Total PE = (-ln(number of empty wells/total number of wells))/number of cells per well x 100%

[4] : Relative PE = PE (test) / PE (vehicle) x 100%

[5] : RTG = Relative SG x Relative PE/100

[6] : Total MF = sum of mutant colonies in all six 96-well plates

[7] : Corrected MF = (-ln(number of empty wells/total number of wells))/number of cells per well x 100/PE

Table G4: Suspension growth (SG), plating efficiency (PE), relative total growth (RTG) and mutation frequency (MF) after 24 hour treatment with metabolic activation.

24h treatment without metabolic activation

Test item	Concentr. (µg/ml)	SG		PE		RTG [5]	MF	
		Total [1]	Relative [2]	Total [3]	Relative [4]		Total [6]	Corrected [7]
Vehicle DMSO	0	14		83			114	133
R207910	1	10	74	85	103	76	150	177
	2.5	8	59	89	108	64	211	255
	5	8	58	87	105	61	203	251
	10	10	70	70	84	59	151	218
	20	12	87	87	105	91	95	104
	30	6	43	100	120	51	63	58
	40	2	16	73	88	14	72	92
	50	1	10	57	69	7	68	110
	60	1	4	-	-	-	-	-
Positive Control (MMS)	7.5	6	42	37	45	19	280	897

[1] : Total SG = (0 hr cells/ml) / 10E5 x ((24 hr cells/ml) / 2x10E5) x ((48 hr cells/ml) / 2x10E5)
[2] : Relative SG = SG (test) / SG (vehicle) x 100%
[3] : Total PE = (-ln(number of empty wells/total number of wells))/number of cells per well x 100%
[4] : Relative PE = PE (test) / PE (vehicle) x 100%
[5] : RTG = Relative SG x Relative PE/100
[6] : Total MF = sum of mutant colonies in all six 96-well plates
[7] : Corrected MF = (-ln(number of empty wells/total number of wells))/number of cells per well x 100/PE

Conclusion

TMC207 showed no evidence of mutagenic potential in the L5178Y Mouse Lymphoma assay.

Study title:	*In vivo* micronucleus test on bone marrow cells of mice
Study no.:	TOX6090
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	June 26, 2003
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	00420075
% purity:	99.83 %

Key Study Findings: TMC207 was nongenotoxic in the mouse micronucleus assay.

Methods

Doses in definitive study:	TMC207 - Low: 40 mg/kg TMC207 - Medium: 160 mg/kg TMC207 - High: 640 mg/kg
Frequency of dosing:	Single
Route of administration:	Oral
TMC207 dose volume:	0.2 mL/10g
Positive control dose volume	0.1 mL/10g
Formulation/Vehicle:	Demineralized water containing 40% hydroxypropyl-β-cyclodextrin (pH 2.0)
Species/Strain:	Mouse (SPF Albino Swiss (CD1)
Number/Sex/Group:	5/sex/dose group
Satellite groups:	Toxicokinetics (3-5/sex/dose group)
Basis of dose selection:	In a previous micronucleus test (Study TOX 5620), in the male SPF Albino Swiss (CD1) mouse, TMC207 was administered once orally at 37.5, 150 and 600 mg/kg, resulting in significantly reduced body weight at 48 hours postdose at 600 mg/kg.
Negative control:	Demineralised water containing 40% hydroxypropyl-β-cyclodextrin (pH 2.0)
Positive control:	Cyclophosphamide (4 mg/kg)

Mice were treated with a single dose of TMC207 or control substance. 24 and/or 48 hours after dosing, mice were sacrificed by cervical dislocation for the preparation of bone marrow smears. The slides were examined by light microscopy (magnification 1000x) and were distributed among 3 different observers. A total of 1000 erythrocytes polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) per mouse were counted to determine the ratio of PCEs to (PCEs + NCEs). A total of 2000 PCEs was counted per mouse and the number of micronucleated PCEs was recorded. At the same time, the number of micronucleated NCEs was also recorded in the fields containing these 2000 PCEs.

Criteria for positive response

The result was considered positive in the micronucleus test if it induced a statistically significant ($p<0.05$) and dose-related increase in the number of micronucleated PCEs either in the combined data for both sexes or in the data for male or female mice separately.

Study Validity

The study was deemed valid because (1) pharmacokinetics data showed good exposure, which were comparable to those seen in the clinic. (2) The single dose study (above) showed mortality and reduction in body weight at 800 mg/kg also supported the conclusion that dosing was adequate (3) Data from the negative and positive control animals demonstrate that they were producing the expected responses and that laboratory is able to reliably detect micronuclei in the test mice. (4) The formulation and administration of the drug was appropriate and (5) positive controls showed appropriate responses.

Results

Toxicokinetics

TMC207 was present in the plasma at exposures ($AUC_{(0-8h)}$) between 5.6 and 52.6 µg*h/mL after single doses between 40 and 640 mg/kg. See Table G5, below. These exposures are acceptable since, in patients, $AUC_{(0-24h)}$ values were about 22 µg*h/mL.

Table G5: TMC207 exposure in male and females mice after single oral doses

Dosage group	Low: 40 mg/kg	Medium: 160 mg/kg	High: 640 mg/kg
Sex	M	M	M
Time (h)	Mean	Mean	Mean
1	993	8153	8374
8	467	3358	5468
$AUC_{0-8\,h}$ ng.h/ml	5604	44365	52632

Dosage group	Low: 40 mg/kg	Medium: 160 mg/kg	High: 640 mg/kg
Sex	F	F	F
Time (h)	Mean	Mean	Mean
1	2086	5493	6997
8	845	3010	3999
$AUC_{0-8\,h}$ ng.h/ml	11300	32507	41984

TMC207 did not result in any biologically significant clinical signs, mortality or body weight change during this study. It also did not induce any increases in micronucleated erythrocytes at doses up to 640 mg/kg.

Table G6: Micronucleated erythrocytes in TMC207-treated mice

Sex : BOTH M+F

Sacr Time	Art. Nature	Dosage Group	No. of animals	Polychromatic erythrocytes			Norm. eryth.	Proportion of PCE to (PCE+NCE)		
				n examined	Micronucleated n	Micronucleated %	Micronucleated n	PCE + NCE n	n PCE	PCE %
24h	Negative control	Negative control	10	20000	26	0.13	10	10000	5654	56.54
24h	Test article	Low	10	20000	32	0.16	17	10000	5619	56.19
24h	Test article	Medium	10	20000	18	0.09	8	10000	5553	55.53
24h	Test article	High	10	20000	15	0.08	9	10000	5702	57.02
48h	Negative control	Negative control	10	20000	22	0.11	14	10000	5441	54.41
48h	Positive control	Positive control	10	20000	428	2.14 ***	125	10000	4997	49.97
48h	Test article	Low	10	20000	28	0.14	11	10000	5870	58.70
48h	Test article	Medium	10	20000	22	0.11	13	10000	6007	60.07
48h	Test article	High	10	20000	19	0.10	22	10000	4932	49.32

Significance computed by Mann-Whitney U test (two-tailed): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Discussion

In the present study, single TMC207 doses between 40 and 640 mg/kg resulted in exposures [($AUC_{(0\text{-}8h)}$) values] between 5 and 52 µg*h/mL in mice. The exposure at the higher concentration is higher than those obtained when TMC207 was used to treat patients, where the exposures $AUC_{(0\text{-}24h)}$ values were 22 µg*h/mL. Since the study was valid and conducted according to established guidelines and controls showed the appropriate responses, it is concluded that TMC207 was nongenotoxic in the mouse micronucleus assay.

8 CARCINOGENICITY

On March 4, 2010, the sponsor submitted a request to IND 69,600 for special protocol assessment of the rat carcinogenicity protocol. The sponsor also included a proposal not to conduct a carcinogenicity study in mice.

