CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

202611Orig1s000

PHARMACOLOGY REVIEW(S)

FOOD AND DRUG ADMINISTRATION
SUPERVISORY MEMO

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	April 25, 2012
Reviewer:	Lynnda Reid, Ph.D. Supervisory Pharmacologist
NDA #/date:	202-611, August 29, 2011
Sponsor:	Astellas Pharma US, Inc.
Drug Product:	Mirabegron (YM178)
Indication:	Treatment of Over Active Bladder

Background: Mirabegron, a new molecular entity, is a beta-3 adrenergic receptor (AR) agonist proposed for use in the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency, and urinary frequency. The maximum recommended human dose (MRHD) is 50 mg/day; 25 mg/day is recommended for patients with renal insufficiency and severe hepatic impairment.

Identified areas of clinical concern, based on increased incidence rates in clinical studies, included effects on cardiovascular parameters (blood pressure and heart rate), increased incidence in overall neoplasia diagnoses, liver toxicity, and hypersensitivity.

The sponsor submitted a complete nonclinical package that included general and safety pharmacology and pharmacokinetics/ADME assessments; single- and repeat-dose toxicology, genetic toxicology, carcinogenicity, reproductive and developmental toxicology studies; and Guinea pig sensitization studies.

Pharmacology: Mirabegron therapeutic activity is attributed to relaxation of the detrusor smooth muscle during the urinary bladder fill-void cycle by activation of beta-3 AR without interfering with the voiding contraction. Studies in overactive bladder (OAB) animal models have shown that mirabegron increases bladder capacity and reduces bladder contractions. Although mirabegron showed very low in vitro binding potential to cloned human beta-1 AR and beta-2 AR, results

of animal studies and cardiac impedance evaluations in humans suggest that some beta-1 AR stimulation may occur at high mirabegron exposures.

Pharmacodynamics: Mirabegron is readily absorbed following oral administration and widely distributed in animals. Mirabegron and its metabolites are eliminated in urine and feces in rats, monkeys, and humans; and enterohepatic recirculation was confirmed in rats.

Systemic exposure to mirabegron during pregnancy was generally 1.5 to 2 times greater in pregnant rabbits compared to non-pregnant rabbits. Pregnancy did not affect exposures in rats. The effect of pregnancy on mirabegron pharmacokinetics in women is unknown.

General Toxicology: Toxicologically targeted tissues and organ systems included the liver, lacrimal and salivary glands, and central nervous and cardiovascular systems.

The liver was the tissue with the greatest exposure to mirabegron in both rats and monkeys. No hepatotoxicity was observed in monkeys at exposures up to 8 times the exposures in humans at the MRHD. In rats and dogs, liver enzymes were moderately elevated at high doses, and hepatic histopathology was only observed at or near the lethal dose with large multiples of the clinical exposure. All findings were reversible in surviving animals.

As expected pharmacology of a β1/β2 agonist, mirabegron promoted salivation and lacrimation in rats and induced atrophy of the secretory cells in the salivary glands in rats and dogs at exposures near clinical levels. High exposures in dogs produced hemorrhaging, atrophy, and necrosis of the salivary gland acinar and ductal cells. However, no adverse histopathology was reported in mice, rats, or in monkeys at relatively high exposures. Findings were generally recoverable or partially recoverable after drug withdrawal.

Adverse signs suggestive of CNS toxicity observed at or near clinical exposures included temporarily decreased activity in mice, rats, and monkeys; prone position in rats; and prone position with slight hyperthermia in mice. At higher exposures, more concerning adverse effects included ptosis, emesis, and staggering in monkeys; and hyperpnea, tremor, and tonic convulsions in rabbits. At near lethal exposures, clonic convulsions were observed in mice and rats. One male monkey dosed at 3 mg/kg IV (13x MRHD based on Cmax) went into coma after the third dose, he later recovered.

At clinically relevant exposures, slight elevations in heart rate were observed in rats after IV dosing, and slight elevations in heart rate and decreases in blood pressure were observed in dogs after oral dosing. At higher exposures, elevated heart rate was observed in rabbits and monkeys, and ventricular tachycardia in dogs and monkeys. One monkey went into ventricular tachycardia and died

within 15 minutes of administrating a 10 mg/kg IV dose (114x MRHD based on Cmax). Similarly intravenous dosing in dogs led to death at IV doses equal to or greater than 10 mg/kg due to ventricular tachycardia that progressed to ventricular fibrillation within 5-10 minutes. PR interval was shortened after oral dosing in dogs at clinically relevant exposures but prolonged in monkeys at exposures equal to or greater than approximately 11 times the human exposures at the MRHD. At supra therapeutic exposures, QRS was slightly prolonged in monkeys, however, no effect on QTc was observed in dogs or monkeys. Cardiac histopathology was not observed.

Genotoxicity: Based on a standard battery of genotoxicity assays, mirabegron is not considered genotoxic. Mirabegron was not mutagenic in the Ames bacterial reverse mutation assay, did not induce chromosomal aberrations in human peripheral lymphocytes at concentrations that were not cytotoxic, and was not clastogenic in the rat micronucleus assay.

Carcinogenicity: Based on 2-year rodent studies, mirabegron is not considered a carcinogen. Mirabegron was not carcinogenic at systemic exposures 38-45 times higher in rats and 21-38 time higher in mice compared to mean AUC values in humans at the MRHD.

Reproductive toxicology: Based on animal data, mirabegron is predicted to have a low risk for major developmental abnormalities in humans. For labeling, the recommended Pregnancy Category is C.

Mirabegron had no adverse effect on development in rats at exposures that were 6 times greater than those in women at the MRHD, or in rabbits at clinically relevant exposures. Reversible adverse developmental findings consisting of delayed ossification and wavy ribs in rats and decreased fetal body weights in rabbits occurred at exposures equal to or greater than 22 and 14 times, respectively, the exposures in women at the MRHD. At maternally toxic exposures decreased fetal weights were observed in rats and rabbits; and fetal death, dilated aorta, and cardiomegaly were reported in rabbits.

Mirabegron was transferred to rat fetuses through the placenta and transferred to rat pups in milk. In lactating rats, the concentration of mirabegron in milk was twice that found in maternal plasma. In the nursing pups, the highest concentrations of mirabegron were found in the lungs, liver, and kidneys. Exposure to mirabegron in utero and through lactation resulted in a slight increase in death in the first few days after birth along with decreased body weight at exposures 22 times that of women at the MRHD.

There were no observed adverse effects on fertility.

Summary: Findings in animals at clinically relevant exposures were characteristic of the expected pharmacologic effects for a mixed beta adrenergic

agonist including decreased frequency of urination, slight decrease in blood pressure, slight increase in heart rate, and increases in salivation and lacrimation. At exposures greater than at the MRHD, the most significant adverse findings included hepatotoxicity, CNS toxicity, cardiovascular toxicity, and reproductive/developmental effects. Adverse effects observed at high multiples of the human exposure were generally reversible and monitorable.

The potential for toxicity in humans appears low at the MRHD based on the lack of significant findings at clinically relevant doses, and the reversibility of findings without any clear evidence of adverse histopathology at higher sublethal doses.

Outstanding nonclinical issues: There are no outstanding nonclinical issues.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Eric Andreasen, that nonclinical data support approval of mirabegron at doses up to 50 mg, to be used daily for the treatment of over active bladder in patients with symptoms of urge urinary incontinence, urgency, and urinary frequency.

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/s/

LYNNDA L REID
04/25/2012
Secondary Review

Comments on NDA 202611
From. A. Jacobs, pharm/tox AD
Date: April 16, 2012

1. The are no pharm/tox approval issues
2. I concur with the pregnancy category and wording proposed for labeling by the reviewer
3. Other comments were discussed with the supervisor and will be addressed as appropriate

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/s/

ABIGAIL C JACOBS
04/17/2012

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 202-611
Supporting document/s:	SN000, DARRTS 1
Applicant's letter date:	August 26, 2011
CDER stamp date:	August 29, 2011
Product:	Mirabegron
Indication:	Treatment of Overactive Bladder
Applicant:	Astellas Pharma US, Inc.
Review Division:	Division of Reproductive and Urologic Products
Reviewer:	Eric Andreasen Ph.D.
Supervisor/Team Leader:	Lynnda Reid, Ph.D.
Acting Division Director:	Julie Beitz, MD
Project Manager:	Nenita Crisostomo

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202-611 are owned by Astellas Pharma US, Inc. or are data for which Astellas Pharma US, Inc. has obtained a written right of reference.

TABLE OF CONTENTS

1 Executive Summary

1.1 Introduction

Mirabegron is a beta-3 adrenergic receptor (β3-AR) agonist indicated for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency, and urinary frequency. Nonclinical and clinical studies suggest that mirabegron also has some β1-AR agonist activity. Mirabegron is a new molecular entity and the first in its class for this indication. Mirabegron has been marketed in Japan under the trade name Betanis® since July of 2011 for a similar indication at a maximum daily oral dose of 50 mg. The maximum recommended human dose (MRHD) proposed by the sponsor in this NDA is also 50 mg once daily. Systemic exposures in animals discussed below are described relative to that in fasted women at least 55 years of age at the MRHD, which represents the subpopulation with the greatest steady state exposure.

1.2 Brief Discussion of Nonclinical Findings

Mirabegron was shown to increase bladder capacity and reduce bladder contractions in nonclinical pharmacology studies. It is readily absorbed following oral administration and widely distributed in animals. Mirabegron and its metabolites are eliminated in urine and feces in rats, monkeys, and humans and enterohepatic recirculation was confirmed in rats.

Findings in animals at exposures similar to the MRHD were characteristic of the expected pharmacologic effects for a mixed beta adrenergic agonist including decreased frequency of urination, slight decrease in blood pressure, slight increase in heart rate, and increases in salivation and lacrimation. Toxicities observed in nonclinical studies at exposures greater than at the MRHD include, but are not limited to, hepatotoxicity, effects on body weight and metabolism, impairment of cardiovascular function, and reproductive/developmental effects. These toxicities as discussed below were generally at high multiples of the human exposure, and were generally reversible and monitorable.

Elevated heart rate was observed in rats after IV dosing at exposures < 2x MRHD and after oral dosing in dogs at exposures ≥ 0.1x MRHD, rabbits at ≥ 9x MRHD, and monkeys at 12x MRHD. Mirabegron also promoted ventricular tachycardia in dogs and monkeys at exposures ≥ 29 to 37 times the MRHD. Mirabegron-induced increases in heart rate were at least partially reversed in rabbits, rats, and dogs that were treated with the β1-AR agonist metoprolol suggesting that increased heart rate in animals may be at least partially related to β1-AR agonism.

The liver was the tissue with the greatest exposure to mirabegron in both rats and monkeys. Elevated liver enzymes, < 2 fold compared to non-treated animals, were noted in rats and dogs at high doses which fully or partially returned to pre-exposure levels after drug withdrawal. Adverse hepatic histology observed in rats included eosinophilic pigment deposition at exposures ≥ 12-17 times the MRHD, along with

hepatocyte swelling and fibrosis at lethal exposures ≥ 130 times the MRHD. Hepatocyte hypertrophy, vacuolation and lipid accumulation were noted in dogs at 25 times the MRHD. Since hepatotoxicity was only observed in rodents and dogs but not monkeys, was reversible, and did not cause adverse histopathology except at or near the lethal dose with large multiples of the clinical exposure, the potential for hepatotoxicity at the dose proposed for marketing appears low.

Exposures near the MRHD temporarily decreased the activity of mice, rats, and monkeys. At high or lethal exposures, more concerning adverse CNS signs were observed including ptosis and staggering (monkeys at 8x MRHD), hyperpenia, tremor, tonic convulsions (rabbits at 21x MRHD), decreased movement, alternates, and muscle tone (mice at 29x MRHD), vomiting and ventricular tachycardia (monkeys at 29x MRHD), clonic convulsions (mice at 64-72x MRHD), clonic convulsions, mydriasis, and tachypenia (rats at 45-160x MRHD). The potential for CNS toxicity in humans appears low at the MRHD based on reversibility of findings without any clear evidence of adverse histology.

Mirabegron and some of its metabolites accumulated in the pigmented tissues of the eyes of rats. However, no adverse drug related ophthalmoscopic or histology findings were observed at large multiples of the clinical exposure in any species evaluated.

Fertility was not affected in male or female rats below the lethal dose or in offspring exposed in utero and during lactation.

Mirabegron had no adverse effect on development in rats at exposures up to 6 times the MRHD, or in rabbits at clinically relevant exposures. However, rat fetuses exposed to mirabegron in utero at maternal exposures ≥ 22 times the MRHD displayed wavy ribs and decreased ossification. Also decreased fetal weight and bone malformations were observed at a dose that was lethal to the mother (96x MRHD). Developmental delays observed in fetal rats were reversible after birth. In utero exposure in rabbits reduced fetal weight at exposures ≥ 14 times the MRHD, and caused cardiomegaly, dilated aortas, and impaired ossification at exposures 36 times the MRHD.

Mirabegron was transferred to rat fetuses through the placenta and transferred to rat pups in milk. Exposure of rats to mirabegron in utero and through lactation resulted in a slight increase in death in the first few days after birth along with decreased body weight at exposures 22 times the MRHD.

Although pregnancy did not affect exposure to mirabegron in rats, the exposure of mirabegron in pregnant rabbits was roughly 2 times greater than in non-pregnant rabbits. The effect of pregnancy on pharmacokinetics in women has not been assessed.

Mirabegron was not genotoxic in a standard battery of in vitro and in vivo studies. Mirabegron related neoplasms were not apparent after two years of daily oral dosing in mice who were exposed up to 21-25 times the MRHD, or in rats who were exposed up

to 25-45 times the MRHD. From a nonclinical perspective the available nonclinical data suggest that mirabegron is not genotoxic or carcinogenic.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support approval of this product for the treatment of over active bladder in adult patients with symptoms of urge urinary incontinence, urgency, and urinary frequency at a maximum daily dose of 50 mg.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Recommended nonclinical revisions to the sponsor's proposed label are provided below. An annotated version of the sponsor's label can be found in Appendix A.

----------INDICATIONS AND USAGE----------
Mirabegron is a beta-3 adrenergic agonist indicated for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency, and urinary frequency.

1 INDICATIONS AND USAGE
Mirabegron is a beta-3 adrenergic agonist indicated for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency, and urinary frequency.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C
There are no adequate and well-controlled studies using mirabegron in pregnant women. [TRADE NAME] should be used during pregnancy only if the potential benefit to the patient outweighs the risk to the patient and fetus. Women who become pregnant during mirabegron treatment are encouraged to contact their physician.

(b) (4)
Based on animal data, mirabegron is predicted to have a low probability of increasing the risk of adverse developmenta (b) (4) above background risk. Reversible adverse developmental findings consisting of delayed ossification and wavy ribs in rats and decreased fetal body weights in rabbits occurred at exposures ≥ 22 and 14 times, respectively, the maximal recommended human dose (MRHD), At maternally toxic exposures decreased fetal weights were observed in rats and rabbits, and fetal death, dilated aorta, and cardiomegaly were reported in rabbits.

Animal Data

In the rat embryo/fetal developmental toxicity study, pregnant rats received daily oral doses of mirabegron at 0, 10, 30, 100, or 300 mg/kg from implantation to closure of the fetal hard palate (7th to 17th day of gestation). Maternal systemic exposures were approximately 0, 1, 6, 22, or 96 times greater than exposures in women treated at the MRHD of 50 mg based on AUC. No embryo/fetal toxicities were observed in rats exposed up to 6 times the human systemic exposure at the MRHD. At systemic exposures equal or greater than 22 times the human systemic exposure at the MRHD, delayed ossification and wavy ribs were observed in fetuses at an increased incidence. These findings were reversible.

In the rabbit embryo/fetal developmental toxicity study, pregnant rabbits received daily oral doses of mirabegron at 0, 3, 10, or 30 mg/kg from implantation to closure of the fetal hard palate (6th to 20th day of gestation). Maternal systemic exposures were 0, 0.7, 14, or 36 times that in women treated at the MRHD of 50 mg based on AUC. No embryo/fetal toxicities were observed in rabbits at systemic exposures that were 0.7-times the human systemic exposure at the MRHD. The embryo/fetal No Adverse Effect Level (NOAEL) of 0.7 times the MRHD was established in this species based on reduced fetal body weight observed at systemic exposures that were 14-fold higher than the human systemic exposure at MRHD. At higher doses, where systemic exposures were 36-fold higher than the human exposure at MRHD, maternal body weight gain and food consumption were reduced, one of 17 pregnant rabbits died, the incidence of fetal death increased and fetal findings of dilated aorta and cardiomegaly were reported.

The effects of mirabegron on prenatal and postnatal development was assessed in pregnant rats dosed at 0, 10, 30, or 100 mg/kg/day from the seventh day of gestation until 20 days after birth. Maternal systemic exposures were 0, 1, 6, and 22 times the exposure in women at the MRHD based on AUC. Rat pups exposed to mirabegron in utero and through 21 days of lactation had no discernable adverse effects at maternal systemic exposures 6 times the MRHD. However, a slight but statistically significant decrease in the survival of pups was observed 4 days after birth at exposures 22 times the MRHD (92.7% survival) compared to the control group (98.8%). However, there was no effect on survival of pups 21 days after birth.

(b) (4)

In utero and lactational exposure did not affect behavior or fertility of offspring at exposures up to 22 times the MRHD.

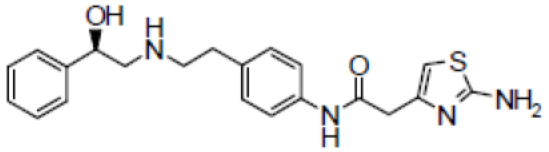
(b) (4)

8.3 Nursing Mothers

It is not known whether mirabegron is excreted in human milk. Mirabegron was found in the milk of rats at concentrations twice the maternal plasma level. Mirabegron was found in lungs, liver, and kidneys of nursing pups. No studies have been conducted to assess the impact of mirabegron on milk production in humans, its presence in human breast milk, or its effects on the breast-fed child. Because mirabegron is predicted to be excreted in human milk and because of the potential for serious 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.

11 DESCRIPTION

Mirabegron is a beta-3 adrenergic agonist. The chemical name is 2-(2-aminothiazol-4-yl)-N-[4-(2-{[(2R)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide having an empirical formula of $C_{21}H_{24}N_4O_2S$ and a molecular weight of 396.51. The structural formula of mirabegron is:



Mirabegron is a white powder. It is practically insoluble in water (0.082 mg/mL). It is soluble in methanol and dimethyl sulfoxide.

Each mirabegron (b) (4) extended release tablet contains either 25 mg or 50 mg of mirabegron and the following inactive ingredients: polyethylene oxide (b) (4) polyethylene glycol (b) (4), hydroxypropyl cellulose, butylated hydroxytoluene, magnesium stearate, hypromellose, ferric oxide yellow, and ferric oxide red (25 mg tablet only).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Mirabegron is an agonist of human beta-3 adrenergic receptor (AR) as demonstrated by in vitro laboratory experiments using the cloned human beta-3 AR. Mirabegron relaxes the detrusor smooth muscle during (b) (4) Although mirabegron showed very low intrinsic activity for cloned human beta-1 AR and beta-2 AR (b) (4) in humans indicate that beta-1 AR stimulation occurs at high mirabegron exposures.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity
Long-term carcinogenicity studies were conducted in rats and mice dosed orally with mirabegron for two years. Male rats were dosed at 0, 12.5, 25, or 50 mg/kg/day and female rats and both sexes of mice were dosed at 0, 25, 50, or 100 mg/kg/day. Mirabegron showed no carcinogenic potential at systemic exposures (AUC) 38 to 45-fold higher in rats and 21 to 38-fold higher in mice than the human systemic exposure at the 50 mg dose.

Mutagenesis
Mirabegron was not mutagenic in the Ames bacterial reverse mutation assay, did not induce chromosomal aberrations in human peripheral lymphocytes at concentrations that were not cytotoxic, and was not clastogenic in the rat micronucleus assay.

Impairment of Fertility
Fertility studies in rats showed that mirabegron had no effect on either male or female fertility at doses up to 100 mg/kg/day. Systemic exposure (AUC) at 100 mg/kg in female rats was estimated to be 22 times the MRHD in women and (b) (4) times the MRHD in men.

2 Drug Information

2.1 Drug

CAS Registry Number: 223673-61-8

Generic Name: Mirabegron

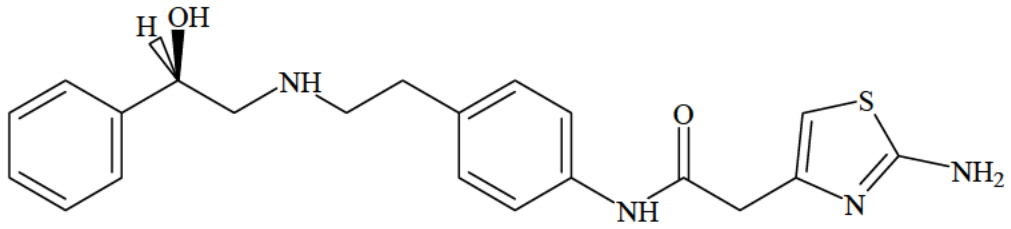
Code Name: YM178

Chemical Name: 2-(2-aminothiazol-4-yl)-*N*-[4-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)phenyl]acetamide

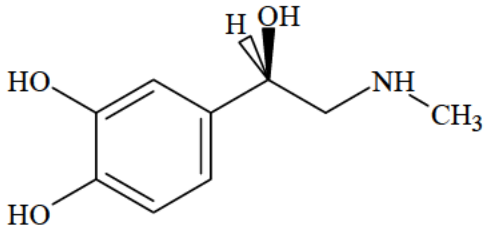
Molecular Formula/Molecular Weight: $C_{21}H_{24}N_4O_2S$ / 396.51

Structure:

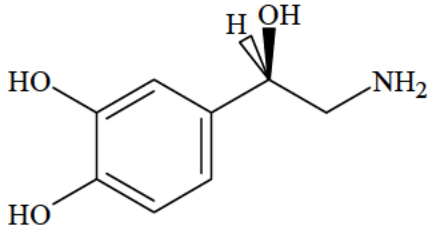
Mirabegron (YM178)



Epinephrine (adrenalin)



Norepinephrine (noradrenaline)



Pharmacologic Class: Beta-3 adrenergic agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

The sponsor developed this drug for this indication under IND 69,416

2.3 Drug Formulation

Ingredient	Quantity (mg/tablet)		Function
	25 mg tablet	50 mg tablet	
YM178	25	50	Active Ingredient
Polyethylene Oxide (b) (4)	(b) (4)		
Polyethylene Glycol (b) (4)			
Hydroxypropyl Cellulose			
Butylated Hydroxytoluene			
Magnesium Stearate			
(b) (4)			
(b) (4)			
(b) (4)			

(b) (4)

Red Ferric Oxide	(b) (4)
Yellow Ferric Oxide	

2.4 Comments on Novel Excipients

None, all excipients have been used in previously marketed products.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Mirabegron is a beta-3 adrenergic agonist indicated for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency, and urinary frequency. The sponsor is seeking a maximum oral dose of 50 mg once daily with or without food. In renal or hepatic impaired patients, a single 25 mg dose is proposed.

2.7 Regulatory Background

Astellas Pharma submitted NDA 202-611 on August 25, 2011. NDA 202-611 contains electronic data intended to support the use of mirabegron for the treatment of overactive bladder (OAB) with symptoms of urge urinary incontinence, urgency, and urinary frequency. Data used to support the NDA was collected and reviewed under IND 69,416 which was opened on June 9, 2006. Pre-NDA meetings were held November 2, 2010 and June 15, 2011 to discuss the data, format, organization, statistical methods, and unresolved issues needed to submit an NDA under 505(b)(1).

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology Studies

Primary Pharmacology

Mechanism of Action

- Binding of YM178 to human beta-adrenoceptors (Study 178-PH-005)
- β-Adrenergic Agonistic Activities of YM178 on CHO Cells Expressing Human Either β1-, β2- or β3-Adrencoeptor (Study 178-PH-003)
- Agonistic Activities of YM178 and Its Metabolites for Human Beta 1-, Beta 2- or Beta 3-Adrenoceptors Expressed in CHO Cells (Study 178-PH-044)
- Agonistic Activity of YM178 on Rat Beta 1-, Beta 2-, or Beta 3-Adrenoceptors Expressed in CHO Cells (Study 178-PH-051)
- Agonistic Activity of YM178 on Dog Beta 1-, Beta 2-, or Beta 3-Adrenoceptors Expressed in CHO Cells (Study 178-PH-052)
- Agonistic Activity of YM178 on Monkey Beta 1-, Beta 2-, or Beta 3-Adrenoceptors Expressed in CHO Cells (Study 178-PH-053)

Primary Pharmacology

- Relaxing Activity of YM178 in Isolated Rat Bladder Smooth Muscle (Study 178-PH-029)
- Effect of YM178 on Rhythmic Bladder Contraction in Anesthetized Rats (Study 178-PH-030)
- Relaxant Effects of YM178 on Isolated Human Detrusor Muscle (Study 178-PH-031)

- Effect of Intraduodenal YM178 on Rhythmic Bladder Contraction in Anesthetized Rats (Study 178-PH-032)
- Effect of YM178 on Bladder Functions in Rats with Bladder Outlet Obstruction (Study 178-PH-033)
- Effect of YM178 on Functional Bladder Capacity in Water-loaded Rats with Cerebral Infarction (Study 178-PH-034)
- Effect of YM178 on the Intravesical Pressure during Urine Storage Phase in Anesthetized Rats (Study 178-PH-035)
- Effect of Intraduodenal YM178 after 2-week Repeated Oral Administration on Rhythmic Bladder Contraction in Anesthetized Rats (Study 178-PH-036)
- Effect of YM178 on Micturition Frequency and Functional Bladder Capacity in Water-loaded Cynomolgus Monkeys (Study 178-PH-037)
- Effect of Mirabegron on Cyclic AMP Accumulation in Urinary Bladder Isolated from Rats (Study 178-PH-055)
- Effects of Beta-Adrenoceptor Antagonists on Mirabegron-induced Relaxation in Isolated Strips of Rat Bladder Smooth Muscle (Study 178-PH-057)
- Effect of Mirabegron on Carbachol-Induced Intravesical Pressure Elevation in Anesthetized Dogs (Study 178-PH-059)

<u>Secondary Pharmacology</u>

- Affinity of YM178 to Various Receptors (Study 178-PH-038)
- Affinity of YM-340790 to Various Receptors (Study 178-PH-039)
- Affinity of YM-538852 Hydrochloride to Various Receptors (Study 178-PH-040)
- Affinity of YM-538853 Trifluoroacetate to Various Receptors (Study 178-PH-041)
- Affinity of YM-382984 to Various Receptors (Study 178-PH-042)
- Affinity of YM-208876 Hydrochloride to Various Receptors (Study 178-PH-043)
- Affinity of YM-538858 to Various Receptors (Study 178-PH-045)
- Affinity of YM-538859 to Various Receptors (Study 178-PH-046)
- Affinity of YM-9636324 to Various Receptors (Study 178-PH-047)
- Affinity of YM-554028 Formate to Various Receptors (Study 178-PH-048)
- Affinity of YM178 to Alpha-1A-adrenergic Receptor, Muscarinic M2 Receptor, Sodium Ion Channel Site 2, Dopamine Transporter, and Norepinephrine Transporter (Study 178-PH-049)

<u>Safety Pharmacology</u>

CNS

- Safety Pharmacology Study of YM178 on the Central Nervous System in Rats (Study 178-PT-003)
- Safety Pharmacology Study of YM178 on the Central Nervous, Cardiovascular, and Respiratory Systems in Monkeys (Study 178-PT-004)
- General Pharmacology Study of YM178 in mice, rats, guinea pigs and dogs (Study 178-PH-019)

Renal and Gastrointestinal

- General Pharmacology Study of YM178 in mice, rats, guinea pigs and dogs (Study 178-PH-019)

Respiratory

- Safety Pharmacology Study of YM178 on the Central Nervous, Cardiovascular, and Respiratory Systems in Monkeys (Study 178-PT-004)

- General Pharmacology Study of YM178 in mice, rats, guinea pigs and dogs (Study 178-PH-019)

Cardiac

- Effects of YM178 and its Five Metabolites on Four Ion Channels Expressed in Mammalian Cells (Study 178-PT-011)
- Effects of YM178 on the hERG Current (Study 178-PT-002)
- Effects of YM178 Metabolites M5 and M16 on the hERG Current in HEK293 Cells (Study 178-PT-006)
- Effects of YM178 Metabolites M11 and M12 on the hERG Current in HEK293 Cells (Study 178-PT-008)
- Effects of YM178 Metabolite M14 on the hERG Current in HEK293 Cells (Study 178-PT-012)
- Effects of YM178 on the hERG Current in HEK293 Cells (Study 178-PT-015)
- Effects of YM178 on Action Potentials in Isolated Guinea Pig Papillary Muscles Study 178-PT-001)
- Effects of YM178 Metabolites M5 and M16 on Action Potentials in Isolated Guinea-Pig Papillary Muscles (Study 178-PT-007)
- Effects of YM178 Metabolites M11 and M12 on Action Potentials in Isolated Guinea-Pig Papillary Muscles (Study 178-PT-009)
- Effects of YM178 Metabolite M14 on Action Potentials in Isolated Guinea-Pig Papillary Muscles (Study 178-PT-013)
- In Vitro Effects of Isoproterenol and YM178 on QT, APD, Tp-e and Arrhythmogenesis in the Canine Ventricular Wedge Preparation (Study 178-PT-010)
- In Vitro Effects of YM178 metabolites, M5, M11, M12, M14 and M16 on QT, APD, Tp-e and Arrhythmogenesis in the Canine Ventricular Wedge Preparation (Study 178-PT-014)
- Effects of YM178 on the Cardiovascular System in Anesthetized Rabbits (Study 178-PT-005)
- Investigational Study for Effects of the Oral Administration of YM178 on Heart Rate in Rabbits (Study 178-TX-048)
- Safety Pharmacology Study of YM178 on the Central Nervous, Cardiovascular, and Respiratory Systems in Monkeys (Study 178-PT-004)
- General Pharmacology Study of YM178 (Effects of YM178 on Cardiovascular System in Cynomolgus Monkeys) (Study 178-PH-020)
- General Pharmacology Study of YM178 in mice, rats, guinea pigs and dogs (Study 178-PH-019)
- Effects of YM178 on Monophasic Action Potential (Study 178-PH-021)
- Effect of Propranolol on the YM178-induced ECG-change in Conscious Dogs (Study 178-PH-022)
- Effects of (b) (4) and YM178 on Canine Cardiovascular Systems (Study 178-PH-023)
- Effect of YM178 on Heart Rate and Examination of its Mechanism of Action in Anesthetized Rats (Study 178-PH-050)
- Effect of Mirabegron on Heart Rate and Elucidation of Its Mechanism of Action in Anesthetized Dogs (Study 178-PH-054)

Pharmacokinetic Studies

<u>Absorption</u>

- Pharmacokinetics of YM178 after Single Intravenous and Oral Administration to Rats (Study 178-ME-005)

- Absorption, Distribution, Metabolism and Excretion after a Single Oral Administration of ^{14}C-YM178 to Albino Rats (Study 178-ME-022)
- Pharmacokinetics of YM178 after Single Intravenous and Oral Administration to Dogs (Study 178-ME-006)
- Pharmacokinetics of YM178 after Repeated Oral Administration to Dogs (Study 178-ME-007)
- Food Effect Study after Single Oral Administration of YM178 to Dogs (Study 178-ME-008)

Distribution

- In Vitro Plasma Protein Binding of YM178 (Study 178-ME-021)
- Estimation of the Major Human Plasma Binding Proteins for YM178 (Study 178-ME-044)
- In Vitro Study for the Transfer of YM178 into Red Blood Cells (Study 178-ME-045)

Rats

- Absorption, Distribution, Metabolism and Excretion after a Single Oral Administration of ^{14}C-YM178 to Albino Rats (Study 178-ME-022)
- Distribution Study after Single Oral Administration of ^{14}C-YM178 to Non-Albino Rats (Study 178-ME-023)
- Pharmacokinetic study of YM178: Placental Transfer in Pregnant Rats after a Single Oral Administration of ^{14}C-YM178 (Study 178-ME-062)
- Pharmacokinetic study of YM178: Transfer into Breast Milk in Lactating Rats after a Single Oral Administration of ^{14}C-YM178 (Study 178-ME-063)
- Pharmacokinetic study of YM178: Tissue Distribution in Rats after Repeated Oral Administration of ^{14}C-YM178 (Study 178-ME-064)
- Pharmacokinetic study of YM178: Distribution of Radioactivity in the Eyeball and Analysis of Composition of Radioactivity in the Eyeball after a Single Oral Administration of ^{14}C-YM178 to Pigmented Rats (Study 178-ME-065)
- Radioactivity in Eyeballs after a Single Oral Administration of ^{14}C-YM178 to Pigmented Rats (Study 178-ME-091)

Monkeys

- Pharmacokinetic study of YM178: Concentrations in Blood and Plasma, Urinary and Fecal Excretion, and Tissue Distribution in Monkeys after a Single Oral Administration of ^{14}C-YM178 (Study 178-ME-061)

Metabolism

Metabolism In Vitro

- Identification of the Human Liver Cytochrome P450 Isoenzymes Involved in the Metabolism of YM178 (178-ME-002)
- In Vitro Metabolic Rate Using Rat, Dog, Cynomolgus Monkey, and Human Liver Microsomes (Study 178-ME-017)
- Comparison of In Vitro Metabolite Patterns of YM178 in Various Species Using Liver Microsomes (Study 178-ME-020)
- Identification of YM178 in Metabolites in Human Plasma (178-ME-034)
- Species Difference of Metabolic Rate of YM178 in Plasma (178-ME-038)
- Characterization of the Human Esterases Involved in the Metabolism of YM178 (178-ME-079)

- Evaluation of the Potential of YM178 to Inhibit the Major Human Liver Cytochrome P450 Isoenzymes (178-ME-009)
- Cytochrome P450 Drug Interaction Studies with YM178 using Human Liver Microsomes (178-ME-015)
- In Vitro Evaluation of YM178 as an Inhibitor of Human Cytochrome P450 Enzymes using Human Liver Microsomes (178-ME-068)
- In Vitro Evaluation of YM178 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes (178-ME-074)

In Vivo Rats

- Identification and Characterization of In Vivo Metabolites of YM178 in Rats (Study 178-ME-018)
- Identification of YM178 Metabolites Purified from Rats with Synthetic Authentic Samples (Study 178-ME-051)
- Structural Elucidation of Unidentified YM178 Metabolites in Rats (Study 178-ME-052)
- Metabolic Profiling in Rats after a Single Oral Administration of ^{14}C-YM178 (Plasma Profiling and Urine and Bile Re-profiling) (Study 178-ME-056)
- Plasma Concentrations of YM178 and Its Metabolites after a Multiple Oral Administration of YM178 to Rats (Study 178-ME-077)
- Identification of YM178 Metabolites in Rats and Humans with Synthetic Authentic Samples (Study 178-ME-083)
- Plasma Concentrations of YM178 and Its Metabolite M16 after a Multiple Oral Administration of YM178 to Rats (Study 178-ME-125)

In Vivo Mice

- Metabolite Profiles in the Plasma after a Single Oral Administration of ^{14}C-YM178 to Mice (Study 178-ME-072)
- Plasma Concentrations of YM178 and Its Metabolites after a Multiple Oral Administration of YM178 to Mice (Study 178-ME-102)
- Plasma Concentrations of YM178 and Its Metabolite M16 after a Multiple Oral Administration of YM178 to Mice (Study 178-ME-124)

In Vivo Rabbits

- Plasma Concentrations of YM178 and Its Metabolites after a Multiple Oral Administration of YM178 to Rabbits (Study 178-ME-103)
- Plasma Concentrations of YM178 and Its Metabolite M16 after a Multiple Oral Administration of YM178 to Rabbits (Study 178-ME-126)

In Vivo Monkeys

- Metabolite Profiles in the Plasma, Urine, and Feces after a Single Oral Administration of ^{14}C-YM178 to Monkeys (Study 178-ME-066)
- Plasma Concentrations of YM178 and Its Metabolites after a Multiple Oral Administration of YM178 to Cynomolgus Monkeys (Study 178-ME-078)

In Vivo Humans

- Metabolic Fingerprinting and Quantitative Analysis of Metabolites in Plasma, Urine, and Feces after a Single Oral Administration of ^{14}C-YM178 to Humans (Study 178-ME-039)
- Identification and Characterization of Metabolites in Urine and Plasma After a Single Oral Administration of ^{14}C-YM178 to Humans (Study 178-ME-046)
- Structural Elucidation of Unidentified YM178 Metabolites in Humans (Study 178-ME-055)

Elimination

- Absorption, Distribution, Metabolism and Excretion of Radioactivity After Oral Administration of ^{14}C-YM178 to Rats (178-ME-016)
- Absorption, Distribution, Metabolism and Excretion after a Single Oral Administration of ^{14}C-YM178 to Albino Rats (Study 178-ME-022)
- Metabolic Profiling in Rats after a Single Oral Administration of 14C-YM178 (Plasma Profiling and Urine and Bile Re-profiling) (178-ME-056)
- Pharmacokinetic study of YM178: Concentrations in Blood and Plasma, Urinary and Fecal Excretion, and Tissue Distribution in Monkeys after a Single Oral Administration of ^{14}C-YM178 (Study 178-ME-061)
- An Open-Label Study to Evaluate the Pharmacokinetics of YM178 After Single Oral Administration of 14C-YM178 in Healthy Male Volunteers (178-CL-007)

Drug Interaction Studies

- Evaluation of the Potential of YM178 to Inhibit the Major Human Liver Cytochrome P450 Isoenzymes (178-ME-009)
- Cytochrome P450 Drug Interaction Studies with YM178 using Human Liver Microsomes (178-ME-015)
- Characterization of the Human Esterases Involved in the Metabolism of YM178 (178-ME-079)
- In Vitro Evaluation of YM178 as an Inhibitor of Human Cytochrome P450 Enzymes using Human Liver Microsomes (178-ME-068)
- In Vitro Transport of YM178 Across Monolayers of Differentiated CACO-2 Cells (178-ME-031)
- In Vitro Inhibition of P-Glycoprotein (MDR1)-Mediated Transport (178-ME-032)
- In Vitro Evaluation of YM178 as an Inhibitor of hOCT1 and hOCT2-mediated Drug Transport (178-ME-086)
- In Vitro Evaluation of hOCT1-, hOCT2-, and hOCT3-mediated Transport of YM178 (178-ME-092)
- In Vitro Evaluation of PEPT1- and PEPT2-Mediated Transport of YM178 (178-ME-130)
- In Vitro Evaluation of P-gp-, MRP2-, and BCRP-mediated Transport of YM178 (178-ME-131)
- In Vitro Evaluation of pH-Dependent Bidirectional Transport of YM178 across Monolayers of Differentiated Caco-2 C3lls (178-ME-132)
- In Vitro Evaluation of OATP1A2- and OATP2B1-Mediated Transport of YM178 (178-ME-133)

Toxicology Studies

Single Dose

- Single Oral Dose Toxicity Study of YM178 in Rats (Study 178-TX-012)
- YM178: Single Dose Oral Toxicity Study in Dogs (Study 178-TX-017)
- Preliminary Single Intravenous Toxicity Study of YM178 in Cynomolgus Monkeys (Study 178-TX-033)
- Measurement of Drug Concentrations in Plasma Samples Originating from “Preliminary Single Intravenous Toxicity Study of YM178 in cynomolgus Monkeys” (Study 178-TX-045)

Repeat Dose

Intravenous Administration

- 2-week Intravenous Dose Toxicity Study of YM178 in Rats (Study 178-TX-035)
- 5-day Dose Range Finding Study for a 2-week Intravenous Dose Toxicity Study of YM178 in Rats (Study 178-TX-044)
- A 2-week Repeated Intravenous Dose Toxicity Study of YM178 in Cynomolgus Monkeys (Study 178-TX-034)

Oral Administration
Mice
- A 2-week Oral Gavage Toxicity Study of YM178 in Mice (Dose Range Finding Study) (Study 178-TX-028)
- A 13-week Oral gavage Toxicity Study of YM178 in Mice (Dose Range Finding Study for Carcinogenicity Study) (Study 178-TX-029)
- Histopathological Examination of "A 13-week Oral Gavage Study of YM178 in mice (dose range-finding study for carcinogenicity study)" (Study 178-TX-041)
- A Preliminary 5-day Repeated Dose Oral Toxicity Study of YM178 in Mice (Study 178-TX-051)
- A Preliminary 2-week Repeated Dose Oral Toxicity Study of YM178 in Mice (Study 178-TX-052)
- A 13-Week Repeated Oral Dose Combination Toxicity Study with YM178 and YM905 in Mice (Study 178-TX-053)

Rat
- 2-Week Oral Toxicity Study of YM178 in Rats Following Recovery Observation (Study 178-TX-013)
- Thirteen-week Repeated Oral Dose Toxicity Study of YM178 in Rats (Study 178-TX-020)
- Determination of Plasma Drug Concentration in "Thirteen-week Repeated Oral Dose Toxicity Study of Ym178 in Rats" (Study 178-TX-042)
- A 26-week Repeated Oral Dose Toxicity Study of YM178 in Rats (Study 178-TX-025)
- Determination of Plasma Drug Concentration in a 26-week Oral Toxicity Study of YM178 in Rats (Study 178-TX-030)
- Electron Microscopic Examination of Livers from a 26-week Repeated Oral Dose Toxicity Study with YM178 in Rats (Study 178-TX-040)

Dog
- YM178: 14 Day Oral Toxicity Study in Dogs (Study 178-TX-018)
- Confirmation of Oral Toxic Effects of YM178 on the Salivary Glands in Beagle Dogs (Study 178-TX-019)

Monkey
- 2-Week Oral Dose Toxicity Study of YM178 in Cynomolgus Monkeys (Study 178-TX-014) (summary review)
- Thirteen-week Oral Dose Toxicity Study of YM178 in Cynomolgus Monkeys, Followed by a 4-week Recovery Observation Period (Study 178-TX-021) (summary review)
- A 52-week Oral Toxicity Study of YM178 in Cynomolgus Monkeys (Study 178-TX-026)

<u>Carcinogenicity</u>
- 104 Week Oral Gavage Carcinogenicity Study of YM178 In Mice (Study 178-TX-031)
- 104-Week Oral Gavage Carcinogenicity Study withYM178 in Rats (Study 178-TX-032)

Genotoxicity

- YM178: Reverse Mutation in four Histidine-Requiring Strains of Salmonella typhimurium and one Tryptophan-Requiring Strain of Escherichia coli (Study 178-TX-003)
- YM178: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes (Study 178-TX-007)
- YM178: Induction of Micronuclei in the Bone Marrow of Treated Rats (Study 178-TX-008)

Reproductive and Developmental Toxicity

Fertility and Embryonic Development

- Study of Fertility and Early Embryonic Development to Implantation in Rats Administered Orally with YM178 (Study 178-TX-015)
- Study of Fertility and Early Embryonic Development to Implantation in Male Rats Treated Orally with YM178 (Study 178-TX-039)

Embryo-Fetal Development

- Effects of YM178 on Embryo-fetal Development in Rabbits by Oral Administration (Study 178-TX-004)
- Effects of YM178 on Embryo-fetal Development in Rats by Oral Administration (Study 178-TX-005)
- A Study for Effects on Embryo-fetal Development in Rabbits Treated Orally with YM178 (Study 178-TX-016)
- Effects of Orally Administered YM178 on Embryo-fetal Development in Rats (Study 178-TX-022)
- Reversibility of Wavy Ribs of Fetuses Induced by YM178 Orally Administered to Rats (Study 178-TX-023)
- Determination of Plasma Drug Concentration in “Effects of Orally Administered YM178 on Embryo-fetal Development in Rats” (Study 178-TX-043)
- Plasma Concentrations of YM178 and its Metabolites after a Multiple Oral Administration of YM178 during Non-Pregnant and Pregnant Periods in Female Rabbits (Study 178-TX-056)
- Investigational Study for Effects of the Oral Administration of YM178 on Embryo-fetal Development in Rabbits: Effects of a beta 1-Blocker (Study 178-TX-047)
- Study for Effects of the Oral Administration of YM178 on Embryo-fetal Development in Rabbits: Effects of a beta 1-Blocker (2) (Study 178-TX-057)
- Investigational Study for Effects of the Oral Administration of DI-Isoproterenol Hydrochloride on Embryo-fetal Development in Rabbits: Effect of a beta 1-Blocker (TX094001)

Pre- and Postnatal Development

- Study for Effects on Pre- and Postnatal Development, including Maternal Function in Rats Treated Orally with YM178 (Study 178-TX-038)

Local Tolerance

- Acute Dermal Irritation Study of YM178 in Rabbits (Study 178-TX-001)
- Eye Irritation Study of YM178 in Rabbits (Study 178-TX-002)
- Intravascular Irritation Study of YM178 in Rabbits (Study 178-TX-036)

Dermal Sensitization

- Skin Sensitization Study of YM178 in Guinea Pigs (Study 178-TX-024)
- A Skin Sensitization Study on YM178 in Guinea Pigs by the Buehler Test (Study 178-TX-027)

Toxicity of Impurity

- A 2-week Repeated Dose Oral Toxicity Study of YM178 (Including (b) (4) of (b) (4) in Rats (Study 178-TX-046)

Other Toxicology Studies

- In vitro Human Blood Hemolysis Test of YM178 for Injection (Study 178-TX-037)
- Effects of YM178 on Analysis of Urinary Protein Levels (Study 178-TX-049)
- Effects of YM178 (mirabegron) and metabolites (M11, M12) on qualitative and quantitative protein analysis in human urine (178-TX-058)

3.2 Studies Not Reviewed

Secondary Pharmacology

- Effects of YM178 on Lipolysis in Isolated Rat Fat Cells (Study 178-PH-002)
- Improvement of Glucose Intolerance by the Treatment of Zucker Fatty Rats with YM178 (Dose-response Study) (Study 178-PH-011)
- Effect of YM178 on Body Temperature in kk/Ay Mice (Study 178-PH-013)
- Effect of YM178 on Energy Metabolism (Heat-Production) in kk/Ay Mice (Study 178-PH-017)

Analytical Methods and Validation Reports

- Validation of a High Performance Liquid Chromatography Method for the Determination of YM178 in Dog Plasma (Study 178-ME-003)
- Validation of a High Performance Liquid Chromatography Method for the Determination of YM178 in Rat Plasma (Study 178-ME-004)
- Validation of a High Performance Liquid Chromatography Method for the Determination of YM178 in Rabbit Plasma (Study 178-ME-011)
- Validation of a High Performance Liquid Chromatography Method for the Determination of YM178 in Monkey Plasma (Study 178-ME-019)
- One-day Validation of a High-performance Liquid Chromatography Method for the Determination of YM178 in Rat Plasma (Study 178-ME-025)
- Validation of an HPLC-UV Method for Determination of YM178 in Rat Plasma (Study 178-ME-026)
- Validation of a High Performance Liquid Chromatography Method for the Detection of YM178 in Mouse Plasma (Study 178-ME-027)
- Validation of a High Performance Liquid Chromatography Method for the Detection of YM178 in Rabbit Plasma (Study 178-ME-028)
- Validation of a High Performance Liquid Chromatography Method for the Detection of YM178 in Monkey Plasma (Study 178-ME-029)
- Validation of a High Performance Liquid Chromatography Method for the Detection of YM178 in Human Plasma (Study 178-ME-030)
- Validation of Analytical Method for the Detection Concentration of YM178 in Mouse Plasma by HPLC (Study 178-ME-033)
- Validation of a High Performance Liquid Chromatography Method for the Determination of YM178 in Rat Plasma (Precision and Accuracy for Changing a Measurement Person-in-Charge, and 25-fold Dilution Measurement) (Study 178-ME-042)
- Validation of a High Performance Liquid Chromatography Method for the Determination of YM178 in Dog Plasma (Precision and Accuracy for Changing a Measurement Person-in-Charge, and 25-fold Dilution Measurement) (Study 178-ME-043)

- Validation of the Analytical Method for the Determination of YM178 in Rat Plasma by HPCL (Study 178-ME-047)
- Validation of a Method for the Determination of YM178 in Rat Plasma by HPLC with UV Detection (Study 178-ME-059)
- Validation of a Method for the Determination of YM178 in Monkey Plasma (Study 178-ME-060)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M11, M12, M13, M14, and M15 in Rat Plasma (Study 178-ME-075)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M11, M12, M13, M14, and M15 in Cynomolgus Monkey Plasma (Study 178-ME-076)
- Validation of an LC-MS/MS Method for the Determination of YM178 in Rat Plasma (Study 178-ME-093)
- Validation of an LC-MS/MS Method for the Determination of YM178 in Rabbit Plasma (Study 178-ME-094)
- Validation of an LC-MS/MS Method for the Determination of YM178 in Cynomolgus Monkey Plasma (Study 178-ME-095)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M11, M12, M13, M14 and M15 in Mouse Plasma (Study 178-ME-096)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M11, M12, M13, M14 and M15 in Rabbit Plasma (Study 178-ME-097)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5, M8, and M16 in Mouse Plasma (Study 178-ME-098)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5, M8, and M16 in Rat Plasma (Study 178-ME-099)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5, M8, and M16 in Rabbit Plasma (Study 178-ME-100)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5, M8, and M16 in Cynomolgus Monkey Plasma (Study 178-ME-101)
- Validation of an LC-MS/MS Method for the Determination of YM178 in Mouse Plasma (Study 178-ME-106)
- Partial Validation of an LC-MS/MS Method for the Determination of YM178 in Rat [Crl:CD(SD)] Plasma (Study 178-ME-114)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5 and M16 in Rat Plasma (Study 178-ME-119)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5 and M16 in Mouse Plasma (Study 178-ME-120)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5 and M16 in Rabbit Plasma (Study 178-ME-121)
- Validation of an LC-MS/MS Method for the Determination of YM178 Metabolites M5 and M16 in Cynomolgus Monkey Plasma (Study 178-ME-122)

3.3 Previous Reviews Referenced

None

4 Pharmacology

Primary Pharmacology Summary

In vitro and in vivo data in animals and humans suggest that mirabegron (YM178) is a β3-adrenergic receptor (β3-AR) agonist. Mirabegron also has potential for off target activation of β1-AR signaling in animals and humans which, at super therapeutic doses, has resulted in changes in blood pressure and heart rate.

The urinary bladder detrusor muscle responds to cholinergic stimulation by contracting and adrenergic stimulation decreases the detrusor contraction. Mirabegron is intended to alleviate overactive bladder symptoms by agonist activation of β3-AR resulting in relaxation of the bladder detrusor muscle.

Evidence for increased bladder capacity and reduced bladder contractions was reported in animals. Mirabegron was able to reduce pre-constricted isolated rat detrusor muscle, reduce distension induced bladder contractions in rats, reduce bladder contractions in a model of bladder outlet obstruction in rats, increase bladder capacity in water loaded rats and monkeys, and reduce intravesical pressure in dogs.

Molecular Pharmacology Summary

There are three beta adrenergic receptor subtypes (β1-, β2-, and β3-AR) which are all transmembrane G-protein-coupled proteins that are activated upon binding norepinephrine and epinephrine (1). The human β3-AR shares roughly 51% and 46% amino acid identity with the human β1-AR and β2-AR, respectively. β3-AR is the primary beta adrenergic receptor in the urinary bladder. Besides the urinary bladder, β3-ARs are located primarily in white and brown adipocytes where agonism can promote lipolysis and thermogenesis (primarily in rodents). β3-AR transcripts have also been detected in the brain, liver, gallbladder, pancreas, stomach, small intestine, skeletal muscle, left ventricle and atrium, lung, kidney, prostate, and corpus cavernosa (2 and 3). Activation of β3-AR may inhibit the contraction of the urinary bladder detrusor, ileum, colon, gallbladder, myocardium, and myometrium, and increase blood vessel dilation.

Upon binding epinephrine or norepinephrine β-ARs undergo a conformational change and activate G-proteins (Gs or Gi) (1 and 2 and 4). Upon activation, the Gsα subunit activates adenylyl cyclase which catalyzes the formation of cAMP. cAMP acts as a second messenger to activate protein kinase A (PKA). Activation of PKA through several steps ultimately results in regulation of thermogenesis and lipolysis in adipocytes. Activation of Gi in the endothelium ultimately results in production of nitric oxide (NO) resulting in vasodilation and negative ionotropic effects in cardiomyocytes.

Prolonged activation of β1-AR or β2-AR results in inactivation and endocytosis of these receptors (desensitization) (3). After ligand binding, Gsα is released resulting in activation of cAMP dependent activation of PKA. Consequently β-adrenergic receptor kinase (BARK) is phosphorylated by PKA. BARK is then drawn to Gsβγ associated with β-AR where it phosphorolates β-AR. Phosphorlyated β-AR then recruits β-arrestin which

inhibits the interaction between G-proteins and the β-ARs and promotes removal of β-ARs from the cytoplasmic membrane by endocytosis. It is unclear if β3-AR is desensitized after prolonged treatment (1 and 3). The β3-AR may resist desensitization because it does not possess the sequence necessary for BARK phosphorylation. Lacking this sequence, β-arrestin can not bind to the ligand bound β3-AR and inhibit its association with G-proteins.

4.1 Primary Pharmacology

Summary of Beta Adrenergic Receptor Selectivity:
Mirabegron is a beta-3 adrenergic agonist. In vitro data suggests that mirabegron is primarily a β3-AR agonist with minimal β1-AR and little β2-AR activity in animals and humans. However, animal models and a clinical study suggest that mirabegron has clinically relevant β1-AR activity in vivo. In vivo, β1 activation is suspected in animals since adverse cardiac effects including increased heart rate in adult rats, rabbits, and dogs were at least partially reversed by metoprolol (β1 antagonist) and/or propanolol (β1/β2 antagonist). The sponsor also noted that mirabegron elevated the heart rate in rabbits and monkeys (β1 effect) without lowering blood pressure (lowering blood pressure is a β3 pharmacologic effect), suggesting that mirabegron may directly activate β1 receptors in cardiomyocytes in these species. Additionally humans administered a single 200 mg dose of mirabegron responded with elevated heart rate and systolic blood pressure which were both repressed by coadministration with propanolol (β1/β2 antagonist) or bisoprolol (β1 antagonist), suggesting that mirabegron activates β1 receptors in humans (Study 178-CI-053). The sponsor acknowledged the potential for off target β1-AR activation in humans at super therapeutic doses (b) (4)

4.1.1 Mechanism of Action – In vitro Beta Adrenergic Signaling Studies:

β-Adrenergic Receptor Competitive Binding Assay – Inconclusive Results:
In vitro competitive binding assays yielded inclusive results (178-PH-005). Although this study suggest that mirabegron may preferentially bind to the human β3-AR in comparison to the β1 and β2 receptors, the poor specificity for the β3-AR confounds comparison in selectivity between receptor types. Mirabegron bound to the three human β-adrenergic receptors (ARs) with binding affinities (Ki) for β1-, β 2-, and β3-ARs of 4200 nM, 1300 nM, and 40 nM, respectively. This was determined by the ability of mirabegron to displace radioligands (β1 and β3 used ^{125}I-cyanopindolol and β2 used ^{3}H-CGP-12177) in cell membrane preparations that expressed one of each of the β-ARs. However, despite the high affinity for the β3-AR, the specificity was poor. Poor specificity is indicated by the gradual slope of the competitive binding curve for the human β3-AR in comparison to the β1 and β2 receptors (See figure below). In addition, although the Ki values differed, the maximal inhibition occurred at a similar mirabegron

concentration for all three β-ARs suggesting that there may not be biologically significant differences in selectivity at some exposure levels. In addition to the poor selectivity for the β3 receptor, it is difficult to definitively compare results with the different receptors in this assay since the assay employed different radioligands, the concentration of the radioligands was not mentioned, and the amount of β-AR protein in each assay was not determined.

Competitive Radioligand Binding with YM178 to Human β-Adrenergic Receptors

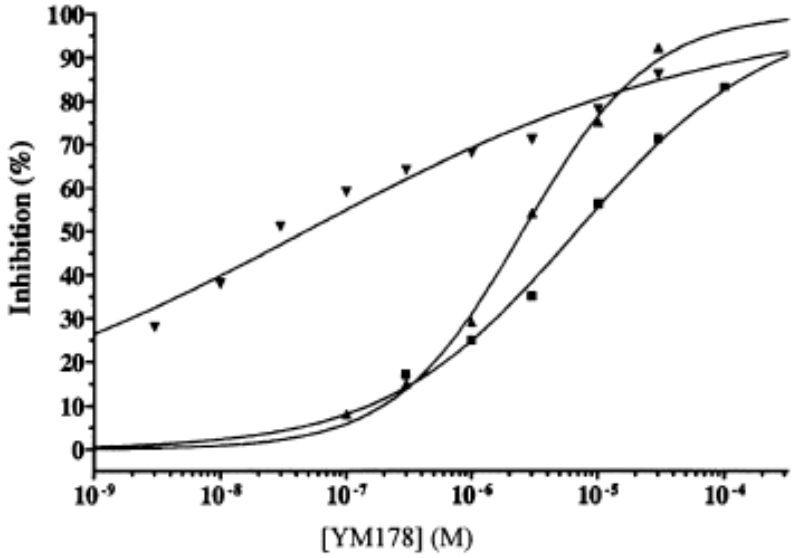


Fig. 1 Inhibition of specific radioligand binding by YM178. ■ Adrenergic β1 (human), ▲ Adrenergic β2 (human), ▼ Adrenergic β3 (human). Mean on 3 separate experiments for each ligand. Test compound tested in duplicate at each concentration. Displacement curves were fitted using non-linear regression analysis.

Figure copied from Sponsor's Figure 1 in study 178-PH-005.

In vitro Activation of Human, Rat, Dog and Monkey β1, β2 and β3-Adrenergic Receptors by mirabegron:

The agonist activity of mirabegron for human, rat, dog and monkey β1, β2 and β3 adrenergic receptors was assessed in Chinese hamster ovary cells (CHO) that individually express one of the recombinant adrenergic receptors from each species (178-PH-044, 178-PH-51, 178-PH-52, 178-PH-53). Receptor activation was quantified by the production of intracellular cyclic AMP (cAMP) due to increased adenylate cyclase activity. The nine most common human metabolites of mirabegron were also assessed in the cells expressing the human β-ARs. Cells were incubated for 30 minutes in the presence of a phosphatase inhibitor (3-isobutyl-1-methylxantine) and 1 to 10,000 nM mirabegron or the non-specific agonist isoproterenol as a positive control. The concentrations of mirabegron encompass the therapeutic Cmax (66.2 ng/mL = 167 nM) at the MRHD (50 mg/day) (178-CL-072). The concentration of mirabegron that was

required to achieve 50% of the maximal cAMP response after isoproterenol treatment (EC_{50}) was calculated for each receptor as a measure of potency. Intrinsic activity (IA) was calculated as the proportion of maximal intracellular cAMP in response to isoproterenol as a measure of maximal efficacy. Comparisons should not be made directly between species or between receptor subtypes within a species since the amount of β-AR protein was not measured in each culture well, data was not normalized to the number of cells or total amount of protein, and the maximal level of isoproterenol activity (IA) may have varied between species and β-AR type. The expression of β1, β2, and β3-ARs protein was determined outside of these experiments and the levels varied significantly between and within species (Table below).

Variable Expression of β-Adrenergic Receptor Protein (fmol/mg protein) (preliminary data collected prior to conducting activity assays)			
	β1-AR	β2-AR	β3-AR
Human	150	630	200
Rat	860	940	730
Dog	580	1,100	200
Monkey	2,200	610	230

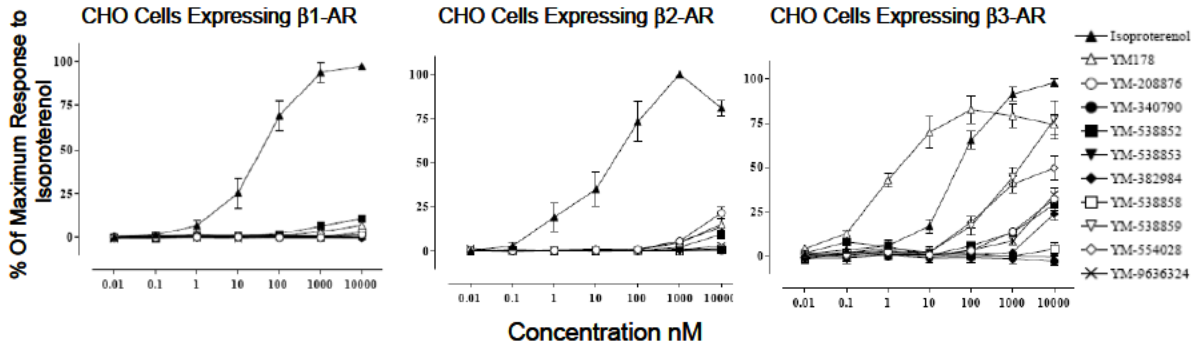
These assays suggest that mirabegron can activate the β3 receptors of all four species but mirabegron is anticipated to only slightly activate the β1 receptor in non-human models while having very little β2 activity in any species. Mirabegron showed roughly 11, 3, 23, and 5 times greater potency than isoproterenol for the human, rat, dog, and monkey β3-AR, respectively (divide isoproterenol EC_{50} by mirabegron EC_{50}). Within each species the maximal efficacy of mirabegron and isoproterenol were similar for the β3-ARs (IA range 0.8-1.0). The IC_{50} for human β3-AR activity is 50 fold less that the mirabegron Cmax in elderly fasted women dosed with mirabegron at 50 mg for 7 days (66 ng/ml or 167 nM) suggesting that serum levels of mirabegron would be expected to be at pharmacologically active concentrations (178-CL-072). In this assay, mirabegron was unable to appreciably activate the β2 receptors in humans, rats, dogs, or monkeys. Although little to no activity was detected with the human β1 receptor, mirabegron did slightly activate the β1 receptor in rats, dogs, and monkeys although the maximal potency was only 20-30% of isoproterenol in dogs and monkeys and 60% in rats.

Summary of In vitro Activation of Beta Adrenoceptors in Rats, Dogs and Monkeys							
		β1-AR		β2-AR		β3-AR	
		EC50 (nM)	IA	EC50 (nM)	IA	EC50 (nM)	IA
Human	Isoproterenol	37	1.0	20	1.0	37	1.0
	mirabegron	-	0.1	-	0.1	3.4	0.8
Rat	Isoproterenol	31	1.0	110	1.0	60	1.0
	mirabegron	610	0.6	-	0.1	19	1.0
Dog	Isoproterenol	80	1.0	39	1.0	180	1.0
	mirabegron	-	0.3	-	0.1	7.9	0.8
Monkey	Isoproterenol	84	1.0	77	1.0	170	1.0
	mirabegron	-	0.2	-	0.1	32	0.8

Table adapted from the sponsor. The values are means of four experiments each conducted in duplicate. **EC50** - the concentration of agonist which is necessary to raise the cAMP level to ½ of that caused by the maximal response to Isoproterenol. **IA**- intrinsic activity (maximal efficacy) = the maximal response divided by the maximal response of Isoproterenol. Similar findings were reported with the human beta adrenergic receptors in a fairly similar study (178-PH-003).