The 24-month repeat-dose oral carcinogenicity study in Sprague Dawley rats is ongoing and is expected to be reported in the 3rd quarter of 2013.

The proposal not to conduct a carcinogenicity study in mice was based partly on data from a 3-month study. In this study in CD-1 mice, the maximum nonlethal bedaquiline dose (20 mg/kg) was about a third of the clinical dose based on AUC comparisons. Also, mice were dying at doses equivalent to half the clinical dose based on AUC comparisons. The sponsor argued that the higher toxicity in mouse will 'jeopardize a successful conduct and completion of such study'. The need to conduct a carcinogenicity study in a second species will be determined by the Division based on its review of the 24 month rat study.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title:	Oral Fertility Study of TMC-207 in the Male and Female Rat.
Report no.:	TMC207-NC115
Study no:	TOX7332
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	August 30, 2005
Date of first dosing:	Males: September 5, 2005 Females: September 19, 2005
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	ZR403323PFA021
% purity:	99.3%
Methods	
Doses:	0, 1.5, 6 and 24 mg.eq/day. Animals were dosed with TMC207 fumarate salt, but doses levels are expressed as amount of TMC207 free base equivalent.
Frequency of dosing:	Males: once daily during a 4-weeks pre-pairing period, during pairing and up to termination. Females: once daily during a 2-week pre-pairing period, during pairing and up to Day 7 of presumed pregnancy [day of copulation = Day 0 of pregnancy]
Dose volume:	5 mL/kg/day
Route of administration:	Oral (gavage)
Formulation/Vehicle:	Aqueous solution containing 20% hydroxypropyl-ß-cyclodextrin (HP-ß-CD)
Species:	Rat (*Rattus norvegicus*)
Strain:	Sprague-Dawley (Crl:CD®)
Number/Sex/Group:	24 rats/sex/dose group
Satellite groups:	None
Deviation from study protocol:	None

Study design:

Male and female rats were dosed with TMC207 for 4 and 2 weeks respectively, after which females were paired (one-to-one) with males from the same dosing group. Each morning, a vaginal smear was prepared from each female to determine if there was evidence of mating (the presence of spermatozoa). Females showing a sperm positive vaginal smear were separated from the males and caged

individually. The female rats remained isolated until sacrifice on Day 14 of presumed pregnancy.

Key Study Findings
TMC207doses up to 24 mg.eq/day resulted in no drug-related effects of on fertility in rats.

Observations and Results

Mortality
Animals were observed at least once daily for mortality and signs of ill health. There were no drug-related deaths during the study. One high dosed male (#299) was sacrificed during the pre-pairing period (on Day 25), but its demise was attributed to gavage error. This death did not impact the assessment of the effects of TMC207 on fertility.

Clinical signs
There were no drug-related clinical signs recorded during this study.

Body Weight
Body weights were recorded weekly during the study. There were no biologically significant differences in body weight in males or females when the drug-treated animals were compared to controls. In high dose males, body weight gain was significantly decreased (by 25 to 60 %) over the dosing period, but the overall effect was to reduce body weight by less than 10 % compared to controls.

Feed Consumption
Feed consumption was measured weekly. High dose males showed food consumption that was about 10 % less than controls throughout the study.

Stability and Homogeneity
The concentration of TMC207 in the formulations fell between 98 and 103 % of the nominal dose when measured two weeks apart and thus complied with the study protocol. Drug preparations were deemed acceptable if the concentrations ranged between 85 and 115 % of nominal concentration and the concentration of the test article in the vehicle formulation must be lower or equal to the LOQ (Limit of Quantification).

Necropsy
Mated females were killed by exsanguination via the carotid artery for examination of their uterine contents on Day 14 post-coitum. Male rats were killed by exsanguination after fertility had been established. The weight of the testes (left and right) of all males was recorded. TMC207 had no effects on estrus cycles or precoital interval at any of the tested doses. There was no drug-related effect on mating. Copulation index was based on the number of sperm-positive vaginal smears and was 100 %. Other statistically significant changes were sporadic and not considered drug-related. Findings are reported on Table R1, below.

Table R1: Adult and litter Fertility Parameters in rats treated with TMC207

Observation		Vehicle 0 mg/kg	Low 1.5 mg/kg	Medium 6 mg/kg	High 24 mg/kg
FEMALE DATA					
Number of pregnant females/ terminally sacrificed	(2)	23/24	21/24	24/24	20/24
Copulation rate	(2)	24/24	24/24	24/24	24/24
Fertility rate	(2)	23/24	21/24	24/24	20/24
Body weight gain (d0 - d7)	(3)	38	37	39	37
Body weight gain (d8 - d13)	(3)	36	36	34	33
Weight gravid uterus	(3)	11.9	13.1 *	12.1	12.1
Corrected mean maternal weight gain	(3)	62.0	59.7	61.5	57.9
Food consumption (d0 - d7)	(3)	194	202	194	186
Food consumption (d8 - d13)	(3)	168	168	162	161
LITTER DATA					
Number of live embryos/pregnant female	(3)	13.4	14.2	13.1	13.6
Mean litter size	(3)	13.4	14.2	13.1	13.6
Number of resorptions/pregnant female	(3)	1.48	0.76 *	0.96	1.65
Pre-implantation loss (%)	(3)	6.95	10.41	16.07	14.43
Post-implantation loss (%)	(3)	9.58	5.17 **	6.50	15.07
Number of implantations/pregnant female	(3)	14.9	15.0	14.1	15.2
Number of corpora lutea of pregnancy/pregnant female	(3)	16.7	16.8	16.8	17.7 *

Significance computed using Fisher Exact Test. *: $p< 0.05$ **: $p<0.01$ ***: $p< 0.001$

Discussion

There were 23/24, 21/24, 24/24 and 20/24 females pregnant in the vehicle, 1.5, 6 or 24 mg eq./kg/day groups, respectively. There was no relationship between dose and pregnancy rate and the 83 % rate observed in the high dose rats was within the range of the applicant's historical control data (Data requested and received from applicant).

The $AUC_{(0\text{-}24h)}$ value in subjects treated with 400 mg TMC207 q.d. for 2 weeks followed by 200 mg tiw. for 22 weeks was 14 µg*h/mL. In the TMC207 toxicology study "TOX6657: Oral developmental toxicity study in the rat", the $AUC_{(0\text{-}24h)}$ value 16/17 was 50 µg*h/mL at 15 mg/kg/day. It is therefore clear that the exposure in rats treated at the high dose in this study at (24 mg/kg) will have achieved exposures surpassing the clinical exposure of 14 µg*h/mL.

Conclusion

TMC207 did not have any adverse effects on the fertility of Sprague Dawley rats exposed at doses up to 24 mg/kg/day. At this dose, blood levels are anticipated to be higher than those observed in patients.

Embryonic Fetal Development

Study title:	Oral developmental toxicity study in the rat
Study no:	TOX6657
Report no:	TMC207-NC104
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	July 6, 2004
Start of mating:	July 12, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #:	00420075
% purity:	99.84

Key Study Findings

TMC207 did not result in adverse effects on embryo-fetal development of rats exposed between gestational Days 6 to 17 at doses up to 45 mg/kg (equivalent to 9 times clinical dose of 200 mg tiw, based on AUC $_{(0\text{-}24h)}$ comparisons).