Effect of YM178 and Its Metabolites on Human Beta Adrenergic Dependent cAMP Production in CHO Cells

YM178 Open Triangles, Isoproterenol Closed Triangle



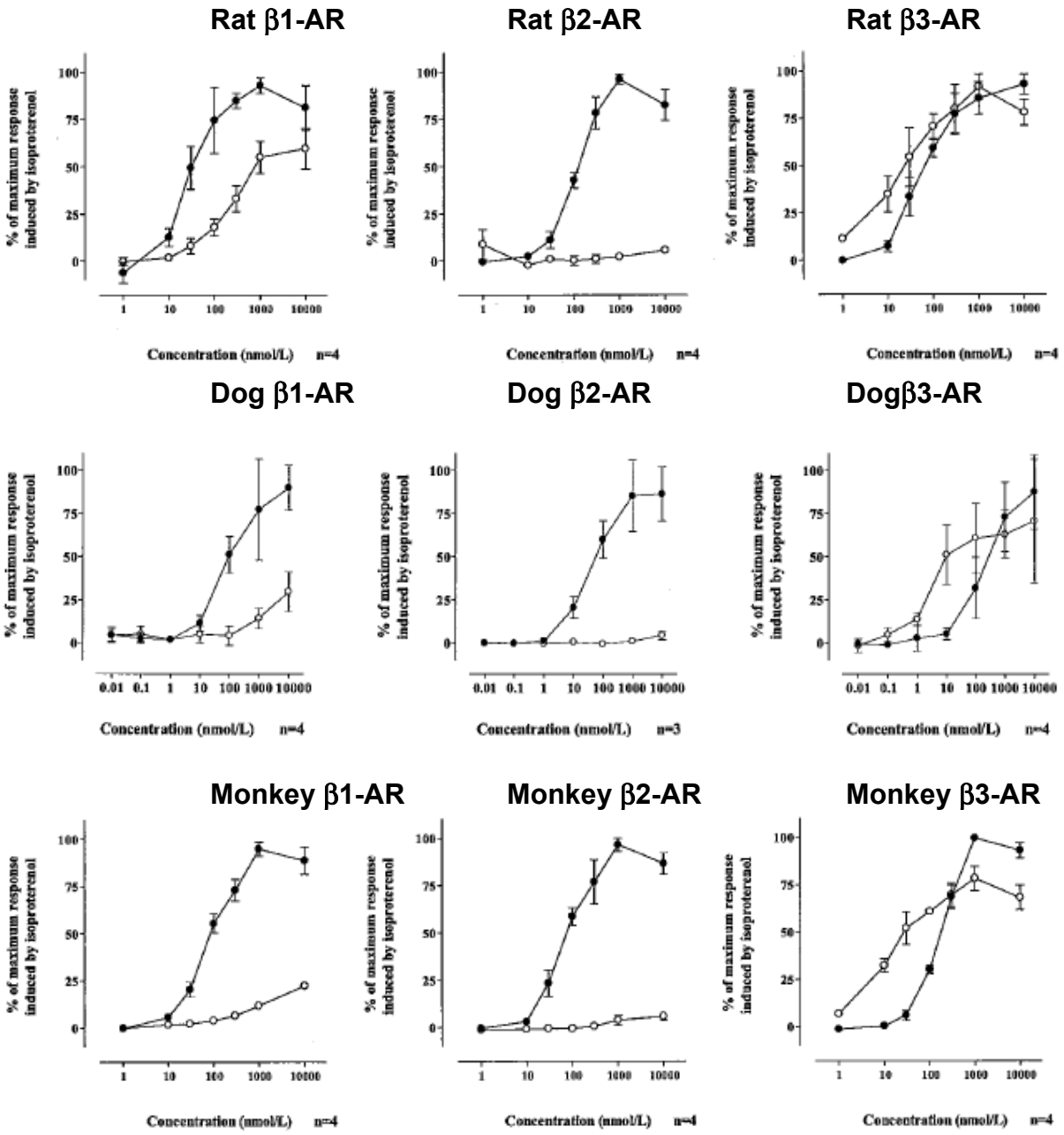
Figures copied from Sponsor (Study 178-PH-044). Each point represents mean ± SEM of 4 independent experiments in duplicate.

Of the metabolites assessed in this assay only M13 and M14 demonstrated β3-agonism with maximal responses being 0.8 and 0.5 times, respectively, that of Isoproterenol (Table below). However, neither M13 nor M14 are suggested to be pharmacologically relevant because both are glucuronide metabolites and the EC50 values were very large. Additionally neither mirabegron nor its metabolites were able to appreciably activate the human β1 or β2 receptors in this assay.

Summary of Agonist Activity of Mirabegron and Human Metabolites for the Human β1, β2, and β3-AR						
Agonist	**β3**		**β1**		**β2**	
	EC50 (nM)	**IA**	**EC50 (nM)**	**IA**	**EC50 (nM)**	**IA**
Isoproterenol	53	1.0	37	1.0	20	1.0
Mirabegron	3.4	0.8	-	0.1	-	0.2
M16 (YM-208876)	-	0.3	-	0	-	0.2
M9 (YM-340790)	-	0	-	0	-	0
M5 (YM-538852)	-	0.3	-	0.1	-	0.1
M8 (YM-538853)	-	0	-	0	-	0
M11 (YM-382984)	-	0.2	-	0	-	0
M12 (YM-538858)	-	0	-	0	-	0
M13 (YM-539959)	1,400	0.8	-	0	-	0
M14 (YM-554028)	-	0.5	-	0	-	0.1
M15 (YM-9636324)	-	0.4	-	0	-	0

Table adapted from the sponsor Table 1 (178-PH-044). EC50 - the concentration of agonist which is necessary to raise the cAMP level to ½ of that caused by the maximal response to Isoproterenol. IA- intrinsic activity (maximal response) = the maximal response for each drug divided by the maximal response of Isoproterenol. The values are means of four experiments each conducted in duplicate.

***In Vitro* Activity of Rat, Dog and Monkey recombinant β1-AR, β2-AR and β3-AR in Response to YM178 (open circles) or Isoproterenol (closed circles)**



Figures copied from the Sponsor (Studies 178-PH-51, 178-PH-52, 178-PH-53). Data presented as percent of maximal response induced by isoproterenol ± SEM of 4 duplicate experiments.

4.1.2 Pharmacodynamic Investigations

Beta Adrenergic Dependent Relaxation of Urinary Bladder Smooth Muscle in Vitro

Initial studies found that mirabegron and isoproterenol (a mixed β1/β2-agonist) were able to relax carbachol induced constriction of smooth muscle isolated from the detrusor of rats and humans in a concentration dependent manner (178-PH-029 and 178-PH-031). In these studies mirabegron was 4 and 28 times less potent than isoproterenol in relaxing the detrusor muscle from rats and human tissues, respectively.

To provide data suggesting that mirabegron relaxes detrusor smooth muscle contraction through β-adrenergic signaling, isolated rat bladder smooth muscle strips were exposed to vehicle or either the β1-AR antagonist CGP-20712A or the β2-AR antagonist ICI-118,551 for ten minutes (Study 178-PH-057). Then the cells were exposed to vehicle or ascending concentrations of mirabegron or the general β1/β2-adrenergic agonist isoproterenol (10^{-9} to 10^{-4} M) and the relaxant response was measured. To determine the maximal relaxant potential, cells in all groups were exposed to papaverine (elevates cAMP) after reaching the maximal mirabegron or Isoproterenol concentration.

Isoproterenol appeared to have better potency (10 fold lower exposure to see relaxation) but was less efficacious (less maximal effect) than mirabegron in relaxing the bladder contraction (see figure below). Pretreatment with either β1- or the β2-AR antagonists failed to impair the relaxation of the bladder strips by mirabegron suggesting that relaxation by mirabegron is mediated by β3-AR signaling or another mechanism. Isoproterenol dependent relaxation was impaired by the β2-AR antagonist but not the β1-AR antagonist. This indicates that there is potential for both β2- and β3-AR dependent relaxation of the bladder detrusor muscle in rats.

Effects of β-Adrenergic Antagonists on the Relaxation Induced by Mirabegron and Isoproterenol in Isolated Strips of Rat Bladder Smooth Muscle

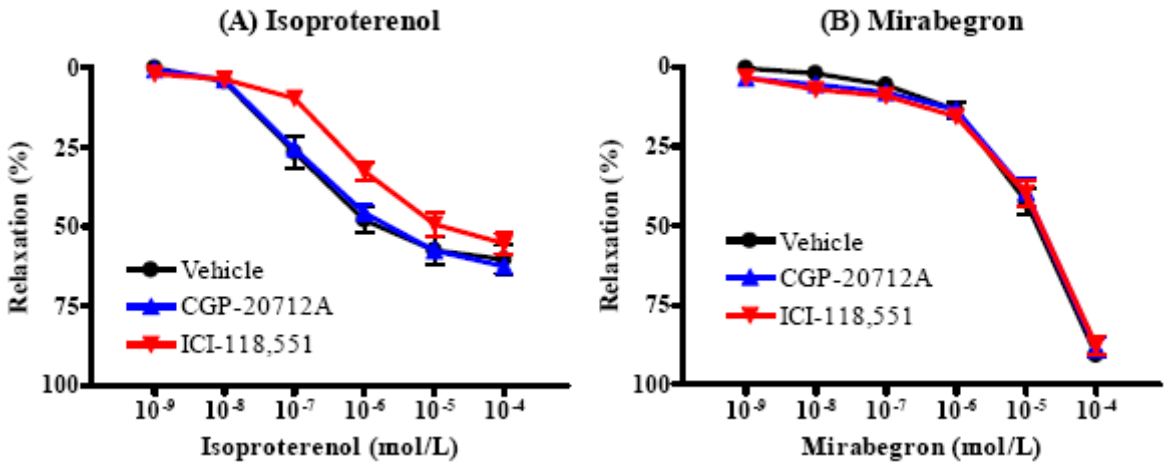


Figure copied from sponsor Figure 1 (178-PH-057). Effects of isoproterenol (A) and mirabegron (B) are shown. Carbachol (10^{-6} mol/L) was added to induce a contractile response. After generated tension had stabilized, CGP-20712A (10^{-7} mol/L), ICI-118,551 (10^{-7} mol/L), or vehicle was added. Ten minutes after the addition of CGP-20712A, ICI-118,551, or vehicle, mirabegron or isoproterenol was cumulatively added in the concentration range of 10^{-9} to 10^{-4} mol/L. Data are shown as the mean ± SEM of 5 independent experiments.

Beta-Adrenergic Signaling in Isolated Rat Urinary Bladder Tissue

Activation of β-adrenergic signaling was assessed by quantification of intracellular cyclic AMP from isolated rat bladders strips exposed to mirabegron (0, 0.1, 1.0, or 10 μM), the general β-agonist isoproterenol (0, 0.001, 0.01, or 0.1 μM), or forskolin (0.3 μM) as a positive control (Study 178-PH-055). Cells were exposed for ten minutes. The study was repeated three times each with six replicates per group. Cyclic AMP was elevated by mirabegron at ≥ 1 μM and isoproterenol at ≥ 0.1 μM suggestive of the presence of beta adrenergic receptors in the urinary bladders of rats.

Elevated cAMP in Isolated Rat Balder Strips Exposed to Mirabegron or Isoproterenol

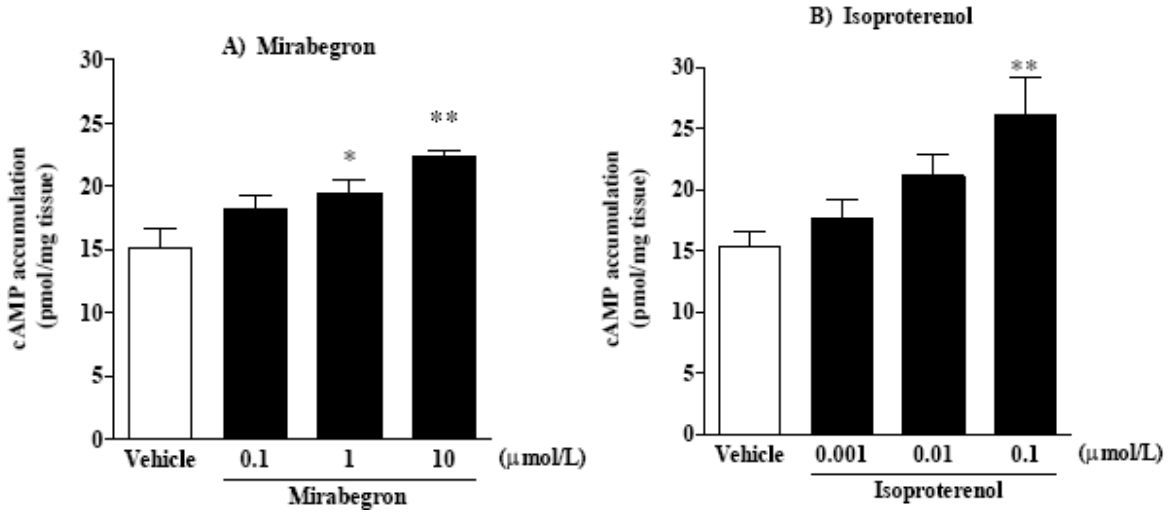


Figure copied from Sponsor Figure 1 (178-PH-055). Effects of mirabegron (A) and isoproterenol (B) are shown. Each column indicates the mean + SEM of 6 independent experiments. *P<0.05, **P<0.01: significant difference compared with vehicle group (Dunnett's multiple comparison test).

Repression of Urinary Bladder Contraction by Mirabegron in Rats and Dogs*:*
In a preliminary study with anesthetized female rats, the frequency but not amplitude of rhythmic bladder contractions induced by instillation of saline into the bladder was reduced by intravenous exposure of mirabegron at 3 mg/kg but not at lower doses (178-PH-030). A follow up study found that intravesical pressure was dose dependently decreased by IV doses of mirabegron at ≥ 0.03 mg/kg (178-PH-035). For these studies, both ureters were tied off and sutured. A urethral catheter was installed and a pressure transducer was placed in the bladder. Saline was installed into the bladder to a pressure of 4.4 mm (178-PH-035) or until rhythmic contractions were recorded (178-PH-030), and after 5-15 minutes the vehicle or mirabegron was administered IV. The sponsor reported that 6-10 mm of pressure is sufficient to stimulate distension evoked bladder contractions in anesthetized rats.

To determine the serum levels necessary to reduce distension induced bladder contractions, rats were administered mirabegron intraduodenally, bladder contractions were monitored 30 to 60 minutes after dosing, and serum levels of mirabegron were measured. Intraduodenal administration of mirabegron at ≥ 3 mg/kg (serum levels ≥ 114 ng/mL) reduced the frequency but not amplitude of saline induced rhythmic bladder contractions in anesthetized rats (178-PH-032). This suggests that low blood levels of mirabegron may increase bladder capacity (decrease contraction frequency) while potentially not effecting micturition reflex (contraction amplitude) in rats. Single and 14 days of intraduodenal dosing in female rats at 30 mg/kg had similar reductions in distension induced frequency without affecting the amplitude of bladder contraction suggesting that the pharmacologic effect may not be altered by repeated dosing in rats (178-PH-036).

Urgency Model in Rats:
The effect of mirabegron on bladder function was assessed in female rats with surgically induced bladder outlet obstruction (proximal urethra was restricted surgically) (178-PH-033). Mirabegron was administered IV at 0.1 to 3 mg/kg (n = 6/group). Pre-micturition contraction was used as the index of urgency. Bladders were infused through a catheter in the apex with saline at 5-15 mL/hr to mimic bladder outlet obstruction or 3.5 mL/hr in sham treated rats. In the bladder obstructed rats, mirabegron at ≥ 1 mg/kg reduced the frequency of pre-micturition contraction but did not affect the amplitude of pre-micturition contraction, micturition pressure, threshold pressure, residual volume, voiding volume, or bladder capacity.

Overactive Bladder Models in Rats, Dogs, and Monkeys:
The effect of mirabegron on bladder capacity was assessed in water-loaded male rats with cerebral infarction (178-PH-034). Cerebral infarction was induced by occlusion of the middle cerebral artery which reduced the bladder capacity from 1.74 mL to 0.71 mL. Rats were orally dosed with vehicle or mirabegron at 0.3 to 3 mg/kg. One hr later they were loaded with water at 30 mL/kg and voided volume was measured for the next 1.5 hrs. Mirabegron at 3 mg/kg increased the bladder capacity.

The ability of intravenous mirabegron (0.0003 to 0.01 mg/kg) to reduce the intravesical pressure induced by the cholinergic carbachol (1.8 μg/kg IV) was assessed in anesthetized male beagle dogs (Study 178-PH-059). Ascending doses of mirabegron were administered every five minutes followed by a carbachol challenge and measurement of the intravesical pressure. Mirabegron dose dependently reduced the intravesical pressure with a 20% reduction at 0.0003 mg/kg and a roughly 75% reduction at 0.01 mg/kg (ED_{30} was 0.0005 mg/kg).

The effect of mirabegron on micturition frequency and bladder capacity was also assessed in water-loaded male Cynomolgus monkeys (178-PH-037). Monkeys were orally dosed with mirabegron at 0, 0.3, 1, or 3 mg/kg. Fifteen minutes later they were orally administered water at 50 mL/kg and micturition frequency and volume was recorded for the following 8 hrs. Mirabegron decreased micturition frequency at ≥ 1 mg/kg and increased bladder capacity (urine volume/micturition frequency) at 3 mg/kg.

Effect of Mirabegron on Carbachol-Induced Intravesical Pressure (IVP) Elevation in Anesthetized Dogs

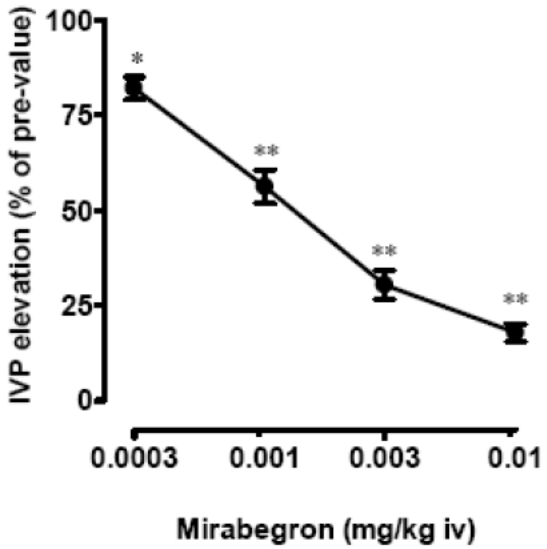


Figure Copied from Sponsor Figure 1. Mirabegron was intravenously administered at increasing doses. At 5 min after each dosing, carbachol (1.8 µg/kg) was intravenously administered. Each point represents the mean ± SEM of IVP elevation relative to pre-values obtained from 6 dogs. *P<0.05, **P<0.01, significant difference from pre-values using actual values (Dunnett's multiple comparison test using within subject error).

4.2 Secondary Pharmacology

Affinity of Mirabegron for Off Target Proteins

Off-target binding to a battery of receptors, ion channels, transporters, and enzymes was assessed in vitro by the ability of a 10 fold excess of mirabegron (10 µM) to inhibit biding of known radiolabeled ligands at 1 µM (178-PH-038). Under the conditions tested, mirabegron inhibited the ability of five known ligands to bind their cognate receptors with greater than 50% inhibition (table below).

Significant Screening Receptor/Transporter Binding Assay Results (178-PH-038)	
Receptor/Transporter	Inhibition of Binding > 40%
α1A-Adrenergic	65.5%
Dopamine Transporter (human)	79.1%
Muscarinic Receptor M1	43.0%
Muscarinic Receptor M2	55.4%
Muscarinic Receptor M3	16.9%
Neurokinin NK1 (human)	41.4%
Sodium Channel Site 2	61.7%
Norepinephrine Transporter (human)	51.9%
Serotonin transporter (human)	45.2%

In vitro filter binding assays determined that the affinity of mirabegron for the rat α1A-adrenergic receptor, human M2 muscarinic receptor, rat sodium ion channel, human dopamine transporter, and human norepinephrine transporter was 1.0, 1.5, 11, 2.1, and 6.6 µM respectively (178-PH-049). Since mirabegron had much greater IC50 and Ki values than the traditional ligands used as positive controls in this assay, it suggests that mirabegron is a relatively weak binder for these targets.

Summary of Receptor/Transporter Filter Binding Assays (178-PH-049)			
Receptor/Transporter	**Agonist**	**Ki (µM)**	**IC50 (µM)**
α1A-Adrenergic Receptor (rat)	Mirabegron	1.01	4.44
	Prazosin	NC	< 0.030
Dopamine Transporter (human)	Mirabegron	1.45	1.70
	GBR12909	NC	< 0.030
Norepinephrine transporter (human)	Mirabegron	11.0	15.0
	Desipramine	NC	< 0.030
M2-Muscarinic (human)	Mirabegron	2.10	7.02
	Atropine	NC	< 0.030
Sodium ion channel site 2 (rat)	Mirabegron	6.59	9.18
	Dibucaine	0.20	0.28
NC = Ki values could not be calculated because EC50 values could not be generated (the positive control was too efficacious). IC50 = the concentration of mirabegron or other agonist needed to displace 50% of the radiolabeled ligand.			

Affinity of Mirabegron Metabolites for Off Target Proteins

Mirabegron metabolites identified in humans were evaluated for their ability to inhibit radioligand binding to a group of ion channels, transporters, and enzymes (178-PH-039 to 178-PH-048). Beta adrenergic receptors were not included in the assay. Mirabegron was in 10 fold excess (10 µM vs. 1 µM) compared to the radiolabeled ligand. Only M5 and M16 inhibited any of the binding assays by more than 50%. M5 inhibited dopamine transporter and opiate receptor binding. M16 inhibited dopamine transporter and norepinephrine transporter binding.

Metabolites of Mirabegron Inhibiting Ion Channels or Transporters by 50%		
	Percent Inhibition	
	YM538852 (M5)	YM-208876 (M16)
Dopamine transporter	83	73
Norepinephrine transporter		68
Opiate (non-selective)	49	
Opiate µ	47	
The following metabolites showed less than 50% inhibition: YM340790 (M9), YM538852 hydrochloride (M5), YM538853 triflouracetate (M8), YM382984 (M11), YM208876 (M16), YM538858 (M12), YM538859 (M13), YM9636324 (M15) and YM554028 (M14).		

Effects in Diabetic Rodent Models

Studies on basal metabolism were conducted because β3-AR agonists have been reported to increase lipolysis in white adipose tissue and increases thermogenesis in brown adipose tissue. Additionally the sponsor reported studies where β3-AR agonists were shown to increase basal metabolism and to reverse hyperglycemia in a diabetic mouse model.

Mirabegron induced lipolysis in isolated fat cells from rats (178-PH-002).

A strain of obese diabetic mice (kk/A^y) were used to study the effect of two weeks of oral mirabegron dosing at 0, 3, 10, or 30 mg/kg on body temperature (178-PH-013), oxygen consumption, glucose, insulin, non-esterified fatty acids, triglycerides, hyperglycemia, and hyperinsulinemia (178-PH-017). This strain of mice was chosen because it is a genetic model of diabetes and obesity and the animals are hyperglycemic and hyperinsulinemic.

Two weeks of dosing kk/Ay mice with mirabegron at ≥ 3 mg/kg resulted in a dose dependent increase in body temperature within 60 minutes of dosing but did not affect basal body temperature (temperature prior to daily dosing) throughout the two week period (178-PH-013). The increased body temperature is likely due to increased lipolysis of brown and white fat. Although basal metabolism was not affected by mirabegron in these kk/Ay mice, energy expenditure (calorie expenditure) was elevated for 1-9 hours at 30 mg/kg from the first to the last dose and for 2-5 hours after one to two weeks of dosing at 10 mg/kg (178-PH-017). After two weeks of dosing kk/Ay mice at ≥ 3 mg/kg, non-esterified fatty acids were reduced (-18 to -58%) and at ≥ 10 mg/kg blood glucose (-50%), plasma insulin (-79 to -95%) and plasma triglycerides (- 36% to -73%) were all reduced (178-PH-017).

Another rodent model of diabetes, fasted Zucker Fatty rats, which are glucose intolerant, were employed to see if mirabegron could increase insulin levels and also reduce glucose levels in response to an oral glucose challenge (178-PH-011). Rats were administered 0, 3, 10, or 30 mg/kg of mirabegron daily for two weeks. They were challenged with oral glucose solution 30 minutes after mirabegron dosing on day 1 and 14. Serum glucose and insulin were assessed -30 to 240 minutes after glucose challenge on the first day of dosing and after two weeks of dosing. A single dose of mirabegron at 3 to 30 mg/kg reduced the elevation of glucose that is normally observed after a glucose challenge (glucose AUC reduced 23-37%). Additionally, prior to glucose challenge, single doses of mirabegron at ≥ 10 mg/kg elevated insulin levels and they remained elevated between 60 to 240 minutes after the glucose challenge (insulin AUC increased 268-288%). Although glucose levels were still reduced (AUC reduced 24-30%) after glucose challenge after two weeks of mirabegron exposure, the dose responsive changes in insulin levels were only observed at 10 mg/kg (insulin AUC increased 47%) and not at 3 or 30 m/kg.

4.3 Safety Pharmacology

4.3.1 CNS Safety Pharmacology

Recoverable clinical signs in mice after single oral doses include prone position with slight hyperthermia at ≥ 10 mg/kg, and decreased alertness, spontaneous movement, and decreased tonus of the limbs and abdominal muscles at 100 mg/kg (178-PH-019). In mice mirabegron did not affect CNS endpoints including the duration of hexobarbital

anesthesia or convulsions induced by electroshock or pentetrazol at single oral doses up to 100 mg/kg (178-PH-019). Pain perception (Randall-Selitto Method) was not effected in rats after single oral doses up to 100 mg/kg (178-PH-019). Assessments of pain perception, use of convulsive agents, and electroshock were not requested by the Agency.

Male rats (n=6/group) were observed for neurological effects (FOB) for 24 hrs after single oral doses mirabegron at 0, 30, 100, or 300 mg/kg (7, 34, 96x MRHD) (178-PT-003). Mirabegron at ≥ 100 mg/kg had slight to moderately suppressive affects on the CNS. The only sign at 30 mg/kg was a single incidence of decreased spontaneous activity 2-8 hrs after dosing. Adverse clinical signs included deep respiration, prone position, decreases in activity, grip strength, body and abdominal tone, and righting reflex, as well as prone position and eyes closed. No adverse findings were observed in the control group. Rats recovered from all adverse effects except that 3/6 HD rats were still respiring deeply 24 hrs after dosing.

Incidence of Adverse Neurological Effects in Rats (Incidence/N)								
Finding	**Grade**	**Dose (mg/kg)**	**Time 0**	**1 hr**	**2 hr**	**4 hr**	**8 hr**	**24 hr**
Deep Respiration		100	-	-	1/6	1/6	-	-
		300	-	4/6	5/6	6/6	6/6	3/6
↓ Activity	+	30	-	-	1/6	1/6	1/6	-
	+	100	-	-	-	1/6	2/6	-
	+	300	-	3/6	1/6	0/6	3/6	-
Laying on Side		100	-	-	1/6	-	-	-
		300	-	2/6	-	-	-	-
Prone Position		300	-	1/6	2/6	2/6	0/6	0/6
↓ Grip Strength	++	100	-	-	-	-	1/6	-
	++	300	-	-	-	-	6/6	-
↓ Righting Reflex	+	300	-	-	-	-	1/6	-
Eyes Closed	+	100	-	-	-	1/6	1/6	-
	+	300	-	-	-	-	6/6	-
↓ Body Tone	++	300	-	-	-	-	6/6	-
↓ Abdominal Tone	++	300	-	-	-	-	6/6	-
Grade: - normal, + slight, ++ moderate. Table adapted from sponsor Table 1 (178-PT-003). Observations included clinical signs, cage side observations, startle response, response to touch, response to holding, and pain response.								

In monkeys, no obvious clinical signs of CNS toxicity were observed after single oral doses up to 100 mg/kg (49x MRHD) (178-PT-004).

4.3.2 Renal and gastrointestinal safety pharmacology

Renal effects: Mirabegron drastically decreased urine volume (75-92%) and concentration of urine Na (72-94%), K (73-81%), and Cl (77-98%) for 0-3 hrs after dosing at ≥ 10 mg/kg in water loaded rats (178-PH-019). These were recoverable findings when urine was sampled from 3-6 hrs after dosing, except that chloride was still repressed at ≥ 30 mg/kg. A NOEL for these effects was 3 mg/kg.

Gastrointestinal effects: Mirabegron did not affect gastric transit time in mice at single oral doses up to 100 mg/kg in mice (178-PH-019).

Mirabegron did not directly affect contractions of ileum tissue isolated from guinea pigs but did inhibit acetylcholine, histamine, $BaCl_2$, and serotonin induced contractions (178-PH-019).

4.3.3 Respiratory safety pharmacology:

In dogs a single oral dose of mirabegron at ≥ 0.3 mg/kg reduced systolic (~30%) and mean (~10%) but not diastolic blood pressure for eight hours (178-PH-019). Perhaps as a response to decreased blood pressure, heart rate increased roughly 100% (60-100 bpm increase over ~ 65 bpm baseline) and respiration rate increased roughly two fold at 3 mg/kg for two hrs and at 10 mg/kg for eight hrs (178-PH-019). Clear effects on arterial O_2, CO_2, and pH were not observed in these dogs at up to 10 mg/kg. Cardiac effects in these dogs are described in Section 4.3.4.2.

In monkeys there was no affect of mirabegron on arterial CO_2, O_2, pH, hemoglobin O_2 saturation, sodium, potassium, calcium, or chloride in monkeys after singles doses up to 100 mg/kg (49x MRHD) (178-PH-004).

4.3.4 Cardiac Safety Studies

General cardiac safety pharmacology was assessed in rats, rabbits, dogs, and monkeys. Mechanistic studies were conducted in rats and dogs to evaluate the mechanism of increased heart rate. To assess proarrhythmic risk of mirabegron and its five major metabolites, the sponsor conducted cardiac ion channel assays, hERG assays, isolated cardiac papillary muscle action potential assays, and canine ventricular wedge assays.

4.3.4.1 Proarrhythmic Evaluation - In Vitro Studies

Effects of M178 and its Five Metabolites on Four Cardiac Ion Channels
The potential for 10 μM mirabegron and its five primary metabolites (M5, M11, M12, M14, and M16) to activate four human cardiac ion channels was assessed by whole cell patch clamp analysis in HEK or CHO cells transfected with the sodium channel (hNav1.5), calcium channel (hCav1.2), and either of two potassium channels (hKvLQT1/hminK or hKv4.3/Kchi2.2) (Study 178-PT-011). The 10 μM concentration of mirabegron is 60 times the mean clinical Cmax (66.3 ng/ml) however this choice of mirabegron exposure may be low since the positive controls ranged from 0.1 to 2,000 μM. Mirabegron inhibited the sodium and calcium channels by 49% and 15% respectively. The only metabolite with greater than 10% inhibition was M16 which inhibited the sodium channel by 11%. Inhibition of the hNAV1.5 sodium channel may not be indicative of QT prolongation since it functions in Phase 0 of ventricular action potential and the QT interval occurs in Phase 2-3.

Effect on Repolarization by the I_{Kr} Potassium Channel (hERG assay)
Since blocking cardiac potassium channels can increase the duration of repolarization of cardiac cells, prolong the QT interval leading to torsade de pointes (TdP) and ventricular fibrillation, the ability of mirabegron and its metabolites to inhibit the rapid delayed rectifier potassium channel (I_{Kr}) was assessed in several hERG assays.

Methods: The potential for mirabegron and its five principal metabolites M5, M11, M12, M14, and M16 to reduce potassium channel currents was assessed in a series of whole cell patch clamp studies in human embryonic kidney cells (HEK293) stably transfected with the human ether-a-go-go related gene (hERG) which codes for the potassium rectifier (I_{Kr}) (GLP Studies 178-PT-002, 178-PT-006, 178-PT-008, 178-PT-012, 178-PT-015). Although there were differences, the study protocols were fairly similar. The change in peak tail currents was assessed prior to exposure and 10-11 minutes after exposure to DMSO vehicle, the positive control E-4031 (0.1 μM), mirabegron (0.03 to 30 μM) or its principal metabolites M5, M11, M12, M14, or M16 (0.3 to 30 μM). Five cells were assessed per group. The dosing solutions were within 96-102% of the desired range. The positive control (E-4031) responded as expected, repressing currents by 73-86% in all experiments. The temperature was maintained between 36.2 and 37.8°C.

Results: The first hERG study indicated that mirabegron did not inhibit hERG currents at up to 30 μM (178-PT-002) (Table below). However, in a second study, hERG currents were suppressed 14% at 30 μM which equates to roughly 180 times the clinical Cmax (178-PT-015). The two major metabolites M11 and M12 did not affect hERG channel currents at any concentration. M14, a minor glucuronide metabolite, only slightly repressed hERG currents at a concentration equivalent to 2,700 times the clinical Cmax. The acetyl conjugate M5 and the phase one metabolite M16 both impaired hERG currents with IC_{50} concentrations of 21 and 31 μM respectively. However these IC_{50} values are more than 1,100 times the clinical Cmax.

Conclusion: These studies are not suggestive of robust repression of potassium rectifier currents by mirabegron or any of its major metabolites at the clinical exposure level.

Summary of Effects of Mirabegron and Metabolites on hERG Current					
Study	**Compound**	**Exposure µM (ng/mL)**	**Multiple of Clinical Cmax†**	**Percent hERG Current Suppression (minus vehicle control value)**	**IC_{50} µM or ng/ml (IC_{50}/Human C_{max})**
178-PT-002	Mirabegron	0.03 (12)	0.2	-0.5	NA
		0.3 (119)	1.8	-2.2	
		3.0 (1,190)	18	0.0	
		30.0 (11,900)	180	-1.2	
178-PT-015	Mirabegron	0.03 (12)	0.2	2.2	NA
		0.3 (119)	1.8	-0.8	
		3.0 (1,190)	18	7.2	
		30.0 (11,900)	180	**14.7****	
178-PT-008	M11 (glucuronide)	0.3 (180)	9.5	6.7	NA
		3.0 (1,800)	95	1.5	
		30.0 (17,990)	950	3.5	
	M12 (glucuronide)	0.3 (198)	23	-1.5	NA
		3.0 (1,983)	230	3.8	
		30.0 (19,834)	2,300	3.0	
178-PT-006	M5 (acetyl conjugate)	0.3 (100)	16.5	3.7	21µM 703 ng/ml (1153)
		3.0 (1004)	165	**17.9***	
		30.0 (10,045)	1,650	**57.4****	
	M16 (Phase I)	0.3 (88)	21	1.8	31 µM 9,135 ng/ml (2,228)
		3.0 (884)	216	11.5	
		30.0 (8,839)	2,160	**49.2****	
178-PT-012	M14 (glucuronide)	0.3 (183)	27	1.0	NA
		3.0 (1,825)	272	-2.4	
		30.0 (18,248)	2,700	**17.3****	

† Multiple of the humans Cmax in elder fasted women at 50 mg (Cmax = 66 ng/ml) (Study 178-CL-072). Clinical Cmax values after 7 days of 50 mg mirabegron dosing in elderly females are M5 (6.1 ng/ml), M11 (18.9 ng/ml), M12 (8.6 ng/ml), M14 (6.7 ng/ml), and M16 (4.1 ng/ml) (Study 178-CL-072). Significantly different from concurrent vehicle control * $p < 0.05$, ** $p < 0.01$. ‡ Suppression of hERG currents minus the suppression in the DMSO vehicle control. NA- not applicable since suppression was insignificant.

Effects on Action Potential of Isolated Guinea Pig Papillary Muscle

Methods: To see if there is a potential physiological effect of mirabegron or its principal metabolites on cardiac action potential, the action potential of isolated guinea pig cardiac papillary muscle was evaluated after perfusion with mirabegron, M5, M11, M12, M14, or M16 (178-PT-001, 178-PT-007, 178-PT-009, and 178-PT-013) (Table below). Isolated guinea pig cardiac papillary tissue was perfused ex-vivo at 5 mL/min for 35 minutes. Action potentials were initiated electronically and the wave form from a single cell was measured with a glass electrode. Five to six replicates were evaluated with the effects on action potential evaluated 30-35 minutes after a 0, 0.3, 3.0, or 30 µM exposure to mirabegron or its metabolites. The positive control E-4031, a hERG inhibitor, was also employed at 0.1 µM. Endpoints measured were the resting membrane potential, action potential amplitude, dV/dt max and the ADP30, ADP50, and ADP90 (time to 30, 50 and 90% repolarization of the action potential). ADP50 was only assessed for mirabegron.

Results: Mirabegron and the glucuronide metabolites (M11, M12, and M14) did not affect action potentials while the positive control prolonged the ADP30, ADP50, and ADP 90 by 11-18%, 25%, and 25-33%, respectively, without affecting the other parameters as expected. Action potentials for the acetyl conjugate M5 and the phase one metabolite M16 both had a 5% prolongation of the ADP90, but the concentration required to do so is roughly > 1,600 times the clinical Cmax at the MRHD. The prolongation of the action potential by M5 and M16 correlates with the impaired hERG currents that were also observed at 30 µM. Mirabegron and its metabolites did not significantly affect the other measured endpoints (resting membrane potential, action potential amplitude, and dV/dt max).

Conclusion: Under the conditions employed, the data here are not suggestive of a robust effect of mirabegron or its major metabolites on action potential at clinical exposure levels. However, it does suggest that M5 and M16 have the potential to slightly prolong action potential if tissue concentrations in the heart are well in excess of the human Cmax.

Effect of Mirabegron and Metabolites on Action Potential of Cardiac Papillary Muscle

Study	Compound	Concentration (µM)	ADP_{30} (% change)	ADP_{50} (% change)	ADP_{90} (% change)	ADP_{30-90} (% change)
178-PT-001	Mirabegron	0	0.3	0.2	-0.4	-
		0.3	-2.2	-0.2	0.4	-
		3.0	-2.3	-1.2	-1.1	-
		30	-3.0	-2.4	-1.4	-
	E-4031	0.1	**18.2****	**25.0****	**24.5****	-
178-PT-007	M5 Acetyl Conjugate	0	-1.3	-	0.1	2.9
		0.3	3.1	-	1.4	-1.9
		3.0	**6.1***	-	1.2	**-7.9****
		30	**5.6***	-	**4.7***	3.4
	M16 Phase I	0.3	2.4	-	2.1	1.2
		3.0	0.8	-	-0.4	-1.9
		30	5.3	-	**5.0***	4.7
	E-4031	0.1	**16.3****	-	**33.3****	**62.6****
178-PT-009	M11 Glucuronide	0	-0.2	-	0.4	1.5
		0.3	-0.5	-	0.1	1.9
		3.0	1.3	-	1.4	1.6
		30	3.5	-	1.4	-2.6
	M12 Glucuronide	0.3	0.7	-	0.5	0.4
		3.0	4.5	-	0.8	-2.7
		30	0.2	-	2.0	2.8
	E-4031	0.1	**12.1****	-	**25.6****	**53.4****
178-PT-013	M14 Glucuronide	0	2.1	-	1.6	0.9
		0.3	2.5	-	2.0	1.0
		3.0	6.5	-	2.0	-5.4
		30	5.4	-	2.8	-2.0
	E-4031	0.1	**11.3****	-	**30.5****	**61.6****

Significantly different from control * $p < 0.05$, ** $p < 0.01$. E-4031 - positive control

Canine Ventricular Tissue Response to Mirabegron and its Primary Metabolites In Vitro
The potential for mirabegron, M5, M11, M12, M14, or M16 to promote QT prolongation and ventricular fibrillation were assessed in perfused canine ventricular wedge tissues (178-PT-010 and 178-PT-014).

Methods: Isolated canine ventricular tissue was treated by arterial perfusion for 30 minutes with solvent control (DMSO), active control (100 μM sotalol, a nonselective β-blocker that prolongs QT and PR intervals), mirabegron at 3, 30, 100, or 300 ng/mL or its metabolites M5, M11, M12, M14, or M16 at 3, 10, 30, or 100 ng/mL or the β1-β2 adrenergic receptor agonist Isoproterenol at 0.3, 2.5, 24.8, or 248 ng/mL (n=6/group). Response to 1,000 and 2,000 ms stimulation was recorded. QT interval was determined. The T wave duration from peak to end (T_{p-e}) was also determined as an index of transmural dispersion of repolarization (TDR). Transmembrane action potential duration was also assessed from the subendocardium and epicardium to determine the ADP90 (time for 90% repolarization of the action potential). Abnormal rhythms indicative of ventricular tachycardia and fibrillation were also noted within five minutes of the 1,000 ms stimulation but not after the 2,000 ms stimulation. A torsades de pointes (TdP) risk score was calculated based upon the change in QT interval, change in Tp-e/QT ratio, and the detection and type of early after depolarization (EAD). Rabbits are the preferred species for this assay. The rational for the choice in species was not mentioned. The sponsor noted that there is no data available to validate their criteria for estimating TdP because they used dogs instead of rabbits.

Results: Under the conditions tested, there was no indication that mirabegron or any of the metabolites have the potential to promote QT prolongation (Table below). The opposite was observed. QT interval and subendocardial ADP90 was slightly reduced by mirabegron at ≥ 100 ng/mL which is a concentration slightly exceeding the mean Cmax in elderly women at the 50 mg clinical dose (66 ng/mL) (178-CL-072). Similarly, M5 exposure reduced the QT interval and ADP90 roughly 8-9% at 100 ng/mL. Exposure to M11 slightly reduced the Tp-e interval (≤ 4%) at ≥ 3 ng/ml and Tp-e was slightly reduced by M16 at 100 ng/mL. Other than this, there were no other significant adverse effects of the metabolites on QT, Tp-e, or ADP90. The positive control Sotalol and the nonspecific β1-β2 agonist Isoproterenol prolonged and shortened the QT interval respectively as expected.

Conclusion: Under the conditions tested, mirabegron and its principal metabolites were unable to prolong the QT interval or promote conditions conducive for ventricular fibrillation at roughly 4.5 times the maximal clinical exposure at the MRHD (50 mg with C_{max} = 66 ng/ml in elderly females study 178-CL-072).

Summary of In Vitro Canine Ventricular Wedge Studies					
	Mirabegron				
	0	3 ng/mL	30 ng/mL	100 ng/mL	300 ng/mL
QT (ms)2000 ms	318.0±2.1	316.8±4.4	314.2±4.1	**309.8±3.7***	**294.8±5.4****
QT (ms)1000 ms	274.8±3.2	278.2±4.0	277.2±3.9	**268.2±4.7***	**262.8±2.8****
Tp-e(ms) 2000 ms	47.0±1.9	46.0±2.1	46.8±2.3	46.0±2.7	45.5±2.8
Tp-e(ms) 1000 ms	38.5±3.1	38.3±3.1	38.5±3.3	38.0±3.0	40.2±3.2
Tp-e/QT% 2000 ms	0.0±0.0	-1.8±0.8	0.7±0.8	0.1±2.4	4.0±1.9
APD90 (ms) 2000 ms	290.6±3.3	288.5±3.0	284.7±2.6	**279.7±1.0***	**275.3±3.1***
APD90 (ms) 1000 ms	257.5±1.9	253.9±1.3	257.4±1.3	**248.2±1.8****	**236.5±2.8****
PVCs	6.7±2.0	2.8±0.9	2.0±0.9	3.8±1.6	4.2±2.2
VT	0	0	0	0	0
TdP score	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.3	0.2±0.3

VT: ventricular tachycardia (≥3 beats); PVCs: premature ventricular complexes. *: $p<0.05$ and **: $p<0.01$ compared with control; n=6 ventricular wedge preparations per concentration. Table adapted from the sponsor's Table 1A in study 178-PT-010.

Summary of In Vitro Canine Ventricular Wedge Studies						
	Isoproterenol (ng/mL)					Sotalol
Isoproterenol	0	0.25	2.5	24.8	248	(100 µM)
QT (ms)2000 ms	306.3	293.8	**274.7***	**227.4****	nd	**445.5****
QT (ms)1000 ms	274.7	262.8	**245.7****	**219.0****	nd	**401.8****
Tp-e(ms) 2000 ms	44.5	44.7	**46.8***	44.5	nd	**85.0****
Tp-e(ms) 1000 ms	39.8	40.7	**41.8***	**42.2***	nd	**68.0****
Tp-e/QT% 2000 ms	0.0	4.5	**17.87****	**33.6****	nd	**29.0***
APD90 (ms) 2000 ms	276.3	270.2	**254.8****	**199.1****	nd	**395.5****
APD90 (ms) 1000 ms	244.6	**236.0***	**225.5****	**190.8****	nd	**359.2****
PVCs	5.5	**7.5***	9.2	**30.2****	nd	1.3
VT	0	0	0	**4 of 6***	**6 of 6****	0
TdP Score	0.0	0.3±0.2	**1.7****	**2.0****	nd	**5.3****

Table adapted from the sponsor's Table 1B in study 178-PT-010. ND – no data obtained due to sustained ventricular tachycardia.

4.3.4.2 Cardiac Safety Pharmacology Study Findings - In Vivo Studies

Rabbits:
The effect of intravenous mirabegron (0, 0.1, 0.3, 1 mg/kg) on blood pressure and heart rate was assessed in pentobarbital anesthetized male rabbits (n=5/group) (178-PT-005). Mirabegron did not affect blood pressure at IV dose up to 1.0 mg/kg. However, heart rate was elevated 6-11% in the 1 mg/kg group for up to 60 minutes after dosing. The double product (heart rate x systolic blood pressure) was also reported since it is an indicator of myocardial work and myocardial O_2 consumption. The double product was statistically elevated 6-9% for up to 10 minutes after dosing at 1 mg/kg but recovered thereafter.

Oral dosing of non-pregnant rabbits at ≥10 mg/kg (n=2/group) may have elevated heart rates for up to 8 hrs after dosing (178-TX-048). Elevated heart rate (6-23%) was also observed for 8-24 hours after oral dosing in pregnant rabbits at 30 mg/kg, which is a dose that caused cardiomegaly and dilated aortas in the fetuses (178-TX-047, 178-TX-057, See section 9.2.3)

Monkeys:
The affects of a single oral dose of mirabegron (0, 3, 10, 30, or 100 mg/kg) on heart rate, blood pressure, ECG, blood gas, body temperature, blood electrolytes, and toxicokinetics was investigated in Cynomolgus monkeys (178-PT-004). The study was a four way cross over design where each animal (n=4) received each dose with a 7-14 day washout period between groups.

The multiples of the 50 mg clinical exposure are 1, 4, 14, and 49 based on AUC and 3, 12, 20, and 52, based on Cmax. Absorption was quick with Tmax values between 1-2.5 hrs. The only clinical sign noted was a single monkey at 100 mg/kg which displayed lateral position and vomiting 5.5 hrs after dosing. There was no affect of mirabegron on body temperature, arterial CO_2, O_2, pH, hemoglobin O_2 saturation, sodium, potassium, calcium, or chloride. There was no affect of mirabegron on systolic, diastolic, or mean blood pressure 1, 2, 4, 8, or 24 hours after dosing. Heart rate was elevated in all exposure groups with the duration of effect being dose dependent (1 hr, 4 hrs, and 8 hrs at 10, 30, and 100 mg/kg, respectively). The elevated heart rate (28-41%) was only statistically significant at ≥ 10 mg/kg. Heart rates recovered within 24 hrs after dosing. PR interval was slightly elevated at 100 mg/kg, but it was not statistically significant. There was no affect on QT or QTc (Bazett's and Fridericia's Correction) interval, however, QRS duration was increased 29-35% in the HD group between 1 and 8 hrs after dosing with no effect 24 hrs post dosing. Similar findings were observed in a similar safety pharmacology study in monkeys at the same doses (178-PH-020).

Toxicokinetics of Mirabegron in Monkeys After a Single Oral Dose (178-PH-004)

Dose (mg/kg)	Mean Tmax (hr)	Mean Cmax (ng/mL)	Mean AUC_{0-24} (ng-hr/mL)	Multiple of 50 mg Clinical Dose†
3*	1	205	499	1
10	1	815	2,145	4
30	1.8	1,305	7,191	14
100	2.5	3,446	25,092	49

* Mirabegron was not detected one animal in the 3 mg/kg group at all time points except 1 hr (7 ng/ml) so it was not included in the average values. † Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072).

Dogs:
The initial cardiac safety study in conscious dogs revealed that mirabegron increases heart rate (~100%, 60-100 bpm increase over ~ 65 bpm baseline) and decreases the mean and systolic blood pressure (~30%) without affecting diastolic blood pressure after single oral doses ≥ 0.3 mg/kg (178-PH-019). Depending on the method of heart rate

correction, QT interval was either reduced at 10 mg/kg (uncorrected), not effected in these dogs at up to 10 mg/kg (Matsunaga's), inconclusively prolonged at ≥ 3 mg/kg (Van de Water's), or more substantially prolonged, 12-37% at 2-8 hrs, at ≥ 0.3 mg/kg using Bazett's correction which is a dose resulting in exposure similar to the human Cmax at the MRHD. The sponsor noted that Matsunaga's and Van de Water's are the common correction methods in dogs but they feel that Matsunaga's method is most appropriate where there is a large change in heart rate so they do not believe that QTc results in dogs are a safety issue. They also noted that the wave forms were not abnormal even at the 10 mg/kg dose. This was supported by a second oral dose study in dogs where blood pressure declined and heart and respiratory rate increased at ≥ 10 mg/kg, ventricular tachycardia was not observed at up to 100 mg/kg, and QTc was either prolonged when using Bazett's correction at ≥ 30 mg/kg or was not effected when using Matsunaga's method at up to 100 mg/kg (178-PH-022).

Similarly, in anesthetized dogs after IV administration, monophasic ventricular action potential duration (MADP90) was shortened at ≥ 0.3 mg/kg while at 3 mg/kg the heart rate was elevated, the T wave was heightened, and QT interval was shortened (178-PH-021). Death was observed in dogs at IV doses ≥ 10 mg/kg due to ventricular tachycardia that lead to ventricular fibrillation within 5-10 minutes (178-PH-022 and 178-PH-023).

These data suggest that mirabegron does not cause QT prolongation in dogs but it could, at high doses, induce ventricular fibrillation due to tachycardia.

The sponsor suggested that the cardiac effects may be partially mediated by β1-AR stimulation since intravenous exposure to propranolol (β1/β2 blocker) partially inhibited the increased heart and respiratory rates and decreased blood pressure observed with oral and IV mirabegron in dogs at ≥ 10 mg/kg (178-PH-02*2*). A mechanistic investigation for the elevated heart rate in rats and dogs was further investigated in non-GLP studies (see Section 4.3.4.3).

4.3.4.3 Mechanist Investigation of Increased Heart Rate

Rats: The sponsor postulated that the elevated heart rates caused by mirabegron may be mediated through the β1-AR in laboratory animals. The effect of mirabegron (0.03, 0.1, or 0.3 mg/kg, IV) on heart rate was assessed in pentobarbital anesthetized male rats (178-PH-050). The heart rate was elevated approximately 18, 25, 30, and 55 beats/min above the mean basal rate of 347 beats/min in the control, LD, MD, and HD groups, respectively, but a statistical difference was only observed in the MD and HD groups (figure below). The mean blood pressure, systolic blood pressure, and diastolic blood pressure were all slightly reduced in the MD and HD groups but not to a statistically significant level.

Effect of IV YM178 on Heart Rate (A) and Blood Pressure (B, C, and D) in Anesthetized Rats

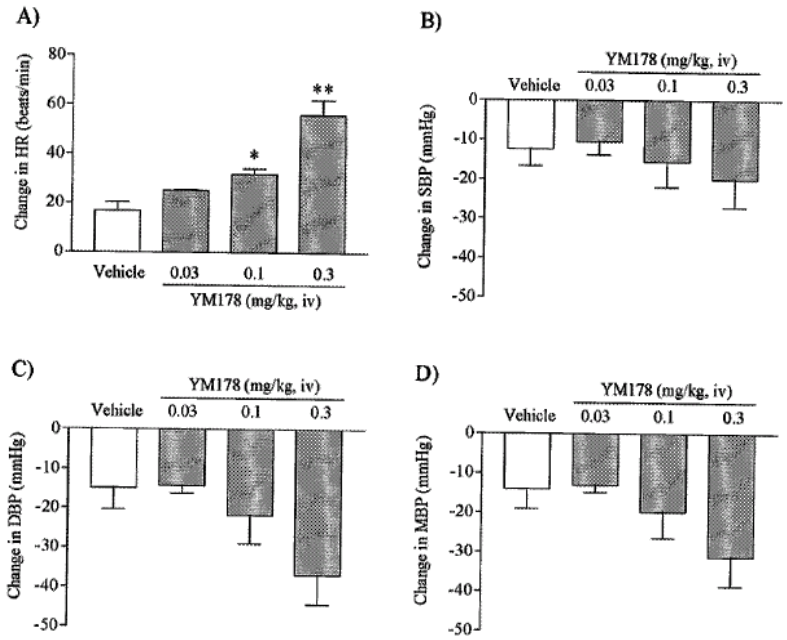
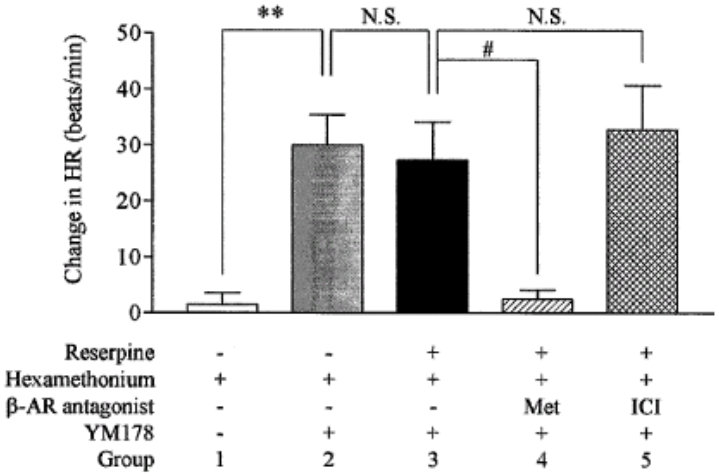


Figure copied from Sponsor's Figure 1 (178-PH-050). Each column represents the mean: SEM of 4 rats. *P<0.05, **P<0.01, significant difference from the vehicle treated group (Dunnett's multiple comparison test). HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure

A mechanistic rational for the mirabegron dependent elevation in heart rate was investigated by pre-treating the rats with a neuronal specific acetylcholine antagonist (hexamethonium), depleting catecholamines (reserpine- depletes catecholamines from peripheral sympathetic nerve endings by blocking their uptake), or treating with a β1-AR antagonist (metoprolol) or a β2-AR antagonist (ICI-118551). First, as a ganglion nerve block, all rats in the mechanistic study were dosed with hexamethonium and the antimuscarinic atropine. This treatment alone had no affect on heart rate (group 1, see figure below) and it did not impair the elevated heart rate caused by mirabegron (0.1 mg/kg) (group 2). The addition of reserpine did not repress the mirabegron effect indicating that elevated catecholamine levels may not be involved. The combination of atropine, reserpine, hexamethonium, and the β2-AR antagonist ICI-118551 also had no affect on mirabegron (group 5). However, the β1-AR antagonist metoprolol in combination with atropine, reserpine, and hexamethonium did reduce the elevated heart rate to a level similar to non-YM78 treated rats suggesting that this affect is mediated through the β1-AR (group 4).

Modulators of YM178 Elevated Heart Rate (Figure Copied From Sponsor)



Each column represents the mean ± SEM of 4 rats. **P<0.01, significant difference from group 1 (Student's t-test). #P<0.05, significant difference from group 3 (Dunnett's multiple comparison test). N.S.: not significant; HR: hem rate; Met: metoprolol (1 mg/kg, IV); ICI: ICI-118551 (0.1 mg/ml/kg, IV); AR: adrenoceptor; +: administration of drug; -: administration of corresponding vehicle. Reserpine (5 mg/ml/kg, ip) was administered on the day before experiment. Hexamethonium (10 mg/0.5 mL/kg, IV) and atropine (1 mg/0.5 mg/kg, IV) were administered prior to injection of β-AR antagonist. YM178 (0.1 mg/ml/kg, IV) was administered 5 min after injection of β-AR antagonist.

The sponsor did not provide data or a rational for the doses of the drugs that were used to ensure that they were used at an effective dose. The dose of ICI-118551 may not have been sufficient to repress β2-AR dependent affects. Alternatively β-AR knockout mice could have been used to demonstrate how responsive heart rate is to activation of each β-AR individually or in combination by mirabegron. This study is informative but not completely convincing because of the lack of proper controls. Further study would be needed to make claims that the cardiac affects are mediated solely through β1-AR and not β2-AR or β3-AR.

Dogs: A study was conducted investigating whether the increased heart rate in dogs is a compensatory response to β3-AR dependent vasodilatation and subsequent reduction in blood pressure (reflex tachycardia) (178-PH-054).

Methods:
The first experiment evaluated the response to mirabegron. Pentobarbital anesthetized dogs were dosed intravenously with ascending doses of mirabegron (0.0001, 0.0003, 0.001, 0.003, 0.01, 0.1, and 1 mg/kg) once every 15 minutes. Plasma levels were determined 5 and 15 minutes after each dose. Blood pressure, heart rate, and ECG were assessed. To determine if the effects on the heart are centrally mediated, a second experiment was conducted where anesthetized dogs were pre-dosed with hexamethonium (10 mg/kg IV), which inhibits the sympathetic inputs to the heart, and atropine (1 mg/kg IV), which inhibits the parasympathetic inputs to the heart. Potential mirabegron related cardiac or vascular effects in the presence of hexamethonium and

atropine would likely be due to direct effects on the cardiac or vascular tissues and would not be secondary to reduced blood pressure or neuronal signaling. After hexamethonium/atropine dosing, ascending IV doses of mirabegron (0.01, 0.03, 0.1, 0.3, or 1.0 mg/kg) were administered at 10 minute intervals. Isoproterenol, a β1-β2 agonist, was administered IV (0.3 μg/kg) 10 minutes after the last mirabegron dose to ensure that the dogs were responsive. In a third experiment the nerve block pretreatment (hexamethonium/atropine) was repeated along with the ascending doses of mirabegron and the β1-antagonist metoprolol (5 mg/kg IV). This study was conducted to determine if the increased heart rate was due to off target β1-AR activation.

Results:
Only graphical data was presented. Intravenous mirabegron decreased left ventricular pressure at 0.001 mg/kg, decreased diastolic and systolic blood pressure at ≥ 0.001 mg/kg and ≥ 0.003 mg/kg, respectively, while a compensatory elevation in heart rate was observed at ≥ 0.001 mg/kg. The exposure to mirabegron in dogs at 0.3 and 1 mg/kg (64-690 ng/mL) was one and ten times the Cmax in older fasted women dosed at 50 mg (66 ng/mL).

Figure 1 Effect of Mirabegron on Heart Rate and Other Cardiovascular Parameters in Anesthetized Dogs

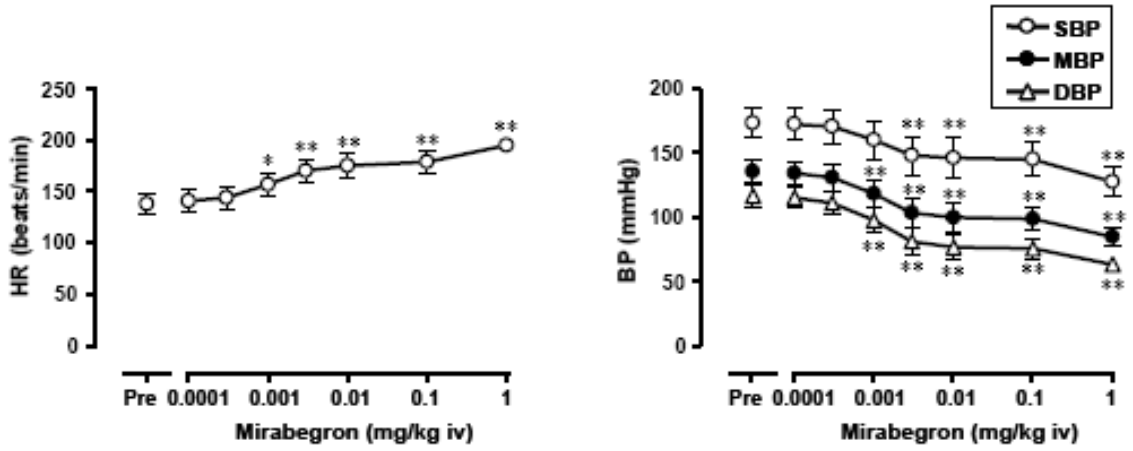


Figure and text copied from the Sponsor (178-PH-054). Each point represents the mean ± SEM of 5 dogs. *P<0.05, **P<0.01, significant difference from pre-values (Dunnett's test using within subject error). HR: heart rate; SBP: systolic blood pressure; MBP: mean blood pressure; DBP: diastolic blood pressure; LVP: left ventricular pressure; +dp/dt(max): maximum positive rate of change in LVP

Plasma Concentration Mirabegron ng/ml after IV Dosing in Dogs (178-PH-60)		
IV Dose (mg/kg)	5 min	15 min
0.0001	0	0
0.0003	0.047	0
0.001	0.38	0.13
0.003	1.48	0.43
0.01	5.5	1.6
0.1	63.9	16.3
1	688.5	256.2
The mean and maximal clinical exposure in fasted elder women dosed at 50 mg at steady state was 66 and 259 ng/mL respectively (178-CL-72).		

As expected, blood pressure was still depressed at mirabegron doses ≥ 0.01 mg/kg in the presence of hexamethonium and atropine since β3-agonists are thought to act directly on the smooth muscles of the vasculature by releasing NO. However, the tachycardic reflex was repressed by the nerve block at low doses of mirabegron. The minimal dose of mirabegron necessary to increase the heart rate increased from 0.001 to 0.1 mg/kg. This suggests that reduced blood pressure may be responsible for the increased heart rate at low doses of mirabegron but high doses of mirabegron overcame the nerve block, so mirabegron may act directly on the heart.