Methods

Doses:	0, 5, 15, 45 mg/kg/day
Frequency of dosing:	Daily from Day 6 to 17 inclusive
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	TMC207 fumarate was formulated in 20% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralised water with HCl/NaOH to pH 2.0±0.1
Species:	Rat (*Rattus norvegicus*)
Strain:	Sprague-Dawley (Crl:CD®)
Number/Group:	24 rats/dose group
Satellite groups:	4 female rats/dose group for toxicokinetics
Study design:	Four groups of pregnant rats were treated orally by gavage, with TMC207 between gestational Days 6 to 17 inclusive. On day 21, main study females were anesthetized and killed by exsanguination. Reproductive parameters are shown on Table R4. Satellite animals were bled on Days 6/7 and 16/17 for toxicokinetics evaluations.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical signs

Animals were checked daily for mortality and clinical signs. There were no treatment-related clinical signs or deaths during this study. One low-dose female was sacrificed on Day 20 after the abortion of its litter. This animal had showed rough coat, weight loss and decreased food consumption beginning on Day 18.

Body Weight

For main study animals, body weight was recorded daily during the dosing period, and on Days 4, 6, 10, 14, 18 and 21 of pregnancy. Body weight gain was significantly reduced sporadically at the high dose (-17% between Days 6-9 and - 20% between Days 18-20. However, the corrected mean maternal weight gain was not significantly different between treated and control animals.

Feed Consumption
Slight reductions in feed consumption were observed in high dose animals. While these changes were statistically significant, they were slight, (< 10 %).

Toxicokinetics

T_{max} (h) values were between 2-4 hours after dosing on Day 6/7 or Day 16/17. C_{max} and $AUC_{(0-24h)}$ values increased in proportion with dose. C_{max} values on Day 16/17 were about double the Day 6/7 values indicating some drug accumulation.

Table R2: Mean plasma concentrations and pharmacokinetics parameters on gestation Day 6/7

Dosage Group	Vehicle	Low (5 mg/kg/day)	Medium (15 mg/kg/day)	High (45 mg/kg/day)
C_{max} (ng/ml	ND	567	1299	4103
T_{max} (h)	ND	4	4	4
$T_{½}$ (h)	ND	10	10	11
$AUC_{(0-24h)}$ ng.h/ml	ND	6224	17448	52538

Table R3: Mean plasma concentrations and pharmacokinetics parameters on gestation Day 16/17

Dosage Group	Vehicle	Low (5 mg/kg/day)	Medium (15 mg/kg/day)	High (45 mg/kg/day)
C_{max} (ng/ml	ND	1133	2990	8753
T_{max} (h)	ND	4	2	4
$T_{½}$ (h)	ND	47	21	53
$AUC_{(0-24h)}$ ng.h/ml	ND	15561	50247	130573

Stability and Homogeneity

The concentration and stability of the test article were evaluated by HPLC after storage at room temperature for 5 days and 2 weeks. Data showed that the test article formulation was stable for at least two weeks since the concentrations measured were between 90 and 110 % of the nominal concentration during the evaluation period.

Necropsy

Cesarean Section Data

Litter data presented on Table R4, show that here were statistically significant decreases in number of parameters at the high dose only. Number of live fetuses/dam (-14 %), mean litter size (-13 %) and weight of live fetuses (-4%) were all reduced compared to control animals (see Table R4). The weight of the gravid uterus was reduced by about 15 % at the high dose.

Comment:

The number of live fetuses at the high dose (mean 12.8) is within the range of historical control data. Historical Control data presented in (http://www.criver.com/sitecollectiondocuments/rm_rm_r_tox_studies_crlcd_sd_br_rat.pdf) show that, in control dams, the range for the number of live fetuses/dam was between 10.8 and 17.5.

Table R4: Litter data for rats dosed with TMC207 between gestational Days 6 to 17.

Dosage Group	Vehicle	Low (5 mg/kg)	Medium (15 mg/kg)	High (45 mg/kg)
Number of live fetuses/dam	14.7	13.5	13.6	12.7*
Number of dead fetuses/dam	0.0	0.0	0.0	0.1
No. of early resorptions/dam	0.8	1.5	0.8	1.4
No. of late resorptions/dam	0.09	0.05	0.08	0.18
Total no. of resorptions/dam	0.9	1.5	0.9	1.6
Pre-implantation loss (%)	8.6	12	11	9
Post-implantation loss (%)	5	10	9	13
No. of implantations/dam	16	15	15	14
Number of corpora lutea/dam	17	17	17	16
Weight of live fetuses	5.4	5.5	5.5	5.2*
Sex ratio (% male fetuses)	50	48	57	50

Table R5: Malformations in offspring from rats dosed with TMC207 between gestational Days 6 to 17.

Dosage Group	Vehicle	Low (5 mg/kg)	Medium (15 mg/kg)	High (45 mg/kg)
Incidence of malformed fetuses	1/324	1/298	1/326	0/279
Number of fetuses examined by sectioning	158	143	155	135
Number of fetuses skeletally examined	166	143	155	135

Malformations and Variations
Sporadic occurrences of abnormalities in external, soft tissue and skeletal examinations were not considered to be related to treatment. Findings included exencephaly, omphalocoele and micrognathia. Low dose pups showed slight increases in the incidence of asymmetrical sternum bone, but the absence of this finding in higher doses led to the conclusion that this finding was unrelated to drug. Bladder distension was found in all groups.

Discussion
The $AUC_{(0\text{-}24h)}$ value in human subjects treated with 400 mg TMC207 q.d. for 2 weeks followed by 200 mg tiw. for 22 weeks was 14 µg*h/mL. High dose animals in this study showed TMC207 exposures ($AUC_{(0\text{-}24h)}$ values) of up to 131 µg*h/mL. There were no effects on embryofetal development in rats at these exposures, which were over 9 times more than the clinical exposures.

Conclusion:
TMC207 did not result in adverse effects on embryo-fetal development in rats at doses up to 45 mg/kg (equivalent to 9 times clinical dose of 200 mg tiw, based on $AUC_{(0\text{-}24h)}$ comparisons).

Embryonic Fetal Development

Study title:	Oral developmental toxicity study in the rabbit
Study no:	TOX6656
Report no:	TMC207-NC105
Study report location:	EDR
Conducting laboratory :	(b) (4)
Date of study initiation:	July 28, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #:	R207910PFA011
% purity:	99.89

Key Study Findings

TMC207 had no adverse effects on embryofetal development in rabbits at doses up to 100 mg/kg/day, a dose equivalent to more than double the clinical dose of 200 mg tiw, based on AUC comparisons.

Methods

Doses:	0, 10, 30, 100 mg/kg/day
Frequency of dosing:	Daily from gestational Day 6 to 19 inclusive
Dose volume:	5 mL/kg
Route of administration:	Oral (gavage)
Formulation/Vehicle:	TMC207 fumarate was formulated in 0.5 % w/v Methocel (hydroxypropylmethylcellulose) in demineralised water.
Species:	Rabbit (*Oryctolagus cuniculus*)
Strain:	New Zealand White
Number/Group:	20 rabbits/dose group
Satellite groups:	3 female rabbits/dose group for toxicokinetics
Study design:	Four groups of pregnant rabbits were treated orally by gavage, with TMC207 or vehicle between Days 6 to 19 inclusive. On day 28, main study females were sacrificed by intravenous pentobarbital. Reproductive parameters are shown on Table R6, below. Satellite animals were bled on Days 6/7 and 19/20 for toxicokinetics evaluations.
Deviation from study protocol:	No significant deviations

Observations and Results

Mortality and Clinical signs

Animals were checked daily for mortality and clinical signs. There were no treatment-related clinical signs or deaths during this study. One high dose animal died on Day 10, presumably due to a dosing accident. Multiple red foci and white-tan discoloration were observed in right lung lobes. A second high dose animal was sacrificed after the abortion of its litter on Day 25. This animal had persistent weight loss and absent fecal output during the doing period.

Clinical signs

Three high dose animals showed reduced/absent fecal output towards the end of dosing and the first days postdose, resulting in a transient (though not statistically significant) reduction in body weight gain between Days 13-19.