Figure 2 **Effect of Mirabegron on Heart Rate and Other Cardiovascular Parameters in the Presence of Hexamethonium and Atropine in Anesthetized Dogs**

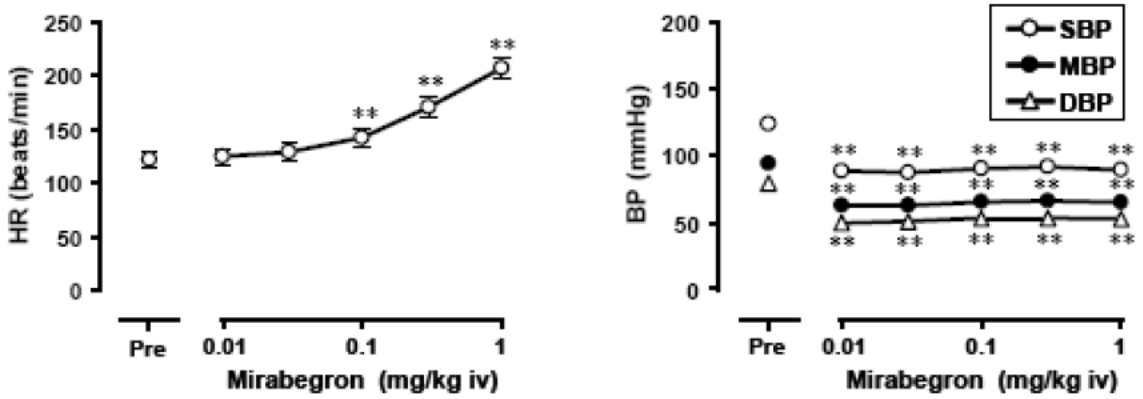


Figure and text copied from the Sponsor (178-PH-054). Each point represents the mean ± SEM of 5 dogs. **P<0.01, significant difference from pre-values (Dunnett's test using within subject error). Hexamethonium (10 mg/kg iv) and atropine (1 mg/kg iv) were given 10 min before administration of mirabegron. HR: heart rate; SBP: systolic blood pressure; MBP: mean blood pressure; DBP: diastolic blood pressure.

The β1-antagonist metoprolol along with hexamethonium and atropine completely blocked the mirabegron dependent elevation in heart rate at up to a 1 mg/kg IV dose of

mirabegron. However, blood pressure (diastolic, systolic, and left ventral pressure) was still reduced by mirabegron at ≥ 0.01 mg/kg (figure below).

Figure 3 Effect of Mirabegron on Heart Rate and Other Cardiovascular Parameters in the Presence of Hexamethonium, Atropine and Metoprolol in Anesthetized Dogs

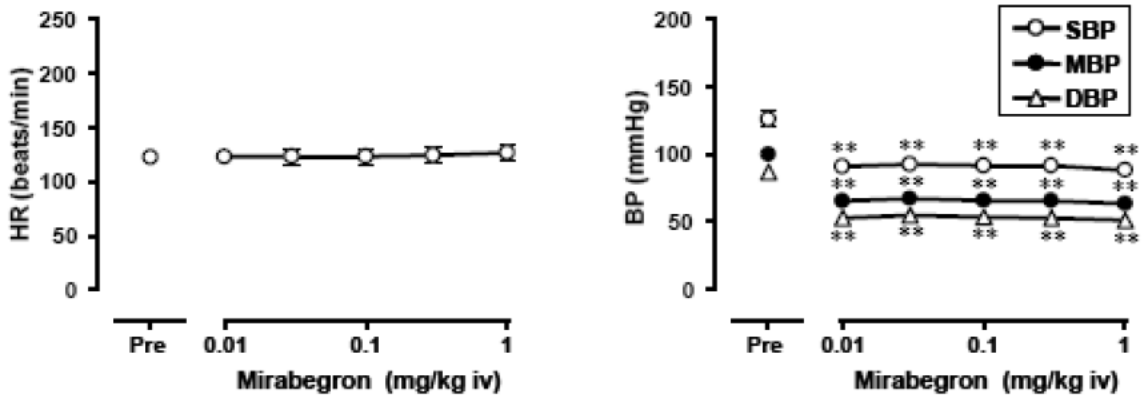


Figure and text copied from the Sponsor (178-PH-054). Each point represents the mean ± SEM of 5 dogs. *P<0.05, **P<0.01, significant difference from prevalues (Dunnett's test using within subject error). Hexamethonium (10 mg/kg iv) and atropine (1 mg/kg iv) were given 5 min before injection of metoprolol (5 mg/kg iv). Mirabegron was administered 10 min after injection of metoprolol. HR: heart rate; SBP: systolic blood pressure; MBP: mean blood pressure; DBP: diastolic blood pressure.

Conclusion:
The findings in dogs suggest that the elevation in heart rate induced by mirabegron may be mediated by direct activation of β1-adrenergic receptors in cardiac myocytes and elevated heart rate may not solely be a compensatory response to reduced blood pressure. The sponsor concluded that the increased heart rate at low doses of mirabegron was a compensatory response to vasodilating effects of β3-AR stimulation (reflex tachycardia), however, at high doses of mirabegron, they concluded that the increased heart rate was attributed to direct chronotropic effects of β1-AR activation.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Absorption

Rats: The absolute bioavailability increased with dose and the Cmax and AUCt-∞ increased proportionally more than the increase in dose (178-ME-005). Absorption of ^{14}C-mirabegron over one hour was only marginally detected from the stomach (7%) but occurred primarily in the duodenum (56%), jejunum (62%), ileum (66%), and colon (15%) (178-ME-022).

Pharmacokinetics of Mirabegron After Oral Dosing in Rats (Study 178-ME-005)					
Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng-hr/mL)	$T_{1/2}$ (hr)	Bioavailability (%)
3	2.0	37.0	243	3.8	23.0
10	4.0	291	1,701	5.0	48.4
30	0.1	1,349	7,980	3.6	75.7
AUC0-∞ following a 1 mg/kg IV dose was 351 ng-hr/mL (178-ME-005). For comparison, AUC_{0-24} was 265, 900, and 3,350 ng-hr/mL after two weeks of IV dosing at 1, 3, and 10 mg/kg respectively (178-TX-035, Section 6.2.1).					

Dogs: Absolute bioavailability after oral doses of 0.25, 0.5, and 1.0 mg/kg were 42%, 65%, and 77% respectively (178-ME-006).

Pharmacokinetics of Mirabegron After Single Oral Dose in Beagle Dogs					
Dose (mg/kg)	C_{max} (ng/ml)	T_{max} (hr)	$AUC_{0-\infty}$ (ng-hr/ml)	$T_{1/2}$ (hr)	Bioavailability (%)
0.25	9.1	0.5	46.7	4.4	41.8
0.5	12.3	4.0	145.3	9.5	64.6
1.0	40.5	0.3	366.2	9.4	77.1
$AUC_{0-\infty}$ was 45.3 ng-hr/mL after a single 0.1 mg/kg IV dose. Table derived for the sponsor's study (178-ME-006).					

Repeated oral dosing of mirabegron resulted in slight elevations in $T_{1/2}$, C_{max}, and AUC suggestive of slight accumulation (178-ME-007, Table below).

Toxicokinetics of Mirabegron After Multiple Oral Doses of 0.5 mg/kg to Beagle Dogs					
Day	T_{max} (hr)	C_{max} (ng/ml)	AUC_{0-24} (ng-hr/mL)	$AUC_{0-\infty}$ (ng-hr/mL)	$T_{1/2}$ (hr)
1	3.5	25.0	147.9	175.3	9.2
8	2.1	25.1	155.5	182.3	8.8
15	1.5	39.4	173.6	218.3	12.4
Table derived for the sponsor's study (178-ME-007). (n=6).					

Food intake reduced exposure as evidenced by a 21.5% and 34.2% reduction in C_{max} and $AUC_{0-\infty}$ respectively (178-ME-008).

Humans:
Bioavailability also increased with dose in fasted young men. A single dose of 50 or 150 mg resulted in 24% and 45% bioavailability, respectively (178-CL-033).

5.1.2 Distribution

In Vitro
In vitro plasma protein binding of ^{14}C-mirabegron was fairly similar between mice (77-78%), rats (79%), and both Japanese (76-77%) and Caucasian (73%) humans (178-ME-021). The rank order of human plasma proteins with the greatest in vitro potential to bind mirabegron were serum albumin > α1-acid glycoprotein > LDL > HDL > γ-globulin

(178-ME-044). ^{14}C-Mirabegron partitions to red blood cells in vitro to a similar extent in rats, dogs, cynomolgus monkeys, and humans since the red cell/plasma ratio ranged between 1.5 to 2.2 (178-ME-045).

In Vivo

Monkeys:

Distribution in male cynomolgus monkeys was not assessed at the time of maximal tissue concentration but rather one week after receiving a single 10 mg/kg ^{14}C-mirabegron oral dose (178-ME-061). The plasma Cmax was 5,500 ng eq/mL and Tmax was 0.5 hrs. Radioactivity was not detected in serum after 96 hours. Due to elimination ($t_{1/2}$ = 24 hrs), only 3.7% of the administered radioactive dose was retained seven days after dosing. The distribution seven days after dosing was in general similar to rats. The rank order of tissue distribution was: liver (1,114 ng eq/g), bile (638 ng eq/g), eye (404 ng eq/g), pancreas (293 ng eq/g), adrenals (265 ng eq/g), kidneys (252 ng eq/g), submaxillary gland (222 ng eq/g), thymus (222 ng eq/g), skin (212 ng eq/g), spleen (203 ng eq/g), bone marrow (195 ng eq/g), S. intestine (142 ng eq/g), thyroid (132 ng eq/g), L. intestine (125 ng eq/g), cecum (99 ng eq/g), testes (92 ng eq/g), lung (79 ng eq/g), heart (69 ng eq/g), stomach (64 ng eq/g), and skeletal muscle (13 ng eq/g). Radioactivity was not detected in the cerebrum, cerebellum, pituitary gland, fat, or gastric contents.

Albino Rats:

Although plasma levels 24 hrs after dosing steadily increased from the first dose (17 ng eq/mL) to 21st daily dose (83 ng eq/ mL) the tissue plasma ratios were nearly similar after the 14th and 21st day of dosing suggesting that steady state had been reached near the 14 day of exposure (178-ME-064). The maximal radioactivity in tissues was generally 4 hrs after each dose. Tissue concentrations tended to increase with repeated dosing with the greatest concentration being observed 4 hrs after the 14th day of exposure with the highest level of radioactivity detected in the liver at 20 times the plasma concentration (16,619 ng-eq/g) followed by the small intestine (14x), kidneys (11x), adrenals (7x), pituitary (7x), thyroid (6x), stomach (5x), cecum (4x), lungs (4x), pancreas (4x), large intestine (3x), spleen (3x), submaxillary glands (3x), bone marrow (3x), heart (2x), testes (2x), skin (1x), and fat (1x). The rank order of tissues with radioactivity less than the plasma level include the thymus, skeletal muscle, blood, eye, cerebellum, and cerebrum. Autoradiography revealed similar finding as well as low levels in the urinary bladder and high levels in the brown fat. High levels of radioactivity were still observed 15 days after the last dose in the kidneys, thyroid, liver, and adrenals. Fifteen days after the last dose less than 12% of the maximal radioactivity remained in intestines, colon, and liver but greater than 40% of the maximal level remained in the thyroid, kidney, testes, and bone marrow suggestive of slow elimination from these organs.

Pigmented Rats: Distribution to Pigmented Tissues (Eyes/Skin)

Accumulation of mirabegron or its metabolites in the eye due to association with melanin was proposed since 68% of the maximal radioactive level was still observed in the eyes of pigmented rats 15 days after ^{14}C-mirabegron dosing (178-ME-023) while

only low levels were observed in albino rats (178-ME-064) (Table below). To further investigate this, the distribution of radioactivity in the eye and identification of ^{14}C-mirabegron metabolites in the eye was assessed in fasted pigmented male rats given a single oral dose of 10 mg/kg (178-ME-065). Another study in pigmented rats similarly dosed with ^{14}C-mirabegron revealed the **half-life of total radioactivity in the eye to be 157 days** (Table below, 178-ME-091). Mirabegron only accounted for 19-26% of all radioactive species in the eye at both time points (178-ME-065).

Radioactive Concentration in the Eye of Male Rats 24 hrs after a single 10 mg/kg Oral Dose of ^{14}C-Mirabegron (ng eq of mirabegron/g eye)				
	Albino (F344)	**Pigmented Rats (Lister Hooded)**		
Day	178-ME-064	178-ME-023	178-ME-065	178-ME-091
1	249	5,556	8,600	4,808 ± 2,530
15	84	5,585	7,100	5,359 ± 947
30	Not Determined	Not Determined	Not Determined	3,369 ± 1,763
90	Not Determined	Not Determined	Not Determined	2,619 ± 929
180	Not Determined	Not Determined	Not Determined	1,738 ± 919

Quantification of Mirabegron and Metabolites in Whole Eye Extracts from Pigmented Male Rats (Lister Hooded) (178-ME-065)				
	24 hrs		**360 hrs**	
Parent or Metabolite	**Percent**	**ng eq/mL**	**Percent**	**ng eq/mL**
YM-178	18.5	1,088	25.5	729
M6	40.6	2,394	36.7	1,046
Unknown	17.1	1,007	21.7	619
M5	5.5	325	12.9	367
M16	1.8	108	3.3	93
Total Identified	83.5	5,892	100.1	2,854
Total Radioactivity		8,599		7,057
M9, M8, M11, M12, M13, M18, and M20 were not detected. Table adapted from the sponsor.				

As in the albino rats, slow elimination from the testes was also observed in the pigmented rats since 23% of the maximal testes concentration was still observed 15 days after dosing (178-ME-023).

Semi-quantitative whole body autoradioluminography was conducted on one rat 24 hours after dosing (178-ME-065). In the eye, the ciliary body, choroid, and conjunctiva of the eye had high levels of radioactivity while medium levels were observed in the iris and trace levels in the vitreous body. In the rest of the body, medium levels of radioactivity were observed in the pituitary gland, thyroid, liver, adrenal, pigmented skin, stomach, cecum, large intestine, and testes. Low radioactivity was detected in submaxillary gland, lungs, kidneys, spleen, pancreas, fat, skeletal muscle, non-pigmented skin, small intestine, and bone marrow. Trace levels of radioactivity were observed in blood, thymus, and heart. Radioactivity was not detected in the cerebrum or cerebellum.

Rats: Placental Transfer
Placental transfer was studied in fasted albino rats 1, 4, and 24 hrs after a single 10 mg/kg oral dose of ^{14}C-mirabegron on gestation day 14 (178-ME-062). After dosing on GD14 plasma levels of radioactivity reached a maximal one hour after dosing and it declined to only 4% of the maximal 24 hrs after dosing. Radioactivity was detected in all of the female reproductive organs and the fetus within one hour of dosing but at concentrations lower than the plasma level (Table below). Maximal levels were observed 4 hrs after dosing. By 4-24 hrs, the level of radioactivity exceeded the plasma levels in ovaries (2x), uterus (2x), placenta (2x), and mammary gland (3x) but not in the fetus (0.2x) or amniotic fluid (0.04x). The concentration in the fetus just slightly exceeded the plasma level 24 hrs after dosing. In the maternal non-reproductive organs, radioactivity exceeded the plasma level at 4 hrs in the liver (16x), kidney (8x), lung (5x), spleen (4x), and heart, and almost undetectable levels were observed in the brain (0.1x).

Tissue Distribution in Maternal Reproductive Organs and Fetus on GD14						
	1 hr		4 hr		24 hr	
Tissue	ng eq/mL	T/P	ng eq/mL	T/P	ng eq/mL	T/P
Plasma	1,437	-	981	-	37	-
Ovary	1,279	0.90	1,979	2.03	525	14.43
Uterus	1,174	0.83	1,999	2.04	367	9.98
Amniotic Fluid	16	0.01	38	0.04		
Placenta	889	0.62	1,477	1.51	412	11.21
Mammary Gland	1,040	0.75	2,458	2.50	637	17.31
Fetus	109	0.08	221	0.23	44	1.20
T/P = Tissue / Plasma ratio. Blood, plasma, brain, heart, lung, kidney spleen, and pancreas were also studied, data not shown.						

In a separate experiment, tissue distribution in the albino dams and their fetuses was qualitatively assessed by whole body autoradioluminography 1, 4, and 24 hrs after dosing (10 mg/kg) on GD19 (178-ME-062). Similar findings were observed on GD14 and GD19. Radioactivity was detected in most all maternal tissues. Tissue levels were generally greatest 4 hours after dosing. Four hours post-dosing high concentrations were observed in the liver, pituitary, brown fat, kidney, and mammary gland. Medium concentrations were observed in the eye, heart, lung, adrenal gland, spleen, pancreas, uterus, placenta, and urinary bladder. Low levels were observed in the blood, ovary, and fetus, while radioactivity was almost undetected in the brain, spinal chord, and amniotic fluid.

Rats: Lactational Transfer
Non-fasted lactating rats were administered a single 10 mg/kg oral dose of ^{14}C-mirabegron 14 days after parturition (178-ME-063). Radioactivity was assessed in blood, plasma, and milk from the lactating rats 1, 4, and 24 hrs after dosing. The number of pups was culled to 8 per litter. Lactating rats were treated with 1 IU/mL/kg of oxytocin 15-30 minutes before milking. Radioactivity was detected in milk 1 and 4 hrs after dosing and in the stomach of pups from 1-24 hrs after dosing with the maximum concentration in the pups stomach at 4 hrs (Table below). The level of radioactivity in

the milk was nearly twice the maternal plasma level at 4 hrs. Radioactivity distributed to the lungs, liver, and kidneys of pups.

The maternal plasma levels of radioactivity in this study (~70 ng eq/mL 1-4 hrs post-dose) were 20-fold lower than previous studies in similarly dosed animals (~1,400 ng eq/mL) (178-ME-062). The only obvious rational for the low maternal exposure is because of a large distribution to milk and/or because the lactating rats were dosed in the fed state in this study since feeding was demonstrated to slightly reduce exposure in dogs (34%). Because of this, the exposure via lactation could be under estimated in this study.

Concentration of Radioactivity in Dams and Pups After Single 10 mg/kg ^{14}C-Mirabegron Dose to Dams

		Concentration (ng eq/g or mL)		
		1 hr	4 hrs	24 hrs
Lactating Dams (n=3/time)	Blood	62	63	BLQ
	Plasma	71	68	BLQ
	Milk	31	115	BLQ
Pups† (8/litter)	Liver	BLQ	5	25
	Kidney	BLQ	BLQ	8
	Lungs	BLQ	BLQ	7
	Milk lumps in stomach	15	92	19

† Radioactivity was not detected in the blood, plasma, brain, heart, or lungs of the pups.
BLQ = below the limit of quantification.

5.1.3 Metabolism

In Vitro Metabolism Studies

Hepatic Metabolism - In vitro
In vitro studies with human liver microsomes suggest that mirabegron may be predominately metabolized in humans by CYP3A4 and to a lesser extent by CYP2D6 (178-ME-002).

Metabolism in Plasma – In Vitro
Radiolabeled mirabegron was metabolized *in vitro* in human plasma to a phenetanol amine, which was thought to be produced by hydroxylation with a carboxylesterase (178-ME-034). Decomposition of mirabegron in mouse, rat, rabbit, dog, cynomolgus monkey, or human plasma in the presence or absence of an esterase inhibitor suggest that mirabegron is metabolized by an esterase in the plasma of mice, monkeys, and humans but not in rats, rabbits, or dogs (178-ME-038). Clearance of mirabegron by esterases was predicted to be five times greater in human plasma in comparison to mice and monkeys.

In vitro hydrolysis of mirabegron to M16 by butyrylcholinesterase (BChE) was observed in human plasma and blood but not in human liver or intestinal microsomes. Metabolism of mirabegron by acetylcholinesterase (AChE), carboxylesterase 1 (CES1) or CES2 solutions were not observed (178-ME-079). Hydrolysis of mirabegron in blood was thought to be principally due to plasma BChE.

Mirabegron was metabolized rapidly in rats. Within one hour of dosing, plasma levels of one metabolite (M6, N-dealkylated product) was more abundant than mirabegron and two others (M16 a suspected product of serum esterase BChE and an unknown) were found at roughly half the concentration of the unchanged drug (178-ME-056).

Inhibition of Human CYP450s – In Vitro
In microsomal preparations expressing specific human CYP450s, mirabegron was a strong inhibitor of the human CYP2D6 and a weak inhibitor of CYP2C19 and CYP3A4 while essentially not inhibiting CYP1A2 or CYP2C9 (178-ME-009). In human liver microsomes, mirabegron was a moderate to weak competitive to mixed type inhibitor of CYP2D6 and a weak non-competitive inhibitor of CYP3A4 (178-ME-015). This was confirmed in another study were only CYP2D6 was inhibited to a great extent (85%) in human liver microsomes by 100 µM mirabegron irrespective of whether the microsomes were pre-incubated with mirabegron for 30 minutes (178-ME-068).

	Direct Inhibition		Time Dependent Inhibition		
	Zero Minute Pre-incubation		30 Min Pre-incubation		
Enzyme	IC_{50} (µM)	Max Inhibition (%)	IC_{50} (µM)	Max Inhibition (%)	Potential Time Dependent Inhibition
CYP1A2	> 100	5.4	> 100	NA	Little or No
CYP2B6	> 100	11	> 100	2.4	Little or No
CYP2C8	> 100	12	> 100	0.8	Little or No
CYP2C9	> 100	NA	> 100	NA	Little or No
CYP2C19	> 100	NA	> 100	7.8	Little or No
CYP2D6	13	85	4.3	86	Yes
CYP2E1	> 100	6.8	> 100	NA	Little or No
CYP3A4/5	> 100	4.2	> 100	31	Yes

Table adapted from the sponsor. IC_{50} concentration of mirabegron necessary to inhibit metabolism of a known substrate by 50%.

Induction of Human CYP450s – In vitro
Three daily treatments of mirabegron (0, 0.1, 1, or 10 µM) did not appreciably induce CYP1A2 and CYP3A4 enzyme activity or mRNA expression in human primary hepatocyte cultures (178-ME-074). Mirabegron only cause a slight increase (↑ 1.8 fold at 10 µM) in CYP1A2 mRNA without effecting enzyme activity.

Metabolism - In Vivo
A total of 18 metabolites were identified in plasma, bile, urine, and/or feces of mice, rats, rabbits, monkeys, and humans (Table below). Eight of the metabolites were observed

in human plasma and an additional two were observed in human urine, none of which were unique to humans (178-CL-007). Toxicokinetics of mirabegron and the eight metabolites in human plasma were assessed in humans, mice, rats, monkeys, and non-pregnant rabbits at steady state (Second table below). The animal doses were the same as those in the mouse carcinogenicity study (178-TX-031), six month toxicity study in rats (178-TX-025), one year toxicity study in cynomolgus monkeys (178-TX-026) and rabbit embryo fetal study (178-TX-016). Only metabolites M11 and M12 (inactive glucuronide conjugates) are considered major metabolites by accounting for more than 10% of the drug related material in plasma of young or older humans at the 50 mg dose (178-CL-072). The metabolite exposure in humans was exceeded by that in at least one species in the chronic toxicity, carcinogenicity (estimated), and reproductive toxicity assays. The sponsor's proposed metabolic pathway for mirabegron is illustrated below.

In monkeys, YM178 was rapidly and extensively metabolized to M11 (glucuronide conjugate) in comparison to humans and other animals. In monkeys, the AUC for YM178 was only 5% of the AUC for total radioactivity while M11 accounted for 65-82% of the total plasma radioactivity (similar finding reported in metabolism section 178-ME-078).

Mirabegron or Its Metabolites Confirmed in Plasma, Urine, Feces or Bile Samples from Humans and Various Animal Species

	Mice	Rats	Rabbits	Cynomolgus monkeys	Humans
Mirabegron	●	● □ ▲	●	● □ ◊	● □ ◊
M1		□			
M3		□			
M5	●	● □	●	● □ ◊	● □
M6		● □		◊	
M8	●	● □ ▲	●	● □ ◊	● □
M9	●	□ ▲		● □	□
M11	●	● ▲	●	● □ ◊	● □
M12	●	● ▲		● □	● □
M13	●	● ▲	●	● □	● □
M14	●	● ▲	●	● □	● □
M15	●		●	● □ ◊	● □
M16	●	● □	●	● ◊	● □
M17					□
M18		□ ▲			
M19		▲			
M20		▲			
M21		▲			
M22		▲			

Table copied from the Sponsor.●: Plasma; □ Urine; ◊: Feces; ▲: Bile.
Source: Studies 178-ME-018, 178-ME-039, 178-ME-046, 178-ME-051, 178-ME-052, 178-ME-055, 178-ME-056, 178-ME-066, 178-ME-072, 178-ME-077, 178-ME-078, 178-ME-083, 178-ME-102, 178-ME-103, 178-ME-124, 178-ME-125, and 178-ME-126.

Sponsor's Postulated Metabolic Pathway for Mirabegron

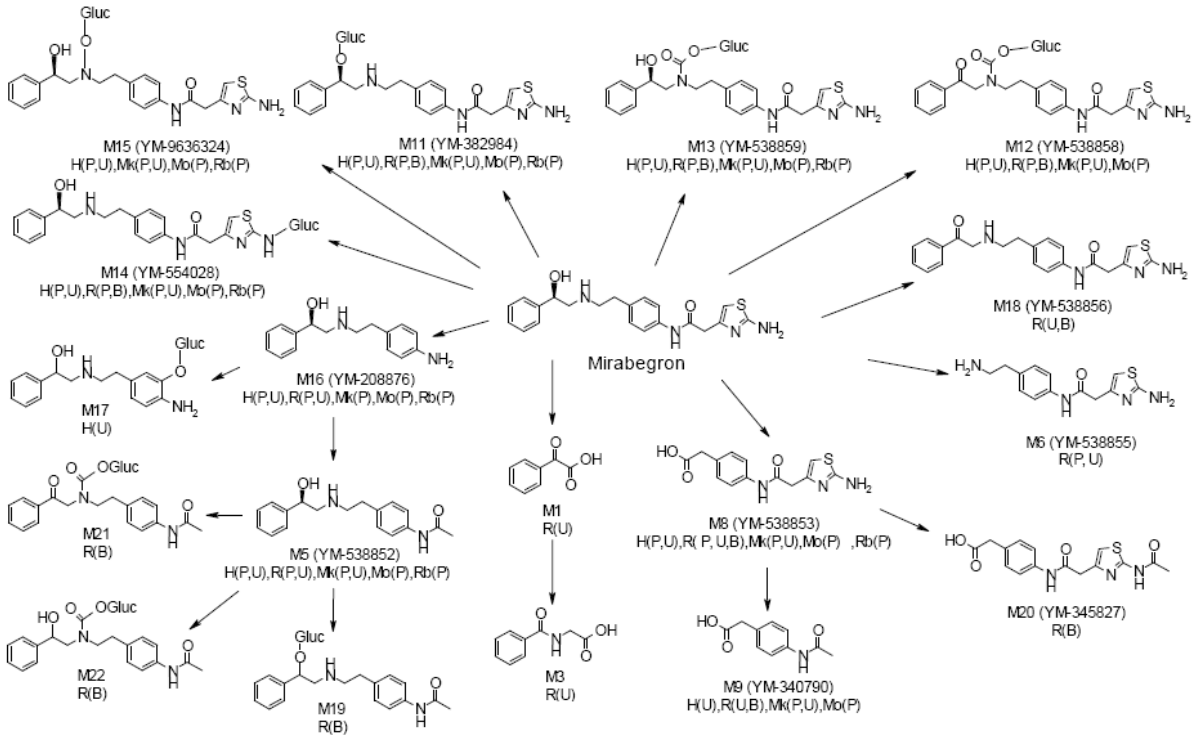


Figure copied from the Sponsor. Gluc: b-D-Glucuronosyl, H: human, R: rat, Mk: monkey, Mo: mouse, Rb: rabbit, P: plasma, U: urine, B: bile.

Metabolite Exposure (AUC_{0-24} ng-hr/mL) At Steady State															
			Human	Rat (mg/kg/day) 178-ME-077 († ME-125)			Mouse (mg/kg/day) 178-ME-102 (†ME-124)			Monkey (mg/kg/day) 178-ME-078			Rabbit (mg/kg/day) 178-ME-103 († ME-126)		
			50 mg	10	30	100	25	50	100	3	10	30	3	10	30
Mirabegron		M	341	468	3,056	20,473	4,134	12,564	35,007	387	1,316	5,709	NA	NA	NA
		F	512	417	2,355	18,821	3,959	11,048	23,877	324	1,382	4,647	160	445	3,220
M11	Phase 2	M	150	1.8	12.9	153	444	1,438	4,153	9,705	34,539	123,581	NA	NA	NA
	Glucuronide	F	201	2.1	15.5	201	348	1,106	2,273	12,945	43,303	131,878	41.1	91.7	838
M12	Phase 2	M	83	1.2	18.4	134	0.0	0.0	0.0	17.0	127	284	NA	NA	NA
	Glucuronide	F	99	0.0	10.8	137	0.0	0.0	0.0	45.8	133	321	0.0	0.0	0.0
M5	Phase 2	M	80	34.5	402	2,085	257	623	1,304	3.7	101	102	NA	NA	NA
	Acetyl conjugate	F	112	26.6	301	1,633	231	676	1,247	6.1	31.4	174	399	3,974	23,556
†M16	Phase 1	M	42	0	30.4	157	NA	NA	1861	3.1	13.2	59.9	NA	NA	NA
		F	70	1.5	22.7	158	NA	NA	1,297	1.0	17.4	70.8	418	NA	10,048
M14	Phase 2	M	69	10.1	76.7	497	91.5	286	821	6.9	46.5	122	NA	NA	NA
	Glucuronide	F	75	10.7	81.4	517	80.3	289	582	18.9	62.3	118	60.2	125	890
M15	Phase 2	M	31	0.0	0.0	0.0	0.0	7.1	49.5	21.1	195	361	NA	NA	NA
	Glucuronide	F	49	0.0	0.0	0.0	9.6	36.6	90.2	46.8	122	376	0.0	2.0	27.7
M13	Phase 2	M	10	0.0	4.0	45.6	11.4	47.3	224	104	524	1,466	NA	NA	NA
	Glucuronide	F	13	0.0	1.6	51.7	14.4	56.5	157	218	588	1,781	10.3	19.0	199
M8	Phase 1	M	**49***	83.7	678	4,044	182	571	1,239	32.7	169	515	NA	NA	NA
		F	**104***	36.2	334	2,534	338	984	1,383	45.9	151	528	3.0	10.1	88.5

Data was derived on day 15 of exposure in mice (178-ME-102), rats (178-ME-077), monkeys (178-ME-078), and rabbits (178-ME-103) and day 7 of dosing in fasted men and women ≥ 55 yrs (178-CL-072,). † In mice, rats, and rabbits the data for M16 was derived from separate two week exposure studies (178-ME-124, 178-ME-125, and 1780-ME-125) because the methods needed to be revised from study 178-ME-077, 178-ME-102. and 178-ME-103. The new methods resulted in reduced M16 exposures in all species (10x in rats, 10x mice, 2-3x in rabbits). As an internal control, mirabegron was assessed along with the reanalysis of M16 and the mirabegron levels were fairly similar to previous analysis. *M8 not quantifiable in humans at the 50 mg dose. NA – not assessed.

Metabolite Exposure At Steady State (Cmax ng/mL)															
			Human	Rat (mg/kg/day) 178-ME-077 († ME-125)			Mouse (mg/kg/day) 178-ME-102 (†ME-124)			Monkey (mg/kg/day) 178-ME-078			Rabbit (mg/kg/day) 178-ME-103 († ME-126)		
			50 mg	10	30	100	25	50	100	3	10	30	3	10	30
Mirabegron		M	43.5	60.3	579	1,952	866	1,957	2,526	89.5	334	1,503	NA	NA	NA
		F	66.3	110	290	1,233	1,275	2,003	2,144	92.8	323	843	58.2	189	958
M11	Phase 2	M	13.5	0.5	3.8	18.3	134	313	368	2,328	9,176	25,735	NA	NA	NA
	Glucuronide	F	18.9	1.4	4.7	26.9	131	267	216	4,229	11,359	29,609	25.3	61.4	370
M12	Phase 2	M	7.4	0.4	4.9	15.4	0	0	0	6.1	37.3	79.4	NA	NA	NA
	Glucuronide	F	8.6	0	2.8	14.8	0	0	0	20.8	40.4	88.3	0	0	0
M5	Phase 2	M	4.5	3.4	42.7	151	49.0	95.7	108	1.3	8.9	12.1	NA	NA	NA
	Acetyl conjugate	F	6.1	3.8	44.8	160	43.0	101	127	1.7	4.7	22.1	82.0	784	2,331
†M16	Phase 1	M	2.6	0	5.5	13.0	NA	NA	197	0.9	2.6	8.6	NA	NA	NA
		F	4.1	0.9	3.8	16.0	NA	NA	149	0.5	3.7	9.3	126	NA	1,108
M14	Phase 2	M	6.1	5.1	36.7	80.8	38.5	71.5	113	2.2	8.4	17.2	NA	NA	NA
	Glucuronide	F	6.7	4.6	37.1	154.3	23.6	65.0	110	3.8	8.3	15.2	17.1	32.6	139
M15	Phase 2	M	3.9	0	0	0	0	3.4	4.9	8.5	68.7	111	NA	NA	NA
	Glucuronide	F	5.5	0	0	0	4.7	10.2	11.0	23.2	45.4	117	0	2.0	13.1
M13	Phase 2	M	1.9	0	2.4	7.9	6.0	11.4	22.8	38.2	189	446	NA	NA	NA
	Glucuronide	F	2.1	0	1.0	7.1	8.0	15.9	14.8	109	196	559	6.4	13.5	76.6
M8	Phase 1	M	**5.6***	14.8	125	346	51.9	115	122	11.0	56.1	117	NA	NA	NA
		F	**10.6***	6.3	53.0	207	137	197	177	19.3	45.4	128	3.0	6.7	34.4

Data was derived on day 15 of exposure in mice (178-ME-102), rats (178-ME-077), monkeys (178-ME-078), and rabbits (178-ME-103) and day 7 of dosing in fasted men and women ≥ 55 yrs (178-CL-072). † In mice, rats, and rabbits the data for M16 was derived from separate two week exposure studies (178-ME-124, 178-ME-125, and 1780-ME-125) because the methods needed to be revised from study 178-ME-077, 178-ME-102, and 178-ME-103. *M8 not quantifiable in humans at the 50 mg dose. NA – not assessed

Chiral Inversion:
Mirabegron was not found to under go chiral inversion in plasma or urine samples from human males orally dosed with ^{14}C-mirabegron (178-ME-041).

5.1.4 Excretion

Rats:
Elimination was extensive in fasted male rats after single 10 mg/kg oral dose of ^{14}C-mirabegron since 81% of the radioactive dose was recovered within 24 hours (16% in urine and 64% in feces) and 91% was recovered within 72 hours (18% in urine and 73% in feces) (178-ME-022). In separate animals, 37% of the radioactivity was excreted in urine and 29% in bile after three days. Enterohepatic circulation was demonstrated since 27% of the radioactivity excreted in bile from ^{14}C-mirabegron dosed rats was reabsorbed after duodenal dosing of bile in naive rats. Fairly similar excretion findings were observed in study 178-ME-016. Unchanged mirabegron accounted for ½ the radioactivity in urine and ¼ of the radioactivity eliminated in bile (178-ME-056).

Monkeys: In monkeys receiving a single 10 mg/kg oral dose of ^{14}C-mirabegron, 97% of the radioactive dose was eliminated within 72 hours (178-ME-061). Radioactivity was equally eliminated in urine (46%) and feces (51%) within 72 hours (178-ME-061). Although plasma levels of mirabegron were below the limit of detection 24 hrs after dosing, only 51% of the total radioactivity was recovered in urine or feces after 24 hours. Similar to plasma, the primary radioactive compound in eliminated in urine over two days was M11 which accounted for 32% of the administered dose while only 5% of the administered dose was excreted in urine as unchanged mirabegron (178-ME-066). Five minor metabolites in urine accounting for less than 2% of the administered dose and one unidentified metabolite accounting for 2% of the dose were also quantified. Excretion of radioactivity in feces was predominantly due to mirabegron which accounted for 33% of the administered dose over 72 hrs. Six other metabolites were identified in feces none of which accounting for more than 6% of the dose.

Humans
In humans, excretion was more similar to monkeys with 55% and 34% of the radioactivity recovered in urine and feces, respectively (178-CL-007).

5.1.5 Potential for Drug Interactions

Drug interaction potential is addressed in the clinical pharmacology review. However, a brief description of the nonclinical findings is described below. Mirabegron may interfere with CYP3A4, CYP2D6, CYP2C19, butyrylcholinesterase, and UDPGTs enzyme activity (see section 5.1.3 above) and may also be transported by organic cation transporters and p-glycoprotein.

Metabolism (see section 5.1.3 above):
Mirabegron was metabolized in vitro by human CYP3A4 and to a lesser extent by CYP2D6. In vitro studies also suggest that YM178 is a strong inhibitor of the human CYP2D6 and a weak inhibitor of CYP2C19 and CYP3A4. In vitro esterase activity

(hydrolysis of YM178 to M16) by butyrylcholinesterase (BChE) was observed in human plasma. Formation of several glucuronide conjugates in animals and humans also suggests that there is a possibility for drug interactions with UDP-glucuronosyltransferases.

Transport:
In vitro assays suggest that mirabegron is a low affinity substrate for transport by human the human P-glycoprotein (P-gp) but under the conditions tested; it was not an inhibitor of P-gp (178-ME-031, 178-ME-032, 178-ME-131 and 178-ME-132). Clinical study also suggest that mirabegron is a weak P-gp inhibitor since exposure to the P-gp substrate digoxin (0.25 mg) increased only slightly in both Cmax (29%) and AUC_{last} (27%) and renal clearance decreased 9% after 14 days of 100 mg of YM178 (178-CL-059). Transport of mirabegron across membrane vesicles was not aided by expression of the human multidrug resistant-associated protein 2 (hMRP2) (178-ME-0131).

In vitro cell culture studies suggest that there is a potential for YM178 to inhibit drug transport by the human organic cation transporter (hOCT1) and minimal ability to inhibit hOCT2 (178-ME-086). Cell culture assays also suggest that YM178 can be transported by the human OCT1, OCT2, and OCT3 (178-ME-092).

In vitro study suggests that mirabegron is a substrate for uptake transport by the human organic anion transporting polypeptide 1A2 (OATP1A2) but not by OATP2B1 (178-ME-0133). Uptake of mirabegron into *Xenopus* oocytes was aided by the expression of organic anion transporting polypeptide (OATP1A2) but uptake of mirabegron was not aided by the expression of OATP2B1 in a human embryonic kidney cell line (HEK293).

Mirabegron was not transported across a monolayer of cells by the human breast cancer resistance protein (BCRP) in vitro (178-ME-132). Likewise transport of mirabegron across vesicles was not aided by expression of the human BCRP protein (178-ME-131).

Cellular uptake of mirabegron was not enhanced by cells expressing proton coupled peptide transporters (PEPT1 and PEPT2) (178-ME-130) which are involved in the absorption of small peptides (di- and tripeptide) or drugs from the intestines.

5.2 Toxicokinetics

Pharmacokinetic Summary in Humans, Mice, Rats, Monkeys and Rabbits						
Population	Duration	Dose (mg/kg)	C_{max} (ng/mL)		AUC_{0-24} (ng-hr/mL)	
			Male	Female	Male	Female
Human Fasted, healthy, young 178-CL-031	Day 10	50 mg	33	46	262	368
		100 mg	72	112	519	800
		200 mg	220	264	1,443	2,046
		300 mg	381	530	2,473	3,888
Human Elder Female 178-CL-072 (n=11)	Day 7 Fasted	50 mg	43.5	**66.3**	341	**512**
Mice 178-TX-029 13-Week Tox	Week 13	50	936	2,083	4,903	6,931
		100	1,939	2,054	14,643	13,899
		200	3,124	2,507	26,340	24,461
Mice 178-TX-031 (Carcinogenicity Study)	Week 52	25	652	1,206	1,975	2,399
		50	1,316	943	4,749	4,676
		100	1,888	1,905	12,905	10,713
Rats 178-TX-025 (26-Week Tox Study)	Week 26	3	23	36	110	113
		10	160	161	1,098	854
		30	719	665	8,542	5,994
		100	2,856	2,313	28,372	30,071
Rats 178-TX-032 (Carcinogenicity Study)	Week 52	12.5	991		3,519	
		25	1,070	1,133	6,168	5,943
		50	2,190	1,953	12,990	12,261
		100	-	3,770	-	23,043
Monkeys 178-TX-026 (1 Year Tox Study)	Week 52	3	116	101	421	342
		10	312	241	1,267	1,092
		30	718	742	4,301	4,031
Dogs 178-TX-018 (2 Week Tox Study)	Week 14	1	49	53	313	269
		3	337	314	1,384	1,408
		10	1,730	1,538	8,561	7,045
	Day 1	20 (lethal)	1,916	2,152	8,404	7,887
Rabbits – non-pregnant 178-TX-056	Day 7	3	-	46	-	127
		10	-	560	-	1,239
		30	-	4,398	-	10,967
Rabbits – pregnant 178-TX-016 (Embryo/fetal Tox) 178-TX-057	GD20	3	-	244	-	364
		10	-	2,753	-	7,230
		30	-	5,938	-	18,277
	GD 20	3	-	83	-	260
		10	-	673	-	1,863
		30	-	6,003	-	18,426

6 General Toxicology

Calculation of the exposure multiples in animals relative to humans dosed at the maximum recommended human dose (MRHD):

Unless otherwise noted, the multiples of systemic exposure (MOE) between animals and humans dosed at the sponsor's maximal recommended human dose (MRHD) of 50 mg are derived by dividing the exposure (AUC or Cmax) in animals by that in fasted women ≥ 55 years old (AUC = 512 ng-hr/mL, Cmax = 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Dose multiples are based on fasted women at least 55 years of age. With respect to age, sex, and fed state, this subpopulation was chosen as a worst case scenario because they were the subpopulation with the greatest steady state exposure (Study 178-CL-072 and 178-CL-041).

6.1 Single-Dose Toxicity

6.1.1 Single Dose Oral Studies:

Summary Findings From Single Oral Dose Studies in Rats and Dogs			
Species No./sex/group Study No	Treatment	Dose (mg/kg)	Main Results
Rat 5/sex/group 178-TX-012	Oral: gavage Single dose followed by 14 days of monitoring	300, 500, or 800	2/5 males and 3/5 females died with in 24 hrs of dosing at 800 mg/kg. Salivation, lacrimation chromodacryorrhea, and hypoactivity ≥ 300 M&F. Prone position ≥ 500 mg/kg M&F. Mydriasis ≥ 800 mg/kg M&F. Pale lobule in liver in dead male at 800 mg/kg. All signs recovered 3 days post dosing. 750 mg/kg was lethal to 1/2 females and 1,000 mg/kg was lethal to 2/2 females and 2/2 males in a preliminary study.
Dog 1/sex/group 178-TX-017	Oral: gelatin capsule. Single dose and 14 days of monitoring	0, 0.3, 3, or 30	The male at 30 mg/kg died within 50 minutes of administration. Extreme skin reddening at ≥ 0.3mg/kg M&F. Vomiting at 30 mg/kg M&F. Gasping and recumbence at 30 M. All survivors normal by 48 hrs post dosing. ↑ Heart rate at ≥ 0.3 mg/kg for 24 hrs w/ 2 fold increase for 1-4 hrs. PR and QT intervals slightly decreased 1-8 hrs post dosing but QTc not effected. Blood pressure may have dropped but it was not clear. Moderate necrosis of zygomatic salivary gland in F at 30 mg/kg and focal acinar dilation/disruption of the zygomatic salivary gland in M at ≥ 0.3 and F at 3 mg/kg. AUC_{0-24} from 0.3 mg/kg to 30 mg/kg for M/F was 45/68, 1,155/732 and not determined/7,730 ng-hr/mL. Cmax for M/F from 0.3 mg/kg to 30 mg/kg for M/F was 15/19, 241/369, and 3,028/551 ng/mL.

6.1.2 Single Dose Intravenous Toxicity Studies

Single Dose Intravenous Toxicity in Monkeys:
A single **10 mg/kg intravenous (IV) dose (114x MRHD based on Cmax) caused** salivation, pale oral mucosa, dyspnea, loss of pupillary reflex and mydriasis, **ventricular tachycardia, and death** in a male monkey within 15 minutes of dosing (178-TX-033). No other animals were given this dose. In the male that died, red dots on the left ventricular papillary muscle and endocardial hemorrhaging were observed. Additionally focal myocardial necrosis and endocardial hemorrhage was noted in a female monkey 10 days after she received a 3 mg/kg (34x MRHD) dose and 3 days after she received a 0.1 mg/kg dose (2x MRHD). PR interval was increased in males for up to one hour ≥ 0.3 mg/kg (5x MRHD) and females at 3 mg/kg (34x MRHD). QRS interval was prolonged in a female after a 3 mg/kg dose but she recovered within 30 minutes (34x MRHD). The NOAEL was 0.1 mg/kg due to ECG findings at ≥ 0.3 mg/kg.

Toxicokinetics in Monkeys after Single Intravenous Dose						
iv Dose (mg/kg)	Tmax (hr)		Cmax (ng/mL)		AUC_{0-24} (ng-hr/mL)	
	Male	Female	Male	Female	Male	Female
0.1	0.05	0.05	122	118	39	39
0.3	0.05	0.05	349	243	155	151
1.0	0.05	0.05	785	787	716	616
3.0	0.05	0.05	2,636	2,237	2,131	2,542
10.0	0.05	Not Dosed	7,561*	Not Dosed	Unknown	Not Dosed
The data is from a single male and female for each dose. * Only one sample at 3 minutes post-dose was taken from the male that died in the 10 mg/kg group. The data was analyzed in study 178-TX-045. Monkeys administered sequential doses of 0.3, 3, and 0.1 mg/kg or 1 and 10 mg/kg with a 7 to 11 day washout between doses (N = 1/sex/sequential dose group).						

6.2 Repeat-Dose Toxicity

6.2.1 Repeat-Dose Intravenous Toxicity Studies

Repeat Dose Intravenous Toxicity in Rats:
A preliminary 5-day repeated IV exposure study was conducted in rats at 3, 10, or 30 mg/kg/day (178-TX-044). **30 mg/kg was lethal to 2/5 females and 5/5 males within 1 hr of the first or second dose. Prior to death the animals displayed mydriasis, tremors, bradypnea, clonic convulsions, gasping, shallow rapid respiration, lacrimation, and salivation**. No abnormal clinical signs were observed at 3 mg/kg, but at ≥ 10 mg/kg prone position, decreased movement, and mydriasis were observed.

Similar findings were observed after two weeks of daily IV exposure to rats at 1, 3, or 10 mg/kg/day (n = 12/sex/group) (178-TX-035). No mortality was observed at up to 10 mg/kg. Clinical signs observed at 10 mg/kg including prone position (M 10/12, F 1/12), decreased movement (M 12/12, F 5/12), and mydriasis (M 12/12, F 12/12). No adverse histopathology was observed despite a slight increase in heart weight at 10 mg/kg in

females (absolute 7% and body weight normalized 5%) and males (absolute heart weight 8%). White adipose lipid droplets were very slightly to slightly decreased at 3 mg/kg (male 3/12, Female 2/10) and 10 mg/kg (male 8/12, female 3/12). There was a minor 1-1.5% decrease in serum chloride at ≥ 1 mg/kg and a consequent elevation in urine chloride at 30 mg/kg. The NOAEL was 3 mg/kg due to clinical signs and heart weight affects at 10 mg/kg.

Toxicokinetics in Rats After IV Dosing (mean values)											
Day	Dose mg/kg	C_0 (ng/ml)		Cmax (ng/ml)		$T_{1/2}$ (hr)		Male		Female	
		M	F	M	F	M	F	AUC_{0-24} (ng-hr/ml)	MOE	AUC_{0-24} (ng-hr/ml)	MOE
1	1	255	182	212	156	2.2	1.7	353	0.7	252	0.5
	3	858	765	728	612	1.7	1.6	1014	2.0	774	4.5
	10	3,408	3,141	2,900	2,637	1.5	1.3	4,074	8.0	3,657	7.1
14	1	311	213	250	174	2.1	1.6	410	0.8	265	0.5
	3	885	834	819	652	1.7	1.7	1,223	2.4	900	1.8
	10	3,835	3,447	3,234	2,723	1.4	1.5	4,782	9.4	3,350	6.5

C_0 – estimated plasma concentration at Time 0.
Multiple of Exposure (MOE) = Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072).
N = 4/sex/time point.

Repeat Dose Intravenous Toxicity in Monkeys:
Two weeks of daily IV exposure in monkeys resulted primarily in adverse cardiac effects and potential mild effects on the thymus, spleen, atretic follicles, and oocytes (178-TX-034). Monkeys were dose at 0, 0.3, 1, or 3 mg/kg/day for two weeks (n=3/group). No obvious adverse drug related effects were observed on body weight, food consumption, ophthalmologic evaluation, urinalysis, clinical chemistries, and hematology. The multiples of exposure for the cardiac findings are based on Cmax and non-cardiac findings are based on AUC. The NOAEL was 0.3 mg/kg (1x MRHD, Cmax) due to tachycardia at ≥ 1.0 mg/kg (5x MRHD, Cmax).

Dosing was not lethal but one male at 3 mg/kg **(14x MRHD based on Cmax) went into coma** after the third dose but he recovered. The sponsor suspected the cause of coma to be due to mirabegron dependent reduction in blood pressure. Specifically, tachycardia was observed within 0.1 hr of dosing in 1/3 males at 1 mg/kg (5x MRHD, Cmax) and also at 3 mg/kg (14x MRHD, Cmax) in 2/3 males and 2/3 females. Heart rate was normal within 1 hr of dosing. ECG assessment was not possible when the animals were experiencing tachycardia. In animals not experiencing tachycardia, the **PR interval and QRS interval were elongated** 0.1 hr after doing from days 1-14 in the male and females at 3 mg/kg (14x MRHD, Cmax). PR and QRS were not clearly affected at 1 mg/kg (3-5x MRHD, Cmax). Very slight focal myocardial degeneration and focal mononuclear cell infiltration were observed in 1 or 2 male monkeys ≥ 0.3 mg/kg (0.2x MRHD, AUC) but not female monkeys.

The spleen of 1/3 males at 3 mg/kg (3x MRHD, AUC) was enlarged and increased in weight. Thymus involution was observed in a single male and female control, however, the incidence increased to 2 or 3 animals per group at ≥ 0.3 mg/kg (0.2x MRHD, AUC)

in females and ≥ 1 mg/kg (1x MRHD, AUC) in males while the severity slightly increased from very slight to slight in the controls to very slight to moderate in the treated animals. Very slight hemorrhaging of the atretic follicle was observed in 1/3 monkeys at ≥ 1 mg/kg (0.6x MRHD, AUC) and 3 mg/kg (2.6x MRHD, AUC). Additionally very slight oocyte mineralization was observed in at 0.2 (1/3), 0.6 (1/3), and 2.5x MRHD (2/3) monkeys.

Toxicokinetics in Monkeys after Intravenous Dosing for 2 Weeks (N=3/group/sex)

Day	*iv* Dose (mg/kg)	Male		Female		$T_{1/2}$ (hr)		Male		Female	
		Cmax (ng/mL)	MOE †	Cmax (ng/mL)	MOE †	M	F	AUC_{0-24} (ng-hr/mL)	MOE †	AUC_{0-24} (ng-hr/mL)	MOE †
1	0.3	68	1.0	71	1.1	1.4	1.4	88	0.2	80	0.2
	1.0	317	4.8	203	3.1	2.7	2.2	620	1.2	301	0.6
	3.0	907	13.7	1,034	15.6	1.8	1.8	1,533	3.0	1,278	2.5
14	0.3	67	1.0	78	1.2	2.2	1.6	92	0.2	90	0.2
	1.0	237	3.6	210	3.2	2.5	1.8	520	1.0	292	0.6
	3.0	938	14.1	904	13.6	2.0	1.9	1,430	2.8	1,338	2.6

† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072).

6.2.2 Repeat Dose Oral Toxicity Studies

6.2.2.1 Repeat Dose Oral Toxicity Studies in Mice

Summary of 2-week and 13-week oral dose toxicology studies mice

A two week toxicity study was conducted with Crj:B6C3F1 SPF mice to determine the appropriate dose for the 13-week toxicity study using the same strain of mice (178-TX-028). Also, later in development, another 2-week toxicity study was conducted using Crlj:CD1(ICR) mice because this strain of mice was to be used in nonclinical combination therapy studies (178-TX-052).

Crj:B6C3F1 mice:
2-week oral gavage dose range-finding toxicology study 178-TX-028
Mice were dosed daily at 0, 30, 100, or 300 mg/kg/day for two weeks (n=10/sex/group). Mirabegron was lethal to 4/10 males and 2/10 females at 300 mg/kg (F 63x and M 72x MRHD based on Cmax) within 30-60 minutes of the first dose in a preliminary two-week repeat-dose study in mice. Decreased activity, lateral/prone position, and colonic convulsions were prodromal signs prior to death and were observed in the surviving animals at 300 mg/kg. Decreased activity was also observed at 100 mg/kg. No adverse signs were observed after the second day of dosing at up to 300 mg/kg. Body weight gain and food consumption increased in females at ≥ 30 mg/kg and males at ≥ 100 and ≥ 30 mg/kg, respectively. No adverse gross pathology was observed in any animal. Histology was not assessed.

Toxicokinetics in CrjB6C3F1 Mice 2-Week Toxicology Study 178-TX-028							
	Dose (mg/kg)	30 mg/kg		100 mg/kg		300 mg/kg	
	Sex	M	F	M	F	M	F
Multiple of Human Exposure (AUC) †		10.2	9.3	51.9	33.8	123	97.7
	(Cmax)	14.2	16.8	32.5	48.0	71.7	62.5
T_{max} (h)	Day 1	2	2	2	2	1	1
	Day 14	1	2	1	2	4	1
C_{max} (ng/ml)	Day 1	889	918	1,966	2,187	3,236	4,458
	Day 14	936	1,113	2,158	3,185	4,756	4,145
AUC_{0-24} (ng-h/ml)	Day 1	3,885	3,525	14,922	12,405	38,918	44,499
	Day 14	5,243	4,755	26,570	17,289	63,127	50,041
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Multiple of exposure based upon week 2 AUC or Cmax levels in mice.							

Hematology was not assessed in the 2-week, 13-week or 2-year carcinogenicity study with Crj:B6C3F1 mice (178-TX-028, 178-TX-029, and 178-TX-031).

Crlj:CD1(ICR) mice:
2-week oral gavage dose range-finding toxicology study (178-TX-52)
The high dose was set at 100 mg/kg/day based on the death of one female Crlj:CD1(ICR) at 300 mg/kg (estimated exposure > 50x MRHD) after displaying decreased spontaneous movement and clonic convulsion after the first dose (178-TX-051).

In this study, mice were dosed at 0, 10, 30, or 100 mg/kg/day for two weeks (n = 6/sex/group).

No deaths were observed after two weeks of dosing up to 100 mg/kg. However, movement decreased immediately post-dose in at least one animal of all groups in a dose dependent manner but disappeared with repeated dosing.

Dose and time dependent increases in food consumption were observed beginning at day 7 in males at ≥ 30 mg/kg and females at 100 mg/kg.

Absolute and body weight adjusted liver weights were elevated 11-27% in males at ≥ 10 mg/kg and 17% of females at ≥ 30 mg/kg. Pale areas of hepatocytes were noted in males only at ≥ 10 mg/kg which was thought to be due to accumulation of glycogen. A NOAEL was not established because of this finding.

The size of lipid drops decreased in the brown fat around the thoracic aorta of both sexes at ≥ 10 mg/kg. Likewise, the adipocytes in the white fat around the mesenteric lymph nodes were small and multivacuolated in both sexes at ≥ 10 mg/kg. These findings are expected pharmacology.

Hematopoiesis was slightly induced at the lowest dose 2x MRHD. Red cell numbers (8-15%), hemoglobin (8-17%), and hematocrit (9-18%) were elevated in males at ≥ 10 mg/kg and females at ≥ 30 mg/kg. Reticulocytes were elevated 40% in males at ≥ 30 mg/kg. This correlated with a 46% increase in spleen weight in males at 100 mg/kg and an increased severity (minimal to mild) of extramedullary hematopoiesis in both sexes at 100 mg/kg.

Toxicokinetics in Crlj:CD1 Mice 2-Week Toxicology Study 178-TX-052							
	Dose (mg/kg)	10		30		100	
	Sex	M	F	M	F	M	F
Multiple of Human Exposure (AUC) †		2.3	2.3	9.6	9.4	29.3	63.2
(Cmax) †		5.3	4.4	16.8	19.9	62.9	52.9
Tmax (hr)	Day 1	2	2	2	2	2	2
	Day 14	1	1	1	2	1	2
Cmax (ng/mL)	Day 1	236	232	778	633	1,630	2,732
	Day 14	352	291	1,111	1,316	4,167	3,508
AUC_{0-24} (ng-hr/mL)	Day 1	817	849	3,649	3,229	13,521	19,227
	Day 14	1,183	1,164	4,893	4,831	14,990	32,374
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron OCAS (Study 178-CL-072). Multiple of exposure based upon week 2 AUC or Cmax levels in mice.							

Summary of A 13-Week Repeated Oral Dose Combination Toxicity Study with Mirabegron and YM905 in Crlj:CD1(ICR) Mice (178-TX-053)

Crlj:CD1(ICR) mice were dosed with 0, 10, or 30 mg/kg of mirabegron for 13 weeks (n = 12/sex/group).

Similar to the two week study with this strain, hematopoiesis appeared to be induced at 10-30 mg/kg. Red blood cells increased (M 9%, F 11%) at 30 m/kg. Likewise hematocrit and hemoglobin was elevated at 30 mg/kg in males (11%) and females at 10 mg/kg (6%) and 30 mg/kg (14-15%). Reticulocytes were also elevated in females at ≥ 10 mg/kg (19-20%). Blood urea nitrogen was elevated in males (28%) and females (22%) dosed with mirabegron at 30 mg/kg. The only clear treatment related histopathology finding was in white fat where the adipocytes were small with microvesicles at ≥ 10 mg/kg. The intensity of this finding was very slight to slight.

Toxicokinetics in Crlj:CD1 Mice 13-Week Toxicology Study 178-TX-053					
	Dose (mg/kg)	10		30	
	Sex	M	F	M	F
Multiple of Human Exposure (AUC) †		1.2	1.2	3.7	5.7
(Cmax) †		3.6	4.0	9.6	14.1
Tmax (hr)	Day 1	1	1	2	2
	Day 91	1	1	2	2
Cmax (ng/mL)	Day 1	264	227	473	567
	Day 91	239	262	636	933
AUC_{0-24} (ng-hr/mL)	Day 1	793	616	2,005	2,747
	Day 91	594	589	1,885	2,932
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Multiple of exposure based upon week 13 AUC or Cmax levels in mice.					

Study title: **A 13-week Oral Gavage Toxicity Study of YM178 (Mirabegron) in Mice (Dose Range-Finding Study for Two Year Carcinogenicity Study 178-TX-031)**

Study no.: 178-TX-029 and 178-TX-041
Study report location: Module 4.2.3.4.2
Conducting laboratory and location: (b) (4)
Date of study initiation: November 15, 2002
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: YM178 (mirabegron), Lot K1780107, 99.4% pure

Key Study Findings

- One female at 200 mg/kg died during week 7 of dosing and two males died in the 200 mg/kg group within the first hour of the initial dose. Cause of death is unknown and gross abnormalities were not reported for these animals. Deaths were assumed to be mirabegron dependent.
- Slight increase in food intake was noted in all dosage groups. Weight gain was increased in males at ≥ 50 mg/kg and females at ≥ 100 mg/kg.
- Decreased spontaneous movement was observed in all males and females at 200 mg/kg within 30 minutes of the initial dose but not upon subsequent dosing. Males and females were observed in the prone position at ≥ 50 mg/kg from the 9th to the 13th week of exposure.
- Minimal hepatocyte hypertrophy was observed at 200 mg/kg (48x MRHD) in both sexes.
- 100 mg/kg was set as the maximum dose for carcinogenicity study based upon mortalities and prone position at 200 mg/kg. The 100 mg/kg dose represents a 27x multiple of exposure in humans based upon an AUC = 512 ng-h/mL for fasted elderly females dosed with 50 mg mirabegron OCAS-M for seven days (178-CL-072).

Methods

Doses:	0, 50, 100, or 200 mg/kg
Frequency of dosing:	Daily for 13 weeks
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Mouse / Crj:B6C3F$_1$ SPF
Number/Sex/Group:	12/sex/group
Age:	6 weeks
Weight:	F 17.2 to 21.7 g and M 20.3 to 24.8 g
Satellite groups:	35 animals of each sex at 50, 100, and 200 mg/kg for toxicokinetics. Blood was pooled from three mice per sex and dose at 1, 2, 4, 8, and 24 hrs after the first dose and after dosing during the 13th week. The responses to drug withdrawal were not assessed.
Unique study design:	Clinical chemistry, hematology, ophthalmoscopy, urinalysis, and organ weights were not conducted. Histological evaluations were conducted on the control and high-dose animals. The mid-dose groups were only evaluated if the sponsor believed that there was an effect at the higher dose. Histology of the following organs was evaluated in the low- and mid-dose groups: adrenal, Harderian gland, kidney, liver, skin (inguinal), spleen, and thymus. The submandibular gland and parotid glands were evaluated in the mid-dose females but not males. Brown adipose tissue in the renal hilum of the kidney and subcutaneous white adipose tissue of the skin were examined to evaluate the effect on brown and white adipose tissue. Periodic acid-Schiff (PAS) staining of the liver and kidneys was also conducted to evaluate glycogen content.
Deviation from study protocol:	Nothing that significantly effected study outcome

Observations and Results

Mortality

One female at 200 mg/kg died within two hours of dosing on day 43. The only adverse response reported for this animal was decreased activity within 30 minutes of the initial dose. In the toxicokinetic group, two males died within the first hour of the initial 200 mg/kg dose. No gross abnormalities were reported for any of these animals and the cause of death was not determined but is considered treatment related at least in the male mice. The high dose in this study was based on mortalities following a single 300

mg/kg dose in 4/10 males and 2/10 females on the first day of dosing in a preliminary two-week repeat-dose study in mice (178-TX-028).

Clinical Signs

Males and females were observed in the prone position at all doses from the 9th to the 13th week of exposure. This occurred 30 minutes after dosing and declined dose dependently throughout the day. Spontaneous movement was decreased in all males and females at 200 mg/kg within 30 minutes of the initial dose on day one.

Incidence of Clinical Signs								
N = 12/sex/group	Male				Female			
Dose (mg/kg)	0	50	100	200	0	50	100	200
Multiple of Human Exposure (AUC) †	0	9.6	28.6	51.4	0	13.5	27.1	47.8
Prone Position (weeks 9-13)		12	12	12		12	12	12
↓ Spontaneous Movement (day 1 only)				12				11
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Multiple of exposure based upon week 13 AUC levels in mice.								

Body Weights

A slight increase (6-7%) in mean body weight was observed at week 13 at ≥ 100 mg/kg in males and females. When analyzed as body weight gain, elevated weight gain was observed in males at ≥ 50 mg/kg and females at ≥ 100 mg/kg.