Body Weight

Main study animals were weighed daily during the dosing period, but body weight gain data were only reported for the following periods of pregnancy: Days 1-3, 4-5, 6-8, 9-12, 13-15, 16-19, 20-21, 22-24, 25-27. Body weight gain was fairly consistently reduced in high dose animals compared to controls. Although these changes were as much as 30 % less than controls (Day 13-19), the changes were not statistically significant.

There were no drug-related differences in corrected mean maternal weight gain.

Feed Consumption
Slight reductions in feed consumption were observed in mid-dose (-10 %) and high-dose (-13 %) animals. These changes were statistically significant in the mid-dose animals only.

Toxicokinetics

T_{max} (h) values were between 5-6 hours after dosing on Day 6/7 and between 3-4 hours on Day 16/17. C_{max} and AUC values increased in proportion with dose up to 30 mg/kg, after which the increase was supraproportional to dose. C_{max} and AUC values on Day 16/17 were generally higher than Day 6/7 values.

Mean AUC values for the N-desmethyl metabolite (R405786) was about 6 times lower than those for parent drug on Day 6/7 and was about 3 times lower on Day 19/20.

Table R6: Mean plasma concentrations and pharmacokinetics parameters on gestation Day 6/7

Dosage Group	Vehicle	Low (10 mg/kg/day)	Medium (30 mg/kg/day)	High (100 mg/kg/day)
C_{max} (ng/ml	ND	74	305	1044
T_{max} (h)	ND	6	5	5
$T_{½}$ (h)	ND	8	6	11
$AUC_{(0-24h)}$ ng.h/ml	ND	NC*	3379	13757

NC* not calculated

Table R7: Mean plasma concentrations and pharmacokinetics parameters on gestation Day 19/20

Dosage Group	Vehicle	Low (10 mg/kg/day)	Medium (30 mg/kg/day)	High (100 mg/kg/day)
C_{max} (ng/ml	ND	94	303	1817
T_{max} (h)	ND	3	4	3
$T_{½}$ (h)	ND	10	9	4*
$AUC_{(0-24h)}$ ng.h/ml	ND	1028	4014	31756

* n=1

Stability and Homogeneity

The concentration, homogeneity and stability of the test article were evaluated by HPLC after storage in the refrigerator for 3 days or 18 days. Data showed that the test article formulation was stable for at least two weeks since the concentrations measured were between 90 and 106 % of the nominal concentration. The predefined acceptable range was 85 to 115 %.

Necropsy

Cesarean Section Data

There was no evidence of drug-related adverse effects in litter data. Pre-implantation loss was increased in high dose rabbits, and this was associated with fewer implantations and live fetuses. However, these decreases were within the range of historical control data from published studies (see Hanley et al, 2000). This effect was also not considered to be drug-related since dosing began after implantation occurred. There were also no drug-related effects on fetus weight, sex ratio or malformed fetuses. The weight of the

gravid uterus was slightly reduced (by about 12 %) at the high dose, but this reduction was not statistically significant.

Hanley, T.R. Jr., Carney, E.W. and Marshall Johnson, E. (2000). Developmental Toxicity Studies in Rats and Rabbits with 3,5,6-Trichloro-2-pyridinol, the Major Metabolite of Chlorpyrifos. *Toxicol. Sci. 53 (1): 100-108.*

Table R8: Litter data for rabbits dosed with TMC207 between gestational Days 6 to 19.

Dosage Group	Vehicle	Low (5 mg/kg)	Medium (15 mg/kg)	High (45 mg/kg)
Number of live fetuses/dam	9.2	8.6	9.1	7.3*
Pre-implantation loss (%)	12	14	10	22
Post-implantation loss (%)	7	7	10	9
No. of implantations/dam	10	9	10	8*
Number of corpora lutea/dam	11	11	11	11
Weight of live fetuses	32	34	31	34*
Sex ratio (% male fetuses)	48	47	46	55
Incidence of malformed fetuses	0/147	1/163	2/164	0/117

Significance computed by Fisher Exact Test. * : $p< 0.05$ **: $p< 0.01$

Fetal observations

Despite isolated instances of major abnormalities (such as scoliosis, brachygnathia, fused ribs), there were no drug-related effects on the incidences of major abnormalities. Statistically significant increases in the incidence of dilated urinary bladder and rudimentary hyoid as well as reduced ossification of the tarsal and metacarpal bones were not considered drug-related since they were not observed in the high dose animals.

Table R9: Malformations in offspring from rabbits dosed with TMC207 Days 6 to 19

Dosage Group	Vehicle	Low (5 mg/kg)	Medium (15 mg/kg)	High (45 mg/kg)
Hyoid: rudimentary	2/76	5/88	9/87*	3/61
13^{th} rib	16/147	31/163*	16/164	10/117
Dilated urinary bladder	3/147	5/163	3/164	15/117*
Reduced ossification of metacarpal bones	9/147	21/163*	27/164**	10/117
Reduced ossification of tarsal bones	0/147	0/163	6/164*	0/117

Comparison of the number of fetuses with a particular major abnormality or variation per litter between

the dosed group and the vehicle group computed by Fisher Exact Test. (* : $p< 0.05$ **: $p< 0.01$

The $AUC_{(0-24h)}$ value in human subjects treated with 400 mg TMC207 q.d. for 2 weeks followed by 200 mg tiw. for 22 weeks was 14 µg*h/mL. High dose animals in this study showed TMC207 exposures ($AUC_{(0-24h)}$) of up to 32 µg*h/mL. There were no effects on embryofetal development in rabbits at these exposures, which were over 2 times more than the clinical exposures.

Conclusion

TMC207 had no biologically significant adverse effects on embryofetal development in rabbits at doses up to 100 mg/kg/day, a dose equivalent to more than double the clinical dose of 200 mg tiw, based on AUC comparisons.

Prenatal and Postnatal Development

Study title:	TMC207-Fumarate: Pre- and Post-Natal Development Study in the CD Rat by Oral (Gavage) Administration
J&J Study no:	TOX9296
Tibotec study number:	TMC207-TiDP13-NC117
Study report location:	EDR
Conducting laboratory:	(b) (4)
Date of study initiation:	23 April, 2009
Experimental start date:	6 May, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, batch #, % purity:	ZR403323PUA051 99.8 %

Key Study Findings

At doses which produced exposures similar to clinical exposures, adverse effects were not observed in rats exposed to TMC207 *in utero* and post-natally. At higher doses, pre- or post-natal exposures resulted in lower body weights and body weight gains in pups.

Methods

Doses:	0, 5, 15, 45 mg/kg
Frequency of dosing:	Once daily from Day 6 after mating to Day 20 of lactation. The F1 generation received no direct administration of the test substance. Any exposure to the TMC207 occurred in *utero* and/or through the milk.
Dose volume:	7.5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	20% w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD), 10N Hydrochloric Acid to pH 1.0 and 10N Sodium Hydroxide to pH 2.0 in reverse osmosis water.
Species/Strain:	Rat (*Rattus norvegicus*)
Number/Sex/Group:	22 pregnant dams
Satellite groups:	Toxicokinetics phase: 3 pregnant F_0 dams/dose level Cross fostering phase: 22 control dams and 18 high dose (45 mg/kg) dams
Deviation from study protocol:	There were no deviations from the protocol

Study design

Main Phase

The F_0 females were bred with stock males, allowed to litter and rear their offspring to weaning and were killed on Day 21 or 25 of lactation. Reflex development of the F_1 offspring was assessed prior to weaning.

F_1 offspring selected at weaning were subjected to functional and behavioral testing after weaning, and were assessed for sexual maturity and reproductive capacity. F_1 females were paired with similarly treated males, allowed to litter and were killed on Day 14 for examination of their uterine contents. Males were killed soon afterwards.