Increased Body Weights After 13 Weeks of Exposure						
Sex	Male			Female		
Dose (mg/kg/day)	50	100	200	50	100	200
Mean body weight at week 13	4%	**7%****	**6%****	3%	**6%****	4%
Body weight gain at week 13	**16%***	**29%****	**28%****	9%	**19%****	**15%****
Significantly different from control, *P<0.05 and **P<0.01						

Feed Consumption

Elevated food consumption was noted at all doses in males and females by the second week of dosing.

Gross Pathology

Adverse pathology was not reported in the single female at 200 mg/kg and two males in the toxicokinetic group at 200 mg/kg that died. The cause of death was not determined. Among the animals that survived the 13 weeks of exposure, a small number of animals in each group at ≥ 50 mg/kg displayed dark red foci in the stomach.

Incidence of Adverse Pathology						
Dose (mg/kg/day)	50		100		200	
Number and Sex	12M	12F	12M	12F	12M	11F*
Glandular Stomach - dark red focus	1	2	2	4	1	4
* One female died on day 43.						

Histopathology
The GLP histological evaluation was reported in a separate study (178-TX-041).

Adequate Battery: Yes, however bone was not assessed.

Peer Review: Yes

Histological Findings:
Attenuation of the adrenal X-zone occurred in females at ≥ 50 mg/kg (75-100%) and not in the controls or males (see Table below). The function of the X-zone is unknown but may have a similar function as the fetal adrenal cortex in humans. The X-zone in female mice undergoes slow regression and ultimately degenerates during the first pregnancy (5).

Perhaps as a sign of stress, minimal to mild porphyrin concretions were noted in all mirabegron dosed animals while only being observed in four female control mice and no male control mice. Similarly minimal thymic atrophy was observed in both males (Con 0, LD 0, MD 3/12, and HD 6/12) and females (Con 0/12, LD 4/12, MD 9/12, and HD 6/12) in a dose responsive manner.

Brown adipose tissue in the renal hilum displayed decreased lipid droplet size in all of the treated males and 11/12 treated females at all doses. This is expected pharmacology for β3-AR agonists. White adipocytes became multivacuolated in the skin of almost all dosed males and females with the severity increasing with the dose of mirabegron.

In the liver, minimal to mild increase in glycogen content was observed in hepatocytes in the mirabegron groups only. This could be in part due to the elevated feed intake in all mirabegron dose groups and the elevated body weight in males at ≥ 50 mg/kg and females at ≥ 100 mg/kg. Minimal **hepatocyte hypertrophy** was observed in 5/12 males and 2/12 females at 200 mg/kg.

In the spleen, the severity of extramedullary hematopoiesis increased from minimal to mild in some mirabegron dosed male and female mice but the incidence was not affected.

Dose dependent occurrence of atrophy of the parotid gland acinar cells was observed in LD (2/12), MD (10/12), and HD (12/12) females but not in control animals or in males. Likewise the incidence and severity of the submandibular acinar atrophy increased with dose in females (Con 2/12, LD 6/12, MD 8/12, and HD 9/12). Males were not affected.

Summary of Adverse Histology (Incidence)										
Organ	**Finding**	**Extent**	**Male (n=12/group)**				**Female (n=12/group)***			
			0	**50**	**100**	**200**	**0**	**50**	**100**	**200**
Adrenal	Attenuated X-zone (unknown function)	Minimal	0	0	0	0	0	6	6	2
		Mild	0	0	0	0	0	2	4	10
		Total	0	0	0	0	0	8	10	12
	Hyperplasia- focal cortex	Mild	0	0	1	0	0	0	0	0
	Hyperplasia - spindle cell	Minimal	0	4	3	1	12	12	9	10
Harderian	Pigmentation porphyrin concretion	Minimal	0	11	6	4	4	9	7	4
		Mild	0	1	6	8	0	3	5	8
		Total	0	12	12	12	4	12	12	12
Kidney	Vacuolation - tubular cortex	Minimal	12	7	2	1	0	0	0	0
	Basophilia - tubular	Minimal	0	1	1	0	1	0	0	0
	↓ Lipid drop size - brown adipose	Minimal	0	12	11	8	0	9	3	2
		Mild	0	0	1	4	0	2	8	9
		Total	0	12	12	12	0	11	11	11
Liver	↑ Glycogen – Hepatocytes	Minimal	0	1	8	8	0	11	4	8
	Hypertrophy - Hepatocyte	Minimal	0	0	0	5	0	0	0	2
Parotid	Atrophy – Acinar	Minimal	0	NE	NE	0	0	1	7	1
		Mild	0	NE	NE	0	0	1	3	11
		Total	0	NE	NE	0	0	2	10	12
	Necrosis – Acinar single cell	Minimal	0	NE	NE	0	0	0	0	1
Skin	Small multivacuolated white adipocyte	Minimal	0	9	4	0	0	4	0	0
		Mild	0	0	8	9	0	7	3	0
		Moderate	0	0	0	3	0	1	9	11
		Total	0	9	12	12	0	12	12	11
Spleen	Extramedullary Hematopoiesis	Minimal	9	11	6	7	8	8	7	7
		Mild	0	0	5	5	2	4	5	5
		Total	9	11	11	12	10	12	12	12
Stomach	Erosion, glandular	Minimal	0	0	0	0	0	0	0	1
Submandibular	Atrophy - acinar	Minimal	0	NE	NE	0	2	4	2	0
		Mild	0	NE	NE	0	0	2	6	9
		Total	0	NE	NE	0	2	6	8	9
	Cell infiltration	Minimal	0	0	0	0	1	0	0	1
Thymus	Atrophy	Minimal	0	0	3	6	0	4	9	6
Thyroid	Ectopic thymus	Minimal	0	0	0	0	1	0	0	1

Table adapted from the sponsor's data. NE – not examined.
*Includes the evaluation of the female in the 200 mg/kg group that died on day 43.

Toxicokinetics

Mirabegron appears to be readily absorbed with T_{max} ranging between 1-4 hrs after the first and last dose. Exposure increased in proportion to the dose at both time points. However, in females the C_{max} levels were similarly independent of dose at week 13. Mirabegron did not appear to accumulate with repeated dosing.

Toxicokinetics in Mice During the 13-Week Toxicology Study							
	Does (mg/kg)	50		100		200	
	Sex	M	F	M	F	M	F
	Multiple of MRDH (AUC) †	9.6	13.5	28.6	27.1	51.4	47.8
T_{max} (h)	Day 1	4	2	2	2	1	1
	Week 13	1	2	2	2	2	2
C_{max} (ng/ml)	Day 1	1,060	1,568	2,060	2,578	4,289	3,691
	Week 13	936	2,083	1,939	2,054	3,124	2,507
AUC_{0-24} (ng-h/ml)	Day 1	6,621	6,773	17,460	13,822	29,434	28,980
	Week 13	4,903	6,931	14,643	13,899	26,340	24,461
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Multiple of exposure based upon week 13 AUC levels in mice.							

Dosing Solution Analysis
Homogeneity of the dosing solutions was assessed at weeks 1, 4, and 13. The mean concentration of all dosing solutions was within 97.0% to 99.5% of the desired concentration. The homogeneity of the upper, middle, and lower fractions was fair with the desired concentration being within 6% of the target for the low (96% to 101%), middle (96% to 99.2%), and high dose (94% to 100%) groups for all time points.

6.2.2.2 Repeat Dose Oral Toxicity Studies in Rats

Summary of 2-Week Oral Dose Toxicity Study in Rats

Rats (F344/DuCrj SPF) were dosed daily for two weeks at 0, 10, 30, 100, or 300 mg/kg (n = 10/sex/group) (178-TX-013). Satellite groups with six rats per sex and group were assessed after two weeks of drug withdrawal and another seven rats per sex and group were used for toxicokinetics. The maximal dose of 300 mg/kg was chosen because of a preliminary 2-week range finding study, which was not submitted, noted that all rats dosed at 500 mg/kg died or became moribund after 9 days of treatment but no adverse effects were observed at 250 mg/kg other than decreased weight gain.

Most findings appeared related to effects on **body weight and metabolism** and were recoverable after drug withdrawal (178-TX-013). The NOAEL was 10 mg/kg (1x MRHD). Adverse hematology, clinical chemistry, urinalysis, body weight, and histology findings (except for white fat effects) were resolved after two weeks of drug withdrawal. There were no deaths at exposure up to 300 mg/kg (136x MRHD). Decreased activity was observed in up to 4/16 males at 300 mg/kg for the first two days and 5/16 females for the first three days. Body weight was reduced 7-10% and body weight gain was reduced in 53% to 60% in males and females at 300 mg/kg seven and 14 days after dose initiation. Male body weight gain was also reduced 24% at 100 mg/kg. Body weight recovered after drug withdrawal. The reduced body weight is correlated with a recoverable reduction in food consumption primarily in the first week of dosing in males (7%) and females (13%) at 300 mg/kg. Probably as a compensatory response to increased feed intake, water intake increased slightly at ≥ 100 mg/kg. Gross pathology

noted recoverable whole body wasting in some animals at ≥ 100 mg/kg. These findings correlated with reduced lipid droplets in white fat at ≥ 10 mg/kg and brown fat at ≥ 100 mg/kg in both sexes. The severity of reduce lipid droplets in white fat increased from slight to marked with increasing dose. Upon recovery all histological findings resolved except for a slight decrease in lipid droplets at ≥ 100 mg/kg in some animals of both sexes.

Severe reduction in **reproductive organ** weights may be a physiological response to reduced body weight. The body weight normalized weights of the ovaries and uterus were reduced 38% and 24%, respectively, at 300 mg/kg. Uterine atrophy was observed histologically at ≥ 30 mg/kg. Also the body weight normalized seminal vesicle weight was reduced at least 44% at ≥ 100 mg/kg, and the prostate was reduced 31% at 300 mg/kg. Histologically the seminal vesicles were described as hyposecretory at ≥ 100 mg/kg.

Recoverable slight to moderate atrophy of the thymus was observed in females at 300 mg/kg along with decreased body weight normalized thymus weight males (-15%) and females (-38%) at 300 mg/kg. **Hematology** findings were apparent but recoverable. Platelet levels were reduced 10-19% at ≥ 100 mg/kg in both sexes. Reticulocytes were reduced 27-33% in both sexes at 300 mg/kg. Coincident with this was a slight to moderate reduction in hematopoiesis in the bone marrow of females at 300 mg/kg. Differential neutrophil counts were elevated 32-36% in both sexes at 300 mg/kg. Lymphocytes were repressed 57% in females at 300 mg/kg. Prothrombin time was slightly reduced in 15% males at ≥ 100 mg/kg and 8% in females at 300 mg/kg. Activated partial thromboplastin time was slightly decreased (10-14%) in males only at ≥ 100 mg/kg.

Although no adverse histology was reported in the **liver**, minimal changes were observed in weight and clinical chemistry parameters. Body weight normalized liver weights increased slightly in males at ≥ 30 mg/kg and females at ≥ 100 mg/kg. Triglycerides were elevated 59-79% in males only at ≥ 30 mg/kg. Cholesterol and phospholipids were elevated 17-22% in males at ≥ 100 mg/kg and 17-20% in females at 300 mg/kg. ALT was elevated in males (15-29%) and females (25-50%) at ≥ 30 mg/kg. ALP was elevated 10-17% in females at ≥ 30 mg/kg and 9-12% in males at ≥ 100 mg/kg. Potassium was elevated 14-15% in males and 9% in females at ≥ 100 m/kg.

Potential effects on the **kidney** are unclear. There was a slight increase in kidney weight but no adverse histopathology was observed. However, the urine concentration of potassium and chloride increased roughly two fold while the amount of sodium, potassium and chloride excreted in urine increased at ≥ 100 mg/kg in both sexes. Urine output increased slightly in males at ≥ 10 mg/kg. Urine protein levels appeared increased at 300 mg/kg in both sexes but this may be a false positive finding because mirabegron itself is misinterpreted as protein a protein with the method used (N-Multistix® SG) (See study 178-TX-49 in Section 10.1). Urine levels of mirabegron were

estimated to be 2 mg/mL after a 300 mg/kg dose and mirabegron is misidentified as protein by this method at concentrations of mirabegron of ≥ 0.25 mg/mL.

Elevated adrenal gland weight and reduced brain weight at 300 mg/kg were not correlated with histopathology and it is not clear if these findings are treatment related.

Ophthalmologic examination and response to whistle (hearing) response were normal.

Change in Body Weight Normalized Organ Weight at Week Two								
	Male				Female			
Dose (mg/kg)	10	30	100	300	10	30	100	300
Adrenals				+14%				+19%
Brain				-11%				-9%
Kidneys		+5%	+9%	+14%			+5%	+6%
Liver		+5%	+5%	+12%			+5%	+11%
Spleen				-13%				
Thymus				-15%				-38%
Ovaries								-24%
Uterus								-51
Prostate				-31				
Seminal Vesicle			-44	-52				
Changes noted if one group is at least 10% different from control. Only statistically significant findings reported above. Since body weight was reduced 9% and 11% in females and males respectively at 300 mg/kg, organ weights that differ from the control by more than 10% are reported normalized to body weight.								

Incidence of Gross Pathology at Week Two										
	Male (n=10/group)					Female (n=10/group)				
Dose (mg/kg)	0	10	30	100	300	0	10	30	100	300
Thymus - atrophy									2	10
Epididymis - nodule				1	2					
Prostate - small				2	4					
Seminal Vesicle - small	2	2	3	6	8					
Uterus – small						0	0	1	3	9
White Fat - decreased				4	10		1		8	10
Whole Body - wasting	0	0	0	1	5	0	0	0	4	6

Incidence of Histology Findings at Week Two										
	Male (n=10/group)					Female (n=10/group)				
Dose (mg/kg)	0	10	30	100	300	0	10	30	100	300
Bone Marrow – ↓hematopoiesis –slight/mod	0	-	-	-	0	0	0	0	0	4
Kidney – mineralization - slight	2	-	-	-	5	4	-	-	-	4
Thymus – atrophy – slight to mod	0	-	-	-	0	0	0	0	0	4
Seminal Vesicle–hyposecretory - slight	3	3	3	6	9	/	/	/	/	/
Uterus – atrophy - slight	/	/	/	/	/	0	0	1	3	9
White Fat – ↓ lipid drops – slight to marked	0	1	10	10	10	0	2	5	10	10
Brown Fat - ↓ lipid drops - slight	0	0	0	3	10	0	0	0	7	10

Toxicokinetics in Rats During the 2-Week Toxicology Study									
		Male				Female			
Dose (mg/kg)		10	30	100	300	10	30	100	300
Multiple MRHD (AUC) †		1.1	3.9	45.2	135.7	0.9	5.7	31.1	156.3
T_{max} (h)	Day 1	2	4	1	8	2	4	8	8
	Day 14	2	2	8	4	2	2	4	1
C_{max} (ng/ml)	Day 1	55	564	1,384	2,760	98	452	1,560	2,096
	Day 14	81	214	1,664	5,333	93	378	1,222	6,042
AUC_{0-24} (ng-h/mL)	Day 1	439	3,450	1,7788	48,922	582	5,318	21,058	39,043
	Day 14	542	2004	23,131	69,464	450	2,901	15,920	80,003

† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron OCAS (Study 178-CL-072). Multiple of exposure based upon week-2 AUC levels in rats.

Summary of 13-Week Oral Toxicity Study in Rats (178-TX-020, TK in 178-TX-042)

As in the 2-week study, the principle effects were on body weight, liver, hematology, kidneys, and reproductive organs and the minimal lethal dose was lowered to 100 mg (44x MRHD). The NOAEL is 10 mg/kg (2x MRHD).

Rats (F344/DuCrj SPF) were dosed daily for thirteen weeks at 0, 10, 30, 100, or 300 mg/kg (n = 10/sex/group). Effects of four weeks of drug withdrawal were assessed in six rats per sex at 0, 100, and 300 mg/kg.

Deaths occurred between weeks 9 and 13 at 100 mg/kg (F 1/16, 44x MRHD) and 300 mg/kg (F 4/16, M 1/16, > 130x MRHD) (See TK table below). Cause of death was not clear but edema in the lungs and heart, along with slight cardiac hemorrhaging was observed in some dead females at 300 mg/kg.

Animals recovered from all clinical signs during the recovery period. Clinical signs in both sexes increased with dose and duration and included salivation at ≥ 30 (M) or 100 mg/kg (F), lacrimation at ≥ 100 mg/kg, and hair loss at 300 mg/kg. Abdominal distension was only observed in females at ≥ 100 mg/kg. One high dose male on day 84 also had clonic convulsions, mydriasis, and tachypnea.

Weight/Metabolism

Dose dependent reductions in body weight (7-24%) and body weight gain (16-55%) were observed in males at ≥ 30 mg/kg. Although males at 300 mg/kg gained three times more body weight during the recovery period than control animals, the absolute body weight was still reduced 11% after four weeks of withdrawal. In females, a recoverable 8% reduction in body weight and a 31% reduction in body weight gain were observed at 300 mg/kg.

Coincident with the reduced body weight and whole body wasting at 300 mg/kg in both sexes, slight to moderate reduction in lipid drops in white fat was observed in males at ≥ 100 mg/kg and females at ≥10 mg/kg. Similarly, lipid droplets were reduced slight to

moderately in the brown fat of males at ≥ 30 and females at ≥ 300 mg/kg. Effects on white and brown fat were fully recoverable.

Despite the reduction in body weight, food consumption was elevated 10% to 22% at ≥ 30 m/kg in males and 15% to 19% in females at ≥ 100 mg/kg during the entire 13 week exposure period. Water intake over the dosing period increased 17% to 49% at ≥ 30 mg/kg in males and 20% in females at 300 mg/kg. Effects on feed and water intake were recoverable after drug withdrawal.

Reproductive organs
Unlike the two-week study, body weight normalized testes weight was increase 10-26% at ≥ 30 mg/kg. Absolute testes weight was not affected and there was no effect on testes weight in the 2-week study. Histologically there was no correlate although minimal seminiferous tubule atrophy and slight hyposecretory seminal vesicle was observed in one male at 300 mg/kg. Reduced prostate and seminal vesicle weight was not observed in the 13-week study as it was in the two-week study.

Body weight normalized uterus weights were decreased 36% and 63% at 100 mg/kg and 300 mg/kg, respectively. This coincided with slight atrophy of the uterus observed at ≥ 100 mg/kg.

Hematology
Hematology findings were recoverable after four weeks of withdrawal.

Minor increases (< 7%) hematology parameters were suggestive of a slight increase in hematopoiesis (hematocrit, hemoglobin, and RBCs) at ≥ 30 to 100 mg/kg. A dose dependent decrease (6-17%) in platelets was observed in both sexes at ≥ 30 mg/kg. Similar to the two-week study, APTT was decreased 13% and 23% in males only at 100 and 300 mg/kg respectively.

Liver
Effects were fairly similar in the two and 13-week studies for liver weight, AST, ALT, cholesterol. However, adverse histology was observed in this study but not in the 2-week study and triglyceride levels were decreased in this study in males while it was increased in the two-week study. Clinical chemistry (except for elevated ALP and reduced triglycerides) and were recoverable after four weeks of withdrawal.

Body weight adjusted liver weights were elevated 5-21% in males ≥ 30 mg/kg and 12-20% in females at ≥ 100 mg/kg. Adverse hepatic histology included, pigment deposition (lipofuscin) (M ≥ 100 mg/kg, F ≥ 30 mg/kg), hepatocyte swelling (M & F at 300 mg/kg), fibrosis (300 mg/kg M & F), and necrosis (1 M at 300 mg/kg). Accumulation of melanin and glycogen were confirmed in the hepatocytes histologically.

Triglycerides were reduced dose dependently 39% to 82% in males only at ≥ 10 mg/kg and remained 35% reduced at 300 mg/kg after four weeks of drug withdrawal. Quizzically, triglycerides were elevated instead of reduced as in the 2-week study.

Unlike the 2-week study, AST was elevated 19% and 4.7 fold at 100 and 300 mg/kg, respectively, in males only. ALT was elevated at 30 mg/kg (M 20%), 100 mg/kg (M 37%), and 300 mg/kg (M 10.2 fold, F 59%). Elevated ALT was much more severe at the high dose in males compared to the two week study. However, the elevated AST and ALT at 300 mg/kg in males was due to a single animal (1/10) with elevated AST and ALT levels 38 and 90 times the control levels. This animal had centrilobular hepatocyte swelling, centrilobular necrosis, and pigment deposition suggesting that the elevated LFT may be treatment related. However, since this was only observed in one animal of one sex, it is unclear if this is truly related to mirabegron exposure. AST and ALT were normal after drug withdrawal. Cholesterol was elevated 16% in males and 21% in females at 300 mg/kg. Cholesterol effect was a recoverable and was not observed at 100 mg/kg as in the two-week study. ALP was elevated in both sexes at 100 mg/kg (M 24%, F 22%) and 300 mg/kg (M 73%, F 89%) and remained elevated in males (17%) and females (44%) at 300 mg/kg after the withdrawal period. As in the two week study, a recoverable 16% decrease in glucose was observed in males only at 300 mg/kg.

Kidney

Renal effects were fairly similar in the two and 13-week studies including elevated kidney weights, serum potassium, and urine chloride and osmotic pressure. A new finding was elevated pigment deposition in the kidney.

Body weight normalized kidney weights increased in males (13-28%) at ≥ 30 mg/kg and females (12-20%) at ≥100 mg/kg. Slight to moderate pigment deposition (lipofuscin) was the only clear treatment related histological finding in males at ≥ 100 and females at 300 mg/kg. Urinary bladder stones were observed in half the males at 300 mg/kg. The composition of the stones was not determined.

Effects on serum electrolytes were mild, not clearly dose-related, and were normal after recovery. Potassium was elevated 11%-17% in males at ≥ 30 mg/kg and in females at 30 mg/kg (17%) and 100 mg/kg (18%) but not at 300 mg/kg. Similar potassium effects were observed in the two week study. Creatinine was slightly repressed in males at ≥ 30 mg/kg (15% to 22%) and females at ≥ 10 mg/kg (10% to 27%) while albumin was elevated 8-9% in both sexes at 300 m/kg. Both of these serum proteins were similar to control after recovery.

In urine, chloride concentration was elevated at week 2, 6, and, 12 at ≥ 30 and 100 mg/kg in both sexes (collected 4PM to 9AM, food and water withheld). At week 12, chloride concentration increased 0.5-fold to 3.3-fold in males at ≥ 30 mg/kg and 90-96% in females at ≥ 100 mg/kg. Likewise, the total amount of chloride excreted in urine increased ≥ 70-80% at 100 mg/kg in males and 60-80% in females at ≥ 100 mg/kg. Osmotic pressure elevation became more apparent with time and was elevated at week 12 in males at 30 mg/kg (46%) and 100 mg/kg (2.9 fold) but not at 300 mg/kg. While in females, osmotic pressure was elevated 41-45% at ≥ 100 mg/kg. Potential elevations in sodium and potassium are difficult to ascribe to treatment because the control value varied between time points and the elevated levels were not necessarily dose related.

The total amount of sodium eliminated at week 12 did, however, appear elevated at least 2-fold at ≥ 100 mg/kg but only in males. Urine bilirubin was detected at ≥ 100 mg/kg in both sexes. Urine pH was slightly decreased (6.0) in some rats of both sexes at ≥ 100 mg/kg during weeks 2-13 of dosing. Urine protein levels appeared elevated at 300 mg/kg throughout the study but this is likely a false positive effect because mirabegron itself reacts with the dipstick method at 0.250 mg/mL, and urine levels at this dose are predicted to be ≤ 23.5 mg/mL (See section 10.1). This assumption is based on an ADME study where 29% of a 10 mg/kg radioactive dose was excreted in urine of rats within 24 hrs of dosing (178-ME-016). Assuming similar elimination rate after a 300 mg/kg dose, 87 mg of drug related compound would be expected to be excreted in a volume of 3.7 mL of urine (23.5 mg/mL).

Stomach – Cecum

At 300 mg/kg, slight hemorrhaging (2M and 1F) and focal necrosis (2M) were observed in the stomach. Also at 300 mg/kg slight pigment deposition (lipofuscin and hemosiderin) (3M and 7F) and vacuolar degeneration (1M and 2F) were observed in the cecum. Rats recovered or were recovering from these findings after drug withdrawal. These findings were not observed in the two-week study.

Other

At 300 mg/kg, special histological staining in the liver, kidney, bone marrow, and cecum was positive for lipofuscin (a lipoprotein pigment) and glycogen. Electronmicroscopy suggested that the lipofuscin in the liver was due to break down of mitochondria. Tissue from the cecum was also positive for hemosiderin (iron-protein pigment) possibly due to macrophage activity after hemorrhaging. These findings persisted after the recovery period. The increased lipofuscin pigmentation may be due to increased beta oxidation of fatty acids. Increased lipofuscin is associated with macular degeneration and lipofuscinosis and may be lethal. However, this is likely an adaptive response and is not a significant safety concern since it was not reported in the six month toxicology study or in the two-year carcinogenicity study.

Elevation in body weight normalized adrenal gland, heart, and brain weights were also observed without correlated histopathology and the relationship to treatment is unclear. Body weight normalized spleen weight was not reduced like it was in the 2-week study.

Fully recoverable slight to moderated reduction in zymogen granules was observed in the parotid gland at ≥ 30 mg/kg in both sexes. In the mandibular gland, eosinophilic granules decreased in 100-300 mg/kg. Neither of these findings was reported in the two-week study.

As in the two week study, ophthalmologic evaluation and hearing assessment (response to whistle) did not reveal adverse findings at week 13.

Change in Body Weight Normalized Organ Weight at Week 13								
	Male				Female			
Dose (mg/kg)	10	30	100	300	10	30	100	300
Adrenals				+29%				
Brain			+10%	+26%				
Heart			+17%	+23%				
Kidneys		+13%	+17%	+28%			+12%	+20%
Liver		+5%	+16%	+21%			+12%	+20%
Lungs			+12%	+22%				
Pituitary								-33%
Testes		+10%	+10%	+26%				
Thymus				-19%				-22%
Ovaries							+14%	-
Uterus							-36%	-63%
Only statistically significant findings at least 10% different from control are reported. Since body weight was reduced 10% in females at 300 mg/kg and 7-25% in males respectively at ≥ 30 mg/kg, organs are reported normalized to body weight. All organ weighs were recovered after one month except for a slight 7% increase in kidney weight at 300 mg/kg in males only.								

Incidence of Gross Pathology at Week 13										
	Male					Female				
Dose (mg/kg)	0	10	30	100	300	0	10	30	100	300
N	10	10	10	10	10	10	10	10	10	8
Thymus - atrophy					8					5
Stomach – red patch					2					
Urinary Bladder - Stone					5					
Seminal Vesicle - atrophic					1					
Uterus – atrophic									1	6
White Fat - decreased			1	9	10					7
Whole Body - wasting		1			7					7
All gross pathology recoverable.										

Incidence of Histology Findings at Week 13 (Includes 2 Female Animals at 300 mg/kg that Died During Dosing Phase)										
	Male (n=10/group)					Female (n=10/group)				
Dose (mg/kg)	0	10	30	100	300	0	10	30	100	300#
Heart – necrosis – focal - slight	2	-	-	-	2	0	-	-	-	2
- edema - slight	0	0	0	0	0	0	0	0	0	2#
- hemorrhage – slight	0	0	0	0	0	0	0	0	0	2#
Kidney – pigment deposit – tubule epithelia – slight to moderate	0	0	0	**2**	**7**	0	0	0	0	**8**
Bone Marrow – macrophage pigment deposit – slight	0	0	0	**10**	**10**	0	0	0	**2**	**10#**
- hemorrhage - slight	0	0	0	0	0	0	0	0	0	1#
Lymph node – pigment deposit -	0	0	0	0	0	0	-	0	0	2
Thymus – atrophy – slight	0	0	0	**2**	**8**	0	0	0	0	**6#**
- hemorrhage – slight to mild	0	0	0	0	0	0	0	0	0	2#
Lung – edema – slight - moderate	0	0	0	0	0	0	0	0	0	2#
- congestion - slight	0	0	0	0	0	0	0	0	0	1#
Stomach – hemorrhage - slight	0	0	0	0	2	0	0	0	0	1
- focal necrosis – slight to moderate	0	0	0	0	2	-	-	-	-	-
Cecum – pigment deposit in macrophages – lamina propria - slight	0	0	0	0	**3**	0	0	0	0	**7**
- vacuolar degeneration - slight	0	0	0	0	1	0	0	0	0	2
Liver – pigment deposit – hepatocellular, macrophage and/or Kupffer cells– slight to moderate	0	0	0	**4**	**10**	0	0	**1**	**5**	**10#**
- hepatocyte swelling - slight	0	0	0	0	**10**	0	0	0	0	**7#**
- fibrosis - slight	0	0	0	0	**3**	-	-	-	-	1#
- necrosis centrilobular - moderate	0	0	0	0	1	0	0	0	0	0
Mandibular gland – ↓ eosinophil granule - slight	0	0	0	**2**	**7**	0	0	0	0	**10#**
- hyposecretory change - min	0	0	0	0	3	0	0	0	0	0
Parotid gland – ↓zymogen granules – slight/mod	0	0	**8**	**10**	**10**	0	0	**8**	**10**	**10#**
Seminal Vesicle – hyposecretory - slight	0	-	-	-	1	/	/	/	/	/
Testes- seminiferous tubule atrophy - min	0	-	-	-	1	/	/	/	/	/
Uterus – atrophy - slight	/	/	/	/	/	0	0	0	1	**8#**
- dilatation lumen - slight	/	/	/	/	/	3	5	2	0	0
Adrenal gland – hemorrhage – slight	0	0	0	0	0	0	-	-	-	1
White Fat – ↓ lipid drops – slight to moderate	0	0	0	**10**	**10**	0	**3**	**10**	**10**	**10#**
Brown Fat - ↓ lipid drops – slight to moderate	0	0	**10**	**10**	**10**	0	0	0	0	**6#**
# Includes data from one or two females that died during the study at 300 mg/kg. Findings were fully or partially recoverable except for pigment deposits in the liver, kidney, cecum, and bone.										

Toxicokinetics in Rats After 1 Day or 13 Weeks of Oral Exposure (178-TX-020, 178-TX-042)									
	Dose (mg/kg)	10		30		100		300	
	Sex	M	F	M	F	M	F	M	F
	Multiple of Human Exposure(AUC) †	1.8	1.6	10.0	6.9	50.0	44.2	159.5	130.4
T_{max} (h)	Day 1	4	2	4	4	4	2	8	1
	Week 13	2	2	2	2	8	2	2	8
C_{max} (ng/mL)	Day 1	96	163	633	421	1,189	1,284	3,254	2,881
	Week 13	157	175	604	602	1,725	1,761	5,768	4,544
AUC_{0-24} (ng-h/mL)	Day 1	566	536	3,739	3,800	16,870	18,312	53,319	43,803
	Week 13	918	818	5,170	3,511	25,648	22,629	81,656	66,745

† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Multiple of exposure based upon week-13 AUC levels in rats. N = 4/sex/time point at 1, 2, 4, 8, and 24 hrs after dosing except after deaths which occurred between weeks 9-13 in TK animals at 100 mg/kg (1F) and 300 mg/kg (1 M, 2F).

Study title: **A 26-Week Repeated Oral Dose Toxicity Study of YM178 in Rats**

Study no.:	178-TX-025 TK also reported in 178-TX-030 Liver EM histology report 178-TX-040
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 24, 2001
GLP compliance:	Yes, (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K1780006, 100.3% pure

Key Study Findings

- No mortality up to 100 mg/kg (56-59x MRHD)
- Male rats appeared to be generally more sensitive
- The NOAEL in males was 3 mg/kg (0.2x MRHD). The NOAEL in females was not established.
  - Lacrimation ≥ 3 mg/kg in Females and 10 mg/kg Males
  - Prone Position ≥ 10 mg/kg in Males and ≥ 30 mg/kg Females
  - Salivation ≥ 30 mg/kg Males and Females
- Reduced body weight ≥ 30 mg/kg in males only, which was recoverable
- Increased food consumption in males at ≥ 10 mg/kg and females at ≥ 30 mg/kg was recoverable
- Increased water intake was observed in males at ≥ 10 mg/kg and at 100 mg/kg in females which was recoverable
- AST and ALT slightly elevated in males at ≥ 30 mg/kg and ALP elevated in males at 100 mg/kg. These findings were recoverable.

- Triglycerides were decreased 45-66% in males at ≥ 30 mg/kg and 62% in females at 100 mg/kg. **Triglycerides remained low after recovery in males at ≥ 30 mg/kg.**
- Slight to marked decrease in lipid droplets in white fat of males and females at ≥ 10 and 30 mg/kg respectively. Observed after 13 weeks of withdrawal at 100 mg/kg.
- Slight decrease in lipid droplets in brown fat at ≥ 30 mg/kg of both sexes. Near full recovery after drug withdrawal.
- Relative liver weights increased at ≥ 30 mg/kg in both sexes while the absolute weight of thymus decreased in males at ≥ 30 mg/kg and absolute and relative weight decreased in females at ≥ 100 mg/kg. Both recoverable findings.
- Slight increases in RBC, HCT, HGB were observed in addition to a decrease in platelets and prothrombin time in males at ≥ 10-30 mg/kg. All were recoverable responses.
- Toxicokinetics did not reveal a sex difference for C_{max} or $AUC_{0\text{-}24hr}$. The blood levels increased greater than the increase in dose.
- **Comparisons between 13 and 26 week exposure studies in rats.** Similar findings were reported during both 13 and 26 week exposure studies in rats. However, in general higher doses were needed to see the same effect during the 13 week study (178-TX-020). The highest dose in the 13 week study was 300 mg/kg compared to only 100 mg/kg in the 26 week study.

Methods

Doses:	0, 3, 10, 30, or 100 mg/kg
Frequency of dosing:	Once daily for 26 weeks
Route of administration:	Oral gavage
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Rats/F344/DuCrj ([redacted] (b) (4)) [SPF]
Number/Sex/Group:	12/sex/group
Age:	7 weeks
Weight:	Female 114-124g and Male 158-176g
Satellite groups:	Recovery: 6 rats per sex at 0, 30, and 100 mg/kg Toxicokinetics: 8 rats per sex at 3, 10, 30, and 100 mg/kg
Unique study design:	None
Deviation from study protocol:	None that significantly effected study outcome

Observations and Results

Mortality

No mortality was observed. However, higher doses used in a 13-week study resulted in the death of four females and one male at 300 mg/kg and one female at 100 mg/kg after 9-13 weeks of exposure (178-TX-020). No cause of death was noted. However, the lungs and heart of the dead females at 300 mg/kg were edematous and slight hemorrhaging of the hearts was noted.

Clinical Signs

Onset of clinical signs began sooner with increasing doses and continued from onset to the end of the dosing period. The duration the sign after onset of was not mentioned.

Off target agonism of α_{1D} adrenergic and β adrenergic receptors are expected to induce lacrimation and off target β1 adrenergic agonism is expected to cause salivation (6-12).

- Lacrimation at ≥ 3 mg/kg in Females and ≥ 10 mg/kg Males
- Salivation at ≥ 30 mg/kg in Males and ≥ 30 mg/kg Females
- Prone Position at ≥ 10 mg/kg in Males and ≥ 30 mg/kg Females
- These adverse effects were no longer observed after one week of drug withdrawal.
- Clinical signs unique to the 13-week study (178-TX-020) include, loss of hair, pale auricles, convulsions, mydriasis (dilated pupils), rapid breathing at 300 mg/kg in males. Distended abdomen was also observed in females at ≥ 100 mg/kg.

Lacrimation:
Males at 3 mg/kg (week 21 and 24, not clearly significant), 10 mg/kg (weeks 19-26), 30 mg/kg (weeks 14-26), and 100 mg/kg (weeks 2-26)
Female at 3 mg/kg (weeks 19-26), 10 mg/kg (weeks 15-26), 30 mg/kg (weeks 5-26), and 100 mg/kg (weeks 2-26)

Salivation:
Males at 10 mg/kg (weeks 20-25, not clear effect), 30 mg/kg (weeks 4-26) and 100 mg/kg (weeks 1-26)
Females at 30 mg/kg (weeks 11-26) and 100 mg/kg (weeks 2-26)

Prone Position: (decreased motor activity, abdomen on floor, lean against cage wall, and/or spread legs)
Males at 3 mg/kg (weeks 23-26, not clearly significant), 10 mg/kg (weeks 12-26), 30 mg/kg (weeks 12-26) and 100 mg/kg (weeks 12-26)
Females at 10 mg/kg (not significant), 30 mg/kg (weeks 12-26) and 100 mg/kg (weeks 12-26)

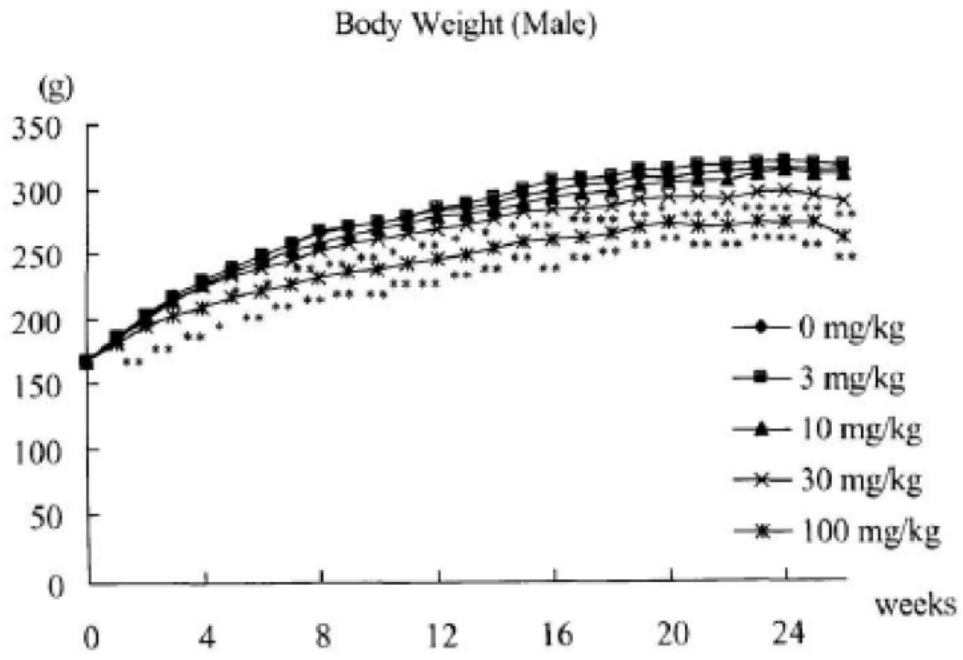
Incidence of Clinical Signs										
Dose (mg/kg)	0		3		10		30		100	
Sex	M	F	M	F	M	F	M	F	M	F
Number of animals (n=12 + 6 recovery 0, 30, 100)	18	18	12	12	12	12	18	18	18	18
Multiple of Human Exposure (AUC) †			0.2	0.2	2.1	1.7	17	12	55	59
Lacrimation		3	2	11	10	11	16	18	18	18
Salivation					2		18	14	18	18
Prone Position			5		12	1	18	18	18	18
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron OCAS for 7 days (178-CL-072).										

Body Weights

- Reduced body weight at ≥ 30 mg/kg in males only
- Weight was reduced in males as early as week 2 at 100 mg/kg and week 6 at 30 mg/kg.
- All recoverable during 13 week recovery period

- Body weight results were also reduced after 13 weeks of exposure in males at ≥ 30 mg/kg and females at ≥ 300 mg/kg (178-TX-020).

Reduced Body Weight in Male Rats					
	Week 26 of Dosing			Week 13 of Recovery	
Dose	Weight (g)	Weight Gain (g)	% Increase from Day 0	Weight (g)	Weight Gain (g)
0	315	147	88%	340	24
3	319	151	90%		
10	312	145	87%		
30	**291****	**123****	**73%**	327	35
100	**264****	**95****	**56%**	335	**64****
**P < 0.01: significantly different from control.					



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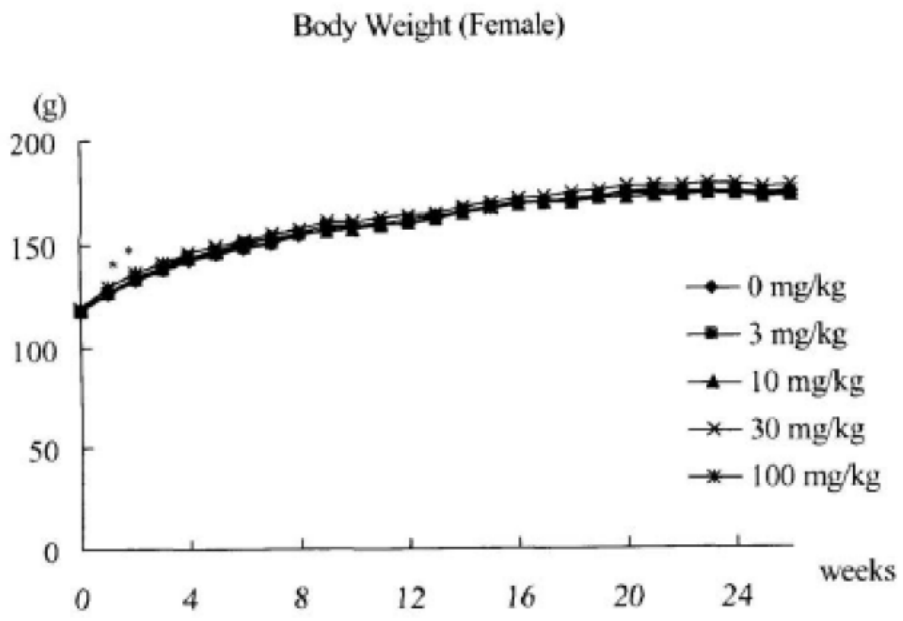

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Feed Consumption

In the 13-week study increased feed consumption occurred in males and females at ≥ 30 and ≥ 100 mg/kg respectively (178-TX-020). In the 26-week study, feed intake increased in males at ≥ 10 mg/kg and at ≥ 30 mg/kg in females. Elevated feed intake was observed in males intermittently at 3 mg/kg and continuously at 10 mg/kg beginning at week 12 and continuously from the first dose to last dose at ≥ 30 mg/kg. Females dosed with ≥ 30 mg/kg had increased feed intake from the second week of dosing until the second week of recovery. Food consumption was similar to controls in all dosage groups within 2 weeks of drug withdrawal except for males at 100 mg/kg who took 5 weeks to recover normal feeding activity.

Increased Cumulative Food Consumption (g)				
Dose	Male		Female	
	Exposure Weeks 1 to 26	Recovery Weeks 1 to 13	Exposure Weeks 1 to 26	Recovery Weeks 1 to 13
0	2678 ± 114	1332 ± 48	1809 ± 52	910 ± 25
3	2801 ± 131		1807 ± 78	
10	**2886 ± 135****		1884 ± 117	
30	**3208 ± 146****	1357 ± 57	**2027 ± 85****	921 ± 33
100	**3522 ± 157****	1435 ± 40	**2238 ± 122****	949 ± 54
**P<0.01: significantly different from control.				

Water Consumption

Increased water intake was observed in males at ≥ 10 mg/kg and females at ≥ 100 mg/kg. The magnitude of increased water consumption was much greater in males. The

time to recovery was not observed but all dosage groups consumed similar amounts of water as the control upon 13 weeks of recovery.

The minimal dose required for increased waster consumption was greater in the 13-week study, 30 mg/kg for males and 300 mg/kg for females (178-TX-020).

Increased Water Consumption (g)						
	Male			Female		
Dose	Week 13	Week 25	Recovery Week 13	Week 13	Week 25	Recovery Week 13
0	16 ± 3	16 ± 2	18 ± 2	14 ± 2	15 ± 3	15 ± 2
3	17 ± 2	16 ± 2		14 ± 3	14 ± 2	
10	18± 2	**18 ± 2***		14 ± 3	13 ± 2	
30	**21 ± 3****	**22 ± 3****	16 ± 1	15 ± 2	14 ± 2	14 ± 2
100	**26 ± 3****	**29 ± 2****	18 ± 2	**16 ± 3***	**18 ± 3****	16 ± 2
**P<0.01: significantly different from control. *P<0.05: significantly different from control.						

Ophthalmoscopy
No treatment related effect

Auditory
No treatment related effect

Hematology
Only very slight hematology effects primarily in males were observed suggestive of increased hematopoiesis. Findings are similar to that seen in the 13-week study. Findings were recoverable after the drug withdrawal period.

Significant Hematology Scores						
	Male				Female	
Dose mg/kg	0	10	30	100	0	100
HCT (%)	45.3		**47.2** (+ 4%)**	**47.7** (+ 5%)**		
HGB (g/dL)	15.9		**16.6** (+ 4%)**	**16.8** (+ 6%)**		
RBC ($x10^6/mm^3$)	9.5	**9.8* (+ 3%)**	**9.8 ** (+ 3%)**	9.6		
MCV (μm^3)	47.6		**48.3** (+ 1%)**	**49.7** (+ 4%)**		
MCH (pg)	16.7		**17.0* (+ 2%)**	**17.5** (+ 5%)**		
PLT ($x10^3/mm^3$)	775	**719** (+ 7%)**	**698** (- 10%)**	**689** (-11%)**	778	**711* (-9%)**

Clinical Chemistry
Similar finding were observed in the 13- and 26-week studies including elevated AST, ALT, ALP, and potassium, along with repressed APTT, creatinine, and triglycerides but the LFT findings were small and did not appear to progress with continued exposure. All parameters were recoverable except for decreased triglycerides in males.

Clinical Chemistry						
Sex	Male				Female	
Dose mg/kg	0	10	30	100	0	100
PT time (sec)	19.3			**18.4* (-5%)**		
APTT (sec)	25.1			**23.4* (-7%)**		
Total Cholesterol (mg/dL)	58			**66** (+14%)**		
Triglycerides (mg/dl)	70	53	**40** (-43%)**	**27** (-62%)**	22	**8** (-66%)**
Potassium (mmol/L)	3.63	**3.99**(+10%)**	**4.07**(+12%)**	**4.14** (+14%)**	3.61	**4.21**(+16%)**
I. Phosphate (mg/dL)	4.93			**5.41** (+10%)**		
Creatinine (mg/dL)	0.28		**0.24** (-14%)**	**0.23** (-18%)**	0.29	**0.24** (-17%)**
AST (U/L)	87	111	**128* (+47%)**	**120**		
ALT (U/L)	54	71	**90** (+67%)**	**88** (+63%)**		
ALP (U/L)	405	427	425	**506** (+25%)**		
* P<0.05, **P<0.01 significantly different from control.						

Urinalysis

Urine was collected at week 13 in two segments (dosing to 4PM and 4PM to 9AM) apparently to determine if effects recovered after exposure to mirabegron dropped. Additionally the effect of restricting food and water on overnight collection of urine (4PM to 9 AM) were assessed at weeks 13 and 25.

The interim week 13 findings with access to food and water include decreased output, decreased pH, and increased bilirubin all of which were recoverable by the afternoon of the same day of dosing. Rats recovered from these effects when they were hydrated. However, when food and water were restricted overnight (4PM to 9AM), urine electrolytes (Na, K, and Cl) became more concentrated and elimination of electrolytes increased while urine became more acetic and bilirubin increased. Overnight sampling at week 25 with restricted food and water access resulted in similar elevations in electrolytes, acidification of urine, and elevated bilirubin.

<u>Week 13- collection from dosing to 4 PM of the same day (food/water ad libitum)</u>

- Urine output decrease in males at ≥ 30 mg/kg and females at 100 mg/kg suggestive of positive pharmacodynamic effect and/or urinary retention.
- pH decreased in males at ≥ 30 mg/kg
- urine protein increased in males at 100 mg/kg but likely a false positive (N-Multistix SG dipstick method, see Section 10.1)
- Bilirubin increased at ≥ 30 mg/kg in males and females
- All findings recoverable by the next day of dosing

<u>Week 13- collection overnight from 4 PM to 9 AM (food/water ad libitum)</u>

- Osmotic pressure increased at ≥ 30 mg/kg in males only

<u>Week 13- collection overnight from 4 PM to 9 AM (water/food restriction)</u>

- Urine volume decreased slightly in males at ≥ 100 mg/kg and was elevated in females at ≥ 3 mg/kg (24-55%)

- Chloride concentration and amount eliminated was elevated in males at ≥ 10 mg/kg and females at ≥ 30 mg/kg.
- Potassium concentration was elevated at ≥ 30 mg/kg (44-87%) and amount elevated (32-50%) at ≥ 10 mg/kg in males only.
- Sodium concentration and amount was elevated ~ 2 fold in males at 100 mg/kg.
- pH decreased below 6.5 at ≥ 30 mg/kg in both sexes.
- Protein increase at 100 mg/kg in males but this may be a false positive finding.
- Bilirubin elevated at ≥ 30 mg/kg in males

Week 25- collection overnight from 4 PM to 9 AM day (food/water restricted)
The utility of overnight collection from 4PM until 9AM is questionable since collection did not start until many hours after drug exposure and dietary/water restriction may have altered the findings.

- Urine volume decreased 44% at 100 mg/kg in males only.
- Chloride concentration but not amount was elevated 2- and 4-fold at 30 and 100 mg/kg respectively in males only.
- Potassium concentration but not amount was elevated 35% and 2.4-fold at 30 an 100 mg/kg in males only.
- Sodium concentration but not amount was elevated 2.2-fold at 100 mg/kg in males only.
- The number of rats with urine pH less than 6.5 increased in both sexes at ≥ 30 mg/kg.
- Elevated urine protein was observed at 100 mg/kg in both sexes but this is likely a false positive.
- Bilirubin was elevated at ≥ 30 mg/kg in both sexes.
- All findings were recoverable after 13 weeks of drug withdrawal.
- Similar findings were observed in the 13-week study (178-TX-020).

In summary, expected pharmacodynamic effects and consequent urinalysis findings and were observed. Composite urine sampling for roughly six hours after dosing in rats with free access to food and water revealed decreased urine excretion, acidification of the urine, and increased urine bilirubin at ≥ 30 or 100 mg/kg (exposures 12-59x MRHD) after 13 weeks of dosing. Rats recovered from these effects when they were hydrated. However, these findings persisted and urine became more concentrated when samples were collected overnight in rats with restricted access to water and food.

Gross Pathology

- Decreased white fat in females at ≥ 10 mg/kg and at ≥ 30 mg/kg in males. White fat was normal after 13 weeks of drug withdrawal.
- Brown fat was discolored dark at ≥ 30 mg/kg males and at ≥ 100 mg/kg females. Occurrence of **dark brown fat was still evident at ≥ 30 mg/kg in males and 100 in females after 13 weeks of recovery.**

Incidence of Adverse Pathology										
	Male					Female				
Dose (mg/kg)	0	3	10	30	100	0	3	10	30	100
White Fat Decreased				**9****	**12****	1	2	**8****	**11****	**12****
Brown Fat Discolored Dark				**6****	**12****			1	3	**12****
Significantly different from control **P<0.01. N = 12 per group. Table modified from the sponsor.										

Organ Weights

- Similar to the two-week and 13-week studies, the body weight normalized **liver** weights were elevated at ≥ 30 mg/kg in both males (11-24%) and females (5-12%). This is likely a treatment related effect since brain normalized liver weights in the high-dose group were elevated 8% and 14% in males and females respectively (mean liver wt/ mean brain wt) and this correlated with increased LFTs and histopathology (eosinophilic change).
- Thymus atrophy and decreased thymus weight was observed at 100 or 300 mg/kg in the 2-week and 13-week studies. In the 26-week study, the absolute and relative thymus weights were also reduced approximately 17% in females at 100 mg/kg while in males the absolute but not relative thymus weights were reduced 14-23% at ≥ 30 mg/kg. Although speculative, this may be a pharmacologic effect since stress (or epinephrine/norepinephrine) can cause thymus involution and repress the maturation of T-cells while beta adrenergic antagonists conversely can promote thymocyte maturation (13-15). Note: concomitant glucocorticoid treatment may exacerbate this condition (16).
- The normalized but not absolute weight of the testes was elevated 10-26% at ≥ 30 mg/kg in the 13-week study and 22% at 100 mg/kg in the 26-week study without a histological correlate.
- Body weight adjusted but not absolute weight of the adrenals was slightly increased in males at 100 mg/kg without a histological correlate. Although not affected during the dosing period, the absolute and body weight normalized adrenals were increased 11-15% in females at 100 mg/kg after the withdrawal period. Body weight adjusted adrenal weight was also increased in the 2-week (M) and 13-week (M, F) studies at 300 mg/kg which could be a compensatory response to sustained adrenergic signaling.
- Also body weight normalized but not absolute weights of the brain, kidneys, heart, lungs, adrenals, spleen, testis, and mandibular gland were elevated in males at 30 or 100 mg/kg. The toxicological significance of these findings is questionable since histological correlates were not reported in these tissues.
- The effects on absolute and normalized organ weights were reversible after 13 weeks of drug withdrawal.

<u>Previous Findings Not Observed</u>

- Body weight normalized weights of the kidneys, heart, and lungs were elevated in the 13-week study but not in the 26-week study.

- Likewise effects on the reproductive organs, other than a questionable increase in testes weight, were not observed as they were in the 2-week and 13-week studies.

Seminal vesicle and prostate weights were reduced in the 2-week (body weight adjusted) and 13-week (absolute weights only) studies at 100 or 300 mg/kg but not in the 26-week study. The prostate and seminal vesicles were visibly small at ≥ 100 mg/kg in the two-week study only. Also, slight atrophy of the uterus at ≥ 30-100 mg/kg and decreased body weight normalized weights of the uterus and ovaries (2-week only) at 100-300 mg/kg were observed in the 2-week and/or 13-week studies but not in the 26-week study.

Effect of Mirabegron on Organ Weights in Male Rats										
	Absolute Weight (g)					Percent of Body Weight				
Dose (mg/kg)	0	3	10	30	100	0	3	10	30	100
Brain	2.09	2.09	2.07	2.07	**2.00** (-4%)**	0.67	0.66	0.66	**0.71** (+7%)**	**0.77** (+15%)**
Liver	6.96	7.24	7.10	7.13	7.15	2.22	2.27	2.28	**2.46** (+11%)**	**2.75** (+24%)**
Spleen	0.55	0.56	0.56	0.52	**0.47** (-15%)**	0.18	0.18	0.18	0.18	**0.18* (+4%)**
Thymus+	81	87	86	**69***	**62** (-23%)**	0.026	0.027	0.028	0.024	0.024
* P< 0.05, **P<0.01: significantly different from control. + Thymus weights are in mg.										

Effect of Mirabegron on Organ Weights in Female Rats										
	Absolute Weight (g)					Percent of Body Weight				
Dose (mg/kg)	0	3	10	30	100	0	3	10	30	100
Brain	1.88	1.89	1.88	1.90	1.87	1.09	1.11	1.10	1.07	1.08
Liver	3.82	3.74	3.95	4.14	**4.32* (+13%)**	2.21	2.17	2.28	**2.32* (+5%)**	**2.47** (+12%)**
Thymus+	80	80	72	80	**67* (-16%)**	0.046	0.046	0.042	0.045	**0.038** (-17%)**
* P< 0.05, **P<0.01: significantly different from control. + Thymus weights are in mg.										

Histopathology

Adequate Battery: Yes

Peer Review: This is not clear but there were several DVMs and MDs that were involved in the pathology evaluation.

Histological Findings

- Slight to marked decrease in white fat lipid droplets in males and females at ≥ 10 mg/kg which was still observed in most animals after 13 weeks of drug withdrawal.
- Slight decrease in lipid droplets in brown fat in males and female rats at ≥ 30 mg/kg that was recoverable.
- The cytoplasm of the hepatocytes had an eosinophilic change of slight intensity at ≥ 30 mg/kg in both sexes which was recoverable. Electron microscopic evaluation of was conducted on two livers from the control and 100 mg/kg groups for both sexes (178-TX-040). EM imaging did not reveal a rational for the eosinophilic findings although large lysosomes containing unusual fine granular material were observed in hepatocytes and Kupffer cells in 3/4 animals at 100 mg/kg. Glycogen depletion

was observed in 1 of 2 males at 100 mg/kg. Cellular degeneration and necrosis were not observed.

- Fully recoverable slight decrease in zymogen granules in the parotid gland at ≥ 100 mg/kg males and at ≥ 10 mg/kg females
- Although pigment deposits were observed in the spleen of all animals, the severity increased from slight to moderate at ≥ 30 mg/kg in some males while it was slight to moderate in all groups of females. This is not a clear treatment related finding.
- Cardiac fibrosis was observed in 2 control and 4 high dose males.
- Notable histopathology signs unique to the 13-week study (178-TX-020) at higher doses include; thymic atrophy at ≥ 300 mg/kg, wasting at ≥ 300 mg/kg, and pigment deposits in the bone marrow, cecum, kidney, and liver at 100-300 mg/kg.

Incidence of Adverse Histopathology										
	Male					Female				
Dose (mg/kg)	0	3	10	30	100	0	3	10	30	100
Heart – fibrosis - slight	2				4					
Liver – hepatocyte cytoplasm eosinophilic – slight				**8****	**12****				**5****	**12****
- hepatocyte lysosomes w/granular material					1/2†					1/2†
- Kupffer cell- lysosomes w/granular material					1/2†					1/2†
Parotid Gland - ↓zymogen granules - slight	1				**12****	2		6	**12****	**12****
Spleen – pigment - slight/mod	12	12	12	12	12	12	12	12	12	12
White Fat - ↓ lipid droplets –slight/marked			3	**12****	**12****	1		**12****	**12****	**12****
Brown Fat - ↓ lipid droplets - slight				**12****	**12****				**11****	**12****
Significantly different from control **P<0.01. N = 12 per group. Table modified from the sponsor. † Electron microscopic evaluation of 2 male and female rats in the control and 100 mg/kg group (178-TX-040).										

Toxicokinetics

Mirabegron was absorbed rapidly with T_{max} between 1-2 hrs. Blood levels increased in proportion greater than the increase in dose. There appeared to be no difference in toxicokinetics parameters between the sexes. Toxicokinetics for this study was also reported in study 178-TX-030. Mirabegron appears to slightly accumulate in rats at doses ≥ 10 mg/kg from the first dose to week 13 in the 13-week toxicology study and further increases after 26 weeks of dosing in this study for a total accumulation of roughly 1.6 to 2.3 fold. Mirabegron did not appear to accumulate further after 52 weeks of dosing in the carcinogenicity study.

Toxicokinetics in Rats After 26 Weeks of Dosing								
Dose	3 mg/kg		10 mg/kg		30 mg/kg		100 mg/kg	
Sex	M	F	M	F	M	F	M	F
Multiple of MRHD (AUC) †	0.2	0.2	2.1	1.7	16.7	11.7	55.4	58.7
T_{max} (hr)	2	2	2	2	2	1	2	2
C_{max} (ng/mL)	23	36	160	161	719	665	2,856	2,313
AUC_{0-24} (ng-h/mL)	110	113	1,098	854	8,542	5,994	28,372	30,071
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). Toxicokinetics also reported in study 178-TX-030.								

Dosing Solution Analysis
Homogeneity of each dosing solution was assessed at weeks 1, 9, 17, and 25. All solutions were within 97 to 107% of the target concentration. The top, middle, and bottom of all dosing solution were within 94% to 110% of the target concentration.

6.2.2.3 Repeat Dose Oral Toxicity Studies in Cynomolgus Monkeys

Summary of 2-Week Oral Toxicology Study in Cynomolgus Monkeys (Study 178-TX-014)

Principle toxicities were **adverse clinical signs** at ≥ 30 mg/kg (8x MRHD) and **ECG findings** at 60 mg/kg (29-34x MRHD). Monkeys were dosed at 0, 10, 30, or 60 mg/kg (n=3/grop) for two weeks. Body weight, food consumption, urinalysis, clinical chemistry, ophthalmology, organ weight, and histology were not clearly affected. The high dose in the 2-week study was set at 60 mg/kg because one of two monkeys dosed at **100 mg/kg died** in a preliminary study after six days of treatment and emesis was observed at 30 mg/kg (preliminary study not submitted but discussed in study 178-TX-014).

Dosing was stopped after the second dose in one male and female monkey at 60 mg/kg due ventricular **tachycardia** and clinical signs including ptosis, pale oral mucosa, decreased spontaneous movement, prone position, and emesis (male only) after the first dose. The two monkeys recovered after drug withdrawal and replacement animals were added to the study. Another male with tachycardia on day one continued treatment and had tachycardia reoccur on days 4, 5, and 14.

In animals that completed the study, ptosis, and pale oral mucosa were observed in 1/3 females at 30 mg/kg (day 1) and 2/3 females at 60 mg/kg (intermittent – to every day) and 1/3 males at 30 mg/kg displayed ptosis for 14 days and pale oral mucosa, decreased spontaneous movement, and prone position on the first and last day of dosing.

Unfortunately ECG was assessed four hours after dosing instead of at Tmax (1-2 hrs). The low number of animals used confounds the results. Heart rate was elevated only in females at 10 and 60 mg/kg 4 hrs after dosing on day one. Although not statistically significant, **QRS and PR interval were elongated** four hours after dosing on day 1 and 14 days at 60 mg/kg in males and females, but the significance in unclear because of the low number of animals used. QT and QTc were not obviously affected in males but **QTc appeared slightly prolonged in females at 60 mg/kg** 4 hrs after dosing on day 14. One male at 60 mg/kg had extremely high elevation in serum CPK on day 7 (35x baseline) and moderate elevation on day 14 (4x baseline). However, a histological correlates were not observed, and CPK, although variable, was not affected in females.

Ulcerous erosion with hemorrhaging was observed in the stomach mucosa of one male at 60 mg/kg.

Apposed to the 52 week study, the weights of the ovaries, uterus, testis, seminal vesicle and prostate weight not effected. All males were slight to moderately immature.

Toxicokinetics in 2-Week Monkey Toxicity Study							
Sex		Male			Female		
Dose of Mirabegron (mg/kg)		10	30	60	10	30	60
Multiple of MRHD (AUC) †		2.9	10.9	34.2	2.9	8.4	28.5
T_{max} (hr)	Day 1	1	3	3	1	1	2
	Week 2	2	2	1	1	1	2
C_{max} (ng/mL)	Day 1	295	1,293	4,126	310	953	2,456
	Week 2	328	1,083	2,970	461	942	2,573
AUC_{0-24} (ng-h/mL)	Day 1	1,390	5,901	19,326	1,157	4,306	13,553
	Week 2	1,461	5,598	17,517	1,462	4,312	14,584
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL) after seven daily 50 mg doses of mirabegron (Study 178-CL-072). The animal AUC for multiples of exposure were based on TK data from week 2.							

Summary of 13-Week Oral Toxicology Study in Cynomolgus Monkeys (Study 178-TX-021)

In the 13-week toxicology study animals (n=3/group/sex) were dosed at 0, 3, 10, or 30 mg/kg/day. Satellite groups of two monkeys per sex were dosed at 0 or 30 mg/kg/day for 13 weeks with a 4 week recovery period. No deaths, or adverse drug related effects were observed on body weight, food consumption, ophthalmologic evaluation, urinalysis, fecal occult blood test, auditory examination, hematology, clinical chemistries, gross pathology, organ weight, or histopathology at doses up to 30 mg/kg. **PR interval was prolonged** at 10 mg/kg (females) and 30 mg/kg (females and males) 2 hrs after dosing at week 13. They recovered 24 hours after administration. One male at 30 mg/kg was said to have a wave form indicative of **tachycardia** after the first dose but he recovered and it was not observed thereafter. Heart histology was not affected. Slight to mild multivacuolated (lipid droplets) small adipocytes were observed in the white adipose tissue around the mesenteric lymph node at 10 mg/kg (M 1/3, F 1/3) and 30 mg/kg (M 2/3, F 2/3). This is likely due to increased lipolysis and is expected pharmacology. The adipocyte histology was not observed after 4 weeks of drug withdrawal at 30 mg/kg.

Toxicokinetics in 13-Week Monkey Toxicology Study							
Sex		Male			Female		
Dose of Mirabegron (mg/kg)		3	10	30	3	10	30
Multiple of MRHD (AUC) †		0.6	3.1	8.6	0.7	2.9	5.6
T_{max} (hr)	Day 1	1	1	2	2	2	1
	Week 5	1	1	2	1	1	2
	Week 13	1	1	2	1	1	3
$T_{1/2}$ (hr)	Day 1	2.3	2.8	3.9	3.0	3.3	3.8
	Week 5	2.7	5.0	4.7	2.6	4.5	4.2
	Week 13	3.1	5.8	5.4	4.8	6.3	5.6
C_{max} (ng/mL)	Day 1	91	300	1,147	50	288	896
	Week 5	62	274	1,102	103	437	1,262
	Week 13	96	392	782	112	556	426
AUC_{0-24} (ng-h/mL)	Day 1	298	1,341	5,710	234	1,172	3,900
	Week 5	233	1,321	5,492	378	1,366	5,094
	Week 13	310	1,593	4,386	369	1,502	2,751
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). The animal AUC for multiples of exposure was based upon AUC from week 13.							

Study title: **A 52-Week Oral Toxicity Study of YM178 in Cynomolgus Monkeys**

Study no.:	178-TX-026
Study report location:	Module 4.2.3.2.1
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	May 18, 2001, First dose June 14-15, 2001
GLP compliance:	Yes according to [redacted] (b) (4) regulations
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K1780005, 99.9% pure

Key Study Findings

Monkeys were dosed at 0, 3, 10, or 30 mg/kg resulting in systemic exposures ≅ 1, 2, and 8 times that in humans at the MRHD.

- No drug related deaths at doses up to 30 mg/kg (8x MRHD) in both sexes
- Cardiac effects- PR interval appeared lengthened at ≥ 30 mg/kg in males and females within 2 hrs of dosing.
- Unspecified ptosis in males and females at ≥ 30 mg/kg. Staggering and decreased locomotion at 30 mg/kg in 1 male occurring within two hours of dosing.
- Epididymal sperm volume and absolute and relative seminal vesicle and testis weights were reduced at ≥ 10 mg/kg but this may be secondary to use of pubescent animals in the effected groups.
- **NOAEL = 10 mg/kg/day which is 2x MRHD** females and 2.5x MRHD in males due to clinical signs and ECG results at 30 mg/kg (8x MRHD). The NOAEL for the 13-week exposure study was 3 mg/kg (~1x MRHD) based upon mild ECG results and histopathology at 10 mg/kg (~3x MRHD).

Methods

Doses:	0, 3, 10, or 30 mg/kg
Frequency of dosing:	Daily
Route of administration:	Oral by catheter to stomach
Dose volume:	4 mL/kg
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	4/sex/group
Age:	3-4 years old (b) (4)
Weight:	Males 2.64-4.26 kg and females 2.04-3.03 kg
Satellite groups:	None
Unique study design:	Toxicokinetic samples were taken before dosing, 1, 2, 4, 8, and 24 hrs after dosing on the first day of dosing and during weeks 13, 27, and 52 of dosing. Blood from control animals was not analyzed.
Deviation from study protocol:	None that effected study outcome

Observations and Results

Dose Selection: The top dose was selected due to abnormal EKG findings (increased PR interval at 10-30 mg/kg) in the 13-week toxicology study.

Mortality

One male control animal died prior to dosing on day 35. The cause of death was not determined. It was replaced with another.

Clinical Signs

Ptosis was observed within one hour of dosing in two males and two females at 30 mg/kg during the first three weeks of dosing in males and the first eight weeks in females. Decreased motor activity, staggering, and pale oral mucosa were observed in one of the males with ptosis at 30 mg/kg within one hour of dosing during the first week of dosing. Soft feces was observed on and off in two females at 10 mg/kg between weeks 12 and 24. These signs were not observed during the 13-week exposure study in monkeys but they were observed in the 2-week toxicity study at 30-60 mg/kg.

Incidence of Adverse Clinical Signs								
Dose (mg/kg)	0 mg/kg		3 mg/kg		10 mg/kg		30 mg/kg	
Multiple of MRHD (AUC) †	0	0	0.8	0.7	2.5	2.1	8.4	7.9
Number of animals (n=4)	4M	4F	4M	4F	4M	4F	4M	4F
Ptosis							2	2
Decreased Activity							1	
Staggering							1	
Pale Oral Mucosa							1	
Soft Feces						2		

† Multiple of a 50 mg mirabegron dose assuming AUC = 512 for fasted elderly females (178-CL-072). The animal AUC data used to calculate multiples of exposure was based on week 52 data.

Body Weights
There was no effect of treatment on body weight or body weight gain in either sex.

Feed Consumption
There was no adverse effect of treatment on feed consumption in either sex.

Ophthalmoscopy
Ophthalmoscopic examination was conducted prior to dose administration during weeks -3, 27, and 51. Eyes were dilated and animals were anesthetized prior to assessment. Light reflex was assessed in response to pen light. The anterior, intermediate optic media, and fundus oculi were assessed with slit lamp ophthalmoscope. The fundus oculi were also photographed.

No adverse drug related effects were reported.

ECG
ECG was assessed during week -2 and day 1, weeks 12, 25, 39, and 51. Assessments were conducted before daily dosing and 2 hours and 24 hours after dosing. The mean value for each variable in each group at each time point was compared to the mean value at the same time in the control group.

Potential effects on ECG were unclear due to the small sample size and large variability.

Main effects:
Heart rate was not affected in males or significantly in females. It was reduced pre-dose during week 51 and 2 hours after dosing during week 52 in females at 30 mg/kg.

In comparison to pre-dose interval or controls, the PR interval was slightly prolonged in males and females 2 hrs after dosing at all time points tested at 30 mg/kg. This is indicative of impaired conductance form the atria to the ventricle. However, the PR interval effect was not statistically significant in males. PR interval appeared lengthened at 10 mg/kg in males as well at weeks 12 and 51, but was not statistically significant.

P wave was not affected in males but was slightly reduced in females 24 hrs after dosing from week 1 to 39 at ≥ 10 mg/kg compared to the control group. However, the significance of this is unclear because it was not always different from the pre-dose level. These potential alterations of the P wave are suggestive of atrial depolarization effects.

Inconsistent effects:
Variability was too large to determine if there was an effect on QT interval. Although not statistically significant QT_C (Bazzet's formula) appeared prolonged two hours after dosing at 30 mg/kg in males compared to pre-dose levels or time matched controls but

this may be an artifact of low control values and high variability and prolonged QTc was not clearly observed in females.

There was an inconsistent tendency towards increased QRS intervals in males at 2 and/or 24 hrs after dosing at ≥ 10 mg/kg. QRS was slightly elevated in males during weeks 12, 25, and 51 at 10 mg/kg, and day 1 and weeks 12, 39, and 51 at 30 mg/kg.

Hematology
Hematology was assessed at weeks -2, 13, 26, 39, and 52.

Transient reduction in neutrophils and elevation in lymphocytes were observed during week 39 in males only at 30 mg/kg when compared to controls. However, these effects were not significant when compared to pre-dose levels.

Clinical Chemistry
Clinical chemistries were assessed at weeks -2, 13, 26, 39, and 52.

There was a tendency towards reduced calcium at ≥ 3 mg/kg in females, but the effects were transient and not obviously dose related. Alkaline phosphatase was elevated during weeks 26 and 39 in females at 30 mg/kg in comparison to time matched controls. This is not considered significant because the alkaline phosphatase levels at 30 mg/kg were not different from pre-dose levels and no effect was seen at week 52. Total cholesterol was elevated from pre-dose to week 52 in females at 10 mg/kg but since the values were not different form the pre-dose level and the high dose group was not statistically elevated, this finding is not considered toxicologically significant.

Clinical Chemistry									
		Male (mg/kg)				Female (mg/kg)			
	Week	0	3	10	30	0	3	10	30
Calcium (mg/dL)	-2	9.7	10.2	9.9	10.1	10.0	9.8	9.9	10.0
	13	9.1	9.5	9.1	9.0	9.6	9.0	9.1	**8.9***
	26	9.8	9.6	9.3	9.2	10.3	**9.1***	9.4	**9.1***
	39	9.9	10.2	9.5	9.6	10.1	9.4	**9.2***	10.0
	52	9.4	9.4	9.2	9.2	9.4	**9.1***	10.0	9.1
* Statistically different from time matched control $p < 0.05$									

Urinalysis
Urinalysis was assessed at weeks -2, 13, 26, 39, and 52.

No adverse effects were observed in either sex at any dose.

Gross Pathology
No clear pathology findings related to mirabegron were observed. Testes were noted as small in the control (2/4), low-dose (3/4) and mid-dose (1/4) groups. The testes size does not correlate with the testes weight or histological description of maturation.

Organ Weights

The body weight normalized liver weights were elevated 18% in males at 30 mg/kg and 19% in females at 10 mg/kg. Absolute liver weights were not different between groups. The toxicological relevance of this is questionable since adverse LFTs and histopathology were not observed.

Dose mg/kg	Liver Weight in Males			
	Male		Female	
	Absolute Weight (g)	Relative Weight (% Body Weight)	Absolute Weight (g)	Relative Weight (% Body Weight)
0	78.4 ± 10.3	1.45 ± 0.05	49.0 ± 4.0	1.69 ± 0.16
3	85.3 ± 8.5	1.52 ± 0.16	49.5 ± 6.3	1.81 ± 0.21
10	79.8 ± 13.4	1.65 ± 0.17	58.6 ± 6.7	**2.01 ± 0.17***
30	87.8 ± 8.2	**1.71 ± 0.08***	58.2 ± 3.1	1.85 ± 0.07
* Significantly different from control P<0.05.				

Absolute and relative uterine weights were reduced ≥ 3 mg/kg, but only absolute weights were statistically significant at 3 and 10 mg/kg. None of the monkeys were reported to be in menses at week 52. Histological pathologies were not noted in the uterus. The sponsor postulated that the reduce weight of the uterus was due to differences in sexual maturation and thought it to be incidental, which is reasonable. Uterine weights were not affected by up to 30 mg/kg after 13 weeks of exposure in monkeys (178-TX-021). Conflicting findings were observed in rats where uterine weights were severely reduced at ≥ 100 mg/kg during a 13 week exposure study but not at 100 mg/kg after 26 weeks of exposure.

Dose (mg/kg)	Uterus	
	Absolute Weight (g)	Relative Weight (% Body Weight)
0	8.05 ± 0.89	0.282 ± 0.063
3	**4.93 ± 1.29****	0.190 ± 0.084
10	**4.92 ± 0.56****	0.172 ± 0.036
30	6.34 ± 1.14	0.201 ± 0.036
**P<0.01: significantly different from control. Organs weighted: Brain, pituitary, thyroid, submandibular gland, heart, lung, liver, spleen, kidney, adrenal, ovary, and uterus. Also the testes, seminal vesicle, and prostate were weighted in males.		

Although not statistically significant, seminal vesicle, and testis weights were lower at ≥ 10 and 30 mg/kg, respectively. The weights of both organs in the 30 mg/kg group were roughly 50% of control weights. The lack of statistical relevance could be due to the small sample size. This could also be due to the use of immature males in the mid (3/4) and high (2/4) dose groups. The maturity differences may not account entirely for this since the high-dose group was characterized as being more mature than the mid-dose group while having smaller seminal vesicles and testis. These effects were not observed after 13 weeks of exposure to monkeys at the same doses (178-TX-021). However, similar responses were observed during 13 weeks of exposure in rats where

absolute but not normalized prostate and seminal vesicle weights were reduced at 300 mg/kg (178-TX-020).

Dose mg/kg	Seminal Vesicle		Testis	
	Absolute Weight (g)	Relative Weight (% Body Weight)	Absolute Weight (g)	Relative Weight (% Body Weight)
0	14.0 ± 9.8	0.25 ± 0.17	33.6 ± 5.7	0.62 ± 0.07
3	13.5 ± 7.8	0.26 ± 0.19	33.8 ± 13.8	0.62 ± 0.29
10	***9.0 ± 10.8***	***0.16 ± 0.14***	*23.4 ± 25.5*	*0.42 ± 0.36*
30	***6.7 ± 2.4***	***0.13 ± 0.05***	***17.2 ± 6.4***	***0.33 ± 0.09***
No responses were statistically different from control weights.				

Histopathology

Adequate Battery: Yes

Peer Review: Unclear

Histological Findings:

It is not clear if any of the histological findings are treatment related other than effects on adipocytes in the heart and mesenteric lymph nodes.

In the heart, slight to moderately severe incidence of small multivacoulated adipocytes (similar to brown adipocytes) were seen in the white adipose tissue of 1 to 2 animals in all groups of females and males at ≥ 3 mg/kg. Myocardial degeneration and necrosis of mild intensity was observed in one high-dose female.

Immature testes were observed in the mid-dose (3/4 moderate to severe) and high-dose (2/4, slight to mild) groups. This correlates with the reduced testes and seminal vesicle weights. Also although the prostate was not affected histologically, all animals had epididymal sperm but volume was reduced at ≥ 10 mg/kg. It is not clear if this is a treatment effect. The monkeys were 4.5 to 5.5 years at necropsy which the sponsor indicated is in the middle of sexual maturation. The sponsor also noted that the background percentage of sexual maturation at this age was 51% in their testing facility.

Slight to moderate thymic involution was noted in all groups. Unfortunately thymus weights were not evaluated.

Incidence of Histopathology								
	Male (n=4/group)				Female (n=4/group)			
Dose (mg/kg)	0	3	10	30	0	3	10	30
Adrenal – cortical nodule - slight				2		3		
Heart – Adipocyte - multivacuolated – slight-moderate		1	1	1	1	1	2	1
- Interstitial cell infiltration - slight			1	1				
- **Myocardial degeneration/necrosis** - mild								1
Intestine/Colon – large thickening muscle mild-mod							1	
Liver – Necrosis – massive area, moderate intensity						1		
- Microgranuloma - slight								1
Lymph Node - mesenteric – S. multivacuolated adipocytes around white adipose - mild			1					
Ovary – dilated oviduct, cystic - slight						1		
Spleen – Capsular thickening - mild			1					
- Follicle - eosinophylic material - slight				1				
- Follicle- focal hyperplasia - slight	1	1	2					
Testes - Immature (mod-severe at 10, slight-mild at 30)			3	2				
Epididymis – Sperm volume, lumen (severe at 0, 3, slight to severe at 10 and 30 mg/kg)	4	4	4	4				
Thymic Involution – slight - mod	3	4	2	2	2	4	4	3

Special Evaluation

Fecal occult blood test and auditory tests were not affected by mirabegron at week 25/26 or week 51/52.

Toxicokinetics

Exposure (AUC and C_{max}) increased roughly or slightly greater than the proportional increase in dosage. At the highest dose, 30 mg/kg, the blood levels (C_{max} and AUC) appear to decrease with repeated dosing suggestive of reduced absorption or increased elimination and metabolism. The half-life was below six hours at all doses. There appeared to be no difference between the sexes. Similar exposure levels were observed in the 13-week toxicity study at the same doses (178-TX-021).

Toxicokinetics							
Sex		Male			Female		
Dose of mirabegron (mg/kg)		3	10	30	3	10	30
Multiple of MRHD (AUC) †		0.8	2.5	8.4	0.7	2.1	7.9
T_{max} (hr)	Day 1	2	1	1	1	1	1
	Week 13	1	1	2	1	1	1
	Week 27	1	1	2	1	2	2
	Week 52	1	1	1	1	2	2
$T_{1/2}$ (hr)	Day 1	1.9	2.7	4.4	2.3	3.0	4.8
	Week 13	2.5	6.1	4.6	2.4	4.1	5.2
	Week 27	3.4	5.3	5.0	3.2	4.7	4.9
	Week 52	2.9	5.0	5.0	4.9	4.3	4.8
C_{max} (ng/mL)	Day 1	135	570	2,390	111	372	2,385
	Week 13	99	423	1,294	118	515	1,545
	Week 27	82	535	1,344	158	371	1,456
	Week 52	116	312	718	101	241	742
AUC_{0-24} (ng-h/mL)	Day 1	367	1,466	7,468	391	1,158	6,941
	Week 13	339	1,494	5,591	369	1,602	5,494
	Week 27	321	1,176	4,895	530	1,530	5,693
	Week 52	421	1,267	4,301	342	1,092	4,031
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). The animal AUC data used to calculate multiples of exposure was taken from week 52.							

Dosing Solution Analysis

Dosing solutions were assessed at day 1 and weeks 13, 26, 39, and 52 for homogeneity and mean concentration. All three dosing solutions were within 99.6 and 105.5% of the desired concentration for all time points. The top, middle, and bottom of each dosing solution were within 5.7% of the desired concentration.

6.2.2.4 Two Week Repeat Dose Oral Toxicity Study in Dogs

Study title: **YM178: 14 Day Oral Toxicity Study in Dogs**
Study no.: 178-TX-018
Study report location: Module 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: April 8, 1999
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: YM178 (mirabegron)
Batch K02, Lot 17801, 95.7% pure prepared in 5% lactose
Batch K02, Lot 17802, 98.1% pure prepared in 50% lactose

Key Study Findings

- Mirabegron was lethal to one female at 20 mg/kg (23x MRHD, Cmax) after 3 days of exposure. A single female at 3 mg/kg (3x MRHD, Cmax) had to be euthanized due to severe eye swelling and resistance to opening her mouth.
- Both ventricular (1M, 2F) and junctional (1F) tachycardias were observed in males and females at 20 mg/kg (29-33x MRHD, Cmax) within 2 hrs of the first dose and not during subsequent time points.
- Heart rates were elevated nearly two fold within two hrs of dosing in male and female dogs at ≥ 10 mg/kg in males (17x MRHD, Cmax) and females (23x MRHD, Cmax) on day 1 of dosing. Heart rate was not clearly affected at day 7 or 14 of dosing at up to 10 mg/kg.
- Dose responsive increase in reddening of the skin was observed at ≥ 1 mg/kg in males and females (1x MRHD, Cmax) within 1-2 hrs of the first dose.
- Dose related inflammation and degeneration of the zygomatic salivary gland was observed in all treatment groups but not in the controls. Incidence and severity increased with dose in male and female dogs at ≥ 1 mg/kg (0.5x MRHD, AUC).
- The low number of animals used may have limited the significance of some responses.
- A NOAEL was not identified since effects were observed at the lowest dose (1 mg/kg/day) which is 0.5X MRHD base on AUC and 1.2x MRHD based on Cmax.

Methods

Doses:	0, 1, 3, 10, or 20 mg/kg.
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	6 mL oral gelatin capsules
Formulation/Vehicle:	Oral gelatin capsules with mirabegron prepared in lactose powder
Species/Strain:	Beagle dog (B and K strain)
Number/Sex/Group:	3/sex/group
Age:	35 to 42 weeks
Weight:	Male 13.6 to 17.4 kg, Female 11.3 to 14.5 kg at first dose
Satellite groups:	Two dogs per sex at 20 mg/kg were used to assess reversibility of findings after two weeks of drug withdrawal but they were only dosed for three days and euthanized on day 6-7 because of the death of one animal in this group.
Unique study design:	Blood was sampled in all dogs for toxicokinetics 1, 2, 4, 8, and, 24 hrs after dosing on day 1 and 14.
Deviation from study protocol:	None that effected results besides early termination of the 20 mg/kg group including the recovery animals.

Observations and Results

Mortality

There was only one death during the study. One female dog in the 20 mg/kg group died 90 minutes after dosing on day 3. The sponsor stated that the death was drug related and the pathology is discussed below. Because of this death, all animals including the recovery animals in the 20 mg/kg group were euthanized on day 6 or 7. A female dog in the 3 mg/kg group was euthanized on day 6 because the dog was reluctant to open her mouth and had swollen eyes, eyelids, and muzzle. These signs were thought to be due to mirabegron exposure.

Clinical Signs

A dose responsive increase in reddening of the skin (hyperemia) was observed at ≥ 1 mg/kg in males and females. This was observed upon the first dose but declined with repeated dosing. This sign may be due to vasodilatation as well as the increased blood flow due to elevated heart rate observed at these doses.

Emesis was observed after the first dose at ≥ 3 mg/kg in females and at ≥ 10 mg/kg in males. The incidence declined with repeated dosing.

Discharge from the eye was observed in females at ≥ 3 mg/kg and males at ≥ 10 mg/kg. Swelling in and around the eye occurred in one male at 20 mg/kg and one female in the 1, 3, and, 20 mg/kg groups. The swelling around the eyes and muzzle was so severe that a female in the 3 mg/kg group was euthanized on day 6.

Incidences of sores, scabbing, red sclera, bleeding and swollen vulva in single individuals were also observed but were not clearly dose related.

Incidence of Clinical Signs										
	Male					Female				
Dose (mg/kg)	0	1	3	10	20#	0	1	3	10	20#
Multiple MRHD (AUC) †	0	0.6	2.7	16.7	16.4		0.5	2.8	13.8	15.4
Number of animals	3	3	3	3	5	3	3	3	3	5
Mortality								1*		1
↓ Activity			1		1				1	2
Resist Dosing				1				1		1
Skin Reddening/Flushing		3	3	3	5		3	3	3	5
Vomiting				2	4			1	3	5
Dosing Salivation		1	1	2	2	1	1	1	1	
Eye - Discharge from				1	1			2	1	1
Eyes - Swelling Below					1		1	1		1
Eyes – Red, Inflammed										1
Eye - Swollen								1		1
Eye - Exophthalmos								1		
Eye - Sclera Red			1					1		
Eyelids Swollen								1		
Muzzle Swollen								1		
Dry Sores on head			1							
Scabs on Head			1							
Vulva Bleeding								1		
Vulva Swollen								1	1	1

† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL) after seven daily 50 mg doses of mirabegron OCAS (Study 178-CL-072). # All animals in the 20 mg/kg group were euthanized on day 6-7 of dosing due to adverse clinical sings and death. *One female at 3 mg/kg was euthanized at day 6 of dosing for humane reasons.

Body Weights
Body weight was not affected by treatment.

Feed Consumption
Feed consumption was not affected by treatment.

Ophthalmoscopy
Ophthalmoscopic evaluation was not well described. It was stated that indirect binocular ophthalmoscopic evaluation was conducted prior to the dosing phase and prior to study termination. Dogs were treated with mydriatic agent prior to evaluation. Ophthalmoscopic findings were not reported. However, clinical signs in mirabegron treated dogs included inflamed, swollen and red eyes, swollen eyelids, red sclera, and discharge from eye. Due to the low incidence and small number of animals used, the findings were not clearly treatment related.

ECG
Two lead ECG was conducted pre-study, one and two hours after the first dose, and 1, 2, and 24 hours after the 7th and 14th daily dose. Animals in the 20 mg/kg group were assessed prior to being euthanized on days 5-7.

Ventricular **tachycardia** was observed in one male and two females at 20 mg/kg within in two hours of dosing on day one. Junctional tachycardia was observed in one female at 20 mg/kg two hours after dosing on day one. Heart rates were elevated nearly two fold within 2 hrs of dosing in male and female dogs at ≥ 10 mg/kg on day 1 of dosing. Heart rates at 1 and 3 mg/kg were also elevated but not statistically different from controls after dosing on day one. Effects on heart rate were not significant after 7 or 14 days of exposure.

PR interval decreased in males and females at all doses within two hours of dosing on day one only. **P wave was prolonged** (> 50 ms) and/or notched at ≥ 10 mg/kg in both sexes within 1-2 hours of dosing on day 1, 7, and 14.

The **QRS interval was slightly increased** within two hours of dosing at ≥ 10 mg/kg in males and females at all time points, but was only statistically elevated in males at 20 mg/kg on day 1 of dosing.

QT interval contracted in females at ≥ 1 mg/kg within 2 hours of the first dose. This may be an artifact of an increased heart rate since QTc was prolonged only at 10 mg/kg in males and females one to two hours after the first dose. Additionally, the potential effect on QTc does not appear treatment related when each individual is compared to their pre-dose value.

T wave amplitude was elevated at 20 mg/kg in males one to two hours after dosing on day one and two hours post-dosing on day one in females. Effects at lower doses are not clear. The T wave amplitude was elevated in males within one to two hours of dosing on days 1, 7, and, 14 in the 10 mg/kg group compared to the control group. However, the effects in the 10 mg/kg group are ambiguous because they were not significant when compared with the values taken prior to initiating the dosing phase within the same group.

Adverse ECG Results Incidence or Percent Change vs. Control (n=3/group/sex)							
		Day 1		Day 7		Day 14	
	mg/kg	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr
Ventricular Tachycardia	20	F 2/3	M 1/3	NA	NA	NA	NA
Junctional Tachycardia	20	0	F 1/3	NA	NA	NA	NA
Heart Rate Increase (Group Mean % increase vs. control)	10	Not Sig	M 76% F 96%	No Effect	No Effect	No Effect	No Effect
	20	M Not Sig F 86%	M 64% F 76%	NA	NA	NA	NA
P Wave Prolonged (> 50 ms)	10	0	M 2/3 F 1/3	M 2/3 F 1/3	M 2/3 F 1/3	M 1/3	M 3/3 F 2/3
	20	0	M 2/3 F 2/3	NA	NA	NA	NA
QRS Prolonged (> 70 ms)	10	F 1/3	F 1/3	-	-	-	M 1/3
	20	0	M 1/3 F 1/3	NA	NA	NA	NA
PR interval Decrease (mean vs. control)	1	M -22% F -21%	M -21% F -23%	ND	ND	No Effect	No Effect
	3	M -23% F -28%	M -23% F -21%	ND	ND	No Effect	No Effect
	10	M -17% F -17%	M -17% F -16%	No Effect	No Effect	No Effect	No Effect
	20	M -25% F -29%	M -11% F -11%	NA	NA	NA	NA
T wave amplitude (mean vs. control)	10	Not Clear	Not Clear	Not Clear	Not Clear	Not Clear	Not Clear
	20	M +12% F +168%	M +115% F +180%	NA	NA	NA	NA

Sponsor's definition of tachycardia.
Ventricular tachycardia – The atria and ventricles beat independently of each other in this arrhythmia which is characterized by rapid, regular heart rate, with a lack of relationship between P waves and QRS complexes (or absence of obvious P wave) and wide though otherwise normal QRS complexes.
Junctional tachycardia – The AV junction acts as the primary pace maker in this arrhythmia which is characterized by rapid, regular heart rate, negative P waves I Lead II, constant and usually normal PR interval, and the QRS complex can be wide and bizarre or normal.
ND – not determined.
NA - not assessed because dosing was ceased at day 3 in the 20 mg/kg group.

Hematology

Hematology was assessed on days 7, 12, 14 and prior to unscheduled termination in the 20 mg/kg group. Hematology was not clearly affected by mirabegron.

Clinical Chemistry

Clinical chemistries were assessed on days 7, 12, 14 and prior to premature termination in the 20 mg/kg group. Clinical chemistries were not clearly affected by mirabegron.

Urinalysis
Twenty four hour samples were collected for urinalysis prior to the first dose and on day 9. Treatment related effects on urinalysis were not apparent.

Gross Pathology
One female dog died 90 minutes after receiving a 20 mg/kg dose of mirabegron day 3. This dog also displayed several pathologies in the heart including subendocardia hemorrhage, white or pale spots in the ventricular wall, and excess pericardial fluid. She also displayed ventricular tachycardia on the first day of dosing. Because of this, the sponsor stated that the **death was drug related**. Additionally slight lymphocytic infiltration of the gallbladder, increased periportal vacuolation, loss of red blood pulp in the spleen, and moderate congestion of the thymus were observed. Due to these responses to mirabegron, all animals dosed with 20 mg/kg were euthanized on day 6 or 7. A female dog in the 3 mg/kg group was also euthanized on day 6 because the dog was reluctant to open her mouth and had swollen eyes, eyelids, and muzzle.

Incidence of Adverse Pathology in the Moribund or Dead Female Dogs

Findings	3 mg/kg	20mg/kg
Eye- swollen	1	
Heart- excess pericardial fluid, discolorations, pale spots		1
Liver- accentuated lobular pattern		1
Liver - pale	1	
Lung- discolored areas		1
Salivary Gland Zygomatic- firm, discolored	1	
Spleen- pale spots or areas		1
Thymus- red spots or areas		1

N = 1 per group.

Dose related gross pathologies in the animals that lived until the scheduled necropsy were not apparent. It is difficult to determine wither pathologies observed were due to mirabegron because of the small number of animals used. However, the zygomatic salivary gland may be a target tissue since it was discolored and firm in two dogs of both sexes at 20 mg/kg.

Incidence of Adverse Pathology								
	0 mg/kg		3 mg/kg		10 mg/kg		20mg/kg+	
	3M	3F	3M	2F*	3M	3F	5M	4F
Heart- excess pericardial fluid							1	
Heart- valve nodule					1			
Kidney- depressed area							1	
Lung- firm area			1					
Lung- dark spots		1		1				
Lung- discolored spots								1
Lymph Node, Rectal- enlarged								1
Lymph Node, Thymus- enlarged								1
Prostate – reduced			1				2	
Salivary Gland, Zygomatic- firm, discolored							2	2
Uterus, distended				1		1		
*One female at 3 mg/kg was euthanized at day 6 of dosing for humane reasons. +All animals in the 20 mg/kg group were euthanized on day 6-7 of dosing due to adverse clinical sings and death.								

Organ Weights
Mirabegron exposure did not appear to alter organ weights.

Histopathology
Adequate Battery:
Yes. Unfortunately the only tissues assessed in the 20 mg/kg group were the zygomatic salivary gland, heart, aorta, liver, and kidneys and specific abnormal lesions.

Peer Review: Unknown

Histological Findings:
The only clear treatment related histological finding was inflammation and degeneration of the zygomatic salivary gland which was observed in all treatment groups with the incidence and severity increasing with dose in both sexes at ≥ 1 mg/kg. The sponsor reported that this was associated with hemorrhage, coagulative necrosis of acini or vacuolar degeneration of acini, inflammatory cell infiltration with variable fibroblastic response, and evidence of regenerative hyperplasia of the duct epithelium.

Other potential drug related effects include lymphocytic infiltration of the gallbladder in females at ≥ 1 mg/kg, focal myocardial degeneration and endocardial hemorrhage in two different females at 20 mg/kg, and periportal vacuolation of the livers of one male and female at 20 mg/kg. Lymphoid hyperplasia and enlarged rectal and thymic lymph nodes were observed two different female dogs at 20 mg/kg.

Incidence of Adverse Histopathology (* includes dogs that died or were euthanized due to morbidity)										
Dose (mg/kg)	0		1		3		10		20 +	
N	3M	3F	3M	3F	3M	3F*	3M	3F	5M	5F*
Salivary gland, zygomatic- degeneration, inflamm – Total			1	1	3	2*	2	1	4	4
- minimal			1			1	1			
- slight				1				1		
- moderate					3		1		1	
- marked						1*			3	4
Salivary gland - parotid – focal atrophy – minimal						2*			?	?
Gallbladder - lymphocytic infilam – slight/min.				1		3*		2	?	?
Heart - myocardial degeneration – focal - minimal										1
- endocardial hemorrhage – focal - moderate										1*
- thrombotic valve cyst							1			
Kidney – congestion										1*
Liver - periportal vacuolation slight- mod.									1	1*
- increased glycogen - moderate						1*				
- congestion										1*
Lymph node – rectal – lymphoid hyperplasia										1
- enlarged										1*
Lymph node – thymic - reactive lymphoid hyperplasia										1
- enlarged										1*
Spleen – loss of blood red pulp – focal - slight										1*
Thymus – congestion – hemorrhage- moderate										1*

* Includes female that was euthanized due to morbidity at 3 mg/kg on day 6 or the female at 20 mg/kg that died on day 3.
\+ All animals in the 20 mg/kg group were euthanized on day 6-7 of dosing due to adverse clinical sings and death.
? – tissue not assessed, histological evaluation of the animals in the 20 mg/kg group was limited to specific lesions and the zygomatic salivary gland, heart, aorta, liver, and kidneys.

Incidence of Adverse Histopathology in the Moribund or Dead Female Dogs		
Findings (N = 1 per group)	3 mg/kg	20mg/kg
Gall Bladder – lymphocytic infiltration - slight	1	
Heart - endocardial hemorrhage – focal - moderate		1
Kidney – medullary calcification	1	1
- congestion		1
Liver – periportal vacuolation – slight		1
- increased glycogen - moderate	1	
- congestion		1
Salivary gland - parotid – focal atrophy – minimal	1	
- missing		1
Salivary gland – zygomatic - degeneration, inflammation, marked	1	
– zygomatic - missing		1
Spleen – loss of blood red pulp – focal - slight		1
Thymus – congestion – hemorrhage - moderate		1

Toxicokinetics

Exposure to mirabegron increased in slightly greater than the proportional increase in dose in the 10 and 20 mg/kg groups for both AUC and Cmax. Dogs were exposed up to 15 to 25 times the clinical exposure. Mirabegron was rapidly absorbed with at Tmax of 1.2 to 3 hours. Toxicokinetics was not affected by the sex. Drug accumulated roughly two fold between the first and 14th day of dosing in the 10 mg/kg group.

Toxicokinetics in Dogs in 2-Week Toxicology Study									
Sex		Male				Female			
Dose (mg/kg)		1	3	10	20	1	3	10	20
Multiple MRHD (AUC) †		0.6	2.7	16.7	16.4	0.5	2.8	13.8	15.4
Multiple of MRHD (Cmax)		1.2	3.3	16.6	28.9	1.4	2.9	23.4	32.5
T_{max} (h)	Day 1	2.0	3.0	1.7	2.0	1.7	1.7	1.0	1.2
	Day 14	1.7	2.0	1.7		2.0	1.5	1.3	
C_{max} (ng/mL)	Day 1	82	218	1,100	1,916	96	195	1,551	2,152
	Day 14	49	337	1,730		53	314	1,538	
AUC_{0-24} (ng-h/mL)	Day 1	300	1,178	4,333	8,404	301	988	4,568	7,887
	Day 14	313	1,384	8,561		269	1,408	7,045	

† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). The animal AUC for multiples of exposure was taken from week 2 except for the 20 mg/kg group where the multiple of exposure is based upon day one of dosing only. Multiples of MRHD (Cmax) were based on day one levels because most of the cardiac effects were first dose findings.

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Summary of Study Investigating Salivary Gland Toxicities in Dogs Dosed Orally with Mirabegron (Study 178-TX-019)

Female beagle dogs were dosed orally with capsules containing 0 or 20 mg/kg of mirabegron for three days and histology was evaluated the day after the last dose and 4 and 13 weeks after dosing cessation.

Key Findings:

No animals died during the study. Mirabegron treated animals exhibited whole body flush and enlarged, yellow livers with moderate hepatocyte hypertrophy vacuolation and glycogen and lipid accumulation. Consistent with the previous cardiac findings and the clinical signs, white and dark foci were observed in the hearts of 25% and 75% of the mirabegron treated group.

Primarily in the Zygomatic salivary glands, mild to moderate edema, hemorrhage, cellular infiltration, acinar cell necrosis and atrophy, duct dilation, necrosis of the duct epithelium, proliferation and mineralization of the ductal epithelium, and thrombosis were observed. These were recoverable findings except for the acinar cell atrophy. During recovery, focal fibrosis with ductal proliferation became apparent and progressively more severe between 4 and 13 weeks after the dosing period.

Clinical signs:

In the dosed group all animals exhibited whole body flush, conjunctiva flush, oral mucosa flush, and 11/14 exhibited emesis after the initial dose. After the last dose, whole body flush (12/14), abdominal flush (13/14), conjunctiva flush (10/14), oral mucosa flush (10/14), decreased activity (1/14), emesis (8/14), and red feces (1/14) were observed in the mirabegron group. These signs dissipated after one week of drug withdrawal.

Body weight/Food consumption: Although food intake was reduced (9-24%) throughout the dosing period, there was no effect on mean body weight and feeding returned to normal during the recovery period.

Gross pathology: One day after the final dosing, white and dark foci were observed in the hearts of 25% and 75% of the mirabegron treated group respectively but not in controls. Ulceration of the duodenum was observed in a single dog dosed with mirabegron. Enlarged and discolored livers were noted in all mirabegron treated dogs. Dark red zygomatic salivary glands were noted in 75% of the dogs. These findings were reversible as no gross pathologies were observed in the mirabegron groups after 4 or 13 weeks of recovery.

Histopathology of the Livers and Salivary Glands: No adverse histopathology findings were observed in the controls one day after the final dosing.

Zygomatic Gland: Almost all mirabegron treated dogs in the main study groups displayed mild to moderate edema, hemorrhage, cellular infiltration, acinar cell necrosis and atrophy, duct dilation, necrosis of the duct epithelium, proliferation and mineralization of the ductal epithelium, and thrombosis. Most of these signs were not apparent by week four of recovery except for atrophy of the acinar cells and reduced frequency of mineralization of the ductal epithelium. During recovery new and progressively worse adverse histopathologies include increased frequency and severity of focal fibrosis with ductal proliferation between weeks 4 and 13 after the dosing period.

Submandibular Gland: Moderate acinar cell atrophy was observed in all animals directly after dosing ceased which declined thereafter.

Parotid Gland: Slight to moderate acinar cell atrophy, cellular infiltration of the ductal epithelium and interstitial cellular infiltration was observed after the final dosing in one or two animals but not after recovery.

Sublingual Glands: Moderate edema of the interstitium was observed in 3 of 4 animals in the major sublingual gland but it was not seen after recovery. Moderate dilation of the duct, dilation of the acinar lumen of serous glands and mucus glands were observed in a single animal after dosing but these were recoverable findings. Moderate cellular infiltration was observed in a single dog after the last dosing and after 13 weeks of recovery but this response was more prevalent in the control animals. Atrophy of the

acinar cells and mineralization of the ductal epithelium were observed in only a single dog only after 13 weeks of recovery in the minor sublingual gland.

Liver: Mild to moderate hepatocyte hypertrophy was observed in all animals after dosing but not after recovery. Mild to moderate hepatic vacuolation was observed in two dogs and retention of pale-brown material in the bile duct was observed in a single dog but neither effect was observed after recovery. Slight deposition of glycogen granules was observed in 3 dogs one day after the last dose and 4 dogs after 4 weeks of recovery, but none after 13 weeks of recovery. Deposition of lipid droplets in peri- and centrilobular hepatocytes was observed in 4 dogs one day after dosing cessation but not thereafter. The sponsor conducted selected clinical chemistries to evaluate liver function but did not evaluate the control animals. Compared to historical controls AIP was elevated 2 to 3 fold in all animals, ALT was elevated in four animals 2 to 3.5 fold, total cholesterol was 1.5 fold increased in one animal. Serum ALP and ALT levels were within background levels after 11 weeks of recovery. Cholesterol appeared elevated in 2/6 dogs at week 11 of recovery.

Incidence of Pathology One Day After the Third Daily Dose		
	0 mg/kg N = 3	20 mg/kg N = 4
Heart - Dark red foci, endocardium, left ventricle. Several	0	3 / 4
- White foci in epicardium , left ventricle	0	1 / 4
Duodenum - Ulcer (4 present)	0	1 / 4
Liver - Enlarged	0	4 / 4
- Yellow Discoloration in all lobes	0	4 / 4
Zygomatic Salivary Gland - Dark red discoloration (focal, bilateral)	0	1 / 4
- Dark red discoloration (bilateral)	0	3 / 4

Incidence and Mean Severity of Histopathology				
	1 Day After Final Dose		Recovery Week 4	Recovery Week 13
	0 mg/kg N = 3	20 mg/kg N = 4	20 mg/kg N = 4	20 mg/kg N = 6
Submandibular Gland				
Atrophy, acinar cell		4/++	2/++	1/++
Parotid Gland				
Atrophy acinar cell		2/+		
Cellular infiltration, ductal epithelium		1/+++		
Interstitium cellular infiltration		1/++		
Major sublingual gland				
Edema, interstitium		3/++		
Dilation, duct		1/++		
Dilation, acinar lumen, serous gland		1/++		
Dilation, acinar lumen, mucus gland		1/++		
Cellular infiltration, interstitium		3/+		1/+
Minor sublingual gland				
Dilation, duct		1/++		1/+
Dilation, acinar lumen, serous gland		1/++		
Dilation, acinar lumen, mucus gland		1/++		
Cellular infiltration, interstitium		1/+		1 /++
Atrophy, acinar cell				1/++
Mineralizaion, ductal epithelium				2/+
Zygomatic gland				
Edema, interstitium		4/+++		
Hemorrhage, interstitium		4/+++		
Cellular infiltration, interstitium		4/++		
Necrosis, acinar cell		4/+++		
Atrophy, acinar cell		3/+	4/+	5/+
Dilation, duct		4/++		
Necrosis and desquamation, ductal epithelium		4/++		
Proliferation, ductal epithelium		4/++		
Mineralization, ductal epithelium	1/+	4/+	1/+	2/+
Thrombus		4/++		
Fibrosis, focal, with ductal proliferation			2/+	5/++
Liver				
Hypertrophy, hepatocyte, perilobular	–	4/+++		
Vacuolation, hepatocyte, diffuse	–	2/+++		
Retention, pale-brown material, bile duct	–	1/++		
Eosinophilic material, hepatocyte	–		1/++	
Liver PAS Stain				
Deposition, glycogen granule, hepatocyte centrilobular	–	3/+	4/+	
Deposition, glycogen droplet, hepatocyte, perilobular	–		4/+	
Liver Oil Red O Stain				
Deposition, lipid droplet, hepatocyte, centrilobular	–	4/++		
Deposition, lipid droplet, hepatocyte, perilobular	–	4/++		
+: slight, ++: mild, +++: moderate, – not evaluated				

Toxicokinetics In Dogs - Day 3	
Dose (mg/kg/day)	20
Multiple of MRHD (AUC) †	25x
T_{max} (h)	2.3 ± 1.0
C_{max} (ng/mL)	1,998 ± 843
AUC_{0-24} (ng-h/mL)	12,855 ± 5,695
† Multiples of MRHD based upon AUC in fasted elderly women (AUC = 512 ng-hr/mL, Cmax 66.3 ng/mL) who received seven daily 50 mg doses of mirabegron OCAS (Study 178-CL-072).	

7 Genetic Toxicology

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

Study title: **YM178: Reverse Mutation in four Histidine-requiring strains of *Salmonella typhimurium* and one Tryptophan-requiring strain of *Escherichia coli*.**

Study no.: 178-TX-003
Study report location: Module 4.2.3.3.1
Conducting laboratory and location: (b) (4)

Date of study initiation: June 2, 1999
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: YM178 (mirabegron), Batch K02, 100.3% pure

Key Study Findings

- Mirabegron was negative in all tests strains for increased mutation frequency.

Methods

Strains: *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2*uvr*A
Concentrations in definitive study: 0, 78, 156, 313, 625, 1250, 2500, or 5000 µg/plate
Basis of concentration selection: An initial range finding study with TA100 ± S9
Negative control: DMSO
Positive control: Without S9 mixture, TA98 used 2-nitrofluorene, TA100 and TA1535 used Sodium azide, TA1537 used 9-aminoacridine, WP2 uvrA used 4-nitroquinoline 1-oxide.
In the presence of S9 mixtures, all salmonella strains used 2-aminoanthracene.
Formulation/Vehicle: DMSO
Incubation & sampling time: Pre-incubate for 20 min at 37°C ± S9 then plate and incubate @ 37°C for 3 days. Rat (Sprague-Dawley) liver S9 fractions were activated with Aroclor 1254.

Study Validity
The study was conducted under GLP conditions and the solvent and positive controls fell within historical or expected ranges.

Results
Following preliminary studies, two definitive replicate studies were conducted. Each study had three plates per exposure concentration and four for the solvent control. Colonies were counted with an automatic colony counter.

5000 µg/plate was completely lethal to all bacterial strains in the absence of S9. In the presence of S9, 5000 µg/plate was completely lethal to only one strain, TA1537. Statistical evidence of increased mutation frequency was not obtained when analyzed by Dunnett's test ($p \leq 0.01$) except for WP2 uvrA in the absence of S9. In the absence of S9 there was only a very slight increase in mutation frequency (1.7 fold) at 2500 µg/plate and a non-statistical increase of 1.5 fold at 625 µg/plate. However, this is within one standard deviation of the historical mean for this strain. The weight of evidence under the conditions of the assays suggests that mirabegron was not genotoxic in this assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: **YM178: Induction of Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes.**

Study no.:	178-TX-007
Study report location:	Module 4.2.3.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 2, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, Purity 100.3%

Key Study Findings

- Mirabegron did not induce chromosomal aberrations in human lymphocytes in a biologically significant or repeatable manner under the conditions employed in this assay.

Methods

Cell line:	Human peripheral lymphocytes harvested from three different non-smoking female donors for the first and second experiments.
Concentrations in definitive study:	Cells were treated for 20 hrs with 88, 110, 137, 172, 215, 268, 336, 419, 524, 655, 819, 1024, 1280, 1600, or 2000 µg/mL of mirabegron in the absence of S9 (analyzed at 0, 110, 172, 215 µg/mL). Cells were exposed to the same concentrations minus the lowest three concentrations for 3 hrs in the presence of S9 followed by 17 hrs of recovery (analyzed at 0, 655, 1024, and 1280 µg/mL). Mirabegron precipitated at concentrations ≥ 819 µg/mL. Mirabegron did not alter osmolality or pH of the media. Negative controls were conducted in quadruplicate all other exposures and the positive controls were conducted in duplicate.
Basis of concentration selection:	The highest concentration that remained dissolved in DMSO that could be exposed to the cells without appreciable exposure to DMSO was set as the highest exposure concentration. The highest concentration for analysis of chromosomal aberrations was one at which at least 50% mitotic inhibition occurred.
Negative control:	DMSO
Positive control:	4-nitroquinoline 1-oxide was used to test cells in the absence of S9 and Cyclophosphamide was used to test cells in the presence of S9.
Formulation/Vehicle:	DMSO
Incubation & sampling time:	See concentrations in definitive study above

Study Validity

Aberrations in the vehicle controls fell within normal historical ranges. Positive controls induced statistically significant increases in structural aberrations in the presence or absence of S9.

Results

A statistical increase in the proportion of cells with structural aberrations excluding gaps was observed in both replicates at the highest evaluable concentration (1,280 µg/mL) when treated with S9 (Control 2/200 vs. high concentration 12/193). However, this just exceeded the historical background level in vehicle controls (0-5%) by roughly 1% and was not repeated in a replicate experiment. Mirabegron did not clearly induce chromosomal aberrations in human lymphocytes in a repeatable manner under the conditions employed in this assay.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: **YM178: Induction of Micronuclei in the Bone Marrow of Treated Rats**

Study no:	178-TX-008
Study report location:	Module 4.2.3.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 1, 1999
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, Purity 100.3%

Key Study Findings

- Mirabegron was lethal to male and female rats at ≥ 500 mg/kg.
- At doses up to 400 mg/kg, mirabegron did not produce an increase in the ratio of polychromatic to normochromatic cells. The frequency of micronucleated PCEs was similar in all dose groups to the vehicle control.

Methods

Doses in definitive study:	Trial 1) Single oral dose of 0, 125, 250, or 500 mg/kg (oral gavage) once daily for two days; 16 rats were dosed with vehicle alone and 8 at each dose of mirabegron or the positive control. Trial 2) Single oral dose of 0, 100, 200, 300, or 400 mg/kg (oral gavage) once daily for two days; 16 rats were dosed with the vehicle alone and 300 or 400 mg/kg mirabegron. 8 rats were dosed with 100 mg/kg, 200 mg/kg, or the positive control.
Frequency of dosing:	Daily for two days Bone marrow collected 24 hrs after the last dose
Route of administration:	Oral gavage
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% aqueous methylcellulose
Species/Strain:	Male and Female rats (F344) approximately 6 weeks old. Only male rats were used in the definitive studies.
Number/Sex/Group:	See dose information above.
Satellite groups:	None
Basis of dose selection:	Clinical signs and mortality observed at ≥ 500 mg/kg in the range finding study
Negative control:	Vehicle (0.5% aqueous methylcellulose)
Positive control:	Cyclophosphamide (80 mg/kg) dosed on day 2 only

Study Validity

The negative and positive controls responded as expected.

Results
Mirabegron was lethal to all males at 1,000 mg/kg during the range-finding study and 5/8 at 500 mg/kg in the first trial. One (1/3) female died at both 500 and 1,000 mg/kg in the range-finding study. Clinical signs at ≥ 500 mg/kg include lethargy, closed eyes, unsteady gate, prostrate position, and reduced body temperature.

In the definitive study, two trials were conducted because of the death of five rats at 500 mg/kg in the first trial precluding assessment of clastogenicity. The second trial had a maximum dose of 400 mg/kg. All animals survived in the second trial at up to 400 mg/kg but clinical signs were observed including lethargy at ≥ 200 mg/kg, prostrate position and piloerection at ≥ 300 mg/kg along with eye closure at 400 mg/kg.

At doses up to 400 mg/kg, mirabegron did not produce an increase in the ratio of polychromatic to normochromatic cells. The frequency of micronucleated polychromatic erythrocytes was similar in all mirabegron dose groups in comparison to the vehicle control.

8 Carcinogenicity

8.1 Two Year Oral Carcinogenicity Study in Mice

Study title: **104 Week Oral Gavage Carcinogenicity Study of YM178 in Mice**	
Study no.:	178-TX-031
Study report location:	Module 4.2.3.4.1.1
Conducting laboratory and location:	Sponsor's Central Toxicology Laboratory, Cheshire UK. Toxicokinetics assessed at (b) (4)
Date of study initiation:	First dose - Males 7-20-05, Females 7-21-05
GLP compliance:	Yes, OECD and UK regulations
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot GLP-K1780211, 99.4% pure
CAC concurrence:	The ECAC concurred with the sponsor's proposed doses of 0, 25, 50, and 100 mg/kg/day in males and female mice dosed via oral gavage based on deaths in both sexes at ≥ 200 mg/kg/day.

Key Study Findings

Adequacy of Carcinogenicity Study
- Appropriate multiples of the clinical exposure at the MRHD were achieved for both male (4, 9, and 25) and female (5, 9, and 21) mice.

- Survival to scheduled necropsy was greater than 73% in all groups without dose-related deaths. Factors that contributed to unscheduled death were not clearly dose-related.
- **Body weight gain was reduced in all mirabegron groups** despite elevated food intake which is an expected pharmacologic effect. At the terminal sacrifice, body weight gain was reduced 8%, 19%, and 21% in low- to high-dose males and 22%, 28%, and 31% in the low- to high-dose females compared to controls.

Appropriateness of Test Models

- The study appears adequate and the species and strain were the same as used in the 13-week toxicity study. Although it is recognized that reduced body weight gain can decrease neoplasm incidence in rodents, the reduced body weight gain observed with mirabegron was not thought to be due to excessive toxicity since it is expected pharmacology of a β3 adrenergic agonist, food intake was dramatically increased in all mirabegron groups during the dosing period, and survival was between 73% and 89% in all groups.

Evaluation of Tumor Findings

- There was no obvious effect of mirabegron on increased neoplasm incidence. The only statistically significant finding was an increase in benign hepatocellular adenomas in female mice in the lowest dose group ($p = 0.0017$, FDA statistical reviewer). The significance of this finding is unclear because it was only statistically significant at the lowest dose evaluated and was not significant in male mice or rats. Benign hepatocellular adenomas were observed in 1.4, 16, 10, and 4% of females in the control to high-dose groups with the historical control rate being 0.6% (range 0-1.4%) and 10, 16, 19, and 14% of males in the control to high-dose groups with the historical control rate of 8% (range 0-12%).
- The Executive Carcinogenicity Assessment Committee meeting minutes regarding the study findings are attached in Appendix B.

Methods

Doses:	0, 25, 50, or 100 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	10 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Basis of dose selection:	MTD – Death and prone position were observed at 200 mg/kg in males and females during 13 week range finding study (178-TX-029) and a single 300 mg/kg dose was lethal to 4/10 males and 2/10 females on the first day of dosing in a preliminary two-week repeat-dose study in mice (178-TX-028). The doses chosen were anticipated to be 0.8, 1.5, and 3 times the anticipated clinical exposure following 150 mg BID. The proposed clinical dose has sense changed to 50 mg once daily resulting in exposure multiples up to 25x in males and 21x in females.
Species/Strain:	Mice / B6C3F1
Number/Sex/Group:	70/sex/group
Age:	36 to 42 days at first dose
Animal housing:	One mouse per cage, 12 day/night, 22±3°C (three single day deviations, 13, 17, and 18°C), 30-70% humidity
Paradigm for dietary restriction:	Not restricted
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	For toxicokinetics 20 per sex at 25, 50, and 100 mg/kg and 4 control mice per sex
Deviation from study protocol:	Seven mice (1 control, 2 LD, 3 MD, and 1 HD) were replaced during days 22 to 33 of dosing because five died and two were euthanized due to morbidity consisting of head tilting and body weight loss or subdued behavior with hunched back, piloerection, and ruptured esophagus. The sponsor did not consider the deaths to be drug related. The replaced animals were dosed for 100-103 weeks.

Observations and Results

Mortality

Survival to week 105 was not affected by mirabegron.

Number Survived to Week 105 (%)								
	Male				Female			
Dose (mg/kg)	0	25	50	100	0	25	50	100
Found Dead	5	0	3	5	2	5	3	4
Euthanized for Humane Reasons	1	1	1	0	1	1	2	2
Euthanized due to Clinical Signs	4	11	4	8	8	13	12	10
Survived to Week 105	**60 (86%)**	**58 (83%)**	**62 (89%)**	**57 (81%)**	**59 (84%)**	**51 (73%)**	**53 (76%)**	**54 (77%)**

Factors that contributed to unscheduled death were not clearly dose-related (Table below). In the animals that died or were euthanized prematurely, the incidence of carcinomas, adenomas, and sarcomas were also not dose-related. Grouping the neoplasms according to NTP criteria also failed to reveal any dose related findings (17). At the time of unscheduled euthanasia, vaginal prolapse was observed in one LD female at week 37 in the toxicokinetics group and two HD females one each at week 70 and 88. The vaginal prolapse in the HD females was secondary to either fatal malignant hepatocellular carcinoma with distended abdomen or fatal benign vaginal stromal cell polyp with vaginal bleeding. There was no mention of other adverse findings in the LD female with vaginal prolapse. Since these effects appear to be secondary in the HD females, they are not considered to be dose related.

Factors that Contributed to Premature Death or Euthanasia								
	Male				Female			
Dose (mg/kg)	0	25	50	100	0	25	50	100
Multiple of MRHD (AUC) †	0	3.9	9.3	25.2	0	4.7	9.1	20.9
Survived to Week 105 (N = 70/group)	60	58	62	57	59	51	53	54
Total Died or Euthanized Prematurely	10	12	8	13	11	19	17	16
Found Dead	5		3	5+1PK	2	5	3+1PK	4
Euthanized Prematurely For Humane Reason	1	1	1	0	1	1	2+1PK	2
Euthanized Prematurely Due Clinical Signs	4	11	4	8	8	13	12	10
Unknown cause of death	-	1	1	5	1	2	3	2
Cervix – squamous cell carcinoma	-	-	-	-	-	1	-	-
Harderian Gland - adenoma	-	-	1	1	1	1	-	1
Liver – vascular ectasia, enlarged hepatocytes	-	-	1	-	-	-	-	-
– hepatocellular adenoma	1	-	-	3	-	-	-	-
– hepatocellular carcinoma	6	2	1	3	1	3	1	2
- cholangiocarcinoma	-	1	-	-	-	-	-	-
– hepatocholangiocellular carcinoma	-	-	-	-	-	-	1	-
Lung – adenocarcinoma	-	1	-	-	-	-	1	-
Lymphatic – malignant lymphoma (lymphosarcoma)	-	2	-	1	4	8	6	6
– mast cell tumor	-	-	-	1	-	1	-	-
– histiocytic sarcoma	-	-	-	-	3	2	1	-
Mammary Gland - adenocarcinoma	-	-	-	-	-	1	-	1
Nasal – Inflammatory cell infiltration (rhinitis)	-	-	1	2	-	-	-	1
Ovary and Oviduct - abscess	-	-	-	-	-	-	1	-
Pancreas – mixed mononuc. inflam. cell infiltration	-	1	-	-	-	-	-	-
Sciatic Nerve - demyelination	-	-	1	-	-	-	-	-
Seminal Vesicle – mixed mononuc. inflam. infil.	-	3	-	-	-	-	-	-
Skin – epidermal follicular cysts	-	-	-	-	-	-	1	-
Stomach – squamous cell carcinoma	-	-	-	-	-	-	1	-
Subcutaneous Tissue – fibrosarcoma	1	-	1	-	-	-	-	-
Tail – Epidermal cysts	-	1	-	-	-	-	-	-
Tongue – squamous cell carcinoma	2	-	-	-	-	-	-	-
Uterus – stromal cell carcinoma	-	-	-	-	-	-	-	1
- hemorrhage	-	-	-	-	1	-	-	-
- hemangiosarcoma	-	-	-	-	-	-	1	-
- leiomyosarcoma	-	-	-	-	-	-	1	-
Vagina – stromal cell polyp	-	-	-	-	-	-	-	1
Vascular - hemangiosarcoma	-	-	1	-	-	-	-	-
Total Carcinomas	8	4	1	3	2	5	4	4
Total Adenomas	1		1	4	1	1		1
Total Sarcomas	1	2	2	1	7	10	9	6
Total Mast Cell "tumor"				1		1		

Adapted from sponsor's Table 6 and 19. PK – includes animals in the pharmacokinetic group.
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072).

Clinical Signs

Noted adverse clinical signs were not obviously dose related.

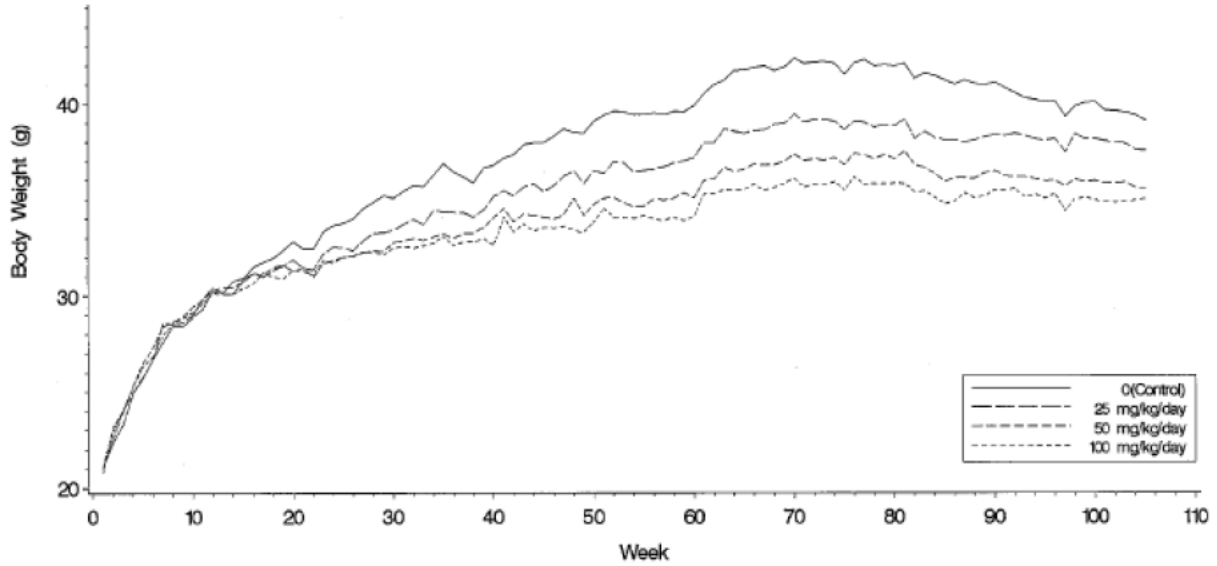
Body Weights

As previously observed in the preliminary 13-week toxicity study, mirabegron stimulated appetite resulting in a slight sporadic increase in body weight during weeks 2 to 7 in males and weeks 3 to 16 in females. Although food consumption remained high, body weight gain was statistically reduced compared to controls in the low- to high-dose groups beginning during week 22, 20, and 18 in males and week 30, 28, and 26 in females at 25, 50, and 100 mg/kg, respectively (Figure below). Thereafter, body weight remained reduced in all mirabegron groups throughout the dosing period (104 weeks). **At the terminal sacrifice body weight gain was reduced 8%, 19%, and 21% in low- to high-dose males and 22%, 28%, and 31% in the low- to high-dose females at 25, 50 and 100 mg/kg, respectively, compared to controls.** However, this is the expected pharmacologic response to a β3 agonist.

Mean Body Weight Gain (g) (Percent Difference From Control)								
	Male				Female			
Dose (mg/kg)	0	25	50	100	0	25	50	100
Multiple of MRHD (AUC)†	0	3.9	9.3	25.2	0	4.7	9.1	20.9
Absolute Body Weight (g) Week 105	39.2	**37.6***	**35.6****	**35.1****	40.0	**35.2****	**33.7****	**32.9****
Weight Gain (g) Weeks 1-105	18.1	16.6 (↓8%)	14.6 (↓19%)	14.3 (↓21%)	21.7	17.0 (↓22%)	15.7 (↓28%)	14.9 (↓31%)

† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). Statistically different from control * $p < 0.05$, ** $p < 0.01$. Statistical analysis was not conducted on the overall body weight change from week 1 to 105.