Toxicokinetic phase

Three females were allocated to each treated dose solely for the purpose of toxicokinetic evaluation. These females were treated identically to those allocated to the Main phase up to Day 6 of lactation, when blood samples were collected from dams and offspring. Milk samples were then collected from the dams on Day 7 of lactation, after which they were sacrificed.

Cross-fostering phase
A Cross-fostering phase was added to assess the influence of exposure in utero and through the milk. Eighteen female rats received TMC207 fumarate by gavage at a dose of 45 mg eq./kg/day from Day 6 after mating to Day 20 of lactation and 22 female rats received the vehicle, at the same dose-volume throughout the same period. Prior to the formal Day 1 of lactation/age designation, at least fifteen litters from the Control group were cross fostered to dams treated at 45 mg eq./kg/day and vice versa. Treatment continued following cross-fostering to Day 20 of lactation and the females were killed on Day 21 of lactation. Blood samples were collected from dams and offspring on Day 12 of lactation for toxicokinetic evaluation and milk samples were collected from the dams on Day 14 of lactation.
Observations and Results

F_0 Dams

Survival:	There were no deaths among F_0 the dams during the study
Clinical signs:	There were no adverse clinical findings.
Body weight:	There were no biologically significant differences between drug-treated animals and controls throughout gestation or lactation. Any statistically significant changes were slight.
Feed consumption:	Feed consumption was 16-21 % lower than controls in the high dose dams all throughout lactation.
Gestation length/index:	All females successfully gave birth with no evidence of dystocia and gestation lengths were within the expected range of 22-23 days. There were no remarkable macroscopic findings at delivery

Toxicokinetics

Exposure to TMC207 was dose-proportional in dams. In pups, exposure was proportional to dose up to 15 mg/kg/day after which it was less than dose proportional. TMC207 levels in pups were always higher than in adults and at all doses studied, the exposures in pups exceeded the exposures seen in patients on TMC207 (14 µg*h/mL). Drug levels on Day 12 in high dose animals were similar to levels on Day 6 in both pups and dams.

Table R10: TMC207 Toxicokinetics in dams and offspring (Day 6)

		Parental female			F_1 offspring		
		Day 6 of lactation			Day 6 of age		
Group	Dose mg eq./kg/day	C_{max} ng/mL	t_{max} h	$AUC_{0-24\ h}$ h.ng/mL	C_{max} ng/mL	t_{max} h	$AUC_{0-24\ h}$ h.ng/mL
2	5	365	3.00	6083	1141	3.67	23433
3	15	1320	2.33	22401	3180	3.33	38148
4	45	2910	2.00	53824	6170	3.00	119546

Table R11: TMC207 Toxicokinetics in dams and offspring (Day 12)

		Parental female			F_1 offspring		
		Day 12 of lactation			Day 12 of age		
Group	Dose mg eq./kg/day	C_{max} ng/mL	t_{max} h	$AUC_{0-24\ h}$ h.ng/mL	C_{max} ng/mL	t_{max} h	$AUC_{0-24\ h}$ h.ng/mL
1	0	BQL	-	-	127	8.00	2465
4	45	2810	1.00	39914	5277	0.00	118066

Table R12: TMC207 Toxicokinetics in milk

Milk concentrations (ng/ml)									
PK Profile		Day 7				Day 14			
Group	Dose (mg eq./kg/day)	TMC207	n	R405786	n	TMC207	n	R405786	n
Group 2	5	2875	2	BQL	2	-		-	
Group 3	15	7600	2	1200	2	-			
Group 4	45	33967	3	12003	3	10430	15	7111	15

Drug levels in milk were approximately dose proportional on Day 7, and were higher than Day 14 levels. On Day 14, drug was not detected in milk at the low- and mid-doses.

Stability: Stability of the drug preparation was demonstrated and documented in study TOX7332

Formulation homogeneity: The concentration of the test substance in the samples was in a range of ± 15 % of the nominal value

F_1 Generation

Litter size, survival, sex ratio: Treatment with TMC207 did not affect mean litter size, offspring survival or sex ratio to Day 21 of age. All litters were raised successfully to weaning with the exception of one 45 mg/kg litter. Six offspring within this litter were found dead or killed between days 4 and 13 and the litter was sacrificed on Day 13. The majority of the offspring showed signs of dark or distended abdomen as well as low body weight gain. The reason for the mortality and failure to thrive was unclear as the mammary tissue of the parent female was active.

Clinical signs: There were no drug-related clinical signs in the offspring

Body weight: Pups from high dose dams showed significantly lower body weights (up to -24 %) and lower body weight gains (up to -32 %) compared to controls between Day 1 and 21.

Table R13 Body weight in F_1 offspring

Bodyweight and bodyweight change - group mean values for Main phase male offspring (F1)

Group	1	2	3	4
Compound	Control	TMC207	TMC207	TMC207
Dose (mg eq./kg/day)	0	5	15	45

Group		Day of age (before cull)		Day of age (after cull)					
		1	4	4	7	11	14	18	21
Statistical test:		Wi	Wi	Wi	Wi	Wi	Wi	Wi	Wi
1	Mean	7.1	9.7	9.8	15.8	24.9	31.4	40.2	51.4
	SD	0.9	1.3	1.4	1.8	2.5	3.1	4.1	5.7
	N	22	22	22	22	22	22	22	22
2	Mean	7.1	9.9	9.9	15.8	25.0	32.0	41.6	52.6
	SD	0.6	1.1	1.1	1.6	2.5	2.9	3.5	4.9
	N	22	22	22	22	22	22	22	22
3	Mean	7.0	9.8	9.9	15.5	24.6	31.4	40.1	51.2
	SD	0.6	1.0	1.0	1.5	2.4	3.0	3.4	4.8
	N	22	22	22	22	22	22	22	22
4	Mean	6.4**	8.4**	8.4**	12.6**	18.3**	23.7**	30.0**	37.9**
	SD	0.7	1.4	1.4	2.2	3.5	4.2	5.3	7.6
	N	22	22	22	22	21‡	21	21	21

‡ Suspected data recording error for one litter; values not reported

Physical development:	In pre-weaning screens, there were no biologically significant adverse effects on surface righting reflex, air righting, pupil reflex and startle response. Surface righting reflex was slightly precocious compared to controls.
Neurological assessment:	Motor activity and performance on the accelerating rotarod were not affected by any dose of TMC207. In the Morris Maze, sector entries were slightly increased at the 45 mg/kg dose in males on Day 3 only. Given the isolated nature of this finding, it was not considered to be related to drug administration.
Sexual maturation	There was no drug-related effect on sexual maturation as assessed by vaginal opening for females and preputial separation in males.
Reproduction:	There were no differences in precoital interval, mating performance and fertility between TMC207-treated and control animals. Data collected at the Day 14 necropsy are shown on Table R14, below. The mean number of live embryos for females in the 45 mg eq./kg/day group was statistically significantly lower than Control (13.0 vs. 15.8 in controls). This was associated with statistically significant low numbers of corpora lutea and implantations, which the applicant considered to reflect low maternal body weight at the time of mating, which would reduce the ovulation rate. This reviewer does not consider the reduction to be drug-related since the change was slight and the number of live embryos was within the range of historical controls, (10.6-16.8 live embryos/litter) based on published data*. The number of implantations (13.8) was also within the range of historical controls (11.0-18.0).
Cross fostering:	Litters born to dams treated with TMC207 at 45 mg eq./kg/day, but cross-fostered by Control dams, showed body weights and body weight changes that were comparable to control pups (born and raised with untreated dams). Litters born to Control dams and reared by dams treated with 45 mg eq./kg/day group up to Day 21 showed body weights that were between 12 and 21 % lower than controls between Days 11 and 21. Body weight changes were also 20 to 29 % lower in litters raised by dosed dams. This is consistent with the lower body weights seen in litters born to high dose dams dosed before and after the birth of their pups. This suggests that pups exposed prenatally are likely to experience low birth weight and pups that receive drug after birth via milk are likely to experience arrested development.

Table R14: Litter data for Main phase females.

Group	:	1	2	3	4
Compound	:	Control	TMC207	TMC207	TMC207
Dose (mg eq./kg/day)	:	0	5	15	45

Group /Sex		Corpora Lutea	Implantations	Resorptions Early	Resorptions Late	Resorptions Total	Live Embryos	Implantation Loss (%) Pre-	Implantation Loss (%) Post-
Statistical test:		Sh	Sh	Wc	Wc	Wc	Sh	Wa	Wa
1F	Mean	17.6	16.6	0.8	0.0	0.8	15.8	6.4	4.8
	SD	2.09	1.47				1.61		
	N	20	20	20	20	20	20	20	20
2F	Mean	16.5	15.4*	0.8	0.0	0.8	14.6	6.3	5.2
	SD	2.04	1.73				1.90		
	N	20	20	20	20	20	20	20	20
3F	Mean	16.4	15.1*	0.3	0.0	0.3	14.7	7.7	2.1
	SD	1.98	2.41				2.45		
	N	19	19	19	19	19	19	19	19
4F	Mean	15.0*	13.8**	0.8	0.0	0.8	13.0*	10.2	5.3
	SD	4.07	4.19				4.17		
	N	20	20	20	20	20	20	20	20

Discussion

Administration of TMC207 to animals in utero and during early postnatal development resulted in adverse effects on the offspring. While there were no significant adverse effects on physical development, neurological assessments, sexual maturation or reproduction, there were measureable effects on body weights in high dose animals only. The exposure (AUC $_{(0\text{-}24h)}$) to TMC207 at the mid dose was 22 µg*h/mL and there were no effects on body weights at that exposure level. This is similar to exposures observed in the clinic (22 µg*h/mL). Adverse effects were observed at the high dose, where exposures were 53 µg*h/mL, levels which were 2-fold higher than the clinical dose.

Conclusion

At doses which produced exposures similar to clinical exposures, adverse effects were not observed in rats exposed to TMC207 *in utero* and post-natally. At higher doses, pre- or post-natal exposures resulted in lower body weights and body weight gains in pups.

* Historical Control Data for Development and Reproductive Toxicity Studies using the CrI:CD®BR Rat http://www.criver.com/sitecollectiondocuments/rm_rm_r_tox_studies_crlcd_br_rat.pdf

TOX9405: TMC207-Fumarate: Toxicity Study in the Juvenile Rat by Oral (Gavage) Administration (July 2009)

Immature Sprague Dawley Crl: CD® (SD) IGS BR rats were treated with 37 daily oral doses of TMC207 at 5, 15 or 45 mg/kg/day from Day 24 to Day 60 of age, inclusive. TMC207 and its metabolites M2 and M3 were measured. Treatment related histopathological changes were seen at 45 mg/kg/day in the liver (hepatocellular hypertrophy and increased (+15%) relative liver weights), kidneys (corticomedullary mineralization) and muscle (myofiber degeneration and inflammation) at 15 and 45 mg/kg/day. AUC values increased approximately in proportion to dose. Mean AUC values in male and female rats were similar at 24 days of age, but by Day 60, values in females were higher than in males.

Table J1: Pharmacokinetics of TMC207 in juvenile rats

Dose (mg/kg/ day)	Day	C_{max} (µg/mL)						$AUC_{0\text{-}24h}$ (µg.h/mL)					
		TMC207		M2		M3		TMC207		M2		M3	
		M	F	M	F	M	F	M	F	M	F	M	F
5	24	0.4	0.4	0.0	0.0	0.0	0.0	5.5	6.2	0.6	0.7	0.0	0.0
	60	0.4	0.9	0.2	0.2	0.0	0.1	4.3	12.6	2.6	4.7	0.9	1.1
15	**24**	**0.9**	**1.1**	**0.1**	**0.1**	**0.0**	**0.0**	**14.0**	**16.5**	**1.9**	**2.0**	**0.2**	**0.2**
	60	**1.2**	**2.5**	**0.5**	**0.7**	**0.3**	**0.2**	**13.1**	**35.6**	**10.5**	**16.3**	**5.8**	**5.3**
45	24	3.2	4.0	0.3	0.4	0.0	0.1	46.5	55.0	6.0	6.9	0.7	0.8
	60	3.5	5.7	1.6	2.4	0.8	0.8	52.1	103.3	34.1	55.0	18.5	17.0

[a] Day 24 of age (after single dosing)
[b] Day 60 of age (after repeated dosing)
Exposure to TMC207, M2 and M3 at NOAEL are indicated in bold
F = female; M = male

Oral administration of TMC207 had no effect on the attainment of sexual maturity, pre-coital interval, mating performance or fertility of Recovery/Reproductive phase animals and no direct effect upon the behavioral development.

TMC207 administration to 24 day old rats resulted in findings similar to those seen in adult rats. The NOAEL was 15 mg/kg, where AUC values were a mean of about 24 µg*h/mL, similar to clinical exposure.

10 Special Toxicology Studies

Study title:	Assessment of immunomodulating effects of TMC207 after repeated dose (4 weeks) oral administration in rats including toxicokinetics
Sponsor reference # no.:	TOX6926
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 12, 2005
GLP compliance:	No
QA statement:	Yes
Drug, batch #:	ZR207910PFA011
Purity (%):	99.5 %
Frequency of dosing:	Single dose
Doses	0, 6, 20 or 60 mg/kg
Route of administration:	Oral gavage
Dose volume:	10 mL/kg body weight
Formulation/Vehicle:	15 % w/v Hydroxypropyl-β-cyclodextrin (HP-β-CD) in demineralised water, (HCl/NaOH to pH 2.0±0.1)
Species/Strain:	Wistar rat
Number/Sex/Group:	8/sex/dose group
Age:	8 weeks old
Weight:	162-248 g
Satellite groups:	Toxicokinetics animals: 3/sex/dose group

Key Study Findings: No evidence that TMC207 induced immunotoxic effects

Methods

This study was designed to evaluate the immunotoxicity and immunomodulating effects of TMC207. Rats were dosed for 28 days at 6, 20 or 60 mg/kg. At each dose, three subgroups, 8/sex/dose were evaluated separately. At each dose, 8 subgroup S1 animals/sex were evaluated for hematology immunophenotyping of white blood cells. Lymphocyte subset analysis was conducted by means of flow cytometry. Selected organs were also preserved for optional electron microscopy and or genomic analysis. Eight animals/sex/dose from subgroup S2 were immunized with sheep red blood cells (SRBC) shortly before the end of the study to investigate the effects on the functionality of the T-cell dependent antibody response. At necropsy, the spleen of these SRBC-immunized animals was collected, weighed and analyzed by means of a Plaque-Forming Cell assay. S3 animals (8 animals/sex/dose) were evaluated for toxicokinetics and to evaluate TMC207 levels in blood, organs, tissues and bronchoalveolar fluid and bronchoalveolar cells.

Pharmacokinetics evaluations (see Table S1, below) showed that TMC207 concentrations increased dose proportionally up to 20 mg/kg, but at higher doses the increase was less than dose proportional. Concentrations of TMC207 and its metabolite M2 were much higher in all tissues compared to plasma. Tissue plasma ratios of TMC207 did not increase with increasing dose but tissue plasma ratios of M2 did increase (data not shown).