Mean Body Weight – Males (g)



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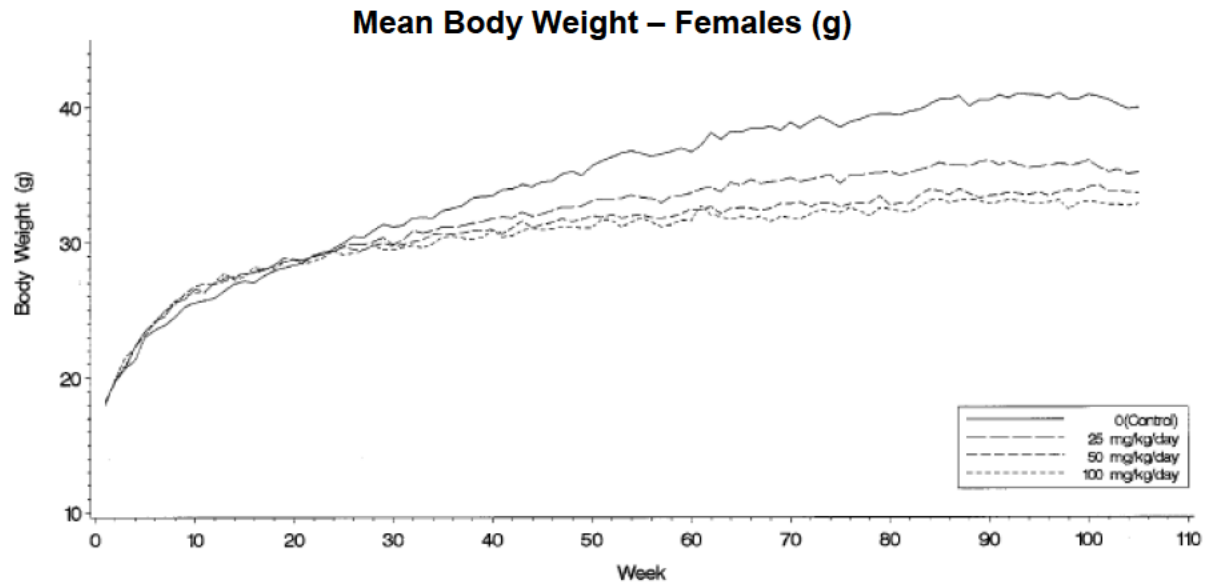

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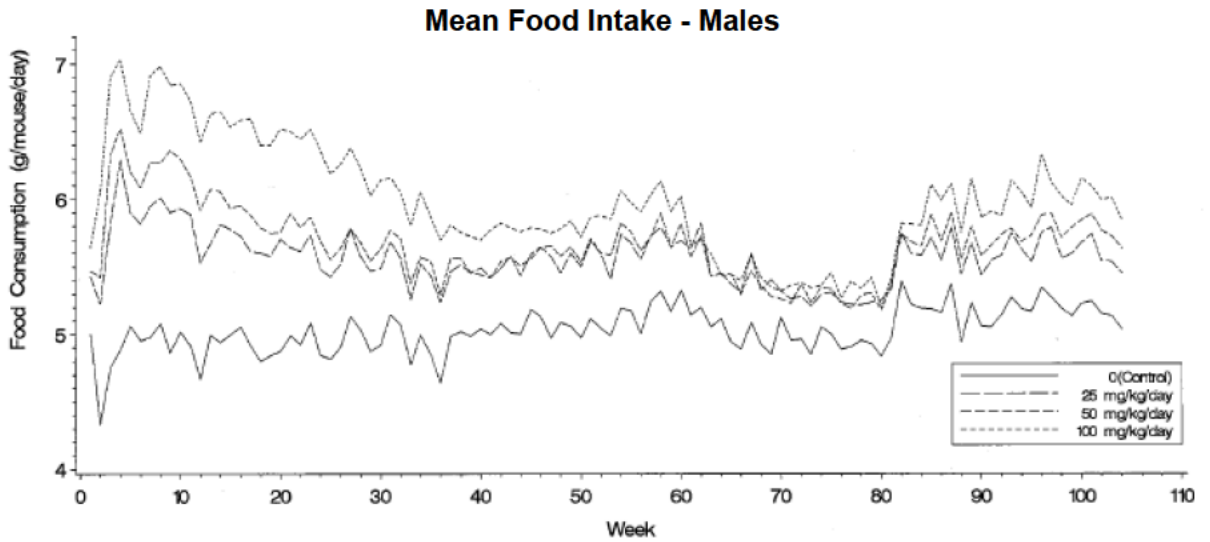
Feed Consumption

Food intake was dramatically increased in all mirabegron groups from the first week of dosing and continuing throughout the dosing period in both sexes (Figure below).

Elevated Feed Intake (Percent Increase Relative to Control)						
	Male			Female		
	25 mg/kg	50 mg/kg	100 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Week 104	110%	112%	118%	111%	113%	116%



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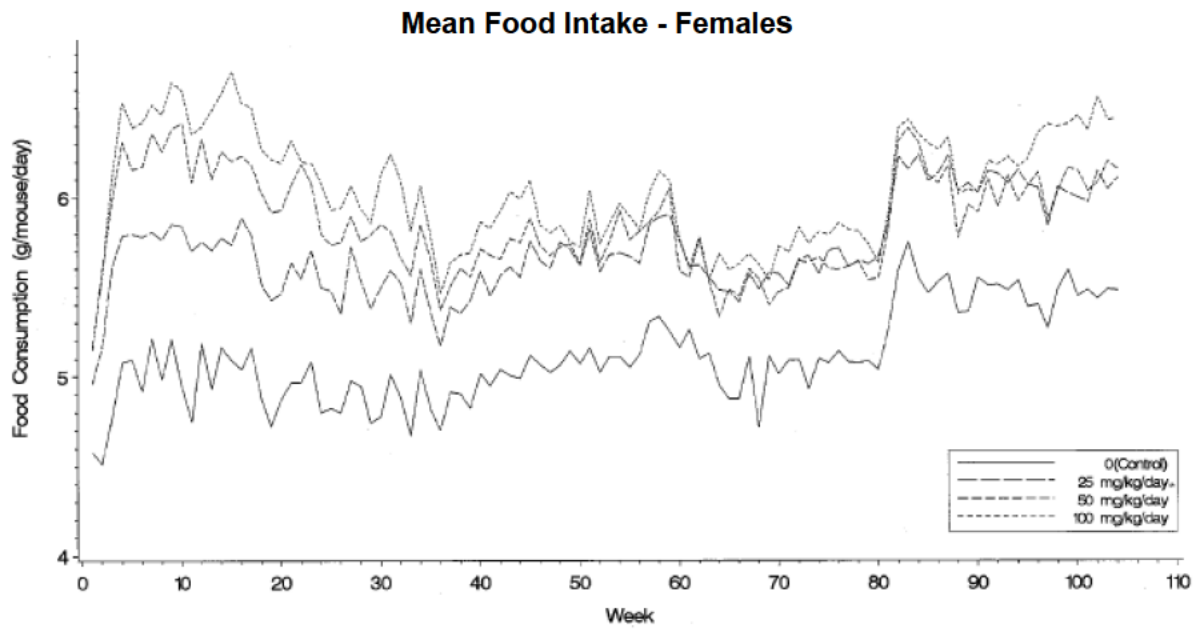

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The conversion of food to increased body weight was diminished in all mirabegron treatment groups in males throughout the dosing period and in females during the first year of dosing.

Conversion of Feed to Increased Body Weight (g Growth / 100 g of Feed)								
	Males				Females			
Dose Week	Control	25	50	100	Control	25	50	100
1-12	2.24	**1.87****	**1.85****	**1.65****	1.94	1.95	**1.79****	1.85
13-26	0.91	**0.50****	**0.33****	**0.35****	0.81	**0.46****	**0.39****	**0.27****
27-39	0.50	0.44	**0.36****	**0.07****	0.70	**0.39****	**0.29****	**0.29****
40-52	0.62	**0.35****	**0.15****	**0.28****	0.69	**0.29****	**0.17****	**0.05****
53-65	0.50	**0.33****	0.38	**0.33****	0.38	0.27	**0.14****	**0.12****
66-78	0.04	0.06	0.09	0.01	0.25	0.12	0.19	0.17
79-91	-0.33	**-0.13***	-0.20	**-0.05****	0.16	0.18	0.05	0.13
92-104	-0.37	**-0.21***	**-0.11****	**-0.13****	-0.16	-0.06	-0.03	-0.02
Statistically different from control * $p < 0.05$, **$p < 0.01$.								

Gross Pathology

Gross pathology did not reveal significant dose responsive adverse effects. Effects were either low incidence or were also observed in the control groups. A clear dose dependent effect on the mice that were euthanized prematurely was also not demonstrated. Cystic bursa in the ovaries/oviduct were observed in 3/17 MD and 2/16 HD mice that were euthanized prior to week 104. Testes were discolored in 3 HD males that were euthanized prematurely.

Adverse Clinical Signs That May Be Dose Related								
	Male				Female			
Dose (mg/kg)	0	25	50	100	0	25	50	100
N	60	58	62	57	59	51	53	54
Kidney – Single Mass			1	2		1		
Liver – Enlarged				1				
- Two Masses		2	2	4				
Lung – Multiple masses				1				
Lymph Node - Hepatic - Enlarged	2	1						4
- Pancreatic - Enlarged				1		1		
- Para-aortic - Enlarged							1	
- Sacral - Enlarged	1			1	1			3
Pituitary – Single Mass					5	5	4	8
Stomach – Single Glandular Mass								1
Data from sponsor's Table 12.								

Histopathology

Peer Review: Yes

Neoplastic:
Since death did not appear to have a dose dependent effect on neoplasms, all neoplasms from animals surviving to week 105 and those that were euthanized prematurely or died were combined if the incidence in the mirabegron groups was greater than the control groups (Tables below). There was a slight decrease in the incidence of all tumors combined, malignant tumors and single tumors in the HD males.

Overall Tumor Incidence of All Mice in Toxicity Groups (Percent Occurrence out of 70 Mice/ Group Independent of Date of Death)								
	Male				Female			
	0	25	50	100	0	25	50	100
Tumors – All	68.6	57.1	54.3	***42.9***	75.7	77.1	77.1	65.7
Single Tumors	51.4	47.1	41.4	***28.6***	54.3	48.6	44.3	50.0
Multiple Tumors	17.1	10.0	12.9	14.3	21.4	28.6	32.9	15.7
Benign Tumors - All	42.9	44.3	41.4	31.4	44.3	42.9	54.3	40.0
Multiple Benign Tumors	5.7	2.9	5.7	10.0	10.0	10.0	15.7	8.6
Malignant Tumors	34.3	18.6	18.6	***17.1****	44.3	54.3	45.7	34.3
Multiple Malignant Tumors	4.3	1.4	1.4	0	2.9	4.3	1.4	2.9
Metastatic Tumors	14.3	8.6	5.7	7.1	37.1	42.9	35.7	30.0
Statistically different from control, * $p < 0.05$, ** $p < 0.01$. Data summarized from sponsor Table 15.								

There was no obvious or statistically significant positive trend in tumor incidence according to the sponsor's Peto trend test analysis or the FDA statistical reviewer's analysis using a poly-k modification of the Cochran-Armitage test. Additionally, the only potentially statistically significant pair wise finding was benign hepatocellular adenomas and benign hepatocellular adenomas or carcinomas, combined, in females in the low

dose group (16% adenomas) compared to the control group (1% adenomas) (p = 0.0017, FDA statistical reviewer).

Although unclear, it is unlikely that the benign hepatocellular adenomas in female mice are a treatment related finding since the incidence was inversely related to dose and no statistically significant increase in the incidence of hepatocellular adenomas or adenomas or carcinomas (combined) were seen in male mice or in rats of either sex in the two-year carcinogenicity study in rats. Additionally, although reduced body weight gain may reduce neoplasms incidence in rodents, body weight gain was reduced in all mirabegron dose groups at 22% to 31%, so decreased body weigh gain would be expected to repress tumor incidence fairly similarly across all mirabegron groups. Furthermore, the historical control incidence of benign hepatocellular adenomas in female mice varies considerably between the conducting laboratory (0-1%), the animal supplier (0-17%), and two studies conducted by the National Toxicology Program (26-28%) (See table below).

Benign renal tubular adenomas, a rare finding in mice, were observed in 1 MD and 2 HD males. It is difficult to determine if this was dose related due to the low incidence and since other dose related pathology was not obvious in the kidney other than tubular dilation. Combining neoplasms according to criteria described by the national toxicology program did not reveal obvious treatment related effects (17).

Historical Control Incidence of Hepatocellular Adenomas in B6C3F1 Mice		
Spontaneous Occurrence Data Source	Male	Female
Concurrent Controls	10% (7/70)	1.4% (1/70)
Conducting Laboratories Historical Control (N = 3 studies)	7.6% (range 0-12%)	0.6% (range 0-1.4%)
Charles River Laboratory Incidence (N=13 studies, 1989)	14% (range 0– 41%)	6% (range 0 – 17%)
National Toxicology Program Incidence (N =2, 2010)	56% (range 48-64%)	27% (range 26-28%)
Data derived from the sponsor's statistical submission (DARRTS 258), the Charles River and NTP web pages.		

Overall Tumor Incidence When Incidence is Greater Than Control Values (Occurrence out of 70 Mice / Group Independent of Date of Death)								
Neoplasm (B- benign, M – Malignant)	Male				Female			
Dose (mg/kg/day)	0	25	50	100	0	25	50	100
Multiple of MRHD (AUC)	-	3.9	9.3	25.2	-	4.7	9.1	20.9
Adrenal – subcapsular adenoma – B	12/70	9/70	10/69	12/70	0/69	0/70	0/70	0/69
- cortical adenocarcinoma – M	0/70	0/70	0/69	0/70	1/69	0/70	0/70	0/69
Bone Marrow – hemangioma – B	-	-	-	-	-	0/2	-	1/1
Brain – meningeal sarcoma – M	0/70	0/70	0/70	0/70	0/70	0/70	0/70	1/70
Cervix – squamous cell carcinoma – M	NA	NA	NA	NA	0/70	1/69	0/69	0/70
Duodenum – adenoma – B	0/70	1/70	0/70	0/70	1/69	0/69	0/70	0/70
Harderian Gland – adenoma – B	2/70	3/69	2/69	2/70	5/68	4/68	4/68	2/70
Kidney – tubular adenoma – B	0/70	0/70	1/70	2/70	0/70	0/70	0/70	0/70
Liver – hepatocellular adenoma – B	7/70	11/70	13/70	10/70	1/70	**11/70**	7/70	3/70
– hepatocellular carcinoma – M	10/70	8/70	8/70	9/70	3/70	5/70	2/70	2/70
- hepatocellular adenomas or carcinomas	17/70	18/70	21/70	17/70	4/70	16/70	9/70	5/70
– cholangiocarcinoma – M	0/70	1/70	0/70	0/70	0/70	0/70	0/70	0/70
- hepatocholangiocellular carcinoma – M	0/70	0/70	0/70	0/70	0/70	0/70	1/70	0/70
– Ito cell tumor - B	0/70	0/70	1/70	0/70	0/70	0/70	0/70	0/70
Lung – adenoma – B	11/70	7/70	7/70	**2/70***	5/70	2/70	4/70	0/70
- adenocarcinoma – M	5/70	2/70	**0/70***	**0/70***	2/70	1/70	2/70	0/70
Lymphatic –lymphoma/lymphosarcoma - M	5/8	3/3	2/2	1/2	23/26	23/28	18/23	17/18
- histiocytic sarcoma – M	3/8	0/3	**0/2***	**0/2***	3/26	5/28	5/23	1/18
- mast cell tumor - M	0/8	0/3	0/2	1/2	0/26	1/28	0/23	0/18
Mammary – adenoma – B	NE	NE	NE	NE	0/67	1/67	0/68	0/65
– adenocarcinoma – M	NE	NE	NE	NE	0/67	2/67	0/68	2/65
Oral – squamous cell papilloma - B	-	-	-	-	0/0	0/0	0/0	1/1
Ovary – granulosa/thecal cell tumor – B	NA	NA	NA	NA	0/70	0/69	1/69	1/70
– cystadenoma – B	NA	NA	NA	NA	0/70	0/69	1/69	1/70
– cystadenocarcinoma – M	NA	NA	NA	NA	0/70	0/69	1/69	0/70
- hemangioma - B	NA	NA	NA	NA	1/70	0/69	0/69	0/70
Pancreas – Islet cell adenoma - benign	0/70	1/70	0/70	1/70	1/70	0/70	0/70	0/69
Pituitary – adenoma - benign	0/70	0/70	0/68	0/67	18/69	17/68	24/67	18/68
Skin – squamous cell papilloma – B	0/70	0/70	0/70	0/70	0/70	1/70	0/70	0/70
– sebaceous adenoma – B	0/70	0/70	0/70	0/70	0/70	2/70	1/70	0/70
Spinal Chord – hemangiosarcoma – M	0/70	0/70	0/70	0/70	0/70	0/70	1/70	0/70
Spleen – hemangiosarcoma – M	0/70	0/70	2/70	0/70	0/70	1/70	0/70	0/70
Stomach – squamous cell papilloma – B	0/70	0/70	0/70	0/70	0/70	0/70	1/70	0/70
- squamous cell carcinoma – M	0/70	0/70	0/70	0/70	0/70	0/70	1/70	0/70
Tail – squamous cell papilloma – B	0/16	0/20	0/20	0/17	0/6	0/8	1/11	0/7
– schwannoma - M	0/16	0/20	0/20	0/17	0/6	1/8	0/11	0/7
Thyroid – follicular cell adenoma - B	0/69	1/69	0/70	0/70	0/70	0/70	0/70	1/70
Tongue – squamous cell carcinoma - M	2/70	0/70	0/70	0/70	-	-	-	-
Uterus – stromal cell polyp – B	NA	NA	NA	NA	2/70	1/70	4/70	4/70
– leiomyoma – B	NA	NA	NA	NA	0/70	1/70	0/70	2/70
– leiomyosarcoma – M	NA	NA	NA	NA	0/70	1/70	0/70	2/70
– hemangioma – B	NA	NA	NA	NA	0/70	0/70	2/70	0/70
– hemangiosarcoma – M	NA	NA	NA	NA	0/70	0/70	1/70	0/70
– fibroma – B	NA	NA	NA	NA	0/70	1/70	0/70	0/70
Vagina – stromal cell polyp – B	NA	NA	NA	NA	0/70	0/68	0/69	1/70
Vascular – hemangiosarcoma - M	1/1	0/0	1/1	1/1	-	-	-	-

Total Hemangioma	-	-	-	-	1	0	2	1
Total Hemangioma & Hemangiosarcoma	1	0	3	1	1	1	4	1
* Statistically different from control, Peto linear trend test (2-sided), p < 0.05. Data from sponsor Table 17 and 18. NA- not applicable. NE – not examined. (-) not reported. Statistically different from control p = 0.0017.								

Non-Neoplastic:
Adverse effects were of low incidence, were not dose dependent, or were not acutely adverse (see Table below). The most common adverse findings were low incidences of inflammatory pathologies noted by endometriosis (F), arteritis (F), lymphoid hyperplasia of the lungs (M/F), rhinitis (M), and inflammatory cell infiltration in the brown fat (F), thyroid (F), and urinary bladders (F).

Inflammatory Pathologies: Minimal to slight mononuclear infiltration in the brown fat of the aorta was observed in females in all mirabegron groups but not in the controls. Slight to moderated arteritis (inflammation of the arteries) was observed in three HD females but not in other groups. Minimal to slight lymphoid hyperplasia was elevated in the lungs of HD males and females. In males rhinitis with inflammatory cell infiltration increased in severity with dose (min-moderate). Minimal to slight mononuclear inflammatory cell infiltration was observed in the thyroids of females. Additional potential dose related findings in female include lymphoid hyperplasia in the urinary bladders and slight to marked endometritis.

Other Pathologies: Pigmentation of the liver, primarily of minimal to slight intensity, increased in the MD (4/70) and HD (5/70) females compared to the slight to marked pigmentation in controls (2/70). Minimal to marked non-glandular hyperplasia of the stomach was observed in LD (1/70), MD (1/70), and HD (3/70) females and 1/70 MD males but not in the control mice. Minimal to marked unilateral tubular degeneration of the testes was also observed at a slightly higher incidence in mirabegron treated mice; however, this is unlikely to be treatment related since the response was not dose-related in animals with bilateral degeneration.

It is not clear why the principal histopathology findings in the 13-week toxicity study were not observed in this two year study. Unlike the 2-year study, adipose tissue findings at exposures 10x MRHD in the 13-week study include multivacuolated white adipose tissue in the skin with decreased lipid droplet size and decreased lipid accumulation in the brown adipose tissue in the kidney. Hepatic glycogen content increased to a minimal or mild extent at ≥ 50 mg/kg (10x MRHD) in the 13-week study but this was not reported in the two year study since histological stains for glycogen were not employed. Of concern in the 13-week toxicity study was minimal hepatocyte hypertrophy which was observed in 2/12 females and 5/12 males at 200 mg/kg (>48x MRHD). However, hepatic hypertrophy did not progress in incidence or severity with two years of exposure at up to 21x MRHD. Hepatic hypertrophy was only observed in 1/70 females at 25 mg/kg (~5x MRHD) and 100 mg/kg (21x MRHD).

Incidence of Non-Neoplastic Lesions - Possibly Treatment Dependent (Occurrence Out of 70 Mice/ Group Independent of Date of Death)								
Sex	Male				Female			
Dose (mg/kg)	0	25	50	100	0	25	50	100
Brown Fat - Heart – mixed/mononuc. infil. (min-slight)	0	0	0	0	0	5	9	6
Epididymes – increased sperm precursor cells (min-slight)	1	1	2	3	NA	NA	NA	NA
Esophagus – mixed mononuc. inflamm. cell infil. (minimal)	0	0	0	0	0	1	0	1
Heart – arteritis (slight-moderate)	0	0	0	0	0	0	0	3
– degenerative cardiomyopathy (min-moderate)	0	0	1	1	0	1	0	1
Ileum - mixed/mononuc. infil. (marked)	0	0	0	0	0	0	0	1
– epithelial necrosis/ulceration/inflame (marked)	0	0	0	1	0	0	0	0
Jejunum – lymphoid hyperplasia (slight)	0	0	0	0	0	0	1	2
Kidney – tubular vacuolation (min-moderate)	1	1	1	3	0	0	0	0
Liver – increased pigmentation (min-marked)	0	0	1	0	2	1	4	5
Lung – lymphoid hyperplasia (min-slight)	0	1	0	4	1	6	5	5
Nasal – rhinitis inflam cell infil (min - mod)	1	1	3	4	0	0	0	1
Ovary / Oviduct - cystic bursa	NA	NA	NA	NA	0	1	1	0
– vascular ectasia (slight)	NA	NA	NA	NA	0	2	3	0
Seminal Vesicle – reduced secretion	0	0	0	2	-	-	-	-
Sternum – myelofibrosis (slight)	0	0	0	0	0	0	0	1
Stomach – hyperplasia – non-glandular (min-marked)	0	0	1	0	0	1	1	4
– hyperplasia – glandular (slight)	0	0	0	1	0	0	0	0
– squamous metaplasia glandular (minimal)	0	1	0	0	0	0	0	0
Testes – unilateral tubular degeneration (min-marked)	1	3	3	3	NA	NA	NA	NA
– bilateral tubular degeneration (min-marked)	2	1	3	1	NA	NA	NA	NA
Thyroid – mixed/monnuc. inflamm. cell infil. (min-slight)	0	0	1	1	1	1	3	4
– follicular cell hyperplasia (min-mod)	0	0	0	2	0	1	1	0
Urinary Bladder – lymphoid hyperplasia (min-mod)	0	1	0	1	5	7	3	11
Uterus – inflamm. cell infil (endometritis) (slight-marked)	NA	NA	NA	NA	2	10	12	5
Data summarized from sponsor's Table 14. NA – not applicable.								

Toxicokinetics

Blood was sampled after 52 weeks of dosing in the TK animals 1, 2, 4, 8, and 24 hrs after dosing (4/sex/group/time). Control animals were assessed 2 hrs after dosing.

Male and female mice were exposed to 4, 9, 25 and 5, 9, 21 times the clinical exposure fasted elderly women at the MRHD respectively. Mirabegron was rapidly absorbed in mice with a T_{max} of one hr for all groups. Exposure (AUC) increased slightly greater than the two fold increase in dose between groups in males (2.4-2.7x) but was essentially dose proportional in females. There did not appear to be an obvious effect of sex on AUC. However, the maximal concentration in females was not dose proportional with levels in the 25 mg/kg group exceeding the 50 mg/kg group. This was due to two females in the LD group with maximal exposures similar to the HD group. Since C_{max} occurred within one hour of dosing the discrepancy between groups may be because Tmax was actually reached at an earlier time. A similar lack of dose

proportionality in maximal exposure level was observed in females during the 13-week toxicity study.

Toxicokinetics at Week 52 of Dosing						
	Male			Female		
Dose (mg/kg)	25	50	100	25	50	100
Multiple of MRHD (AUC) †	3.9	9.3	25.2	4.7	9.1	20.9
T_{max} (hr)	1	1	1	1	1	1
C_{max} (ng/mL)	652	1,316	1,888	1,206	943	1,905
AUC_{0-24} (ng-h/mL)	1,975	4,749	12,905	2,399	4,676	10,713
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron OCAS for 7 days (178-CL-072).						

Pharmacokinetic Summary in Humans and Mice						
Population	Duration	Dose	C_{max} (ng/mL)		AUC_{0-24} (ng-hr/mL)	
			Male	Female	Male	Female
Human Fasted, healthy, young 178-CL-031	Day 10	50 mg 100 mg 200 mg 300 mg	33 72 220 381	46 112 264 530	262 519 1443 2473	368 800 2046 3888
Human Elder Female 178-CL-072 (n=11)	Day 7 Fasted	50 mg	43.5	**66.3**	341	**512**
Mice 178-TX-029 (13 Week Tox Study)	Week 13	50 mg/kg 100 mg/kg 200 mg/kg	936 1939 3124	2083 2054 2507	4903 14,643 26,340	6931 13,899 24,461
Mice 178-TX-031 (Carcinogenicity Study)	Week 52	25 mg/kg 50 mg/kg 100 mg/kg	652 1,316 1,888	1,206 943 1,905	1,975 4,749 12,905	2,399 4,676 10,713

Dosing Solution Analysis

Dosing solution was assessed every six months and was within 8% of target concentration. The homogeneity between the top, middle, and bottom of the dosing vials and was within 1% of the mean for all layers combined. Mirabegron at 0.6 and 200 mg/mL in 0.5% methylcellulose was stable at room temperature for eight days.

8.2 Two Year Oral Carcinogenicity Study in Rats

Study title: **104 Week Oral Gavage Carcinogenicity Study of YM178 in Rats**	
Study no.:	178-TX-032
Study report location:	Module 4.2.3.4.1.1
Conducting laboratory and location:	(b) (4) Pathology peer review was conducted by (b) (4)
Date of study initiation:	First dose 8-17-05
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), lot GLP-K1780211, 99.4% pure prior to first dose and 100% at week 102
CAC concurrence:	Yes, based upon MTD (lethality in females at 300 mg/kg and ↓BW in males at 100 mg/kg)

Key Study Findings

Adequacy of Carcinogenicity Study

- Appropriate multiples of the clinical exposure at the MRHD were achieved for both male (7, 12, and 25) and female (11, 24, and 45) rats based upon AUC and a 50 mg clinical dose and acceptable survival in all groups (≥ 24/60).
- In males, decreased weight gain (↓17%) was observed at the highest dose but survival was significantly higher in this group (65%) compared to controls (48%).
- In females, decreased survival without an effect on body weight was observed at the high dose (control 73% vs. HD 40%) and the time to death in the high-dose group appeared dose dependent, suggesting that the MTD may have been exceeded in this group.

Appropriateness of Test Models

- The choice of species was the same as in the chronic toxicity study and the study results adequately assessed the carcinogenicity potential.

Evaluation of Tumor Findings

- Mirabegron dosing at up to 25 (M) or 45 (F) times the exposure at the MRHD did not increase the incidence of neoplasms after up to two years of daily oral administration.
- The Executive Carcinogenicity Assessment Committee meeting minutes regarding the study findings are attached in Appendix B.

Methods

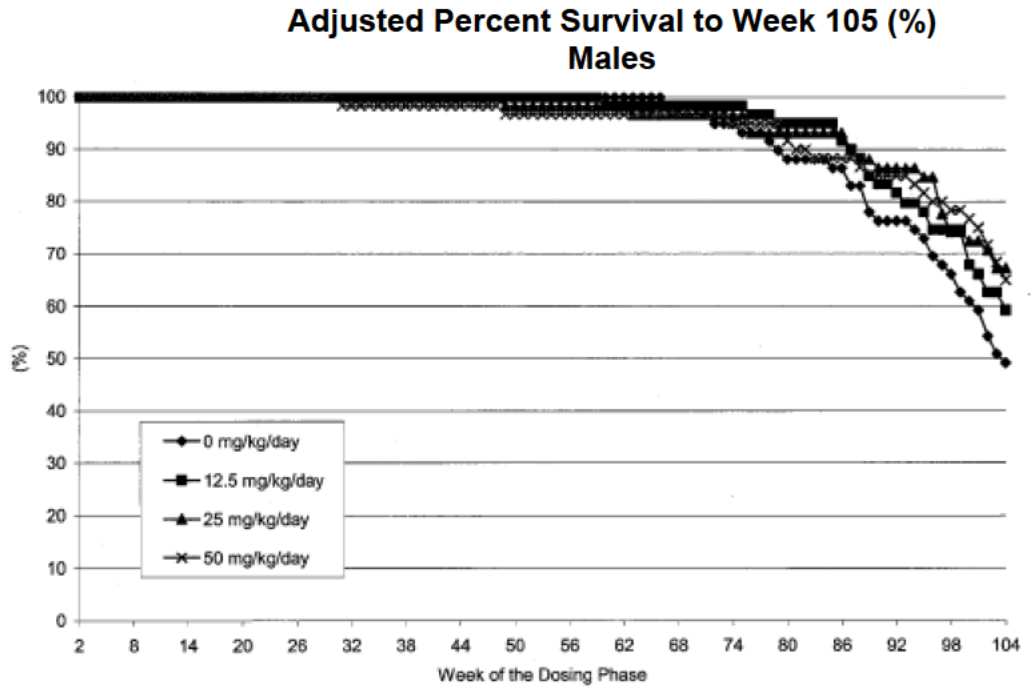
Doses:	Male: 0, 12.5, 25, or 50 mg/kg/day Female 0, 25, 50, or 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose in reverse osmosis water
Basis of dose selection:	The ECAC recommended oral gavage doses of 0, 25, 50, and 100 mg/kg/day in females based on deaths at 300 mg/kg/day in the 13-week study. This was expected to achieve systemic exposures 0.2, 1.3, and 5 times the proposed therapeutic dose of 150 mg BID. For males, the ECAC recommended doses of 12.5, 25, and 50 mg/kg, based upon 16% and 35% reduction in body weight gain at 30 and 100 mg/kg respectively in the 26-week toxicity study. This was expected to achieve systemic exposures 0.07, 0.2, and 0.8 times the 150 mg BID clinical dose. The sponsor's maxim proposed clinical dose is 50 mg once daily which resulted in exposure multiples > 25x.
Species/Strain:	Rats / CDF® F-344/DuCrl strain
Number/Sex/Group:	60/sex/group
Age:	40-46 days at first dose
Animal housing:	Individually housed in stainless steal cages with environmental enrichments unless adverse health called for polycarbonate cages with hardwood bedding; 18-26°C, 13-86% humidity and 12 hr light/dark
Paradigm for dietary restriction:	No, feed available ad libitum
Dual control employed:	No
Interim sacrifice:	No
Satellite groups:	4 control and 12/sex/group were included for TK analysis at week 52. They were euthanized after sampling at week 52.
Deviation from study protocol:	None that severely altered study outcome. Documentation for the intermediate calibration standards (on 8-25-06) and the quality control preparations (8-29-06) could not be located. The sponsor indicated that the study was not adversely affected since very detailed methods were followed and the results were acceptable.

Observations and Results

Mortality

There was no adverse effect of mirabegron dosing on the survival of males. In fact the survival of the MD and HD male groups was increased in comparison to the control. However, there was a large increase in mortality at the HD females where only 40% survived to week 105 vs. 73% of the controls (Figures and table below). The time to death in the high-dose female group appeared dose dependent, suggesting that the MTD was exceeded in this group.

Survival to Week 105					
N = 60/group	0 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Male	29 (48%)	35 (58%)	**39 (65%)+**	**39 (65%)+**	NA
Female	44 (73%)	NA	38 (63%)	36 (60%)	**24 (40%)****
Three accidental deaths occurred in males (1 each in the control, LD, and MD groups) and females (1 each in the LD, MD, and HD groups). + Increased survival compared to control p < 0.05. ** Decreased survival compared to control p < 0.01.					



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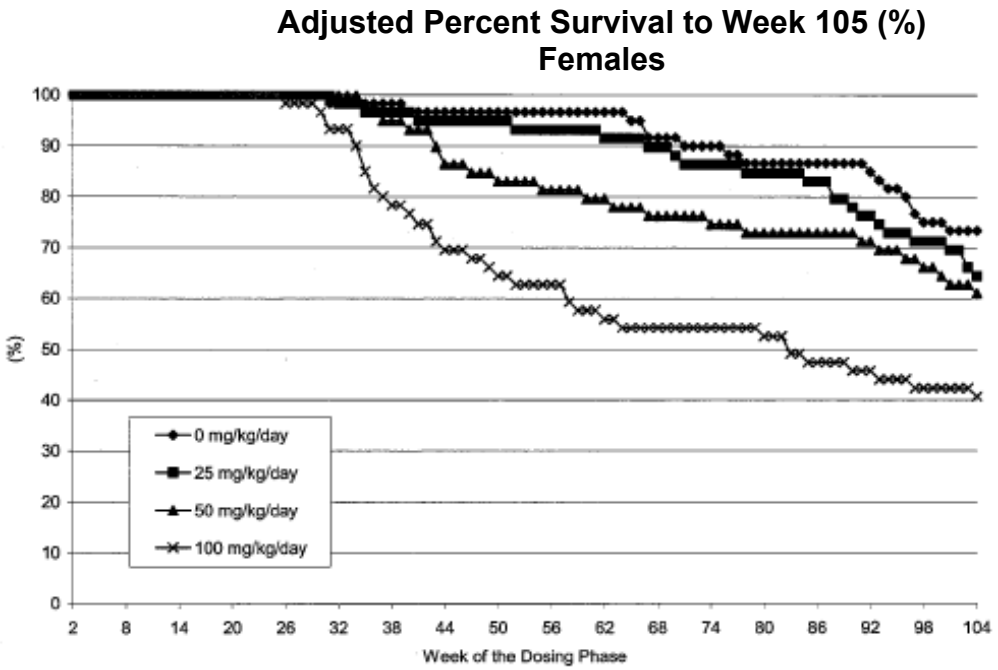

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There was no clear causative effect of mirabegron in the unscheduled deaths (Table below). The causes of unscheduled deaths were not determined for 4, 5, 5, and, 10 males or 4, 9, 14, and 27 females in the control, LD, MD, and HD groups, respectively. The incidence of total carcinomas, adenomas, and sarcomas leading to unscheduled death was not dose responsive.

Factors that the Sponsor Contributed to Unscheduled Death or Euthanasia								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
N	60	60	60	60	60	60	60	60
Survived to Week 104	28	35	39	39	43	37	36	24
Unknown cause of death	4	5	5	10	4	9	14	27
Arthritis	1	0	0	0	0	0	0	0
Aspiration Pneumonia	0	0	0	0	0	0	1	0
Carcinoma	0	0	0	0	0	1	0	0
Chronic Progressive Nephropathy	0	2	0	0	0	0	0	0
Dermatitis	0	1	0	0	0	0	0	0
Endometrial Stromal Polyp	0	0	0	0	0	1	0	0
Endometrial Stromal Sarcoma	0	0	0	0	1	0	0	0
Fibroma	0	0	0	0	0	0	1	0
Fibrosarcoma	2	2	0	1	0	0	1	0
Bone Fracture	1	0	1	0	0	1	1	0
Gavage Related Death	0	1	1	0	0	0	0	1
Haemangiosarcoma	0	0	1	0	0	0	0	0
Hepatocellular Adenoma	0	0	1	0	0	0	0	0
Hepatocellular Carcinoma	-	-	-	-	-	-	-	-
Histiocytic Sarcoma	1	0	0	0	0	2	0	1
Large Granular Cell Leukemia	16	9	10	7	7	6	5	4
Liposarcoma	0	1	0	0	0	0	0	0
Malignant Pheochromocytoma	0	0	0	1	0	0	0	0
Malignant Schwannoma	0	0	1	0	0	0	0	0
Mammary Carcinoma	0	0	0	0	0	0	0	1
Mammary Fibroadenoma	0	0	0	0	0	1	0	1
Mesothelioma	0	0	0	1	0	0	0	0
Neural Crest Tumor	0	0	0	0	0	0	1	0
Ophthalmitis	0	0	0	0	1	0	0	0
Papilloma	0	0	0	0	1	0	0	0
Pneumonia	0	1	0	0	0	0	0	0
Pituitary Adenoma	5	2	0	0	2	1	0	1
Rhinitis	0	1	0	0	0	0	0	0
Salivary Gland Carcinoma	0	0	0	1	0	0	0	0
Sarcoma	1	0	0	0	0	0	0	0
Squamous Cell Carcinoma	1	0	1	0	0	1	0	0
Transitional Cell Carcinoma	0	0	0	0	1	0	0	0
Total Deaths Related to Carcinomas	1	0	1	1	1	2	0	1
Total Deaths Related to Adenomas	5	2	1	0	2	1	0	1
Total Deaths Related to Sarcomas	4	3	0	1	1	2	1	1
Adapted from sponsor's Table 12.								

Clinical Signs

Signs were generally not consistent between the sexes. However, a clear oral discharge (presumably salivation) was observed in males at ≥ 12.5 mg/kg and females at ≥ 50 mg/kg, while a red oral discharge was only observed in males at ≥ 25 mg/kg.

Dose dependent increases in non-formed feces were observed in females at ≥ 25 mg/kg, while most control and mirabegron treated males had non-formed feces. The incidence of sore and scabbed mouths was elevated in females at 100 mg/kg (22/60) compared to the control group (8/60). Two of the main clinical signs observed in the 26-week toxicity study, i.e., lacrimation and prone position, were rare and not dose responsive here.

Number of Rats with Potentially Adverse Clinical Signs								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Multiple MRHD (AUC) †	0	7	12	25	0	12	24	45
Head Tilt – Left	2	2	6	5	3	2	2	1
– Right	2	2	3	5	1	1	2	1
Oral Discharge – Clear	0	12	50	59	0	0	10	11
– Red	0	0	2	25	0	0	0	0
Nasal Discharge – Red	0	0	0	5	1	1	0	0
Nose – Swollen	0	0	0	0	0	0	2	2
Feces – Few	18	6	6	5	6	5	4	5
– Liquid	2	0	2	2	0	3	3	9
– Non-formed	41	41	45	49	6	22	21	32
Mouth – Sore-Scabbed	8	8	4	5	8	8	6	22
Masses	23	25	12	12	8	13	11	11
Tail – Skin Scaly	3	0	1	14	2	0	1	2
Summary of sponsor's Table 4. † Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072).								

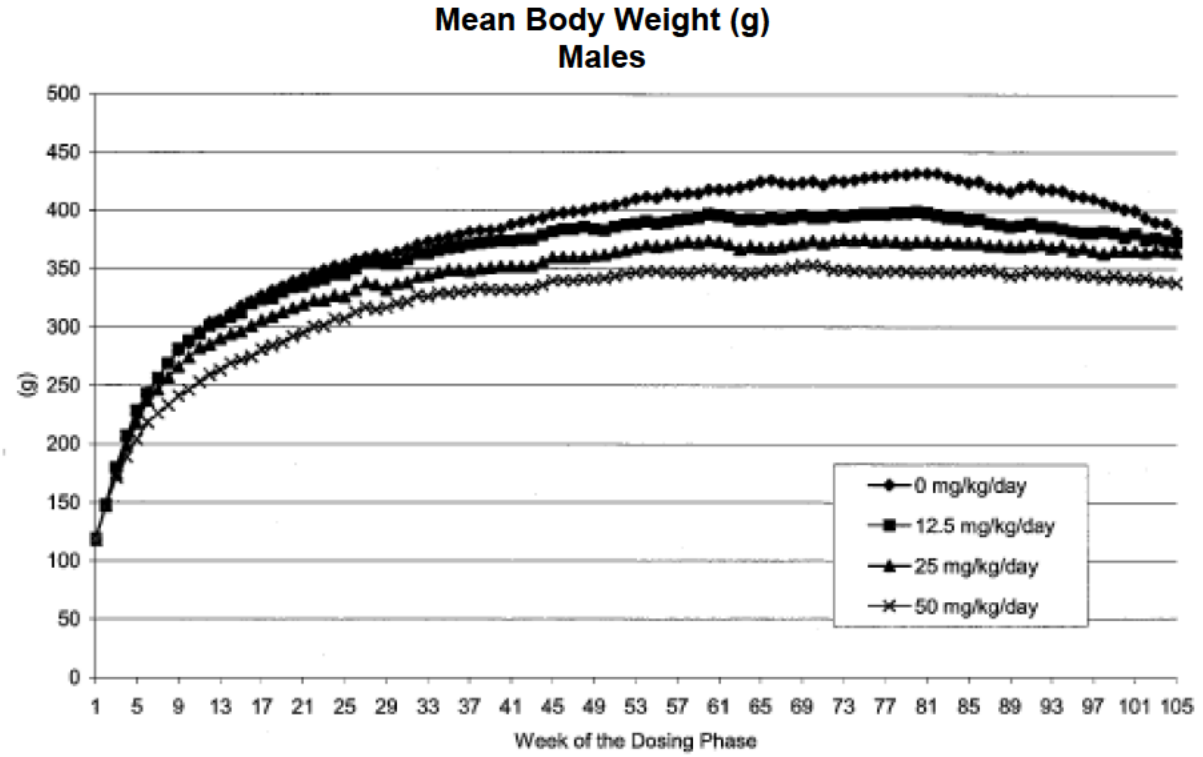
Body Weights

Body weight gain from week 1 to 105 was decreased 17% in HD males. This was due to a reduction in body weight gain from weeks 2 to 14 which was also observed in the mid-dose group. Weight loss was observed in all groups of males in the second year of dosing but it was greater in the control males than the mirabegron treated males.

There was no significant effect of mirabegron on the body weight or body weight gain in females from week 1 to 105. However, during the last year of the study, body weight gain was reduced 18% and 26% in the MD and HD females.

Mean Body Weight Gain (g) (Percent Difference From Control)								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Multiple MRHD (AUC)†	0	7	12	25	0	12	24	45
Weeks 1-53	292	**271*(↓7%)**	**250*(↓14%)**	**229*(↓22%)**	108	112	111	111
Weeks 53-105	-28	**-13*(↑53%)**	**-2*(↑93%)**	**-7*(↑75%)**	50	45	**41*(↓18%)**	**37*(↓26%)**
Weeks 1-105	264	254	246 (↓7%)	220 (↓17%)	158	157	152	148 (↓6%)
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). *Statistically different from control $p < 0.05$. Statistical analysis was not conducted on the overall change from week 1 to 105.								

Absolute Mean Body Weight (g) at Weeks 53 and 105 (% Difference From Control)								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Multiple MRDH (AUC)†	0	7	12	25	0	12	24	45
Week 53	410	389*(↓5)	**368* (↓10)**	**347*(↓15)**	206	209	208	207
Week 105	382	373	364*(↓5)	**338*(↓12)**	254	254	249	245
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). *Statistically different from control p < 0.05.								



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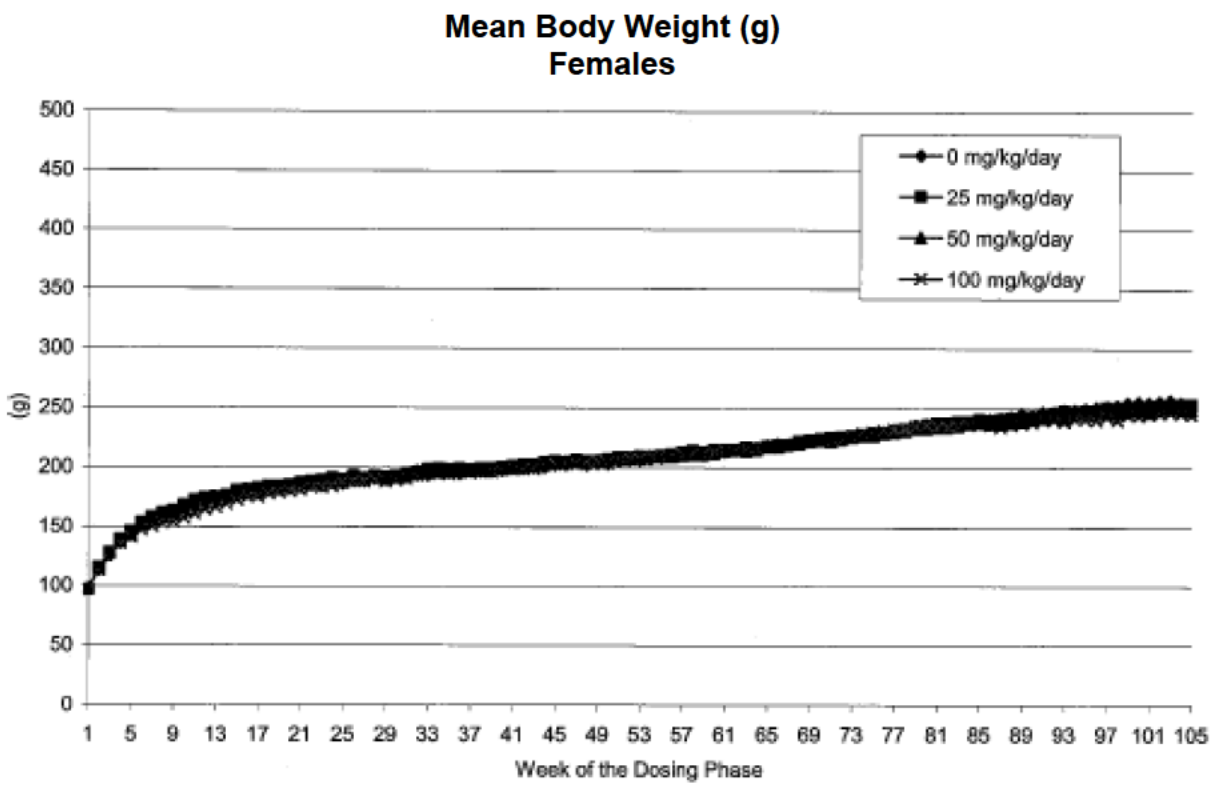

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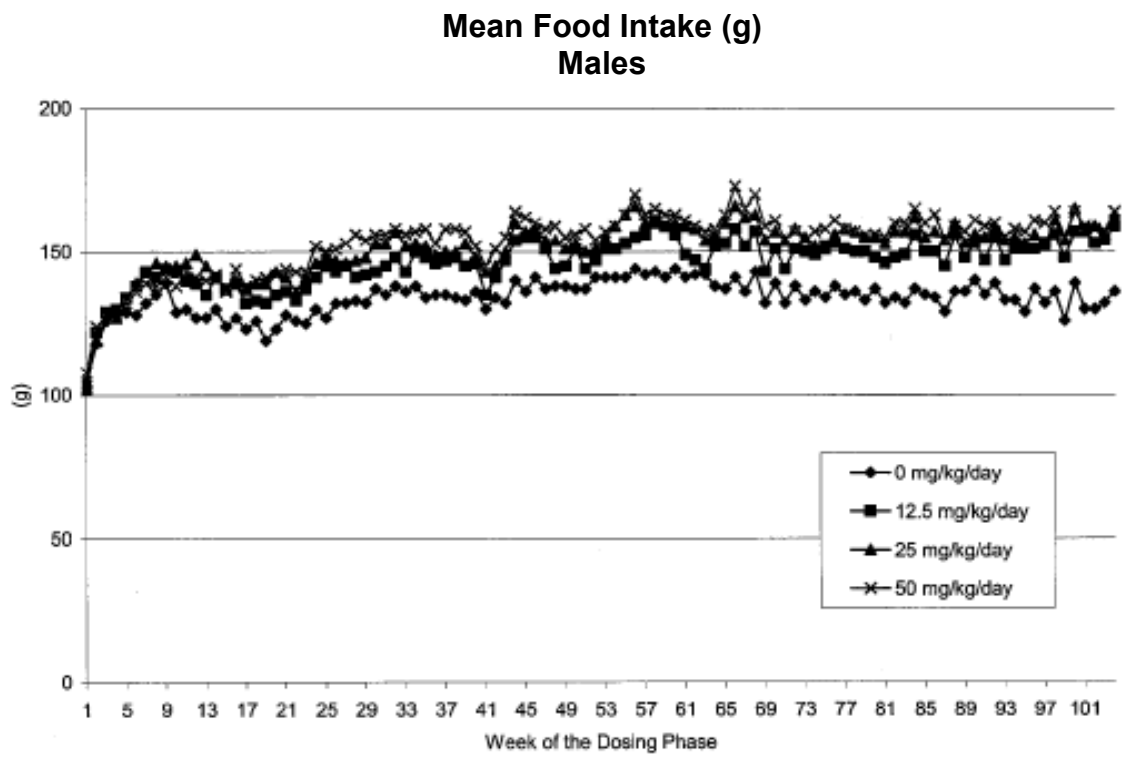
Feed Consumption

There was a dose dependent increase in feed intake in males and females in all mirabegron groups beginning within the first few weeks of dosing and it continuing throughout the 105 weeks of exposure.

Mean Feed Intake (% Increase)								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Week 53	141	147*(↑5)	151*(↑7)	154*(↑9)	93	108*(↑16)	109*(↑9)	113*(↑22)
Week 105	136	159*(↑17)	162*(↑19)	164*(↑21)	105	127*(↑21)	120*(↑14)	126*(↑20)
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron OCAS for 7 days (178-CL-072). *Statistically different from control $p < 0.05$.								



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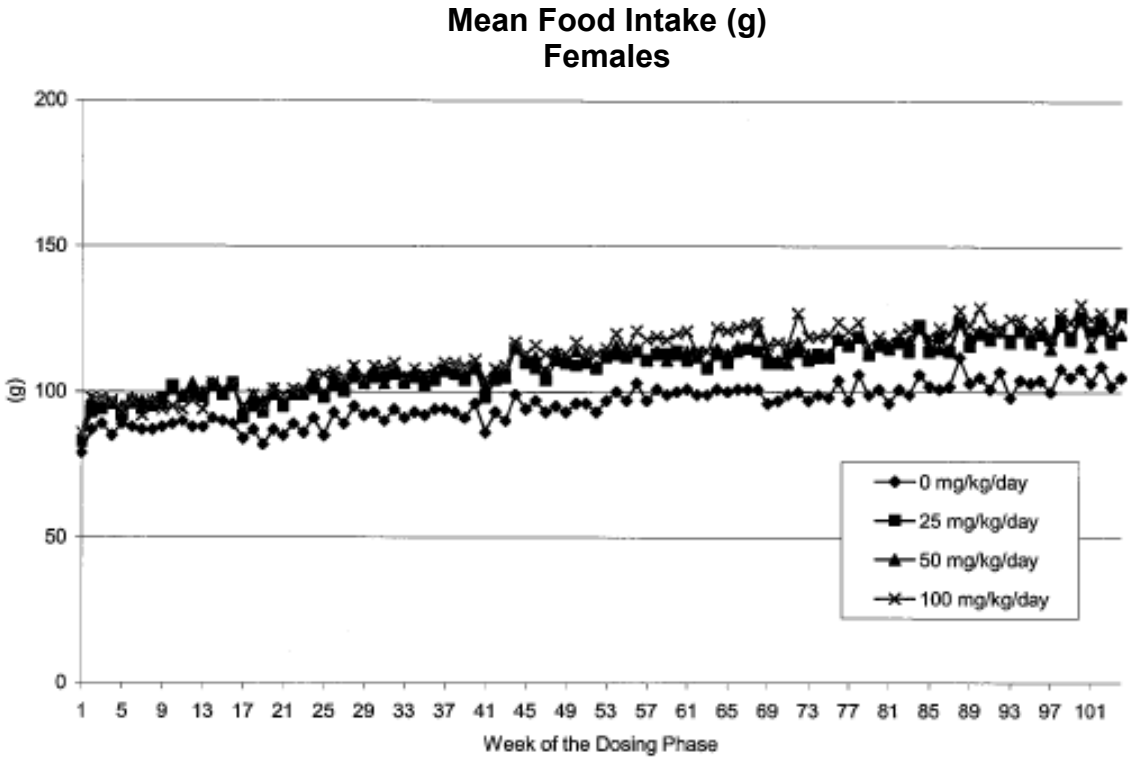

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Gross Pathology

Pathology data from animals surviving to scheduled necropsy and rats euthanized prematurely were combined (Table below). Gross pathology did not reveal excessively adverse pathologies related to mirabegron. The potential effects of mirabegron include a slight increase (2-4 animal increase compared to control) in the incidence of discolored thymus (M at ≥ 25 mg/kg), distended colon (M at 50 mg/kg, F at 100 mg/kg), discolored cecum (M at 50 mg/kg, F at 100 mg/kg), discolored eye (M at ≥ 25 mg/kg, F opposite effect), nodule in the prostate (at 12.5 and 50 mg/kg), uterus containing fluid (at ≥ 50 mg/kg), and discolored uterus (at 100 mg/kg).

Incidence of Adverse Pathology (All Animals to Term and Unscheduled Deaths Combined)

	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Multiple of MRHD (AUC) †	0	7	12	25	0	12	24	45
Thymus – Discolored	0	0	2	2	0	0	0	0
Colon – Distended	0	1	1	3	0	1	0	3
Cecum – Discolored	0	1	1	3	0	0	2	1
Eye – Discolored	3	4	9	7	6	4	4	1
Prostate – Nodule	0	3	1	2	NA	NA	NA	NA
Testes – Large	15	20	27	26	NA	NA	NA	NA
– Small	12	3	7	8	NA	NA	NA	NA
Epididymis - Discolored	0	2	1	4	NA	NA	NA	NA
Uterus – Contains Fluid	NA	NA	NA	NA	0	1	2	3
– Discolored	NA	NA	NA	NA	0	1	0	2
Bone – Fracture	1	0	1	0	0	1	1	0

Data from all animals (those surviving to week 104 and those that died or were euthanized prematurely) † Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072).

Histopathology

Peer Review: Yes, all tissues from 10% of the control and high dose group animals. Also all target tissues, neoplasms and lesions of interest from all animals were peer reviewed.

Neoplastic:

Toxicologically significant increases in neoplastic findings were not observed in the mirabegron groups (Table below). The FDA statistical reviewer did not find positive trend or an increase in neoplasms between the control and high-dose groups. The low-dose females had an increase in endometrial stromal polyps when the uterus and cervix data were combined (7/60 con vs. 13/60 LD). However, this was not significant in the mid- and high-dose groups or in either organ alone. The rate of endometrial stromal polyps combined from the uterus and cervix just slightly exceeds the range of the laboratory's historical control rate for the uterus alone (average 10%, range 7-21%). Although benign thyroid follicular cell adenomas were observed in the MD (3/60) and HD (2/60) males and HD females (1/59), the incidence was not statistically significant.

Incidence of Neoplastic Findings (Only Showing Neoplasms where Difference From Control > 2 in at Least One Sex) (Includes All Animals to Term and Animals Euthanized Prematurely)								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Multiple of MRHD (AUC) †	0	7	12	25	0	12	24	45
Survived to Week 104	28	35	39	39	43	37	36	24
Whole Body- malignant large granular cell leukemia	27	20	27	13	13	12	11	7
Liver- benign hepatocellular adenoma	3	2	3	0	-	-	-	-
Mammary Gland – benign fibroadenoma	-	-	-	-	3	6	5	3
– malignant carcinoma	-	-	-	-	0	0	0	1
– fibroadenoma + carcinoma	-	-	-	-	3	6	5	4
Pituitary – benign adenoma	30	**20***	**13****	**11****	30	16	23	11
– malignant carcinoma	-	-	-	-	0	1	0	0
– adenoma + carcinoma	30	**20***	**13****	**11****	30	**17***	23	**11***
Skin – benign fibroma	1	4	2	0	0	1	1	0
– malignant fibrosarcoma	2	4	0	1	0	0	1	0
– combined fibroma + fibrosarcoma	3	8	2	1	0	1	2	0
– benign squamous cell papilloma	0	0	0	2	2	0	0	0
– malignant sarcoma w/bone formation	-	-	-	-	0	0	0	2
Testes – benign interstitial cell tumor	50	55	52	55	NA	NA	NA	NA
Thyroid - C-cell adenoma – benign	12	7	8	7	8	3	4	4
– C-cell carcinoma – malignant	0	1	0	0	0	0	0	0
– C-cell adenoma + carcinoma	12	8	8	7	8	3	4	4
- Follicular Cell adenoma - benign	0	0	3	2	0	0	0	1
Uterus – benign adenoma	NA	NA	NA	NA	0	3	0	1
– malignant carcinoma	NA	NA	NA	NA	0	1	1	0
– adenoma + carcinoma	NA	NA	NA	NA	0	4	1	1
- benign endometrial stromal polyp	NA	NA	NA	NA	7	10	8	3
Uterus / Cervix – benign leiomyoma	NA	NA	NA	NA	0	0	2	1
– benign endometrial stromal polyp	NA	NA	NA	NA	7	**13***	9	3
– malignant endometrial stromal sarcoma	NA	NA	NA	NA	3	0	1	1
– endometrial stromal polyp + sarcoma	NA	NA	NA	NA	9 [A]	13	10	4

Summary of sponsor's Text Table 3-4 & Table 15. Sponsor indicated statistically different from control * $p < 0.05$, ** $p < 0.01$. † Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). Data from all animals (those surviving to week 104 and those that died or were euthanized prematurely) [A] – one control animal had both an endometrial stromal polyp and sarcoma so it was counted only once. For statistical purposes the sponsor counted the incidences certain neoplastic findings per animal instead of by tissue (granular cell leukemia, haemangiomas, lipoma, fibroma, fibrosarcoma, and osteosarcoma) and they also combined the incidences of endometrial stromal findings (polyps and sarcoma) and liver findings (hepatocellular adenoma and carcinoma) for each animal.

Non-Neoplastic

Adverse histopathology findings are listed in the table below. There was a dose related slight increase in the incidence of a number of findings in males which may be due to the increased survival of the mirabegron groups. Dose related increase in the incidence of lymphocyte/macrophage infiltration in the heart (M&F) and mesenteric lymph nodes (M&F), vessel mineralization of the heart (male) and lungs (M), mineralization of the eye (M) and renal findings including increased protein casts (M), and mineralization of the renal pelvis (M) and tubule (M) were observed. Findings uniquely elevated in females

include dose related slight to moderate multifocal hemorrhaging of the thymus in HD females and lymphocyte/macrophage infiltration of brown adipose tissue in all females. The thymic hemorrhaging is likely due to post-death trauma during necropsy and not drug related since it was only observed in animals in the high dose group that were found dead and not in rats surviving to scheduled necropsy or animals euthanized due to morbidity (18).

Incidence of Non-Neoplastic Histopathology (All Animals to Term and Unscheduled Deaths)								
	Male				Female			
Dose (mg/kg)	0	12.5	25	50	0	25	50	100
Multiple of MRHD (AUC) †	0	7	12	25	0	12	24	45
Survived to Week 104	28	35	39	39	43	37	36	24
Heart – lympho/macro infiltration	33	36	38	42	17	27	23	21
– vessel mineralization	2	4	6	13	1	2	3	0
Aorta - mineralization	36	45	45	46	31	31	29	21
Lung – vessel mineralization	8	18	20	22	9	11	11	7
Thymus - hemorrhage	0	0	0	1	3	2	5	19
Kidney – protein cast	4	4	7	8	10	8	5	6
– pelvis mineralization	11	19	26	21	28	37	34	28
– tubule mineralization	21	28	32	34	54	50	50	48
Stomach - mineralization	0	1	4	6	1	1	0	0
LN Mesenteric – pigmented. macrophage infil.	37	47	45	48	44	44	43	30
LN Mandibular- pigmented macrophage infil.	1	7	9	15	1	20	18	15
LN Mediastinal - pigmented	6	6	3	8	6	12	20	12
Eye – cornea mineralization	4	12	12	10	22	15	20	13
Bone – femur - thickened trabeculae	0	0	0	0	18	26	10	3
– sternum – thickened trabeculae	0	0	0	0	20	24	10	3

Summary of sponsor's Table 12. † Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072).

Thickening of the trabecular bone is a normal pathology in aged rats. The incidence of trabecular thickening in the sternum and femur declined dramatically in MD and HD females in a dose responsive manner. Premature deaths in the MD and HD groups could partially account for the reduction in this finding but it is not clear what else contributed to this finding or if high doses of mirabegron would affect bone strength. This is likely not a significant toxicity due to the high multiple of exposure and the lack of an effect of mirabegron on fracture incidence. However, there is very small body of nonclinical and conflicting clinical data suggesting that β1 and β2-AR antagonists may help to prevent fractures in osteoporotic patients and promote fracture healing (19 and 20). The role that a β3-AR agonist may play in bone is not well understood, however, one limited study suggests that β3-AR agonists may promote bone resorption (21). If mirabegron were to promote bone loss, it would likely have been observed in the standard histopathology screen in the carcinogenicity studies and chronic rat (26 weeks) and monkey (52 weeks) toxicity studies along with increased fracture incidences. This has not been observed.

Toxicokinetics

Rats were exposed to large multiples of the MRHD. Compared to exposure in humans at the maximum human dose, the multiples of exposure based upon AUC were 7, 12, and 25 in males and 12, 24, and 45 in females. Exposure increased proportionally with dose in both males and females. The half-life was 4-5 hrs in all groups except for the high-dose females where it was elevated to 7 hrs. There was no affect of sex on exposure.

Toxicokinetics at Week 52 of Dosing						
	Male			Female		
Dose (mg/kg)	12.5	25	50	25	50	100
Multiple of MRHD (AUC) †	6.9	12.0	25.4	11.6	24.0	45.0
$T_{1/2}$ (hr)	5.0	4.1	3.7	4.1	3.8	6.6
T_{max} (hr)	2	2	2	1	1	2
C_{max} (ng/mL)	991	1,070	2,190	1,133	1,953	3,770
AUC_{0-24} (ng-h/mL)	3,519	6,168	12,990	5,943	12,261	23,043
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072).						

Pharmacokinetic Summary in Humans and Rats						
Population	Duration	Dose	C_{max} (ng/mL)		AUC_{0-24} (ng-hr/mL)	
			Male	Female	Male	Female
Human Fasted, healthy, young 178-CL-031	Day 10	50 mg OCAS-M **100 mg OCAS-M** 200 mg OCAS-M 300 mg OCAS-M	33 72 220 381	46 **112** 264 530	262 519 1,443 2,473	368 **800** 2,046 3,888
Human Elder Female 178-CL-072 (n=11)	Day 7 Fasted	50 mg	43.5	**66.3**	341	**512**
Rats 178-TX-025 (26 Week Tox Study)	Week 26	3 mg/kg 10 mg/kg 30 mg/kg 100 mg/kg	23 160 719 2,856	36 161 665 2,313	110 1,098 8,542 28,372	113 854 5,994 30,071
Rats 178-TX-032 (Carcinogenicity Study)	Week 52	12.5 mg/kg 25 mg/kg 50 mg/kg 100 mg/kg	991 1,070 2,190	 1,133 1,953 3,770	3,519 6,168 12,990	 5,943 12,261 23,043
The maximal anticipated clinical dose is a single 50 mg dose.						