Table I1: Mean maximum plasma concentrations and pharmacokinetics parameters on Days 0 and 27

Dosage Group	**6 mg/kg/day**		**20 mg/kg/day**		**60 mg/kg/day**	
	Day 0	Day 27	Day 0	Day 27	Day 0	Day 27
C_{max} (ng/ml	480	604	1795	1834	3015	2950
AUC $_{(0\text{-}24h)}$ (ng.h/ml)	5.4	7.6	26	28	52	55

Table I2: Tissue to plasma ratio in rats for selected tissues at the end of a 28-day dosing regimen

	TMC207	M2
Lymph nodes	314	197
Thymus	54	70
Lung	42	296
Spleen	39	311
Liver	15	79

Levels were always 1.3 to 6 times higher in females compared to males. In most tissues, M2 levels were higher than TMC207 levels. Only a small percentage (0.4 to 2.7 %) of the total amounts of TMC207 present in the lung before lavage was present in the bronchoalveolar lavage. The amounts of M2 in BALC and BALF were 5 to 17 times the amounts of TMC207.

Data form the immunophenotyping of white blood cells into T cells, T helper cells, cytotoxic T cells and total natural killer cells revealed that TMC207 did not result in any change in the these cells as a percentage of the number of lymphocytes. There was also no dose-related change in the number of plaque-forming cells in TMC207-treated animals. Positive control animals showed statistically significant decreases in the number of PFC's per 10^6 cells and total numbers of PFC's per spleen.

Conclusion:
Despite treatment for 4 weeks with TMC207, rats showed no evidence of immunotoxicity, as evaluated by immunophenotyping or a Plaque-Forming Cell assay. There was also no evidence of immunotoxicity of from nonclinical study *TOX7484: Assessment of immunomodulating effects of R403323 (TMC207 fumarate) in a host resistance assay with Listeria monocytogenes after repeated dose (4 weeks) oral administration in rats.* In this study, TMC207 treatment had no adverse effect on the nonspecific immune reaction to Listeria infection.

Intermittent Dosing

TOX5731: 1-Month Intermittent Dose Oral Toxicity Study in the Rat.

Various intermittent dosing regimes and various dose levels were evaluated in rat for a period of one month. Lower twice weekly or five times weekly doses resulted in few toxic effects. The emergence of toxicities appeared to depend on the total weekly dose, since toxicities similar to those seen in previous studies (such as liver and lung effects) were seen when (high) weekly doses are administered.

TOX5669: 10-week Intermittent Dose Oral Toxicity Study in the Rat (2002)

As a result of excessive mortality in a previous toxicology study of 100 mg/kg/day, intermittent dosing of TMC207 was evaluated. 10 weeks of TMC207 in rats, 100 mg/week resulted in no deaths and AUC values 6 times clinical values. Adverse effects were qualitatively similar to previous studies and included increases in relative liver weight (18%); centrilobular hepatocellular hypertrophy, fatty-like vacuolation (midzonal), single cell/focal necrosis. There was also swelling/proliferation of cells of the MPS (liver, lungs, and lymph nodes).

Mechanistic studies

TOX7539: 5-day repeated dose oral toxicity study of TMC207-fumarate/M2 in the C57BL/6N Mouse

In this 5-day study, M2, the major metabolite of TMC207 was directly administered to mice at 100 and 130 mg/kg to evaluate the toxicity of this compound. TMC207 was administered to two groups of mice at 80 and 100 mg/kg for comparison. Dosing had to be stopped in the M2 mice due to excessive toxicity. The maximum nonlethal dose of M2 was less than 100 mg/kg. Toxic effects treated with M2 mice were qualitatively similar to those observed in this and other studies of TMC207. The amount of M2 in the plasma was several folds higher than TMC207, which is unlike the situation in humans where TMC207 is higher. The sponsor therefore concluded that the mouse is not a good model for predicting human toxicities.
Table M1

Dose (mg/kg/day)	C_{max} (µg/mL)				AUC_{0-24h} (µg.h/mL)			
	TMC207		M2		TMC207		M2	
	M	F	M	F	M	F	M	F
80 (TMC207)	2.2	1.9	-[a]	-[a]	20.3	13.7	92.9	69.5
100 (TMC207)	1.8	2.0	-[a]	-[a]	18.4	16.4	119	78.8
100 (M2)	-	-	-[a]	-[a]	-	-	172	108
130 (M2)	-	-	-[a]	-[a]	-	-	-[b]	-[b]

[a] No C_{max} could be determined
[b] No AUC_{0-24h} could be calculated because of sparse plasma concentration data
F = female; M = male

Impurities

TOX9398: *In Vitro* Bacterial Reverse Mutation Test with TMC207 fumarate spiked with (b) (4) in *Salmonella typhimurium*

TMC207-fumarate spiked with (b) (4), dissolved in DMSO, was negative in the bacterial reverse mutation assay (Ames test) using the plate incorporation method with *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537 in the absence and presence of S9 metabolic activation (Aroclor-induced rat liver S9).

TOX 9394. Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the Microtitre fluctuation technique.

TMC207-fumarate spiked with (b) (4), dissolved in DMSO, did not induce mutations at the TK locus in L5178Y mouse lymphoma cells in both the absence and the presence of S9 metabolic activation (Aroclor-induced rat liver S9).

TOX7562: 2-Week Repeated Dose Oral Toxicity Study in the Rat

In a two week toxicology study of TMC207 spiked with (b) (4), differences between spiked and non-spiked groups were minimal.

TOX9483: *In Vitro* Bacterial Reverse Mutation Test with TMC207 fumarate spiked with (b) (4) in *Salmonella typhimurium*

TMC207-fumarate spiked with (b) (4) dissolved in DMSO, was negative in the bacterial reverse mutation assay (Ames test) using the plate incorporation method with *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537 in the absence and presence of S9 metabolic activation (Aroclor-induced rat liver S9).

TOX6327: *In Vitro* Bacterial Reverse Mutation Test with *Salmonella typhimurium*

TMC207-fumarate spiked with (b) (4) and (b) (4) and dissolved in DMSO, was negative in the bacterial reverse mutation assay (Ames test) using the plate incorporation method with *Salmonella typhimurium* tester strains TA98, TA100, TA102, TA1535 and TA1537 in the absence and presence of S9 metabolic activation (Aroclor-induced rat liver S9).

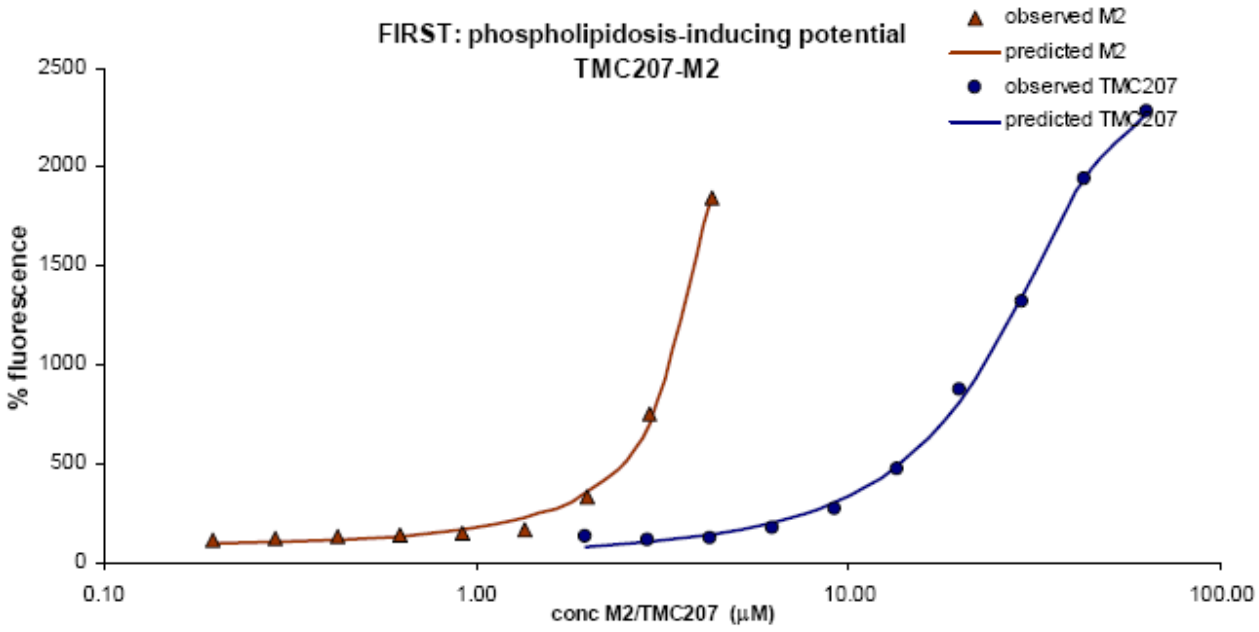
TOX8271: Comparison of the cytotoxicity and phospholipidogenic potency of TMC207and R405786, the N-desmethyl metabolite of TMC207 in an *in vitro* phospholipidosis screening assay.

THP-1 cells, a human monocyte cell line, cells were incubated with the TMC207 or its metabolite M2 for 48 hours. Cells were then stained with ethidiumbromide and calcein and the percentage of dead and live cells respectively was determined. M2 was therefore determined to be more cytotoxic on the THP-1 monocytes than the parent TMC207. For M2, IC_{50} values ranged between 4.86 µM and 5.58 µM, while the IC_{50}- for TMC207 could only be estimated as > 93 µM, since the highest concentration of TMC207 soluble (93 µM) only caused a slight decrease (-20%) in the viability of cells.

To analyze the phospholipidosis-inducing potential, fluorescent phospholipids were added to the medium containing THP-1 cells, for 24 hours, during which the cells were loaded with the fluorescent phospholipids. Fluorescent phospholipids were then removed from the incubation medium followed by another incubation period of 24 hours in which the fluorescent phospholipids could be degraded by lysosomal phospholipases. Phospholipidosis-induction was then assessed by determining the percentage of increase of fluorescence of the treated cells compared to the fluorescence of the control cells.

M2 caused phospholipidosis at concentrations ranging between 1.17 µM and 1.30 µM, while TMC207 induced phospholipidosis at concentrations ranging between 6.18 µM and 8.49 µM. M2 was therefore identified as a stronger phospholipidosis-inducer than TMC207.

Figure P1. Phospholipogenicity dose response curves of TMC207 and M2.



M2 causes phospholipidosis-induction at lower concentrations than TMC207 (table 4, figure 6). There is a 5-fold difference between the 2-fold fluorescence increase values, 6.18 µM and 1.17 µM for TMC207 and M2 respectively.

Integrated Summary and Safety Evaluation

Janssen has applied for the accelerated approval of SIRTURO for the treatment of MDR TB based on the results of Phase II trials. A phase III trial will be conducted to provide additional efficacy and safety data to support traditional approval. Nonclinical studies have revealed several adverse events of special concern. The relevance of these findings to humans will become clearer as we acquire and review data from patient exposures and the Phase III trial.

Summary Table: Safety margins for adverse events of special interest

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Toxicity	Species	NOAEL (mg/kg) M/F	Safety Margin Based on AUC*
QT prolongation	Dog	18 mg/kg	7x
Phospholipidosis*	Dog	No NOAEL	None
	Rat	No NOAEL	None
	Mice	No NOAEL	None
Liver	Mice	20 mg/kg	0.3
	Rat	10 mg/kg	0.13
	Dog	18 mg/kg	7x
Pancreatitis	Dog	10 mg/kg	3x
	Mouse	30 mg/kg	0.5
	Rat	20 mg/kg	3x
Myopathy	Mouse	10 mg/kg	0.3
	Rat	10 mg/kg	1x
	Dog	10 mg/kg	3x

*AUC in human: 22 µg.hr/ml at 200 mg, three times weekly. In animal studies where treatments were at least six months, there was no NOAEL for phospholipidosis. Although less severe and less frequent, changes were present at the lowest doses.

As illustrated on the Summary Table (above). The mouse was the most sensitive species and the applicant ascribed this to greater production of the more cytotoxic M2.

In most of the nonclinical studies animals were treated with SIRTURO dissolved in hydroxypropyl-β-cyclodextrin (HPβCD) and phospholipidosis was widespread. Published literature has shown that HPβCD itself is associated with phospholipidosis, characterized by vacuolation and foamy macrophages (Gould, S, and Scott, R., 2005. 2-Hydroxypropyl-b-cyclodextrin (HP-β-CD): A toxicology review. Food and Chemical Toxicology 43 (2005) 1451–1459. While phospholipidosis was increased in these animals compared to (HPβCD) controls, the effects in the bedaquiline-treated animals was likely the result of the additive effect of two phospholipidogenic compounds. Since phospholipidosis has been seen in many drugs (e.g. fluoxetine and posaconazole), and has been shown to be reversible without any adverse long

term sequelae, the level of concern over this finding is balanced by the sometimes fatal result of tuberculosis. Also, since there is no HPβCD in the clinical formulation of bedaquiline, the risk to patients may be reduced.

QT prolongation was detected at high doses (40 mg/kg) in dogs treated with bedaquiline for 2 months at approximately 7 times the clinical exposure, but it has also been seen in patients in the Phase II trial. These increases in QT interval are consistent with the drug's demonstrated ability to inhibit the IKr-like membrane potassium current in the hERG assay. However, a thorough QT study in humans was negative and the findings seem to be dose-related in dogs. In a separate dog study, where dogs were treated with bedaquiline at lower doses (18 mg/kg) for 9 months (about 8x clinical AUC) there were no increases in QT intervals. Data collected during the Phase III trial should help to characterize the risk to patients taking bedaquiline. Patients and their doctors should be made aware of the QT effects via the label.

Increased liver enzymes and hepatocellular hypertrophy were observed in animals treated with bedaquiline, but, at low doses, the findings were reversible and not severe. Also, liver function can be monitored. It should however be noted that liver toxicity (hepatocellular hypertrophy and necrosis) developed very quickly at very high (800 mg/kg) single doses in the mouse. Patients and prescribing physicians should be made aware of these findings (via the label) and appropriate monitoring instituted as needed.

Pancreatitis was observed in dogs and mice, and at least one patient. There were also increases in amylase in the Phase II trial. There should be appropriate education of the doctor and patient about these effects (via labeling) with monitoring where necessary. Data regarding any pancreatic findings in the Phase III will add to our understanding of the risks to patients.

Musculopathy was a dose related findings seen mice, rats and dogs, but this finding was reversible and was not seen in recovery animals. Data regarding rhabdomyolysis in the clinical trials/patients should be made available to patients and prescribing physicians.

SIRTURO has 2 main safety risks, liver toxicity and QT prolongation, and both are moderate, reversible, and can be monitored with routine ECG and laboratory testing.

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/s/

OWEN G MCMASTER
12/11/2012

WENDELYN J SCHMIDT
12/12/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 204384 **Applicant:** JANSSEN **Stamp Date:** June 29, 2012

Drug Name: TMC207 **NDA Type:** PRIORITY

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?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
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)?	√		
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).	√		
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?	√		
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?	√		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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?	√		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			See Chemistry Review
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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.

OWEN G MCMASTER — 7/26/2012
Reviewing Pharmacologist — Date

WENDELYN SCHMIDT — 7/26/2012
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

OWEN G MCMASTER
07/26/2012

WENDELYN J SCHMIDT
07/30/2012