Dosing Solution Analysis

Homogeneity analysis was conducted on the first dosing solution from each new batch of drug (Weeks 0, 1, 27, 52, 79, and 104) prior to dosing. The overall mean values for samples from the top, middle and bottom of the dosing solutions were within 7% of the target concentration. One of the two replicate samples from the top of the 2.5 mg/ml solution (low-dose) was 126% of the desired concentration at week 79 but the other replicate was 102%.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

9.1.1 Fertility and Early Embryonic Development – Female Rats

Study title:	**Study of Fertility and Early Embryonic Development to Implantation in Rats Administered Orally with YM178**
Study no.:	178-TX-015
Study report location:	Module 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 28, 2000
GLP compliance:	Yes, according to (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, Purity 100.3%

Key Study Findings

- Mortality or morbidity of 3/20 dams at 300 mg/kg
- Reduced food consumption and decreased body weight before mating and during gestation at 300 mg/kg
- Prolonged diestrus (≥ 4 days) was noted in 10/18 dams prior to cohabitation at 300 mg/kg. Also a decrease in the mean number of corpora lutea was observed at 300 mg/kg. These findings are suggestive of adverse effects on the ovaries.
- Consequent to the decrease in the mean numbers of corpora lutea at 300 mg/kg, the number of implantations and live fetuses also decreased at 300 mg/kg.
- **NOAEL was 100 mg/kg** for female reproductive function and general toxicity in the pregnant rats.

Note: Impaired reproductive outcomes and estrus cycling may be secondary to effects on feeding and body weight.

Methods

Doses:	Only females dosed at 0, 30, 100, or 300 mg/kg. High dose was selected based 100% lethality/morbidity in a preliminarily 2-week toxicity study at 500 mg/kg with no deaths at 250 mg/kg.
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Sprague-Dawley (Crj:CD, SPF)
Number/Sex/Group:	20/sex/group

Study design:

Females were dosed daily for two weeks prior to cohabitation with males, during cohabitation and until day 7 of gestation (GD7). Cohabitation did not exceed two weeks. Females with successful copulation were subjected to cesarean section on GD13.

Parameters and endpoints evaluated: Clinical signs of the females were noted daily throughout the experiment and three times daily during the dosing period and prior to necropsy. Untreated males were monitored daily until they were excluded form the study. Vaginal smears were taken daily 9 days prior to dosing and until successful copulation. Body weight was measured prior to initiating of dosing during cohabitation, and on days 0, 1, 2, 3, 4, 5, 6, 7, 10, and 13 of gestation. Food consumption was determined at the same time points except during cohabitation. Dams were euthanized on GD13 and their abdominal cavities observed for lesions, and the ovaries and uteri were removed to note the number of corpora lutea and number of implantations. Both pre- and post-implantation losses were scored as well as copulatory and fertility indices. Necropsies of male rats whose partner were not pregnant were conducted to assess their reproductive organs, thoracic, and abdominal cavities.

Deviation from study protocol: None that effected study outcome. One male in the control group which had with visibly small testes and epididymides was replaced because of unsuccessful copulation.

Observations and Results

Mortality

Two females died prior to cohabitation at 300 mg/kg on day 2 and 19 of dosing. The female that died on day 2 of dosing displayed decreased locomotor activity, lacrimation, and soiled coat. The female that died after dosing on day 19 displayed decreased activity, prone position, and tremor prior to death. This rat also lost 68 g of weight from the fourth day of treatment to death. Another female at 300 mg/kg was euthanized due to moribundity at day 10 of treatment. Prior to being euthanized she displayed decreased activity, prone position, and tremor before becoming moribund. No abnormalities were observed in these three rats upon necropsy.

Clinical Signs
No signs were observed at ≤ 100 mg/kg. At 300 mg/kg all females had transient reduction in activity directly after dosing which became increasingly less prevalent after 10 days of dosing and was not observed throughout the last six days of gestation. Tremor was observed in one animal at 300 mg/kg after treatment on days 9, 10, and 16. Other signs in the high-dose group include soiled coat (4), lacrimation (2), and hypothermia (1) which were generally not observed during the gestational period. No signs were noted in males except a fractured tooth in one male that did not affect his behavior.

Body Weight
Body weight was reduced 8-15% at 300 mg/kg in the pre-copulation period from the 8th day of dosing and 9-12% until the end of the dosing period at gestation day 7.

Feed Consumption
Food consumption was reduced 45% after the initial 300 mg/kg dose. Although reduced food intake continued through out much of dosing phase during gestation, it was less drastic, and recovered after dosing cessation (GD 10 to GD 13). Feed intake increased at 100 mg/kg after 10-13 days of dosing and after dosing cessation (GD10-GD13). Elevated feed intake has been observed in repeat dose toxicity studies.

Food Consumption Prior to Copulation and During Gestation			
Dose (mg/kg)	**0**	**100**	**300**
Before copulation			
Day 3 of dosing	18.4 ± 2.2	17.0 ± 1.6	**10.2 ± 2.7****
Day 7 of dosing	19.5 ± 2.3	20.5 ± 2.1	**13.0 ± 3.8****
Day 10 of dosing	19.2 ± 2.3	21.5 ± 1.6**	**13.8 ± 4.4****
Day 13 of dosing	19.7 ± 2.8	23.2 ± 2.2**	**16.1 ± 4.6****
Gestation Day			
GD1	19.7 ± 2.9	21.9 ± 3.8	20.5 ± 6.0
GD2	24.3 ± 3.0	25.6 ± 3.5	23.4 ± 6.1
GD3	24.4 ± 3.5	25.6± 3.5	21.6 ± 4.4
GD4	24.6 ± 3.3	25.8 ± 2.7	22.4 ± 6.5
GD5	24.1 ± 3.8	24.5 ± 1.6	**19.5 ± 4.7***
GD6	25.3 ± 3.1	26.3 ± 2.9	**20.3 ± 6.2***
GD7	24.9 ± 3.2	27.0 ± 2.9	**19.7 ± 5.5****
GD10	25.7 ± 3.3	28.3 ± 3.5*	27.5 ± 2.6
GD13	26.5 ± 3.3	28.9 ± 3.5*	30.5 ± 2.4**
Statistically significant * P < 0.05, ** P < 0.01			

Toxicokinetics
Not assessed

Necropsy
Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

Three rats were removed from the study at 300 mg/kg because of mortality or morbidity. Of the 17 remaining rats at 300 mg/kg, two who had normal estrus cycles did not become pregnant. A **prolonged diestrus** (≥ 4 days) was noted in 10 of 18 rats prior to cohabitation at 300 mg/kg. Prolonged diestrus was not observed in other groups. The time to and number of successful copulations was the same in all treatment groups. The pregnancy rate (fertility index) was not statistically different among treatment groups. However, the **mean number of corpora lutea slightly decreased** at 300 mg/kg consequently resulting in a proportional decrease in number of implantations and live fetuses. Post-implantation loss was not different among groups.

Summary of Fertility Parameters (Mean Litter Value ± SD)				
Dose	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Number of Dams	20	20	20	17
Number of Pregnant Dams	20	20	20	15
Fertility Index	100%	100%	100%	**88%**
No. corpora lutea (mean ± SD)	18.7 ± 2.4	18.0 ± 1.9	18.7 ± 3.5	**15.0 ± 1.5 ****
No. implantations (mean ± SD)	17.2 ± 2.7	15.8 ± 3.1	16.3 ± 2.6	**13.7 ± 1.7 ****
No. Pre-implantation loss (mean ± SD)	8.4 ± 7.7	12.2 ± 15.3	11.7 ± 14.2	8.5 ± 6.2
Post-implantation loss				
Total (%, mean ± SD)	5.0 ± 6.0	8.6 ± 11.0	5.3 ± 7.3	6.6 ± 6.9
Early (%, mean ± SD)	1.7 ± 3.3	1.7 ± 3.7	0.3 ± 1.2	2.0 ± 4.7
Resorbed (%, mean ± SD)	3.2 ± 3.9	7.0 ± 10.9	5.0 ± 7.3	4.6 ± 6.8
Dead (%, mean ± SD)	0	0	0	0
No. live fetuses (mean ± SD)	15.3 ± 2.7	14.6 ± 3.6	15.5 ± 3.0	**12.9 ± 2.1 ****
Statistically significant * P < 0.05, ** P < 0.01. This table was adapted from sponsor's table 7.				

Dosing Solution Analysis
Although not located in the raw data, it was mentioned in description of the dosing solution that the dosing solutions were 98-103% of the target on the first and last day of dosing.

9.1.2 Fertility and Early Embryonic Development – Male Rats

Study title:	**Study of Fertility and Early Embryonic Development to Implantation in Male Rats Treated orally with YM178**
Study no.:	178-TX-039
Study report location:	Module 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 13, 2005
GLP compliance:	Yes, according to (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot GLP-K1780211, 99.4% pure

Key Study Findings

General Toxicity

- Exposures ranged from 34 to 171 times the exposures in adult men at MRHD.
- MTD exceeded at HD: 14 of 20 rats died at 300 mg/kg (171x MRHD) between days 3 and 14 of dosing after showing tremor and decreased movement.
- Adverse gross pathology including red discolorations of the lungs was only reported in the animals that died at 300 mg/kg.
- Male body weight gain was reduced in all mirabegron groups (14-49%) after 43 days.
- Male food consumption was reduced initially at all doses but all groups recovered by the 14th day of dosing.

Fertility

- No clear effect of mirabegron on male fertility or mating was observed at sublethal doses (exposures up to 77x MRHD).
- Questionable effects of mirabegron on male fertility outcomes were observed at the lethal dose (exposure 171x MRHD) which may be secondary to overt toxicity, reduced food intake, decreased weight gain, or low number of surviving animals.
  - All males copulated and there was no effect of treatment on the time to copulation
  - Pregnancy rate was only (50%) at 300 mg/kg (only 3/6 mating pairs became pregnant). However, the males with non-pregnant mates had motile sperm at necropsy and their mates had no visible implantation sites.
  - Slight decrease in the number of implantations and number of live fetuses at exposures 171x MRHD which could be due to the proportional reduction in corpora lutea in mated females and not clearly due to exposure of males to mirabegron.
- NOAEL for male fertility and embryonic development = 100 mg/kg (exposures up to 77x MRHD) based upon decreased male fertility at 300 mg/kg (171x MRHD).

Methods

Doses:	0, 30, 100, or 300 mg/kg
Frequency of dosing:	Males dosed daily for two weeks prior to mating through the mating period and continuing until females were in mid gestation (GD14) for a total of 42 days of dosing. Females were not dosed.
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous methylcellulose
Species/Strain:	Sprague Dawley SPF rats [Crl:CD(SD)]
Number/Sex/Group:	20/sex/group
Satellite groups:	Toxicokinetics assessed in 8 males/dose group and 4 for vehicle control. Blood samples were taken 1, 2, 4, 8, and 24 hrs after dosing. Sampling occurred after the first and last administration in addition to the pre-administration period and prior to the first mating.

Study design:

Mating: A single male and female were housed together. Vaginal plugs or the presence of sperm in the vaginal spears were indicated as the initiation of gestation (GD0). If mating was not successful, male sperm motility was assessed and the epididymis and testis were collected for histological analysis.

Necropsy: Males and females were necropsied at mid gestation (GD14). Internal and external macroscopic observations were conducted on males and females. The number of corpora lutea, number of implantations, number of live embryos, and the number of dead embryos were recorded. For females without visible implantation sites, the uterus was treated with NaOH to reveal implantation sites. Ovaries and uterus of non-pregnant females were stored for histological analysis.

Deviation from protocol: None that effected study outcome.

Observations and Results

Mortality

Fourteen of 20 male rats dosed at 300 mg/kg died between the third and the 14th day of dosing.

Clinical Signs

There were no adverse clinical signs observed in the untreated mated females.

In males, clinical signs were only observed at 300 mg/kg, and were predominantly observed during the first 14 days of dosing prior to mating. Reduced spontaneous movement became increasingly frequent with a maximum observed in 10/14 individuals on day 8 at 300 mg/kg. This was not observed after day 21 however only six animals in the 300 mg/kg group survived the 14 day treatment period prior to mating. Tremor was also observed at 300 mg/kg and the time course and frequency mirrored the reduced spontaneous movement with a maximal occurrence of 8/18 on day 6 with no incidences occurring after day 21.

Incidence of Adverse Clinical Signs in Male Rats				
Dose (mg/kg)	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Multiple of MRHD (AUC) †	0	34x	77x	171x
Number of animals (n=20)	20	20	20	20
Death				14
Decreased spontaneous movement				10
Tremor				8
Fractured incisors		1		
† Multiples of MRHD based upon AUC in fasted men 18-45 years old (AUC = 413 ng-hr/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072).				

Body Weight

Body weight gain was reduced at all doses. The minimal time for a significant reduction in weight was 39 days at 30 mg/kg, 25 days at 100 mg/kg and 4 days for 300 mg/kg. Once there was a significant reduction in weight it continued until test termination, day 43. The overall reduction in weight gain over the 43 day period was 14%, 32%, and 49% for the 30, 100, and 300 mg/kg groups respectively.

Female body weight was not affected during gestation.

Feed Consumption

Food consumption was only assessed during the 14-day pre-mating period. Food intake was initially reduced on day 2 at 30 and 100 mg/kg, but was elevated by day 8 compared to controls. At 300 mg/kg, food consumption was drastically reduced (44% to 71%) from day 2-11, but it recovered in the rats that survived to day 15.

Food Consumption						
Dose (mg/kg)		Day Post Administration				
		2	4	8	11	15
0	Survival	20	20	20	20	20
	g/rat ± SD	30.7 ± 3.7	32.2 ± 3.2	31.2 ± 2.4	31.2 ± 2.8	31.0 ± 2.3
30	Survival	20	20	20	20	20
	g/rat ± SD	**26.9 ± 3.2***	32.6 ± 3.9	**34.6 ± 3.3***	**34.8 ± 3.1***	32.9 ± 4.0
100	Survival	20	20	20	20	20
	g/rat ± SD	**22.2 ± 3.7***	**27.1 ± 7.0***	**34.9 ± 2.8***	**36.7 ± 2.6***	**36.9 ± 3.0***
300	Survival	20	**18**	**14**	**10**	6
	g/rat ± SD	**9.8 ± 5.2***	**9.8 ± 5.4***	**9.1 ± 8.0***	**17.5 ± 13.1***	36.5 ± 8.9
* Statistically different from the control group $p < 0.05$.						

Necropsy

Histopathology was not conducted. Adverse gross pathology was detected only in the rats that died at the high dose. Parts of the lungs were discolored red (14/20) and there were dark red foci in the glandular stomach of one of the dead males (1/14). No adverse gross pathology was reported in the dams.

Gross Pathology in Male Rats at Study Termination					
Dose	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	
Condition at Termination	Alive	Alive	Alive	Alive	Dead
Number	20	20	20	6	14
Lung Discolored dark red					6
Area, dark red					7
Focus, dark red					1
Glandular Stomach - Dark red foci					1

Fertility Parameters
There was no effect of treatment on fertility parameters at 30 or 100 mg/kg.

The days until copulation (2.3 to 2.8 days in all groups) and the incidence of copulating mating pairs (100% all groups) was not altered by mirabegron. However, only three of the surviving six rats at 300 mg/kg were able to impregnate their mate (fertility index = 50% vs. 95-100% for all other groups). Also one female at 30 mg/kg did not become pregnant. The males (1 at 30 mg/kg and 3 at 300 mg/kg) with non-pregnant mates had motile sperm at necropsy. The dams that did not become pregnant had no visible evidence of implantation sites. The reproductive organs (testes, epididymis, ovaries, and uterus) of the mating pairs that did not become pregnant were saved for histological evaluation but the results were not reported.

A slight decrease in the number of implantations and number of live fetuses was reported at 300 mg/kg. The reduced number of implantations and live fetuses could be due to the slight reduction in corpora lutea at 300 mg/kg which would not be due to exposure of males to mirabegron.

Summary of Fertility Parameters				
Dose	0 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Number of Mating Pairs	20	20	20	**6**
Number of Pregnant Dams	20	19	20	**3**
Fertility Index	100%	95%	100%	**50%***
No. corpora lutea (mean ± SD)	15.1 ± 1.5	15.2 ± 1.5	15.2 ± 1.7	**12.0 ± 4.0***
No. implantations (mean ± SD)	14.4 ± 1.6	14.1 ± 2.6	14.5 ± 1.8	**10.3 ± 4.7***
Pre-implantation loss (mean ± SD)[1]	4.3 ± 5.6	7.2 ± 14.4	4.3 ± 7.2	16.7± 19.1
Post-implantation loss				
Total (%, mean ± SD)[2]	7.0 ± 5.0	4.7 ± 4.1	6.2 ± 7.6	6.7 ± 11.5
No. Resorbed	17	9	13	1
No. Placental remnant	3	4	6	0
No. live fetuses (mean ± SD)	13.4 ± 1.7	13.4 ± 2.4	13.6 ± 1.7	**10.0 ± 5.3***

Values represent means per dam.
Mean values statistically significant compared to control at * $P < 0.05$.
[1] Pre-implantation loss index [(#corpora lutea- # implantations)/ (# corpora lutea)] x 100.
[2] Post-implantation loss (# dead embryos/# implantations).

Toxicokinetics
Male rats received 0, 34, 77, or 171 times the exposure in young men at the maximum anticipated clinical dose. Mirabegron was not detectable in the vehicle control samples. Tmax was reached between 1 and 8 hrs post-dosing.

Toxicokinetics in Male Rats During the Pre-mating Period				
Dose of mirabegron		30 mg/kg	100 mg/kg	300 mg/kg
Multiple of MRHD (AUC) †		34x	77x	171x
T_{max} (hr)	Day 1	1	8	8
	Day 15	4	8	ND
C_{max} (ng/ml)	Day 1	930	1,800	4,270
	Day 15	967	2,420	4,491
AUC_{0-24} (ng-h/mL)	Day 1	10,400	26,200	70,800
	Day 15	14,100	31,800	ND
† Multiples of MRHD based upon AUC in fasted men 18-45 years old (AUC = 413 ng-hr/mL) who received seven daily 50 mg doses of mirabegron (Study 178-CL-072). Multiple of exposure based on day 15 AUC data in rats except for the high dose where day 1 data was used. ND - not determined because only one rat at 300 mg/kg was assessed at day 15.				

Dosing Solution Analysis
Homogeneity of dosing solutions was assessed for first and last preparation. The upper, middle, and lower layers of all dosing solutions were within 99% to 102% of the target concentration.

Embryonic Fetal Development

9.2.1 Embryofetal Toxicity - Rats

Study title: **Effects of Orally Administered YM178 on Embryo-fetal Development in Rats**	
Study no.:	178-TX-022 (Toxicokinetics 178-TX-043)
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 26, 2000
GLP compliance:	Yes, (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, 100.3% pure

Key Study Findings

Maternal Findings
- Lethal at 300 mg/kg to 3/20 dams which displayed tremor, decreased movement, decreased respiratory rate and dark focal reddening in the lungs
- Body weight gain during the dosing period was reduced 74% in dams at 300 mg/kg

- Food intake was reduced in dams at 300 mg/kg (40-50%) from the second (GD8) to the fourth (GD12) days of dosing but recovered upon drug withdrawal.

Fetal Findings
- Both male and female fetal body weights were reduced (18-20%) at 300 mg/kg.
- Fetal Skeletal Findings Include:
  - Slight decrease in ossification of the metatarsi at ≥ 100 mg/kg
  - Slight decreases in ossification of the sternebrae and vertebrae at 300 mg/kg
  - Misshaped bend of the scapula, ulna, and radius at 300 mg/kg (14% litters)
  - Wavy ribs in 40% and 36% of the litters at 100 and 300 mg/kg respectively
    Wavy ribs were not reversible at 100 mg/kg when pups were allowed to live for four days after natural birth.

Maternal NOAEL = 100 mg/kg (22x MRHD) due to decreased weight gain, food intake and death at 300 mg/kg
Fetal NOAEL = 30 mg/kg (6x MRHD) due to ossification problems and misshapen bones at ≥ 100 mg/kg and reduced fetal weight at 300 mg/kg

Similar findings were observed in a dose range-finding study at the same doses (178-TX-005). Previous findings include maternal death (2/10) after morbidity and tremor at 300 mg/kg and fetal bone findings including wavy ribs at 100 mg/kg and bending of the scapula, humerus, ulna, and radius at 300 mg/kg and reduced ossification of the sternebrae, metatarsi, and sacral and caudal vertebrae at 300 mg/kg. One unique finding in study 178-TX-005 was maternal bilateral adrenal enlargement at 300 mg/kg in 1/10 dams. A follow up study, reviewed after this study, was conducted to determine if fetal bone findings resolve after birth (178-TX-023).

Methods

Doses:	0, 10, 30, 100, or 300 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous methylcellulose
Species/Strain:	Sprague-Dawley SPF rats (Crj:CD)
Number/Sex/Group:	20/pregnant females per group

Study design:
Pregnant rats were dosed daily from GD7 to GD17, the period from implantation to closure of the hard palate. Dams and fetus were euthanized at GD20. Maternal body weight, food intake, and clinical signs were assessed. Gross pathology of the thoracic and abdominal organs was conducted on the dam. Maternal parameters evaluated included the numbers of live and dead or resorbed fetuses, pre- and post-implantation losses, number of corpus lutea, and placenta weight. On GD20 fetuses were examined externally, weighed. Half of each litter was assessed for gross pathology at 0 and 300 mg/kg and a skeletal examination were conducted on the other half of each litter in all treatment groups.

Satellite groups:

Recovery- Additional 20 pregnant rats at 0 and 100 mg/kg were followed until day 21 of lactation to see if fetal wavy ribs were reversible. Dams were followed for birthing success, clinical signs body weight, and food consumption. Litters were culled to 4pups/sex/litter at postnatal day four. Pup weight was assessed throughout the postnatal period. On postnatal day 21, gross pathology was conducted on the dams and pups and the number of implantation sites was noted. Skeletal assessment was assessed on postnatal day four only and not on day 21 because the PND 21 tissues were not stored properly.

Toxicokinetics- Additional 7-8 pregnant rats per dosage group (4 per time point) were used for toxicokinetics at the initial dosing (GD7) and after the last dose (GD17).

Observations and Results

Mortality

Three dams in the 300 mg/kg group died. Two during the dosing period on GD9 and GD12, and another one day after dosing ceased on GD18.

Clinical Signs

No signs were observed in dams at ≤ 100 mg/kg. Generally only animals that died showed adverse clinical signs except a single incidence of tremor in one dam (GD12) and peri-genitourinary smudge in another dam (GD16-20) at 300 mg/kg. The animals that died on GD9 and GD12 displayed tremor, decreased movement, and oligopnea during the dosing period. The rat that died on GD18 exhibited signs including tremor, vaginal hemorrhage, and smudge of the peri-genitourinary area from GD15 until death. Upon necropsy all three of the dams that died displayed dark focal reddening in the lungs.

Body Weight

At 300 mg/kg, body weight was significantly reduced in dams from the third day of dosing (GD9) until necropsy resulting in a 74% reduction in weight gain during the dosing period. Body weight gain was reduced 14% during the dosing period at 100 mg/kg in the recovery group but this was recoverable and was not observed in the main group at this dose.

Feed Consumption

Food intake was reduced only at 300 mg/kg throughout the dosing period but only statistically reduced (40-50%) from the second (GD8) to the fourth (GD12) days of dosing. Food intake at 300 mg/kg recovered upon drug withdrawal even exceeding the control value two days after dosing ceased. Very slight increase in food intake was observed at 30 and 100 mg/kg from GD14 to GD20.

Toxicokinetics

Dams were exposed to broad multiples (1 to 96x) of the exposure at the maximum proposed dose for marketing. Mirabegron was absorbed readily with T_{max} values of 1 to 4 hrs. Exposure was fairly proportional to dose except from 10 to 30 mg/kg where it increased 7 times vs. only a three fold increase in dose. Mirabegron did not accumulate

with repeated dosing. Toxicokinetic findings were reported in a separate report (178-TX-043). In comparison to the 2-, 13- and 26-week toxicology studies, pregnancy did not affect exposure to mirabegron in rats.

Toxicokinetics					
Dose of Mirabegron		10 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg
Multiple of MRHD (AUC) †		0.9x	6.1x	21.5x	95.6x
T_{max} (hr)	GD7	4	4	1	2
	GD17	4	2	2	4
C_{max} (ng/mL)	GD7	74	503	1,682	3,366
	GD17	43	400	1,290	3,067
AUC_{0-24} (ng-h/mL)	GD7	636	5,911	20,214	48,532
	GD17	457	3,166	11,015	48,948
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). Animal AUC was from GD17.					

Necropsy
All three of the dams that died displayed dark focal reddening in the lungs. These were the only adverse pathology signs reported in any of the dams.

Cesarean Section Data
No effects were observed on the average number of corpora lutea, implantations, pre-implantation losses, number of resorbed fetuses, and average number of live fetuses or sex ratio. The placental weights were slightly increased in the mid-doses (13-17%) without adverse pathology, and there was no placental weight at 300 mg/kg.

Fetal Findings
Both male and female fetal body weights were reduced (18-20%) in the 300 mg/kg group. The only unique finding upon fetal visceral examination was a “variation” in the left umbilical artery in 2 fetuses from the same litter at 300 mg/kg. Flexure (misshaped bend) of the scapula (2/14 litters), ulna, and radius (2/14 litters) was reported at 300 mg/kg (one litter common for these findings). Wavy ribs were noted in 8% (8/20 litters) and 20% (5/14 litters) of the fetuses at 100 and 300 mg/kg respectively and single fetuses at 10 and 30 mg/kg. Very slight decreases in ossification of the sternebrae and vertebrae were also observed at 300 mg/kg, and slight decrease in the ossification of the metatarsi observed at ≥ 100 mg/kg.

The sponsor suggested that the effects of mirabegron on fetal bone development may be a pharmacologic effect of beta adrenergic signaling in rats and that they should resolve as the rat ages postnatally (22-24). Wavy ribs, defective mineralization in the paws, and bending of the long bones and scapula have been observed in fetal rats exposed to in utero to the β-agonists buphenin and doxaminol and these bone and mineralization findings were prevented with maternal exposure to the beta blocker carazolol (23).

Effect of Mirabegron on Fetal Bone Shape (Fetal Incidence/ % of Litters)					
Dose (mg/kg)	0	10	30	100	300
Multiple of MRHD (AUC) †	0	1	6	22	96
No. Fetuses Examined	154	158	158	169	111
No. Litters Examined	19	20	20	20	14
Fetuses with anomaly	0	0	0	0	**10 (21% litters)***
Fetuses with flexure of scapula	0	0	0	0	**9 (14% litters)***
Fetuses with flexure of ulna & radius	0	0	0	0	**5 (14% litters)***
Fetuses with flexure of tibia &fibula	0	0	0	0	4 (7% litter)
Fetuses with wavy ribs	0	1	1	**14 (40% litters)***	**19 (36% litters)***

† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072). Fetal incidence statistically different from control * $p < 0.05$.

Effect of Mirabegron on Progression of Fetal Ossification				
Dose (mg/kg)	0	30	100	300
No. Fetuses Examined	154	158	169	111
No. of ossified sternebrae (Mean ± SD)	5.91 ± 0.21	5.87 ± 0.19	5.84 ± 0.22	**5.13 ± 1.04***
No. of ossified metatarsi (Mean ± SD)				
Right	4.97 ± 0.07	4.90 ± 0.16	**4.80 ± 0.20***	**4.59 ± 0.41***
Left	4.98 ± 0.01	4.91 ± 0.14	**4.82 ± 0.18***	**4.54 ± 0.44***
No. of ossified Sacral & caudal vertebrae (Mean ± SD)	8.34 ± 0.50	8.41 ± 0.65	7.95 ± 0.51	**6.85 ± 1.20***

Statistically different from control * $p < 0.05$. Data from the 10 mg/kg group not shown and was not significantly different from control.

Postnatal Recovery Findings

To see if rats recover from the wavy ribs after in utero exposure to mirabegron, the offspring from 20 pregnant rats who were exposed in utero to mirabegron at 0 or 100 mg/kg from GD7 to GD17 were followed for 21 days after live birth. Pups were culled to 4/sex/litter where possible. Unfortunately, due to improper storage of the tissues in a potassium hydroxide storage solution, the bones of the pups degraded and the reversibility of wavy ribs was only analyzed 4 days after birth and not at 21 days as the protocol called for.

Effects on the Dams Postnatally: There were no clinical signs in the dams dosed at 100 mg/kg from the dosing period throughout the 21 day postnatal period. Mirabegron at 100 mg/kg was not associated with changes in number of live births, gestation period, number of implantations, number of still born, number of live births, or sex ratio. No gross pathology was observed in the dams at 0 or 100 mg/kg at postnatal day 21.

Effects on Pups 4 Days Postnatally: The weight of female pups was slightly greater (5-7%) at 100 mg/kg compared to controls for the first 17 postnatal days. The pups in both groups displayed no gross pathologies 21 days after birth. Fusion of the sternebrae (4% of pups) and wavy ribs (6% of pups) were significantly increased in pups 4 days after

birth at 100 mg/kg. Rat pups did not recover from the wavy rib malformation within four days of birth.

Effect of Mirabegron on Skeleton at Postnatal Day 4			
Dose (mg/kg)	0	100	
Individual pups / # of Litters	160/20	135/18	
Malformation	Incidence (%)	Incidence (%)	Litter Incidence
Fusion of sternebrae	0	**5 (4% ± 15%)***	**1 of 18 litters**
Wavy ribs	0	**8 (6% ± 12%)***	**5 of 18 litters**
Statistically different from control * $p < 0.05$.			

Dosing Solution Analysis
Homogeneity of dosing solutions was assessed before the fist dose and during the last week of dosing. The bottom, middle, and top fractions of each dosing solution were within 92% to 99% of the target concentration.

Study title: **Reversibility of Wavy Ribs of Fetuses Induced by YM178 Orally Administered to Rats**

Study Number: 178-TX-23
Study report location: Module 4.2.3.5.2.1
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: December 19, 2000
GLP compliance: Yes, according to [redacted] (b) (4) protocols
QA statement: Yes
Drug, lot #, and % purity: YM178 (mirabegron), Lot K02, 100.3% pure

Key Study Findings
In addition to previous studies with mirabegron (178-TX-005 and 178-TX-022), wavy ribs, reduced ossification, and misshaped bones were reported in fetuses exposed *in utero* to drugs including β-agonists (22-24). Wavy ribs have been reported to resolve after birth and withdrawal from beta adrenergic agonists. It has been speculated that wavy ribs and defective mineralization induced by beta agonists could be due to activation of osteoclasts, enhanced prostaglandin synthesis, and increased myometrial tone (23). To see if the skeletal findings are reversible after *in utero* exposure to 0 or 100 mg/kg of mirabegron (22x MRHD), the offspring from 20 pregnant rats were assessed for skeletal abnormalities at gestation day 20 and postnatal days 4 and 63. The dose chosen is the lowest dose where the adverse findings were previously reported. Significant findings include;

- Slight decrease in body weight of the dams during the dosing period and also from 11-17 days post-birth, whereas maternal food intake was slightly reduced only after the initial dosing.
- The placental weights at GD20 were significantly greater in the dosed group (24%).

- Wavy ribs were not noted in fetuses of the control group but were observed in fetuses from 6 of 19 dams (8% of the all fetuses: 32% of litters) along with reduced ossification of the sternebrae and metacarpi at 100 mg/kg. **Wavy ribs and reduced ossification appeared to resolve and were not observed in the control or mirabegron exposed pups evaluated at 4 and 63 days after birth.**

Methods

Doses:	0 or 100 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Rats, Sprague Dawley [Crj:CD(SD)]]
Number/Sex/Group:	20 pregnant dams/group for maternal necropsy at GD20 & another 20/group for postnatal study with necropsy at PND 21
Satellite groups:	None

Study design:

Pregnant rats were dosed daily from GD7 to GD17, the period from implantation to closure of the hard palate. One group of dams (n=20/group) and their fetuses were examined after cesarean section on GD 20. Pups from a second group of dams (n=20/group) were culled to 4/sex/litter four days after birth and weaned 21 days after birth (postnatal day 21, PND21). These pups were then allowed to develop until postnatal day 63. One control and 100 mg/kg mirabegron-treated rats were not impregnated, and were not included in analysis.

Dams: Clinical signs were noted 4 times before administration and 2 times within 2 hrs of dosing daily. Body weight of the dams was measured on gestation days 0, 4, 7-20 and on days 0, 4, 7, 11, 14, 17, and 21 post-delivery. Food consumption was measured on gestation days 1, 4, 7, 8, 10, 12, 14, 16, 18, and 20 and also on days 2, 4, 7, 11, 14, 17, and 21 post-delivery. Dams that had cesarean sections were euthanized at GD20 and examined macroscopically for thoracic and abdominal abnormalities. Ovaries and uteri were examined for numbers live and dead or resorbed fetuses. Placental weights were determined. Number of pre- and post-implantation losses was calculated. Dams in the lactation group were followed for birthing success and euthanized after weaning on PND 21.

Fetuses: Fetuses that were alive at termination on GD20 were sexed, examined externally, weighed, and examined for skeletal defects.

Pups: were weighed, sexed, and examined for abnormalities upon birth. Weight was recorded on 0, 4, 7, 11, 14, 17, 21, 28, 35, 42, 49, 56, and 63 days after birth. Pups in excess of 4/sex/litter were euthanized at 4 days post-birth and their skeletal abnormalities were noted. Additionally pups that were raised to 63 days post-birth were examined for wavy ribs.

Deviation from study protocol: None that significantly effected study findings

Observations and Results

Mortality (dams)
No deaths were reported.

Clinical Signs (dams)
No adverse clinical signs were reported during gestation or during the postnatal period.

Body Weight (dams)
There was a very slight decrease in body weight gain (6%) at 100 mg/kg from GD7 to GD18 but no statistical difference was noted on any single day of dosing. During the postnatal period, body weight was only reduced (3-6%) from postnatal days (PND) 11 to 17 and dams recovered by PND 21.

Feed Consumption (dams)
Feed intake was only reduced (24%) for one day (GD8) during the dosing period at 100 mg/kg and was not affected during the postnatal period.

Toxicokinetics
Not assessed

Necropsy
No gross pathologies were noted in dams on GD20 or PND 21.

Cesarean Section Data
No effects were observed on the average number of corpora lutea, implantations, pre-implantation losses, resorbed or dead fetuses, average number of live fetuses, or sex ratio. However, the placental weights were elevated 24% at 100 mg/kg.

Fetal Findings
Fetal body weights were not affected by mirabegron. Significant external malformations of the fetuses were not noted. Wavy ribs were noted in 8% of the fetuses from 6 of 19 dams at 100 mg/kg and were not observed in controls. Very slight decreases in ossification of the sternebrae and metacarpi were also observed at 100 mg/kg.

Effect of Mirabegron on Fetal Bone Development and Ossification (GD20)		
Dose (mg/kg)	0	100
Multiple of MRHD (AUC) †	0	22
No. Examined (Fetuses/Dams)	156/19	149/19
Wavy ribs, number of fetuses (% ± SD)	0	**12 (8 ± 13)***
Wavy ribs, incidence/litter	0	**6/19***
No. of ossified sternebrae (Mean ± SD)	5.50 ± 0.35	**4.81 ± 0.42***
No. of ossified metacarpi (Mean ± SD) - Right	3.81 ± 0.21	**3.36 ± 0.28***
- Left	3.78 ± 0.23	**3.36 ± 0.29***
† Multiple of exposure in fasted elderly women (AUC = 512 ng-h/mL) dosed at 50 mg mirabegron for 7 days (178-CL-072) assuming a maternal AUC of 11,015 ng-h/mL in rats (178-TX-022). Statistically different from control, * $p < 0.05$		

Postnatal Maternal Reproductive Findings: Although not statistically significant, the number of implantations and number of liveborn was reduced 7-8% in the mirabegron treated group. The number of still-born and sex ratio was not significantly different between groups. Mirabegron was not associated with changes in number of live births, gestation period, number implanted, number of still-born, number of live births, or sex ratio. No gross histopathology was observed in the dams at PND 21.

Effects on Pups (PND4 and PND63):
No statistically significant defects in the skeleton were observed in pups of the control or mirabegron treated dams at 4 or 63 days after birth. Wavy ribs were not observed in pups of control or mirabegron treated dams 63 days after birth. Body weight was not altered in pups after birth. No gross pathologies were noted in control or mirabegron exposed pups at birth or 63 days after birth.

Dosing Solution Analysis
Homogeneity of dosing solutions was assessed before the fist dose and during the last week of dosing. The bottom, middle, and top fractions of each dosing solution were within 94% to 100% of the target concentration.

9.2.2 Embryofetal Toxicity – Female Rabbits

Study title: **A study for Effects on Embryo-fetal Development in Rabbits Treated Orally with YM178**	
Study no.:	178-TX-016
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Mating initiated August 14, 2000
GLP compliance:	Yes, (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, 100.3% pure

Key study findings:
Effects of mirabegron on pregnant rabbits and fetal development were examined after dosing from implantation to closure of the hard palate. Dams were treated with 0, 3, 10, or 30 mg/kg/day (0.7, 14, or 36x MRHD) from GD6 to GD20. Significant findings include;

- Maternal body weight gain and food consumption were reduced at 30 mg/kg.
- A significant decrease (10-14%) in mean fetal weight was observed both sexes at ≥ 10 mg/kg.
- **Dilated aorta in** 75% of litters (24% of fetuses) at 30 mg/kg with none in the control

- **Cardiomegaly** in 13% of litters (3% of fetuses) at 30 mg/kg with none in control
- Post-implantation losses increased at 30 mg/kg which were primarily accounted for by a significant increase in dead fetuses.
- Adverse effects on ossification include fusion of the sternebrae in 56% of the litters at 30 mg/kg in comparison to 10% of the control litters and slight decrease in ossification of the fingers and toes in 44-63% of the litters at 30 mg/kg.

NOAEL for fetuses = 3 mg/kg/day (0.7x MRHD) decreased fetal weight at ≥ 10 mg/kg
NOAEL for dams = 10 mg/kg/day (14x MRHD) decreased food intake, decreased body weight gain, and death at 30 mg/kg.

Methods

Doses:	0, 3, 10, or 30 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	5 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous methylcellulose vehicle
Species/Strain:	New Zealand White rabbits [Kbl:NZW(SPF)]
Number/Sex/Group:	22/females/group. (17 to 22 per group were pregnant)
Satellite groups:	5 females per group (3, 10, or 30 mg/kg/day) for toxicokinetics
Study design:	Pregnant rabbits were dosed daily from GD6 to GD20. Fetuses were allowed to develop until GD29 when the does and fetus were euthanized.
Parameters and endpoints:	Adults: Mortality, clinical signs, body weight, food intake. Necropsy was preformed on GD29. Ovaries and uterus were weighted on GD29. Number of corpora lutea, implantations, resorptions, live or dead fetuses and placental weights were assessed. Toxicokinetics were evaluated on GD6 and GD20, the first and last days of dosing. Offspring: Sex, fetal weights, external abnormalities and visceral defects were assessed. One half of the fetal heads were examined. Skeletal defects were assessed.
Deviation from study protocol:	None reported

Observations and Results

The doses were chosen based on a range finding study in non-pregnant rabbits where mirabegron was 100% lethal after single doses ≥ 250 mg/kg and 1/3 rabbits died after a single 100 mg/kg dose. Prodrome to death included hyperpnea, tremor, dyspnea, and tonic convulsions. Marked reduction in food consumption was observed at 60 mg/kg after 5 days of dosing. Also in a preliminary study with pregnant rabbits, decreased food intake, and weight gain was observed at 10 mg/kg and 30 mg/kg respectively

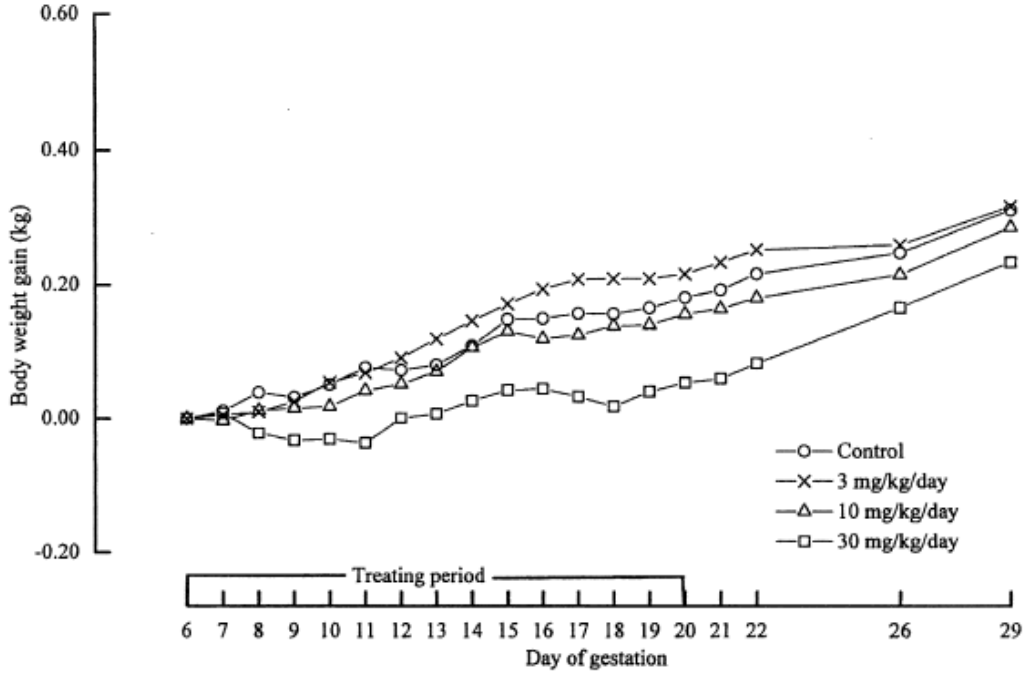
without obviously affecting the pregnancy outcomes (178-TX-004). Because of these findings the high dose was set at 30 mg/kg.

Mortality
One dam at 10 mg/kg died due to gavage error on GD14. One dam at 30 mg/kg died of apparent gavage error on GD16 approximately 30 minutes after dosing. She displayed lateral position and dyspnea prior to death and showed red patches in the lungs. In the toxicokinetic group, one animal died one hour after the last dose on GD20 and another was euthanized due to abortion on GD20.

Clinical Signs
No adverse effects were observed at 3 or 10 mg/kg. At 30 mg/kg, bloody discharge from the vagina was observed in two animals before (GD4) and after (GD 29) the dosing period.

Body Weight
Body weight gain, but not mean total weight, was reduced at 30 mg/kg from the second day of dosing (GD8) until two days after dosing ceased (GD22). It recovered thereafter.

Group Mean Body Weight Gain



Copy of Sponsor's Figure 2

Feed Consumption
Food intake was decreased roughly 24% to 44% at 30 mg/kg throughout the dosing period from GD7 to GD20. It recovered thereafter. A slight but not statistically significant decrease in food consumption occurred at 10 mg/kg during the dosing period as well.

Necropsy
One animal in the 30 mg/kg group had a discolored liver. A pale change in the kidney was noted in one animal at 30 mg/kg as well.

Fertility Parameters:
A significant decrease (10-14%) in mean fetal weight was observed in males and females at ≥ 10 mg/kg. The number of implantations, pre-implantation losses, and live fetuses were similar in all groups. However, the number of post-implantation losses increased at 30 mg/kg accounting for 20% of the total number of implantations compared to 4% in controls. At least one post-implantation loss was reported in 75% (12/16) of the litters at 30 mg/kg and 5 of 16 does had multiple losses vs. only 4% of the control litters. These losses were accounted for primarily with a significant increase in dead fetuses. Fetal death increased from 1% in the controls to nearly 11% at 30 mg/kg. Likewise the number of litters with a fetal death increased from 5% in controls to 56% at 30 mg/kg. Placental weight was not affected. Uterine weight was not measured.

Although data was not provided, the sponsor indicated that similar findings were seen in the satellite group used for toxicokinetics where the post-implantation loss was 17% at 30 mg/kg. One animal in the toxicokinetic group at 30 mg/kg aborted prior to dosing on GD20 and another died one hour after the last dose on GD20.

Significant Observations upon Cesarean Section					
Dose (mg/kg)		0	3	10	30
No. of Animals		20	22	18	16
Fetal Weight, mean (g)	Male	46.5 ± 7.4	45.2 ± 4.5	**42.1 ± 4.8***	**39.9 ± 4.3****
	Female	45.2 ± 5.8	44.1 ± 3.7	**39.7 ± 6.2****	**39.3 ± 6.0****
Corpora lutea (mean)		9.2	9.8	10.4	9.8
Implantations (mean)		7.9	8.3	9.3	8.6
Live Fetuses (mean)		7.6	8.0	8.5	6.9
Pre-Implantation Losses, No. (%)		25 (14%)	33 (15%)	21 (11%)	20 (13%)
Post-Implantation Losses					
Total No. (%)		7 (4.4%)	8 (4.4%)	14 (8.4%)	**27** (19.7%)**
Litters w/ loss (%)		5 (25%)	4 (18%)	7 (39%)	**12 (75%)**
Early Implantation sites, No (%)		1 (0.6%)	0	4 (2.4%)	1 (0.7%)
Resorptions, No. (%)		5 (3.2%)	4 (2.2%)	3 (1.8%)	11 (8.0%)
Dead fetuses, No. (%)		1 (0.6%)	4 (2.2%)	7 (4.2%)	**15 (10.9%)****
Litters w/ dead fetuses, No. (%)		1 (5%)	1 (5%)	4 (22%)	**9 (56%)**
Statistically different from control,* $p < 0.05$, ** $p < 0.01$.					

Offspring: No external anomalies were noted. There was a significant increase in adverse fetal viscera malformations at 30 mg/kg primarily in the lung and heart. An absence of an accessory lobe in the lung was elevated at 30 mg/kg (38% of litters/8% fetuses) in comparison the control (15% of litters/2% fetuses). More strikingly, dilated aortas were observed in 75% of litters (24% of the fetuses) at 30 mg/kg without being observed in the control group. This was reported in at least one fetus in 12 of 16 litters and multiple fetuses in 6 of the 16 litters. The sponsor stated that this was considered to be attributable to the pharmacology of mirabegron. Very low incidences of other cardiovascular effects were observed only at 30 mg/kg including cardiomegaly (13%

litters/ 3% fetuses), membranous ventricular septum, overriding aorta, and narrowed pulmonary trunk at 30 mg/kg.

Significant Observations in Fetal Viscera Incidence (%)								
Dose (mg/kg)	0		3		10		30	
Incidence per fetus or litter	Fetus	Litter	Fetus	Litter	Fetus	Litter	Fetus	Litter
No. of Examined	151	20	175	22	153	18	115	16
Total No. Visceral Defects	3 (2)	3 (15)	6 (3)	6 (27)	8 (5)	5 (28)	**32 (28) ****	**13 (81)**
Absent lung accessory lobe	3 (2)	3 (15)	3 (2)	3 (14)	8 (5)	5 (28)	**9 (8) ****	**6 (38)**
Dilated Aorta	0	0	0	0	0	0	**27 (24) ****	**12 (75)**
Cardiomegaly	0	0	0	0	0	0	3 (3)	2 (13)
Membranous ventricular septum	0	0	0	0	0	0	1 (1)	1 (6)
Overriding aorta	0	0	0	0	0	0	1 (1)	1 (6)
Narrowed pulmonary trunk	0	0	0	0	0	0	1 (1)	1 (6)
Statistically different from control, * $p < 0.05$, ** $p < 0.01$								

The sternebrae were fused in 56% of the litters (14% of fetuses) at 30 mg/kg in comparison to 10% of the litters in the control group. The historical incidence of fused sternebrae was not reported. Dose related effects on the ribs and vertebrae were not observed. A slight delay in ossification (indicated by the number of bones) of the middle phalanges and metacarpi was observed at 30 mg/kg in 44% to 63% of the litters. Various other developmental effects on the skeletal system occurred in less than 1% of the fetuses.

Significant Fetal Skeletal Defects/Variations			
Dose (mg/kg)	0	10	30
No. of Fetuses Examined	151	153	115
No. of Does	20	18	16
Total No. Defects	9 (6%)	9 (6%)	**24 (21%) ****
Sternebrae – Fused	2 (1.3%) [2/20 litters]	5 (3.3%) [4/18 litters]	**16 (14%) ** [9/16 litters]**
- Misshapen	1 (0.7%)	0	0
Total Number of Variations	51 (34%)	48 (31%)	42 (37%)
Number of bone in manus			
- distal phalanges	10	10	10
- middle phalanges	8	8	**7.5** [10/16 litters]**
- proximal phalanges	10	10	10
- metacarpi	10	9.8	**9.5 ** [10/16 litters]**
Number of bones in hind foot			
- distal phalanges	8	8	8
- middle phalanges	8	8	**7.7** [7/16 litters]**
- proximal phalanges	8	8	8
- metatarsi	8	8	8
- tarsi	4	4	3.9
Significantly different from control ** $p < 0.01$. Data for the 3 mg/kg group was not provided.			

Toxicokinetics

Mirabegron was absorbed rapidly with T_{max} values within 2 hrs of dosing. Exposure to mirabegron increases greater than proportional increase in dose from 3 to 10 mg/kg. C_{max} and AUC values increased roughly 15 fold. However, a less than proportional

increase in AUC occurred from 10 to 30 mg/kg. Mirabegron did not appear to accumulate with repeated dosing.

The variability was very large between animals with the levels being as large as the standard deviation. It can not be determined if fetuses with dilated aortas or cardiomegaly were from does with higher than average exposures because the toxicokinetic data was acquired from satellite animals.

Toxicokinetic Values ± SD				
Dose of Mirabegron		3 mg/kg	10 mg/kg	30 mg/kg
Multiple MRHD (AUC) †		0.7	14.1	35.7
	N	5	4	5
T_{max} (hr)	GD6	1	1	2.4
	GD20	1	1	1
C_{max} (ng/ml)	GD6	165 ± 218	3,124 ± 1,949	3,872 ± 4,166
	GD20	244 ± 400	2,753 ± 1,614	5,938 ± 7,071
AUC_{0-24} (ng-h/ml)	GD6	452 ± 537	9,390 ± 5,672	16,871 ± 18,809
	GD20	364 ± 454	7,230 ± 4,434	18,277 ± 25,470
† Multiple of exposure in elderly fasted women administered 50 mg of mirabegron for seven days (AUC = 512 ng-h/mL) (178-CL-072). Multiple of MRHD based on animal AUC from GD20.				

Dosing Solution Analysis
Dosing solution was assessed for homogeneity and concentration prior to the first and last dose. The mean of the lower, middle, and upper layers of all three dosing solutions were within 93.8 to 102.5% of the target concentration. Homogeneity between the layers was within a 5% confidence interval.

Study title: **Plasma Concentrations of YM178 and its Metabolites after a Multiple Oral Administration of YM178 during Non-Pregnant and Pregnant Periods in Female Rabbits**

Background: The effect of pregnancy on toxicokinetics was investigated because exposure to mirabegron was roughly six fold lower in non-pregnant rabbits (178-ME-097) in comparison to pregnant rabbits in an embryo-fetal toxicity study (178-TX-016) despite administration of similar oral doses. Additionally, two dedicated toxicokinetic studies in non-pregnant rabbits (178-ME-103 and 178-ME-126) found the exposure to be 3 to 16 fold lower than in the pregnant rabbits despite being dosed similarly. The sponsor suggested that the high exposure data in pregnant rabbits may be due to high animal to animal variability. In any event, this appears to be a species specific effect since pregnancy in rats did not appear to have an effect on mirabegron exposure (studies 178-TX-022 vs. 178-ME-077, and 178-ME-125).

Toxicokinetics of Mirabegron Pregnant/Non-Pregnant Rabbits									
	Pregnant Rabbits				Non-Pregnant Rabbits				
	178-TX-016 (Gestation Day 6 and 20)				178-ME-103 (Dosing Day 15)			178-ME-126 (Dosing Day 15)	
Dose of mirabegron		3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	3 mg/kg	30 mg/kg
Multiple of Human Exposure (AUC) †	Day	0.7	14.1	35.7	0.3	0.9	6.3	0.3	9.1
C_{max} (ng/ml)	GD6 GD20	165 244	3,124 2,753	3,872 5,938	58	189	958	46	1,281
AUC_{0-24} (ng-h/ml)	GD6 GD20	452 364	9,390 7,230	16,871 18,277	160	445	3,220	146	4,643
† Multiple of exposure in fasted elderly females receiving 50 mg mirabegron assuming AUC = 512 ng-h/mL (178-CL-072). MOE determined from data on GD20.									

To address the effect of pregnancy in rabbits on the exposure to the mirabegron and 7 metabolites, the sponsor conducted this toxicokinetic study at steady state in non-pregnant and pregnant rabbits dosed orally at 3, 10, and 30 mg/kg/day. These doses are the same doses used in the embryo/fetal developmental toxicity study in rabbits. Males were not treated. Toxicokinetics were assessed in non-pregnant rabbits after seven days of daily dosing. Then after an eight day washout period the same rabbits were mated and toxicokinetics were assessed again after the first (GD6) and last dose (GD20).

Study no.: 178-TX-056
Study report location: Module 4.2.3.5.1
Conducting laboratory and location: (b) (4)
Date of study initiation: First Dose April 2, 2010
GLP compliance: Yes, (b) (4) Guidelines
QA statement: Yes
Drug, lot #, and % purity: YM178 (mirabegron), Lot GLP-K1780211, 100.2% Pure

Key Study Findings

- Study confirmed exposures to mirabegron during pregnancy increased 1.5-2 fold
- All metabolites should be considered qualified or covered by other nonclinical studies
- Previous toxicokinetic studies in non-pregnant rabbits may have underestimated the exposure to mirabegron in non-pregnant rabbits

Methods

Doses: 3, 10, or 30 mg/kg/day
Frequency of dosing: Non-pregnant rabbits – 7 consecutive days
Pregnant rabbits- daily from GD6 to GD20
Dose volume: 5 mL/kg
Route of administration: Oral gavage

Formulation/Vehicle: 0.5% Methylcellulose in water
Species/Strain: Rabbits (Kbl:NZW) / Females 15 weeks old at first dose
6/females/group
Study design: Non-pregnant females dosed for seven days at 3, 10, or 30 mg/kg and pregnant rabbits dosed from GD6 to GD20. Toxicokinetics of parent and seven metabolites assessed on days 1 and 7 in non-pregnant rabbits and on GD6 and GD20. Fertility endpoints evaluated but assessment of fetuses was not conducted.
Deviation from study protocol: No major deviations.

Observations and Results

Mortality
No deaths reported.

Clinical Signs
No adverse clinical signs were reported in either phase of the study.

Body Weight
No effect of dosing on body weight during the non-pregnant or pregnant periods

Feed Consumption
A slight reduction in feed intake was observed in the high dose group from days 4-7 in non-pregnant rabbits but the variability was so large between animals that it is was not clearly treatment related. Feed intake was not affected by dosing during the pregnancy phase.

Gross Pathology
No adverse pathology was reported when dams were euthanized on GD20.

Fertility Parameters
There was no effect of treatment on the number of corpora lutea, number of implantations, number of live fetuses, or the number of embryofetal deaths.

Fertility Parameters			
N = 6/group	3 mg/kg	10 mg/kg	30 mg/kg
Aborted or Total Resorptions	0	0	0
Mean No. Corpora Lutea	8.5	9.3	9.0
Mean No. Implantations	8.3	8.8	8.2
Mean No. Live Fetuses	8.3	8.7	8.0
Mean No. Embryofetal Deaths	0.2	0.2	0.2

Toxicokinetics
Toxicokinetics were assessed 1, 2, 4, 8, and 24 hours after dosing. Non-pregnant animals were evaluated after the first dose on day 1 and after the last dose on day 7.

Exposure in pregnant rabbits was evaluated after the first (GD6) and last dose (GD20). The doses (3, 10, and 30 mg/kg) are same doses used in the embryo/fetal developmental toxicity study in rabbits.

This study confirmed the toxicokinetic findings in previous studies in pregnant rabbits with the exception that exposure in the mid-dose group was reduced in this study (178-TX-056) compared to that in the previous study (178-TX-016). This study (178-TX-056) revealed that exposure in non-pregnant rabbits was elevated roughly two to three fold compared to previous studies in non-pregnant rabbits (178-ME-103, 178-ME-126).

In this study, exposure to mirabegron and the metabolites was generally twice as high in the pregnant rabbits compared to the non-pregnant rabbits. The ratio of mirabegron exposure (AUC) in pregnant rabbits on GD20 to non-pregnant rabbits on day 7 was 2.0, 1.5, and 1.7 in the low to high dose groups. Exposure (AUC) increased greater than the proportional increase in dose in pregnant and non-pregnant rabbits. Also, exposure increased slightly (< 2x) with repeated dosing in non-pregnant and pregnant rabbits. Tmax for mirabegron was 1 to 1.2 hrs and 1 to 2.7 hrs for all metabolites for all groups independent of dose, number of exposures, or pregnancy state.

Toxicokinetics of Mirabegron in Pregnant and Non-Pregnant Rabbits											
	Pregnant Rabbits							Non-Pregnant Rabbits			
	178-TX-016 (Gestation Day 6 and 20)				178-TX-056 (Gestation Day 6 and 20)			178-TX-056 (Day 1 and 7 of Dosing)			
Dose		**3 mg/kg**	**10 mg/kg**	**30 mg/kg**	**3 mg/kg**	**10 mg/kg**	**30 mg/kg**		**3 mg/kg**	**10 mg/kg**	**30 mg/kg**
MOE (AUC) †		**0.7**	**14.1**	**35.7**	**0.5**	**3.6**	**36.0**		**0.3**	**2.4**	**21.4**
C_{max} (ng/ml)	GD6	165	3,124	3,872	63	652	4,149	Day1	43	321	2,459
	GD20	244	2,753	5,938	83	673	6,003	Day7	46	560	4,398
AUC_{0-24} (ng-h/ml)	GD6	452	9,390	16,871	175	1,600	13,001	Day1	118	831	8,958
	GD20	364	7,230	18,277	260	1,863	18,426	Day	127	1,239	10,967

† Multiple of exposure in for fasted elderly females at 50 mg mirabegron dose assuming AUC = 512 ng-h/mL (178-CL-072). MOE determined from data on GD20 or dosing day 7.

The metabolite profile was similar in pregnant and non-pregnant rabbits (Table below). The acetyl conjugate, M5, and the phase one metabolite, M16, were the principal metabolites in rabbits. Exposure to M5 was similar to or twice as high as the parent, while M16 was detected at levels similar to the parent except at the high dose were it was roughly 1/3 as high as the parent. Exposures to the glucuronide metabolites (M11 to M15) were much less than the parent.

There are two major metabolite in humans (M11 and M12) which comprise slightly more than 10% of the circulating drug related material in men or women, and one (M5) that is just slightly less than 10% of the drug related material. Exposure to M11 in pregnant rabbits in the mid- and high-dose groups exceeded that in humans. Exposure to M5 in rabbits, independent of pregnancy state and dose group, exceeded that in humans by at least four fold. Exposure to M12 at the high dose in pregnant rabbits was significantly

less than that in humans. However, since M12 is a glucuronide conjugate, is pharmacologically inactive in in vitro studies, and the exposure in rats in the fertility, embryofetal, and peri-postnatal toxicity studies met or exceeded that in humans, there is no strong rational for conducting additional toxicology studies on this metabolite alone.

Exposure (AUC_{0-24}) to Mirabegron and Metabolites in Humans and Pregnant/Non-pregnant Rabbits

			Human	Rabbit Non-pregnant Day 15 178-ME-103 († 178-ME-126)			Rabbit Non-pregnant Day 7 178-TX-056			Rabbit Pregnant GD20 178-TX-056		
			50 mg	3	10	30	3	10	30	3	10	30
Mirabegron		M	341	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	512	160	445	3,220	127	1,239	10,967	260	1,863	18,426
M5	Phase 2 Acetyl conjugate	M	80	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	112	399	3,974	23,556	410	2,511	16,052	698	3,984	19,518
M8	Phase 1	M	**49***	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	**104**	3	10	89	ND	ND	ND	ND	ND	ND
M11	Phase 2 Glucuronide	M	150	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	201	41	92	838	11	135	786	30	258	1,835
M12	Phase 2 Glucuronide	M	83	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	99	0	0	0	0	0	2.7	0	0.5	5.5
M13	Phase 2 Glucuronide	M	10	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	13	10	19	199	3.8	40	315	12	95	703
M14	Phase 2 Glucuronide	M	69	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	75	60	125	890	14	187	993	57	341	2,077
M15	Phase 2 Glucuronide	M	31	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	49	0	2.0	13.1	0	14	69	0.3	11	97
†M16	Phase 1	M	42	ND	ND	ND	ND	ND	ND	ND	ND	ND
		F	70	418	ND	10,048	143	1,157	4,871	247	1,658	6,218

Data was derived on day 15 of exposure rabbits (178-ME-103) and day 7 of dosing in fasted men and women ≥ 55 yrs (178-CL-072). ND – not determined. † The data for M16 was derived from separate two week exposure studies (178-ME-126) because the methods needed to be revised from study 178-ME-103. *M8 data in humans only available from the 100 mg dose. See PT review 7 3-29-11.

Dosing Solution Analysis

Samples from the top, middle, and bottom of the dosing solution vials for each dose were within 96.9 to 100.3% of the target on the first day of dosing. Dosing solutions were between 102.2% and 102.8% of the target on gestation day 20.

9.2.3 Mechanistic Evaluation of Maternal Heart Rate and Fetal Cardiotoxicities in Rabbits

Studies were conducted in rabbits to see if the adverse maternal cardiac affects and development affects were in part due to off target β1-AR activation.

Study title:	**Investigational Study for Effects of the Oral Administration of YM178 on Embryo-fetal Development in Rabbits: Effects of a beta 1-Blocker**
Study no.:	178-TX-047
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	Drug Safety Research Laboratories, Astellas Pharma Inc., Osaka, Japan
Date of study initiation:	April 13, 2007. First dose May 20, 2007
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	YM178 (mirabegron), Lot GLP-K1780211, 99.4% pure Metoprolol (β1 blocker), Sigma Lot 35K1557

Background

Pregnant rabbits were dosed with vehicle alone, mirabegron (30 mg/kg), or mirabegron (30 mg/kg) + the β1 antagonist metoprolol (3 mg/kg) once daily from GD6 to GD20 and the fetuses were examined on GD29 for adverse cardiovascular toxicity. Antagonizing the β1 receptor with metoprolol may have transiently reduced the elevated heart rate in does caused by mirabegron and also reduced the incidence of abnormal cardiac development. However, the data did not demonstrate that the adverse developmental affects are solely mediated by β1 receptor activation.

Key Study Findings

- At roughly 36 times the clinical exposure at the MRHD, mirabegron elevated the maternal heart rate (12-15%) for 4 to 8 hrs after dosing but they recovered by 24 hrs. Metoprolol transiently repressed the mirabegron dependent elevation in heart rate (7-13% reduction from baseline) from 1 to 4 hrs post-dosing. The affect of metoprolol on heart rate was transient and was not evident by 8 hrs post-dosing.
- Mirabegron treatment resulted in 100% of litters (51/71 fetuses) with dilated aortas and 55% of the litters (16/71 fetuses) with cardiomegaly. The incidence of dilated aorta and cardiomegaly was reduced to 80% of litters (21/72 fetuses) and 20% of litters (2/72 fetuses), respectively, with the co-treatment of mirabegron and metoprolol. Metoprolol was not 100% effective in inhibiting developmental cardiovascular toxicities of mirabegron. This may be because metoprolol has a short half-life and does not effectively reduce the mirabegron mediated increase in heart rate 8 hours after dosing and/or because these fetal toxicities may be mediated by mechanisms other then β1 activation.
- This study suggests that the developmental cardiotoxicities in rabbits may be mediated at least in part by activation of the β1-AR.

Methods

Doses:	0 or 30 mg mirabegron ± 5 mg metoprolol. No metoprolol alone group.
Frequency of dosing:	Daily, GD6 to GD20
Dose volume:	10 mL/kg total volume
Route of administration:	Oral gavage
Formulation/Vehicle:	Aqueous 0.5% methylcellulose
Species/Strain:	Kbl:New Zealand White rabbits (SPF), same as in study 178-TX-016
Number/Sex/Group:	5 control and 10 per treatment group
Deviation from study protocol:	None significant

Study design: Females were mated with a single male. Pregnant rabbits were dosed once daily from GD6 to GD20 and necropsy was preformed on GD29. The three exposure groups were a vehicle control, mirabegron (30 mg/kg), and a combination of mirabegron (30 mg/kg) and the β1 antagonist metoprolol (3 mg/kg). The sponsor chose a 3 mg/kg of metoprolol because it reduced the elevated heart rate caused by mirabegron (30 mg/kg) in two male rabbits in a preliminary study (Study not submitted). The dose of mirabegron was the dose previously associated with dilated aorta and cardiomegaly in fetal rabbits (178-TX-016).

Dosing Groups					
Group	N	Mirabegron		Metoprolol	
		Dose (mg/kg)	Volume (mL/kg)	Dose (mg/kg)	Volume (mL/kg)
Control	4	0	10	0	0
Mirabegron	9	30	10	0	0
Mirabegron + Metoprolol	10	30	5	3	5
One rabbit in the control group and mirabegron group did not become pregnant so the N was 4 and 9 respectively in these groups.					

Parameters and endpoints evaluated:

Does: Mortality, clinical signs, body weight, food intake, heart rate (measured in three dams/group 1, 4, 8, and 24 hrs after dosing on GD15), placental weights, gross lesions, number of corpora lutea and implantations.

Fetus: External and visceral abnormalities, skeletal defects, fetal weights, number of live and dead fetuses, post-implantation loss, incidence of external abnormalities, and sex ratio were tabulated. Gross internal examination was restricted to the aortic arch and hearts which were examined with a stereomicroscope.

Observations and Results

Mortality

No deaths. One of 17 does died at 30 mg/kg in the previous study (178-TX-016).

Clinical Signs

No clinical signs were noted.

Body Weight
There was no statistically significant affect of dosing on weight at the measured time points. However, body weight gain between GD6 and GS21 was slightly reduced in the mirabegron group with body weight gains being 0.20, 0.08, and 0.12 kg for the control mirabegron, and mirabegron/metoprolol combination dose groups, respectively.

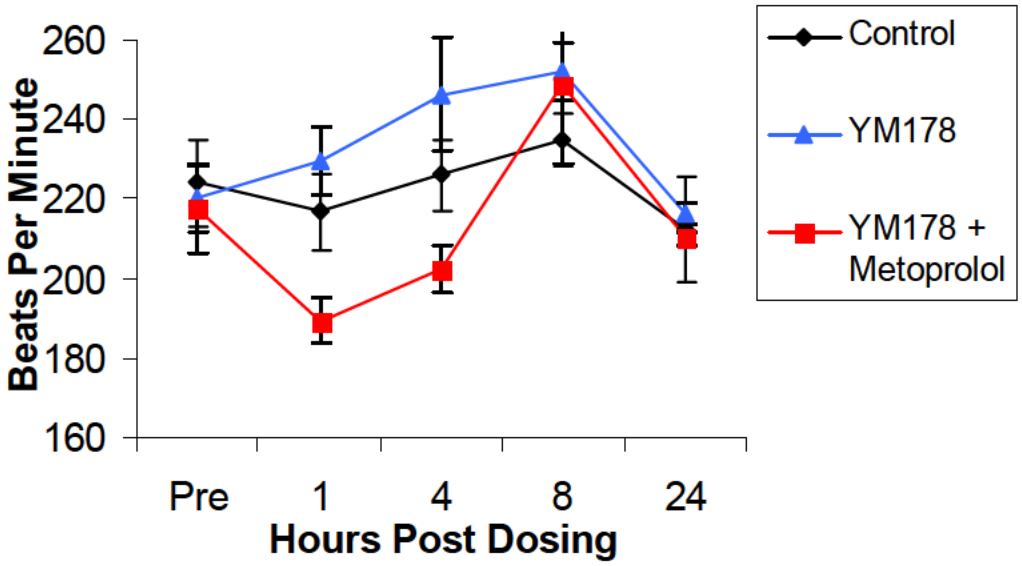
Feed Consumption
Food intake was reduced 20-41% during the dosing period (GD6 to GD21) in the mirabegron group. Food intake was also reduced in the combination dose group (23-31%) from GD6-GD17 but it was apparently not statistically significant. Also the combination dose group seemed to recover from the reduced feed intake during the dosing period since there was no affect at the end of the dosing period (GD17-21).

Heart Rate
Maternal mean heart rate was slightly elevated (12-15% from baseline) in the mirabegron group at 4 and 8 hrs after dosing but returned to baseline by 24 hrs. The β1 antagonist apparently repressed the mirabegron enhanced heart rate (7-13% reduction from baseline) 1 to 4 hrs after dosing. However, the metoprolol affect was transient with heart rates elevated 14% from base line at 8 hrs. This transient effect of metoprolol could be due to its short half-life. Statistical analysis was not conducted by the sponsor. Arrhythmia was noted in one dam in the mirabegron + metoprolol group four hours after dosing.

See section 4.3.4 for studies investigating cardiac toxicities.

Affect of Mirabegron (YM178) and Metoprolol on Heart Rates (beats/min)					
	Pre-Dose	**1 hr**	**4 hrs**	**8 hrs**	**24 hrs**
Control	224.0	216.7	226.0	235.0	212.3
Mirabegron	220.0	229.7	246.3	252.0	216.3
Mirabegron +Metoprolol	217.7	189.3	202.5	248.7	210.3
Table adapted from the sponsor. Statistical analysis was not conducted. N = 3 / time point/group.					

Effect of Mirabegron (YM178) and Metoprolol on Heart Rate (± SE) on GD15



Necropsy

Maternal - Fertility Parameters:

No adverse gross pathology was observed in any doe in any treatment group.

Neither mirabegron nor the combination of mirabegron and metoprolol affected fertility indices including number of corpora lutea, number of implantations, number of implantation sites, number of resorptions, sex or the number of dead fetuses.

Fetal Necropsy:

Mean fetal weight was not different among the treatment groups. External fetal abnormalities were only observed in one fetus in the combination treatment group. This fetus had exencephaly, open eyes, club foot, and paw hyperflexion.

None of the control fetuses had cardiac abnormalities. However, mirabegron treatment resulted in 51 of the 71 fetuses with dilated aortas and 16 of 71 fetuses with cardiomegaly. The incidence of dilated aorta and cardiomegaly in fetuses was reduced in the co-treatment group to 21/72 and 2/72 respectively. In the mirabegron group, all litters had offspring with dilated aortas and 5/9 litters had offspring with cardiomegaly. The number of litters with dilated aortas (8/10) and cardiomegaly (2/10) was slightly reduced with co-administration of metoprolol. Not all of the dead fetuses were examined for cardiovascular abnormalities but at least one dead fetus in the mirabegron group had dilated aorta.

Incidence of Significant Fetal Abnormalities in Live Fetuses (Litter Incidence %)			
	Control	**Mirabegron**	**Mirabegron+Metoprolol**
No. Does	4	9	10
No. Litters with Visceral Abnormality	0 (0%)	**9 (100%)****	**8 (80%)***
No. of Fetuses	28	71	72
Total Number Visceral Abnormality	0 (0%)	**51 (74%)****	**21 (27%) *, ##**
Dilated Aorta	0 (0%)	**51 (74%)****	**21 (27%) *, ##**
Cardiomegaly	0 (0%)	16 (31%)	2 (3%)
Significantly different from Control * $p < 0.05$, ** $p < 0.01$. ## Significantly different from mirabegron $p < 0.01$.			

Dosing Solution Analysis
No assessed

Study title: **Study for Effects of the Oral Administration of YM178 on Embryo-fetal Development in Rabbits: Effects of a beta 1-Blocker (2)**

Study no.:	178-TX-057
Study report location:	Module 4.2.3.5.2.1
Conducting laboratory and location:	Drug Safety Research Laboratories Astellas Pharma Inc. Osaka, Japan
Date of study initiation:	First Dose November 10, 2010
GLP compliance:	No statement on GLP compliance was provided.
QA statement:	A quality assurance statement was only provided for the toxicokinetic assessment.
Drug, lot #, and % purity:	YM178 (mirabegron), Lot# GLP-K1780211, 100.2% pure Metoprolol tartrate, Lot# BCBB6169, 100% pure

Background
This study was conducted to determine if the adverse developmental effects in rabbits are mediated by off target β1 adrenergic activation. A previous study determined that the β1 antagonist metoprolol (MET) can reduce but not completely eliminate the incidence of dilated aortas (178-TX-047). The study below increased the frequency of metoprolol dosing from 3 mg/kg once daily to 3 mg/kg BID to see if the incidence of dilated aortas and cardiomegaly can be completely eliminated with sustained repression of off target β1-signaling. Also, the heart rate was investigated in this study since mirabegron elevated the maternal heart rate and metoprolol reduces it.

Key Study Findings
- Metoprolol decreased the maternal exposure to mirabegron to levels below that known to cause fetal toxicities. Because of this, **the contribution of off target β1 signaling on these developmental toxicities is still unknown**.

Methods

Doses:	0, 30 mg/kg mirabegron, 30 mg/kg mirabegron + metoprolol tartrate (3 mg/kg/BID) The dose of MET was chosen because it previously was shown to decrease elevation in heart rate caused by mirabegron (178-TX-47).
Frequency of dosing:	Vehicle and mirabegron dosed once daily Metoprolol tartrate BID (First dose 24-40 min prior to mirabegron dosing, Second daily dose 6 hrs after first)
Dose volume:	5 mL/kg for vehicle and mirabegron Each dose of metoprolol was 2.5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% aqueous methylcellulose
Species/Strain:	Kbl: New Zealand White Rabbits (SPF). Females 15-16 weeks at mating.
Number/Sex/Group:	Vehicle (6), mirabegron alone (11) mirabegron+Metoprolol (12)
Satellite groups:	None
Study design:	Pregnant does were dosed from GD6 to GD20. Body weight and food consumption assessed throughout the study. Heart rate of the does was assessed on GD6 and GD20 in 3 vehicle control does and 5 does in the other groups. Does were euthanized on GD29. The numbers of live and dead fetuses, number of corpora lutea, and implantations were recorded from does that survived to GD29 only. Fetuses were examined externally, weighed, sexed, and the heart was examined for dilated aorta and cardiomegaly. Toxicokinetics assessed on GD6 and GD20 in five dams/group excluding the controls
Deviation from study protocol:	Storage of TK samples exceeded -10°C for 10 min but it did not affect results.

Observations and Results

Mortality
There was no maternal mortality.

Clinical Signs
No adverse signs were reported in any group.

Body Weight
Body weight gain was repressed during the dosing period in both the mirabegron group and the combination group but increased after the dosing period in all groups.

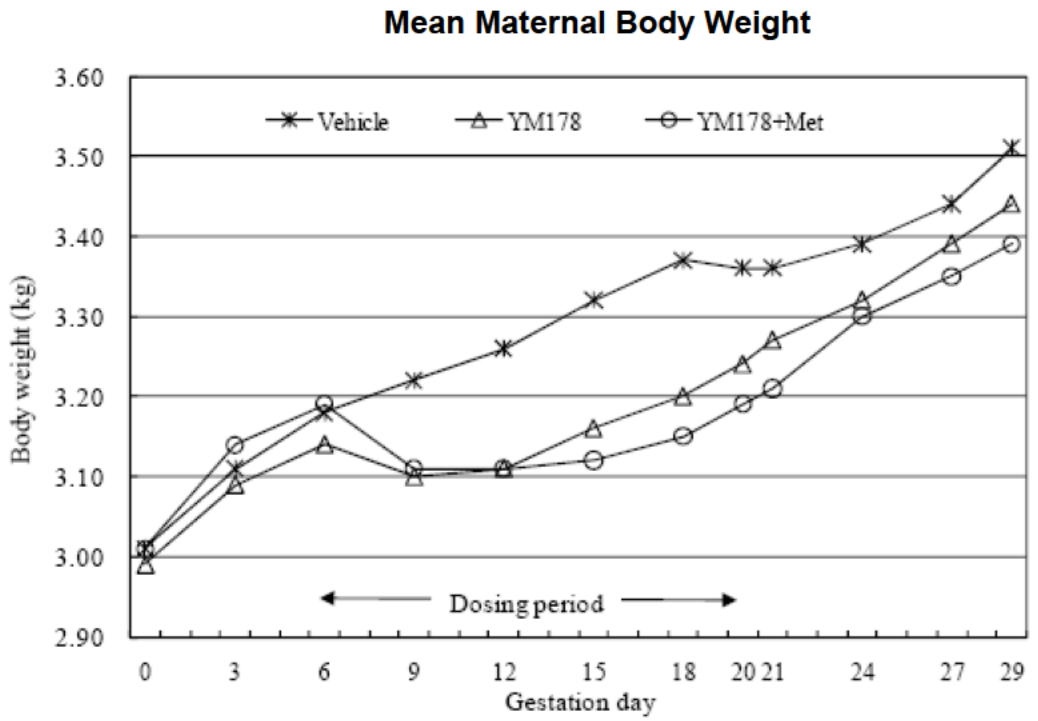


Figure is a copy of Sponsor's Figure 1.

Feed Consumption

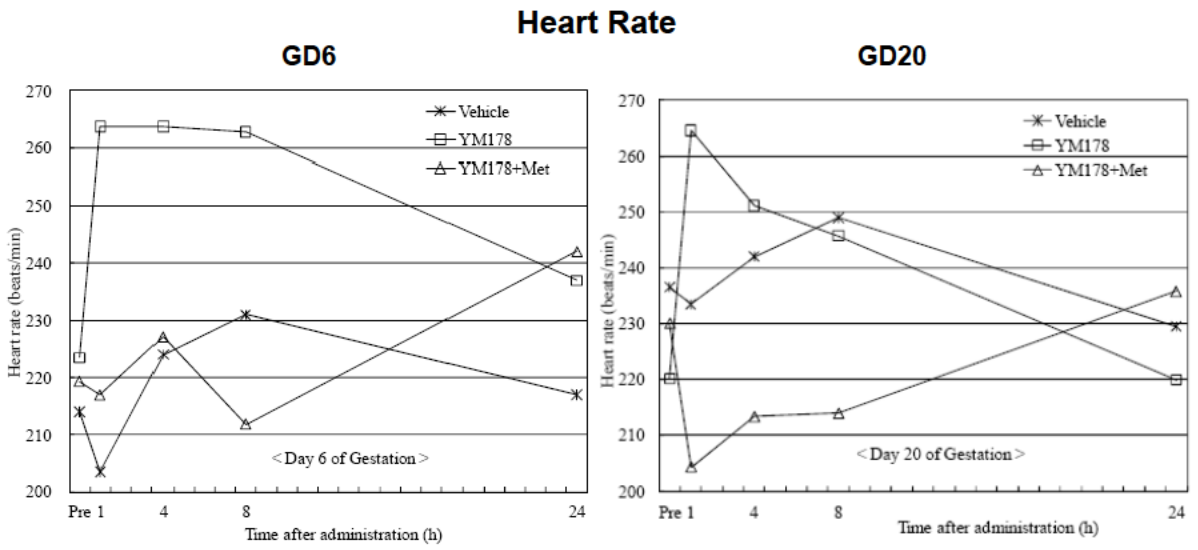
Food consumption was reduced during the dosing period in the mirabegron alone and mirabegron + MET groups compared to the controls but it was only statistically significant in the mirabegron + MET group from GD18-20 (-34%).

Heart Rate

Mirabegron increased heart rate for 8-24 hrs (6-23%) on the first and last day of dosing. The addition of MET suppressed the mirabegron mediated elevation in heart rate for eight hours but not 24 hours on GD6. The suppressive effect of MET exposure was even greater on GD20 where it reduced heart rates 7-11% below baseline for eight hours.

Heart Rate Beats per Minute (Change From Baseline)						
Day	Dose	Pre-Dose	1 hr	4 hrs	8 hrs	24 hrs
GD6	Vehicle	214	204 (-5%)	224 (+4%)	231 (+7%)	217 (+1%)
	Mirabegron	224	265 (+18%)	264 (+23%)	263 (+18%)	237 (+6%)
	Mirabegron + MET	219	217 (-1%)	227 (+4%)	212 (-3%)	242 (+11%)
GD20	Vehicle	237	234 (-1%)	242 (+2%)	249 (+6%)	230 (-2%)
	Mirabegron	220	265 (+20%)	251 (+14%)	246 (+12%)	220 (0%)
	Mirabegron + MET	230	204 (-11%)	213 (-7%)	214 (-7%)	236 (+3%)

Green indicates increased heart rate from baseline.
Red highlight indicates decreased heart rate from baseline.



Figures copied from the Sponsor Figure 3.

Necropsy

There was no gross pathology reported in the does attributed to treatment in any group on GD29.

Cesarean Section Data

There was no difference between groups on the number of corpora lutea, number of implantations, number of implantation sites, number of resorptions, number of dead fetuses, number of live fetuses, difference in sex ratio or fetal weight. However, post implantation loss (embryofetal deaths/ number of implantations x 100) was slightly elevated in the mirabegron group (17.6%) versus the control (5%) and combination (7.6%) groups but this was not statistically significant.

Offspring

External abnormalities were not reported in any fetus in any group. Mirabegron exposure resulted in dilated aortas in 29% of the fetuses and 73% of the litters. The incidence of dilated aortas was reduced to 3% of the fetuses and 17% of the liters with MET co-treatment. Cardiomegaly was observed in 6% of the fetuses and 27% of the litters in the mirabegron group. Cardiomegaly was also reduced to 1% of the fetuses and 8% of the litters in the MET co-treatment group.

Visceral Abnormalities in Fetuses			
	Vehicle	Mirabegron	Mirabegron + MET
No. Does	6	11	12
No. Fetuses	50	85	93
Dilated Aorta – No. Fetuses	0	25 (29%)	**3* (3%)**
- No. Litters	0	8 (73%)	2 (17%)
Cardiomegaly – No. Fetuses	0	5 (6%)	1 (1%)
- No. Litters	0	3 (27%)	1 (8%)
* Significantly different from mirabegron alone group p < 0.01			

Toxicokinetics

The mean exposure to mirabegron was similar to previous studies in pregnant rabbits at the same dose (178-TX-016 and 178-TX-056) (Table below).

Exposure (Mean AUC_{0-24}) to Mirabegron and Metabolites in Humans and Pregnant Rabbits								
			Human	Rabbit Pregnant GD20 Study 178-TX-056			Rabbit Pregnant GD20 Study 178-TX-057	
			50 mg	3 mg/kg	10 mg/kg	30 mg/kg	30 mg/kg	30 mg/kg + MET 3 mg/kg BID
Mirabegron		F	512	260	1,863	18,426	21,875	4,004
M5	Phase 2 Acetyl conjugate	F	112	698	3,984	19,518	15,777	25,225
M8	Phase 1	F	**104***	ND	ND	ND	ND	ND
M11	Phase 2 Glucuronide	F	201	30	258	1,835	2,865	568
M12	Phase 2 Glucuronide	F	99	0	0.5	5.5	13	0
M13	Phase 2 Glucuronide	F	13	12	95	703	957	247
M14	Phase 2 Glucuronide	F	75	57	341	2,077	2,237	776
M15	Phase 2 Glucuronide	F	49	0.3	11	97	156	22
M16	Phase 1	F	70	247	1,658	6,218	6,148	11,627
Human exposure was determined on day 7 of dosing in fasted women ≥ 55 yrs (178-CL-072). ND – not determined. * M8 data in humans only available from the 100 mg dose.								

The sponsor indicated that there may be vast differences in the rabbit's ability to metabolize mirabegron. In the mirabegron alone group in this study 2/5 rabbits in the toxicokinetic group were apparent poor metabolizers of mirabegron to M5 and M16 throughout the study (data in brackets in tables below). When the results from poor metabolizers (numbered 21011 and 21013) were removed (data in parentheses in tables below), the TK is similar between the mirabegron group and the mirabegron plus MET treatment group. However, the mirabegron exposure with the two poor metabolizers is removed (5,050 ng-hr/mL) is much lower than in the previous study where animals were dosed at 30 mg/kg (17,000 to 19,000 ng-hr/mL) (Study 178-TX-16 reviewed above or 178-TX-056). Curiously the two animals (21011 and 21013) with the greatest mirabegron exposure did not appear to be adversely effected by the higher exposure to mirabegron. They did not have weight gain problems, both had a slight reduction in food consumption, there were no obvious effects on cesarean observations, none of their offspring had dilated aorta or cardiomegaly, and none of their fetuses died during gestation.

Toxicokinetics in Pregnant Rabbits on GD6 178-TX-057 (n = 5 per group)							
		Mirabegron (30 mg/kg)			Mirabegron (30 mg/kg) Metoprolol (3 mg/kg BID)		
		Tmax (hr)	Cmax (ng/mL)	AUC_{0-24} (ng-hr/mL)	Tmax (hr)	Cmax (ng/mL)	AUC_{0-24} (ng-hr/mL)
Mirabegron		1.4	5,750 (3,194) † [9,584] *	18,438 (5,050) † [38,520] *	1.6	729	3,106
M5	Phase 2 Acetyl conjugate	2.2	1,391 (1,623) † [1,044] *	11,040 (16,897) † [2,255] *	4.4	1,631	16,205
M11	Phase 2 Glucuronide	1.2	743 (414) †	1,940 (616) †	1.2	143	416
M12	Phase 2 Glucuronide	1.3	3 (1) †	9 (1) †	ND	ND	ND
M13	Phase 2 Glucuronide	1.2	223 (136) †	628 (208)†	1.6	40	158
M14	Phase 2 Glucuronide	1.6	266 (153) †	1,524 (580) †	2.2	69	557
M15	Phase 2 Glucuronide	1.2	47 (28) †	153 (36) †	1.4	5	14
M16	Phase 1	2.0	714 (886) † [455] *	4,939 (7,770) † [693] *	2.0	974	8,332

Green indicates increased AUC in the mirabegron + MET group compared to mirabegron alone.
Red highlight indicates decreased AUC in the mirabegron + MET group compared to mirabegron alone.
† Numbers in parenthesis exclude animals 21011 and 21013 because the sponsor believes they are outliers.
* Numbers in brackets are for animals 21011 and 21013 only.
Toxicokinetics was not GLP compliant. Toxicokinetics was conducted on five animals per group and blood was not sampled from the remaining animals in each group.

Toxicokinetics in Pregnant Rabbits on GD20 178-TX-057 (n = 5 per group)							
		Mirabegron (30 mg/kg)			Mirabegron (30 mg/kg) Metoprolol (3 mg/kg BID)		
		Tmax (hr)	Cmax (ng/mL)	AUC_{0-24} (ng-hr/mL)	Tmax (hr)	Cmax (ng/mL)	AUC_{0-24} (ng-hr/mL)
Mirabegron		1	5,796 (658) †	21,875 (2,958) † [50,250] *	1.4	833	4,004
M5	Phase 2 Acetyl conjugate	3.6	1,481 (2,347) †	15,777 (24,822) † [2,209] *	3.6	2,090	25,225
M11	Phase 2 Glucuronide	1	1,133 (140) †	2,865 (419) †	1.2	170	568
M12	Phase 2 Glucuronide	1	4 (0) †	13 (0) †	ND	ND	ND
M13	Phase 2 Glucuronide	1	334 (60) †	957 (189) †	1.6	67	247
M14	Phase 2 Glucuronide	1.8	264 (86) †	2,237 (709) †	2.4	103	776
M15	Phase 2 Glucuronide	1	54 (6) †	156 (17) †	1.2	7	22
M16	Phase 1	1.6	685 (1,108) †	6,148 (10,005) † [362] *	2.2	1,151	11,627

Green indicates increased AUC in the mirabegron + MET group compared to mirabegron alone.
Red highlight indicates decreased AUC in the mirabegron + MET group compared to mirabegron alone.
† Numbers in parenthesis exclude animals 21011 and 21013 because the sponsor believes they are outliers.
* Numbers in brackets are for animals 21011 and 21013 only.

Metoprolol Appears to Reduce Mirabegron Exposure

Co-treatment of mirabegron at 30 mg/kg with 3 mg/kg MET BID reduced the exposure to mirabegron by six-fold after the first (GD6) and last (GD20) dose (Tables above). This may be due to increased metabolism of mirabegron primarily to M5 and M16 which are inactive metabolites at physiological concentrations in vitro. This completely confounds the results of this study and the previous embryo-fetal study in rabbits using metoprolol (178-TX-047) because the exposure to mirabegron was reduced after co-treatment with MET to levels that did not cause cardiomegaly or dilated aortas in the first embryofetal study (178-TX-016, reviewed above). In the previous study, dilated aortas and cardiomegaly were not observed in rabbits dosed with mirabegron at 10 mg/kg where the mean AUC was 7,000 to 9,000 ng-hr/mL (178-TX-016). Because MET appeared to reduce the maternal exposure of mirabegron to non-fetotoxic levels (3,000 to 4,000 ng-hr/mL) in this study, it is still unknown if off target β1 activation and/or elevated heart rates contribute to these developmental toxicities. The sponsor confirmed that metoprolol did not interfere with the assay for mirabegron.

If enzyme induction by MET reduced the mirabegron exposure in this study, it is unknown what enzymes were induced. However, metoprolol is a CYP2D6 substrate

and in vitro studies suggest that mirabegron is metabolized primarily by CYP3A4 and to a lesser extent by CYP2D6 (178-ME-002).

Beta antagonists in general do not appear to decrease exposure to mirabegron in humans. Decreased exposure to mirabegron was not reported in clinical studies after co-therapy with bisoprolol, metoprolol, or propranolol. The sponsor conducted a clinical drug-drug interaction study with metoprolol where they found that mirabegron increases the metoprolol exposure (AUC) three-fold suggesting that mirabegron and MET are both metabolized by CYP2D6 (178-CL-005). Although AUC levels were not located in the report, MET increased, rather than decreased, the trough levels of mirabegron in humans (178-CL-005). Pharmacokinetics of mirabegron did not appear to be effected in humans exposed to a β1 (bisoprolol) or a mixed β1/β2 antagonist (propranolol) along with a single 200 mg dose of mirabegron (178-CL-053).

Metabolites

Due to the high variability and small number of animals, it is difficult to form conclusions regarding the involvement of metabolites in the toxicity. Although not likely, data from the mirabegron alone group suggests that metabolites could be implicated in the fetal toxicity because exposure to M5 and M16 but not mirabegron was correlated with embryo-fetal toxicities in animals used for TK assessment. The maternal exposure to M5 and M16 were elevated at least 8-fold while exposure to mirabegron was at least 8-fold lower in the three rabbits with fetal cardio defects in the mirabegron alone group (data in parenthesis), in comparison to the two does without fetal toxicities in the same group (data in brackets). Never the less, it is unlikely that metabolites are involved in the fetal toxicities. Metabolites M11 to M15 are not expected to have contributed to the toxicity since they are glucuronide conjugates and were only present at low levels. Although the exposure to M5 and M16 in rabbits is exceedingly high compared to humans, rats, mice, and monkeys which implicate them in the fetal cardio toxicities (Tables above and Section 5.1.3 cross species metabolism), they did not have appreciable in vitro agonist activity for the human β1-AR, β2-AR, or β3-AR at pharmacological concentrations (see Section 4.1.1). Additionally M5 and M16 are likely not causative of these fetal toxicities because similarly high levels of M5 and M16 were observed in both treatment groups while fetal cardio toxicities were almost eliminated in the mirabegron + MET group (compare group mean values or values in parentheses in the mirabegron group to the combination group).

Dosing Solution Analysis

Dosing solution homogeneity was not assessed.

Title: **Investigational Study for Effects of the Oral Administration of Dl-Isoproterenol Hydrochloride on Embryo-fetal Development in Rabbits: Effect of a beta 1-Blocker (Non-GLP study TX094001)**

Key Findings:

- Similarly to mirabegron, in utero exposure to the nonspecific β-agonist DL-isoproterenol (ISO) caused dilated aorta (83% litters/49% fetuses) and cardiomegaly (50% litters/3% fetuses) which was partially inhibited with co-administration to the β1-antagonist metoprolol. Metoprolol reduced the dilated aorta incidence from 83% to 44% of litters and cardiomegaly incidence declined from 50% to 11% of the litters.
- Maternal heart rate was elevated ~30-40% by ISO exposure but it recovered within 8-24 hrs. Met exposure impaired the ISO induced heart rate increase such that heart rate was not clearly elevated.
- These findings suggest that developmental cardiotoxicities in rabbits may be mediated in part by activation of the β1-adrenergic receptor.

Methods:

The effects of the nonspecific β-agonist DL-Isoproterenol (ISO) on maternal and fetal toxicity was assessed (Study TX094001). New Zealand White rabbits were dosed orally from GD14 to GD20 with vehicle (water) (n=5) or 50 mg/kg ISO with or without co-administration of the β1 antagonist metoprolol tartrate (Met) at 16 mg/kg (n=10/group). Maternal heart rate was assessed prior to dosing and 1, 4, 8, and 24 hrs after dosing on GD14 and GD20. Necropsy and fetal evaluation was conducted at GD29.

Results:

The dose of ISO was chosen because it had previously been shown to cause dilated aorta and cardiomegaly in fetuses. The dose of metoprolol was chosen because it was the highest dose without post-implantation loss in a published report.

All does in the control and combination groups survived. In the ISO group, two does died within 26 minutes of the first dose and one doe in the ISO group aborted on GD26 and another had no fetuses despite having 12 implantation sites. One dam in the ISO + Met group aborted on GD27. No gross pathology was observed in any of the dams including the ones that died.

Incidence of Fetal Cardiomegaly and Dilated Aorta			
Dose Group	Vehicle	ISO	ISO + Met
Dams (N)	5	10	10
Maternal Death	0	2	0
Dam Aborted	0	1	1
Total Litter Loss after Implantation	0	1	0

Mean maternal body weight gain during the dosing period was reduced 47g and 29g in the ISO and ISO + Met groups respectively compared a 9g increase in the control group.

Heart rate was not clearly affected by treatment in the control or ISO + Met group. However, after the first dose heart rate was elevated ~80 bpm (40%) in the ISO group but it declined within 8-24 hrs of dosing. Heart rate was similarly elevated ~60bpm (27%) in the ISO group on GD20 but it recovered within eight hours of dosing.

Maternal Fertility Parameters:
There was no difference in mean number of corpora lutea, number of live fetuses, sex ratio, fetal weight, number of resorptions, and number of dead fetuses. However, the mean number of implantation sites was reduced in the ISO + Met group (0%) compared to control (8%) and ISO (16%).

Fetal Necropsy:
Dilated aorta and cardiomegaly were not observed in fetuses from the control group. Dilated aorta incidence in the ISO group decreased from 83% of the litters to 44% with concomitant Met dosing. Likewise the incidence of cardiomegaly in the ISO group decreased from 50% to 11% of the litters with Met co-treatment.

Incidence of Fetal Cardiomegaly and Dilated Aorta			
Dose Group	Vehicle	ISO	ISO + Met
Dams (N)	5	6	9
Fetuses (N)	38	42	53
Dilated Aorta	0	17 fetuses (49%) 5 litters (83%)	7 fetuses (18%) 4 (44%)
Cardiomegaly	0	4 fetuses (13%) 3 (50%)	2 (3%) 1 (11%)

Toxicokinetics: Toxicokinetics was not assessed. It is unknown if metoprolol reduced the maternal exposure to isoproterenol to levels that were not fetal toxic as it did with mirabegron.

Prenatal and Postnatal Development – Rats

Study title: **Study for Effects on Pre- and Postnatal Development, Including Maternal Function in Rats Treated Orally with YM178**

Study no.:	178-TX-038
Study report location:	Module 4.2.3.5.3.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 15, 2006
GLP compliance:	Yes, (b) (4) regulations
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot GLP-K1780211, 99.4% pure

Key Study Findings
Rats were exposed to mirabegron at 0.9, 6.1, and 21.5x times the MRHD based upon toxicokinetic data from gestational rats (178-TX-022) and elderly fasted women dosed with 50 mg of mirabegron (178-CL-072).

F_0
- Mortality in the F_0 generation at 100 mg/kg. 2F at GD21-GD22 (cause unknown).
- Recoverable reduction in food intake and decreased weight F_0 at ≥ 30 mg/kg during gestation.

F_1
- Elevated death of F_1 pups between PND0 and PND4 at 100 mg/kg. 7.5% died at 100 mg/kg vs. 1.2% in controls.
- Decreased body weight of F_1 males and females at 100 mg/kg during the post weaning period.
- No adverse effects on behavior or learning
- No significant adverse effects on reproductive endpoints

NOAEL for F_0 Maternal toxicity = 10 mg/kg (1x MRHD) based upon decreased feed intake and reduced weight at 30 mg/kg
NOAEL for F_0 Fertility = 30 mg/kg (6x MRHD) based upon decreased viability of offspring between PND0 and PND4 at 100 mg/kg
NOAEL for F_1 Toxicity = 30 mg/kg (6x MRHD) based upon post weaning deaths males (2/36) and females (1/36) at 100 mg/kg
NOAEL for F_1 Fertility ≥ 100 mg/kg (22x MRHD) based on no affects on F_1 fertility

Methods

Doses:	0, 10, 30, or 100 mg/kg
Frequency of dosing:	Daily
Dose volume:	5 mL/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% methylcellulose
Species/Strain:	Sprague-Dawley rats SPF [Crl:CD(SD)]
Number/Sex/Group:	20/females/group
Satellite groups:	None, toxicokinetics was not assessed
Study design:	A single male was mated to a female in proestrus. The day following successful copulation was considered gestation day 0 (GD0). Dams were dosed daily from GD7 to postnatal day 20 (PND20). This correlates with implantation to weaning. The dams were euthanized on PND21.

Parameters and endpoints evaluated:
Dams: Clinical signs were taken four times daily. Body weight was measured on gestation days 0, 4, and 7-20 and on postnatal days 0, 4, 7, 11, 14, 17, and 21. Food consumption was measured on gestation days 1, 4, 8, 11, 14, 17, and 20 and on postnatal days 2, 4, 7, 11, 14, 17, and 21. The day of delivery was designated as

postnatal day 0. Rats nursed their pups until weaning on PND21. Gross pathology was conducted on all dams at PND21.

Pups: were weighed, sexed, and examined for abnormalities at birth. Still born fetuses were fixed in 70% ethanol. Five days after birth the pups were culled to 4/sex/litter. Excess litter mates were examined for gross pathology. Mortality was noted daily with the dead pups being stored for necropsy. Pup weight was recorded on days 4, 7, 11, 14, 17, and 21 after birth. Pups were observed for pinna detachment, incisor eruption, opening of the eyes, righting reflex, air righting reflex, pupillary reflex, Preyer's reflex, and pain reflex. On PND21 2/sex/litter were selected one each for behavioral testing and reproductive testing, see below. The remaining pups were euthanized and examined macroscopically.

F1 animals post weaning: Postnatal weights were recorded on PND 28, 35, 42, 49, 56, 63, and 70. Sexual development was noted for females on days 35 and 42 (opening of the vagina) and days 42 and 49 in males (cleavage of the balanopreputial gland). Behavioral tests were conducted on 1 pup/sex/litter including, open field test at 5 weeks of age and water filled multiple T-maze during weeks 7-8. These animals were euthanized after reaching 10 weeks of age and examined macroscopically. In addition to behavioral testing, an additional single pup sex/litter were tested for mating success during weeks 10-12 after birth. Males and females from the same dose group (not litter mates) were housed together. F1 offspring used for reproductive study were euthanized 12 weeks after birth. Major organs were examined macroscopically. F1 females were weighed on days 0, 4, 7, 11, and 14 of gestation, and euthanized on GD14. F1 females were examined macroscopically at necropsy on GD14. Ovaries, uterus, and vagina were examined for implantation, number of corpora lutea, and number of live and dead fetuses. Females who did not show implantation sites were further studied by clearing the tissue with 10% NaOH to confirm the lack of implantation. Ovaries and uterus with vagina from non-pregnant F1 rats were stored in 10% formalin.

Deviation from study protocol: None that significantly effected study findings

Homogeneity: Dosing solutions were 96.5 to 103.3% of the target.

Observations and Results

The high dose of 100 mg/kg was chosen because of maternal deaths at 300 mg/kg in the embryo-fetal study (178-TX-022) and decreased body weight, food consumption, and impaired bone development in the fetuses at ≥ 100 mg/kg.

F_0 in-life:

Mortality: Two F_0 dams in the 100 mg/kg group died, one each on GD21 and GD22. These dams did not display any adverse signs or gross pathology and their fetuses appeared normal. The cause of death was unknown.

Clinical Signs: No clinical signs were reported in any group during gestation and lactation.

Body Weight: Dams in the 100 mg/kg group had reduced weight (4%) during GD 11-17 and during postnatal days 0 to 11 (4-6%) but recovered thereafter.

Feed Intake: Feed intake was reduced on GD 8 in the 30 (12%) and 100 mg/kg (29%) groups and on GD 11 in the 100 mg/kg group (13%). Intake was normal after this time point.

Reproductive Success: There was no affect of mirabegron on the mean numbers of live births, still born pups, gestation period, and implantation sites. There was no affect of mirabegron on sex ratio or birth weight of the pups. None of the pups had external malformations at birth.

F_0 necropsy: No gross pathology was observed in the dams.

F_1 physical development:
During lactation: Viability between postnatal days 0 and 4 was reduced at the high dose (92.5% survived to postnatal day 4 vs. 98.8 in the control). Death of 1 to 3 pups per litter occurred during the first four days after birth in 2/19 litters at 0 mg/kg and 11/18 litters at 100 mg/kg. Viability was similar across groups from PND4 to PND 21. The time to detachment of the pinna, eruption of lower incisors, opening of eyelids, vaginal opening, and cleavage of the balanopreputial gland was not altered by mirabegron exposure. Gross pathology was not affected by mirabegron either 4 or 21 days of birth.

Post-weaning: After weaning, three F_1 deaths occurred at the high dose. Two F_1 males died, one each 61 days (1 of 18 in the behavioral testing study) and 81 days after birth (1 of 18 in the reproductive function study) along with one female (1 of 18 in the reproductive function study) 68 days after birth. The male that died 61 days after birth displayed dark red area in the lungs (1/18) and retention of food in the pharynx. Adverse pathology was not observed in the other animals that died after weaning. Adverse pathology was not reported animals that lived to PND70 except for one male in the high dose group who had enlarged unilateral testis.

Body weight: Body weight was reduced 6% to 18% from postnatal day 4 to 70 in the high dose males and 7% to 14% in high dose females from postnatal day 4 to 35.

F_1 behavioral evaluation:
There was no affect of mirabegron on righting reflex, air righting reflex, pupillary reflex, Preyer's reflex, or pain reflex. In the open field test at week 5, latency, ambulation, rearing, grooming, and defecation were not altered by mirabegron in females but defecation was slightly increased in males at the high dose. Mirabegron did not appreciably affect the results from the water-filled multiple T-maze in males or females at weeks 7-8.

F_1 fertility:
Since both the male and female F_1 rats of each mating pair were from the same dose group, any potential affects could not be linked to the fertility of one specific sex alone.

The fertility index (number of pregnant females/number of females copulated x 100) was reduced at the high dose (82%) vs. the control (100%) although not to a statistically significant extent. This was within the range of the historical control data (78.9% to 100%) generated from 19 studies.

Fertility parameters of F_1 females at GD 14 were not affected by mirabegron. The mean number of corpora lutea, number of implantations, preimplantation loss, post implantation loss and number of live embryos were similar across groups.

Body weight was not affected in pregnant F_1 dams.

There were no adverse gross pathologies in the F_1 females used for the reproductive studies.

F_2 findings: The mean number of live embryos on GD14 was the same across groups.

10 Special Toxicology Studies

10.1 Local Tolerance

10.1.1 Acute Dermal Irritation – Rabbits

Study title: **Acute Dermal Irritation Study of YM178 in Rabbits**

Study no.:	178-TX-001
Study report location:	Module 4.2.3.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 6, 1999
GLP compliance:	Yes, (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, 100.3% pure

Key study findings:
Rabbit skin showed no signs of irritation after four hours of dermal contact to an aqueous paste of mirabegron (0.5 g over 2.5 cm^2) applied to a lint cloth next to the skin.

Methods:
Three male (b) (4) white rabbits (Kbl:JW) at 16 weeks of age were used in the study. Bilateral dorsal regions (2.5 cm^2) were shaven for dermal application. An aqueous paste of mirabegron (0.5 g in 1 mL in distilled water for each application) was spread over a 2.5 cm^2 piece of lint which was taped to the left dorsal side of the rabbit. The right side of each rabbit served as a control site that was covered with lint lacking mirabegron paste. The lint was held in place with a bandage. After four hours, the bandage and lint were removed and the application site was washed clean. Exposure sites were graded

for the presence and extent of erythema and edema before exposure and 1, 24, 48, and 72 hrs after exposure.

Results:
No irritation (erythema or edema) was observed in non-treated or mirabegron treated skin patches at any time point.

10.1.2 Ocular Irritation Investigation in Rabbits

Study title: **Eye Irritation Study of YM178 in Rabbits**

Study no.:	178-TX-002
Study report location:	Module 4.2.3.6.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 6, 1999
GLP compliance:	Yes, (b) (4) guidance
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K02, 100.3% pure
Formulation/vehicle:	No vehicle was used but it was passed through a 300 µm mesh to remove large particles.

Key study findings: mirabegron was minimally irritating to the eye but flushing the eyes reduced the severity to practically nonirritating under the conditions used in this assay.

Methods:
Six 10 week old (b) (4) white rabbits (Kbl:JW) were administered 100 mg of mirabegron to the conjunctival sac of the right eye. The eye lids were held together for one second after exposure. The left eye was not exposed. Thirty seconds after dosing, the eyes of three of the dosed rabbits were washed twice with 100 mL of water while eyes of the other three rabbits were not washed. Gross observation of the cornea, iris, and conjunctiva were conducted prior to exposure and 1, 24, 48, and 72 hrs after exposure. Comparisons were made to the non-treated eye and graded on a 110 point scale.

Results:
No adverse affects were observed in the non-treated left eyes in both groups.

Mirabegron was observed one hour after exposure in the eye of 1/3 rabbits in the non-washing group but was not observed at 24 hrs. When the eyes were not washed, mild reddening of the conjunctivae (1/3 animals) and chemosis (swelling/edema) were observed in 2/3 animals one hour after exposure. One day after exposure the only sign in the non-washed group was red conjunctivae in 1/3 rabbits and no further signs were reported thereafter.

In the rabbits that had their eyes flushed after exposure, mild reddening of the conjunctivae was observed in 2/3 animals one hour after exposure and no further signs were reported thereafter.

Overall, 100 mg of mirabegron was minimally irritating for one hour without washing and practically nonirritating for one hour if they eyes were flushed after exposure.

Mean Irritation Score ± SD in Mirabegron Treated Eyes							
Treatment	N	Pre-exposure	1 hr	24 hr	48 hr	72 hr	Evaluation
No washing	3	0	3.3 ± 1.2	0.7 ± 1.2	0	0	Minimally irritating
Washing	3	0	1.3 ± 1.2	0	0	0	Practically nonirritating

10.1.3 Intravascular Irritation - Rabbits

Study title: **Intravascular Irritation Study of YM178 in Rabbits**

Study no.: 178-TX-036
Study report location: Module 4.2.3.6.1
Conducting laboratory and location: (b) (4)
Date of study initiation: Dose initiation on March 9, 2005
GLP compliance: Yes (b) (4) guidance
QA statement: Yes
Drug, lot #, and % purity: YM178 (mirabegron), Lot K1780211, purity 99.8%
Formulation/vehicle: 8.4% sucrose solution in 20 mM citrate buffer (final pH 5.1) with a final mirabegron concentration of 10 mg/mL.
Dosing Solution: The solution was 100.6% of the target.

Key findings

Intravenous injection of mirabegron (0.5 mg) only resulted in mild edema which resolved within 8 hrs of treatment. Perivenous injection of mirabegron (2 mg) resulted in mild local irritation and what appeared to be inflammation and impaired wound healing that had not resolved within 4 days of treatment. Rabbits recovered from mirabegron dependent injection site edema and erythema within 48 hrs but histology of the injection site revealed evidence of minimal to slight edema, hemorrhaging, inflammatory cell infiltration, and granulation tissue formation 96 hours after dosing.

Methods

Three 10 month old male (b) (4) White rabbits (Kbl:JW, SPF) (2.1-2.2 kg) were dosed intravenously with 0.05 mL (0.5 mg) of mirabegron or saline into the posterior auricular vein. A 0.2 mL (2 mg) paravenous dose of mirabegron or saline was injected into the subcutaneous tissue near the auricular vein. The injection sites were grossly observed 0.5, 1, 4, 8, 24, 48, 72, and 96 hrs after administration, and the size of any lesions were recorded. Histological assessment of the injection sites occurred at 96 hrs.

Route	N	Right Auricle	Left Auricle
Intravenous	3	0.5 mg mirabegron	Saline
Paravenous	3	2 mg mirabegron	Saline

Results: No adverse clinical signs or death occurred.

Intravenous Treatment: Intravenous administration of mirabegron resulted in edematous lesions (10x13 mm) in 3/3 rabbits within 0.5 hrs of dosing which resolved within 8 hrs of administration. There were no gross findings in the intravenous saline control sites. No adverse histology was recorded at the intravenous injection site four days after exposure to vehicle or mirabegron.

Paravenous Treatment: Paravenous injection of saline resulted in only 1/3 rabbits with erythema which resolved within one hour of administration. Paravenous administration of mirabegron resulted in edematous lesions in 3/3 rabbits (mean of 37 x 13 mm) and erythema in 2/3 rabbits within 0.5 hrs of dosing which all resolved by 48 hrs. However, purpura (red/purple lesions) became apparent in one rabbit at 48 hrs after dosing and it did not resolve by 96 hrs after dosing. Histology revealed that 3/3 of the mirabegron dosed sites had edema (minimal to slight), hemorrhaging (minimal to slight), inflammatory cell infiltration (minimal to slight), and granulation tissue formation (minimal) 96 hrs after dosing. At the control injection site, only 1/3 animal had slight granulation tissue formation.

10.2 Dermal Sensitization Studies in Guinea Pigs

Study title:	**Skin Sensitization Study of YM178 in Guinea Pigs**
Study no.:	178-TX-024
Study report location:	Module 4.2.3.7.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 21, 2001
GLP compliance:	Yes, (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K1780006, 100.3% pure
Formulation/Vehicle:	Liquid paraffin was used as the vehicle.
Dosing solution homogenization:	Dosing solution samples from the top, middle, and bottom were within 92-104% of the target.

Key Study Findings

- Mirabegron caused primary skin irritation and moderate sensitizing potential (Grade III) in guinea pigs after challenge with 2% or 10% mirabegron.

Methods

Exposure: A preliminary range finding study exposed the shaven lateral abdomen of guinea pigs to patches containing 1.0 mL of mirabegron at 0.4%, 2%, or 10% after which the sites were covered with adhesive plaster. No skin reactions were observed

after 24 hrs so the sensitization dose in the main study was set as the highest concentration possible (10%) and a lower dose of 1%. The challenge concentrations for mirabegron in the definitive study were set at 2% and 10%.

Species/strain: Three week old Slc:Hartley male guinea pigs (SPF) six weeks old at sensitization weighing 325-407 g.

Study design:
Sensitization- The neck of the guinea pigs were shaved and used as the injection site for sensitization. Freund's complete adjuvant (FCA, in an oil/water emulsion) was injected (0.1 mL) intradermally into the four corners of the shaven skin patch. The skin was then irritated with an injection needle. The injection sites for each guinea pig were then covered with paraffin mixtures containing one of the following; 0%, 1%, or 10% mirabegron (n = 10/group) or 2,4-Dinitrochlorobenzene (DNCB) in acetone (n = 5/group) as the positive control. This treatment was repeated on three consecutive days. Six days after the initial exposure, the injection sites were covered with a 10% sodium lauryl sulfate petrolatum mixture. The injection sites were wiped clean on the following day and each injection site was overlaid for 48 hrs with filter paper containing the same concentration of test substance used in the initial exposure.

Challenge- Twenty one days after the final sensitization, the right and left lateral abdomens were shaved. The right abdomen of each guinea pig was exposed to 2 cm^2 patches containing 0.1 mL of 2% mirabegron or acetone in the case of the positive control group for 24 hrs. The left abdomen of each guinea pig was similarly challenged with 10% mirabegron or the positive control (DNCB) for 24 hrs. Challenge sites were occluded with an adhesive plaster.

Skin reactions: Skin reactions (erythema, eschar, and edema) were assessed 24 and 48 hrs after challenge exposure using a graded scale. The range of scores for erythema and eschar were 0 for non-detected to 4 for severe. The range of edema was 0 to 3 for severe edema. Intensity scores for an individual of at least two were required for a positive result. A cumulative grade for each dosage group was based upon the percentage of animals in each group with positive sensitization.

Gradation of Skin Sensitization		
Positive Ratio %	Grade	Evaluation
0	-	Non
1-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

Clinical Signs: Clinical signs were monitored daily.

Body weight: Weight was assessed weekly.

Results:
Ten percent mirabegron alone did not irritate the skin unless animals were pretreated with Freund's complete adjuvant (FCA) and later challenged with mirabegron. FCA pretreatment was used in all experiments. Animals sensitized with vehicle and challenged with vehicle did not respond with adverse skin reactions (Table below). Animals sensitized with 1% or 10% mirabegron and challenged with vehicle did not respond with adverse skin reactions. Twenty percent of the animals sensitized with the vehicle responded to a 2% or 10% mirabegron challenge with very slight to well defined erythema but no edema **suggesting that mirabegron causes primary skin irritation**. Guiney pigs sensitized with 1% mirabegron and then challenged with 2% or 10% mirabegron responded with mild-moderate skin reactions (very slight to moderate erythema/eschar and very slight edema) in 80% of the animals 24 and 48 hrs after the challenge. Animals sensitized with 10% mirabegron responded to a 2% or 10% mirabegron challenge with mild to moderate skin reactions (very slight to moderate erythema/eschar and very slight edema) in 80% or 90% of the animals 24 or 48 hrs after challenge respectively.

In the groups with primary skin irritation (vehicle sensitization group with mirabegron challenge), the skin reactions were similar 24 and 48 hours after challenge. However, in groups sensitized with mirabegron and challenged with mirabegron, the severity of the reactions was greater and also increased between 24 and 48 hrs after challenge primarily due to increased incidence of edema which suggests that **mirabegron may not only be a skin irritant but also a skin sensitizer** in this assay. Scores indicated that mirabegron has moderate sensitizing potential (Grade III) after challenge with 2% or 10% mirabegron in this assay.

Animals sensitized and challenged with the positive control (DNCB) responded with 100% of the animals with moderate to strong adverse skin reactions (moderate erythema and very slight to moderate edema) 24 or 48 hrs after challenge.

Summary of Skin Sensitivity to Mirabegron (YM178)

Sensitization	N	Challenge	24 Hrs After Challenge			48 Hrs After Challenge		
			No. Positive Animals	Percent Positive	Mean Response	No. Positive Animals	Percent Positive	Mean Response
Vehicle	10	Vehicle	0	0	0	0	0	0
		2% YM178	2	20	0.5	2	20	0.5
		10% YM178	2	20	0.5	2	20	0.5
1% YM178	10	Vehicle	0	0	0	0	0	0
		2% YM178	8 (1)	80 (10)	1.9	8 (6)	80 (60)	2.5
		10% YM178	8 (1)	80 (10)	2.0	8 (6)	80 (60)	2.6
10% YM178	10	Vehicle	0	0	0	0	0	0
		2% YM178	8 (2)	80 (20)	1.9	8 (5)	80 (50)	2.4
		10% YM178	9 (2)	90 (20)	2.1	9 (5)	90 (50)	2.6
DNCB	5	Vehicle	0	0	0	0	0	0
		DCNB	5	100	3.4	5	100	4.4

Numbers in parenthesize are the number of individuals with a sensitivity score of at least 3 as being positive instead of at least 2. Table adapted from sponsor's Table 1.

Study title: **A Skin Sensitization Study on YM178 in Guinea pigs by the Buehler Test**

Study no.:	178-TX-027
Study report location:	Module 4.2.3.7.1.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 18, 2002
GLP compliance:	Yes, (b) (4) protocols
QA statement:	Yes
Drug, lot #, and % purity:	YM178 (mirabegron), Lot K1780006, Purity 100.3%
Formulation/Vehicle:	Liquid paraffin was the solvent for mirabegron. Acetone was the solvent for the positive control, 2,4-Dinitrochlorobenzene (DNCB)(0.1%).
Dosing solution homogenization:	Dosing solution samples from the top, middle, and bottom were within 91-109% of the target

Key study findings:

- Mirabegron displayed Grade III moderate skin sensitization potential in Guinea pigs in both the 1% and 10% sensitization groups challenged with either 2% or 10% mirabegron.

Methods

Exposure: The exposure concentrations for sensitization and challenge were based on the positive findings in study 178-TX-024.

Species/strain: Three week old Hartley male guinea pigs (SPF) four weeks old at sensitization weighing 302-352 g.

Study design:

Number of Animals	Sensitization	Challenge	
		Right Side (one site)	Left Side (two sites)
10	Paraffin Solvent	Mirabegron 2.0%	Solvent, Mirabegron 10%
10	Mirabegron 1.0%	Mirabegron 2.0%	Solvent, Mirabegron 10%
10	Mirabegron 10%	Mirabegron 2.0%	Solvent, Mirabegron 10%
5	DNCB 0.1%	Acetone	DNCB 0.1%

Sensitization: The neck of the guiea pigs were shaven and used for the application site. Filter paper containing 0.1 mL of dosing solution (paraffin solvent, 1.0% mirabegron,

10% mirabegron or 0.1% DNCB) was applied to the shaven areas for 24 hrs. Exposure was repeated every other day (3 times a week) for three weeks accounting for a total of 9 exposures.

Challenge: 13 days after the final sensitization exposure, hair was shaved from two left and one right patch (2 cm^2) in the lateral abdomen. The right abdomen of each guinea pig was challenged acetone vehicle control for DNCB or 2% mirabegron for 24 hrs. The left abdomen of each guinea pig was challenged for 24 hrs at one site with 10% mirabegron and either paraffin solvent control or the positive control (DNCB) at the other site.

Clinical Signs: Guinea pigs were observed daily for clinical signs of toxicity. Necropsies were not conducted.

Body weight: Measured weekly

Skin reactions: Skin sites were assessed 24 and 48 hrs after challenge dose. Erythema, eschar, and edema were graded on a scale. The range of scores for erythema and eschar were 0 for non-detected to 4 for severe. The range of edema was 0 to 3 for severe edema. Intensity scores for edema, erythema, and eschar for an individual of at least two were required for a positive result. A cumulative grade for each dosage group was based upon the percentage of animals in each group with positive sensitization (table below).

Gradation of Skin Sensitization		
Positive Ratio %	Grade	Evaluation
0	-	Non
1-8	I	Weak
9-28	II	Mild
29-64	III	Moderate
65-80	IV	Strong
81-100	V	Extreme

Results:
Animals sensitized with vehicle did not respond to vehicle challenge. After vehicle sensitization and 2% or 10% mirabegron challenge only slight erythema was observed in 2/10 animals at the 24 hr time point suggesting that mirabegron causes a weak primary skin irritation.

Animals sensitized with 1% mirabegron did not respond to a vehicle challenge however a 2% or 10% mirabegron challenge resulted in 40% responders at both 24 and 48 hrs after challenge with a slight increase in mean response from 1.0 to 1.3 during that time interval. The increased response at 48 hrs was due to increased incidence of very slight grade edema.

Animals sensitized with 10% mirabegron did not respond to the vehicle control. Sensitization with 10% mirabegron and challenge with 2% or 10% mirabegron resulted

in 50% of the animals responding at both 24 and 48 hrs after the challenge dose. The mean response level also slightly increased between 24 and 48 hrs. The increase mean response at 48 hrs was due to the slight to moderate edema and increased severity of erythema and eschar. This was evaluated as a Grade III skin sensitization potential.

Scores following both 2% and 10% mirabegron challenges were indicative of a Grade III skin sensitization potential.

Sensitization with the positive control and challenge with vehicle resulted in no response but 100% response was observed when the guinea pigs were challenged with DCNB.

Summary of Skin Sensitivity to Mirabegron (YM178)

Sensitization	N	Challenge	24 Hrs After Challenge			48 Hrs After Challenge		
			No. Positive Animals	Percent Positive	Mean Response	No. Positive Animals	Percent Positive	Mean Response
Vehicle	10	Vehicle	0	0	0	0	0	0
		2% YM178	0	0	0.2	0	0	0
		10% YM178	0	0	0.2	0	0	0
1% YM178	10	Vehicle	0	0	0	0	0	0
		2% YM178	4	40	1.0	4	40	1.3
		10% YM178	4	40	1.0	4	40	1.3
10% YM178	10	Vehicle	0	0	0	0	0	0
		2% YM178	5	50	1.1	5	50	1.8
		10% YM178	5	50	1.3	5	50	2.0
DNCB	5	Vehicle	0	0	0	0	0	0
		DCNB	5	100	2.8	5	100	3.2

Table adapted from the sponsor's Table 1. For an individual animal, intensity scores for edema (moderate), erythema, and eschar (well defined) of at least two were required to be considered positive.

10.3 – Drug Substance Impurities

Three impurities were identified in the drug substance above the (b) (4) identification threshold in production batches. The concentration impurities anticipated by the sponsor in the drug substance are (b) (4) for (b) (4) (b) (4) for (b) (4) and not more than (NMT) (b) (4) for (b) (4) is an (b) (4) and was found at (b) (4) production batches. The sponsor's proposed specification for (b) (4) exceeds the qualification threshold of (b) (4) however the sponsor's specification was based on the mean plus the upper confidence limits for two batches according to ICH Q3A and ICH Q6A. From a toxicological perspective, the proposed specifications for drug related impurities in the drug substance are reasonable.

The proposed drug substance specifications for the drug related impurities are

(b) (4): NMT (b) (4)

(b) (4) or other Single Impurity: NMT

Total:

Proposed specification for the (b) (4)

(b) (4) (b) (4)): NMT

To investigate the safety of [redacted] (b) (4) the sponsor conducted a two-week oral dose toxicity study in male and female rats with mirabegron containing [redacted] (b) (4) of the impurity [redacted] (b) (4) (178-TX-046). The mirabegron doses were 0, 3, and 10 mg/kg (n=10/group). The 10 mg/kg dose of mirabegron was chosen because it was the NOAEL in the 2-week toxicology study with mirabegron (178-TX-013). No clear adverse effects were observed at 3 mg/kg and at 10 mg/kg only slight increase in fibrinogen (23%) in males and minor increases in AST (6%) and ALT (30%) in females, minimal reduction in adipocyte size in females and a slight increase in food intake in females were observed. The NOAEL was 3 mg/kg.

Theoretical Impurities that are Ames Positive

Four theoretical impurities were identified by CMC and the sponsor as being genotoxic due to positive Ames assay findings (Table below). [redacted] (b) (4) However, they are considered theoretical impurities because [redacted] (b) (4) of the threshold limit of 1.5 µg/day for genotoxic impurities in chronic use products assuming a 50 mg dose of mirabegron (Draft Guidance for Industry, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, 2008). The sponsor's proposed specification for these theoretical impurities in the drug substance is [redacted] (b) (4) which equates to [redacted] (b) (4) in a 50 mg dose of mirabegron. This is acceptable since the threshold dose for genotoxic impurities is 1.5 µg/day for chronic use products. The sponsor proposed to [redacted] (b) (4) The chemistry reviewer recommends continuous monitoring for these theoretical impurities since they are genotoxic. This is a reasonable proposal from a toxicological perspective.

Theoretical Impurities that were Ames Positive			
Code Name	Substance	Formula	Structure
[redacted] (b) (4)			

10.4 – In vitro Mirabegron Hemolysis Study

In order to support safety for intravenous administration, the potential of mirabegron to lyse red blood cells was assessed in human blood samples (178-TX-037). Mirabegron (10 mg/mL in 8.4% sucrose: 20mM citrate buffer) or control (saline solution) was mixed in a 10:1 ratio with human blood and incubated at 37°C for 30 minutes. Physiological saline was used as a negative control and water was used as a positive control. The solution was centrifuged and the grade of hemolysis was characterized based upon the color of the supernatant (clear- no hemolysis, pale red – weak hemolysis, red – hemolysis).

No hemolysis was observed in the physiological saline control or in the mirabegron samples. The placebo was weakly hemolytic in all samples and water was hemolytic in all samples. The weak hemolytic action of the placebo was considered to due to the low pH (3.0) while the mirabegron test solution had a pH of 5.1. Mirabegron was not hemolytic under these conditions.

Hemolysis Exposure Groups (n=5/group)	
Treatment	Hemolytic Grade
Mirabegron (10 mg/mL)	None
Placebo (vehicle control)	Weak in all samples
Physiological Saline (negative control)	None
Water (positive control)	Strong in all samples

10.5 Interference of Mirabegron with Detection of Protein in Urine

Elevated urinary protein was detected in rats in the 2- and 13-week toxicity studies in the 300 mg/kg group. The sponsor postulated that the findings in rats were false positive since adverse histopathology or other adverse urinary endpoints were not observed.

To address this, experiments were conducted with several urinary protein detection methods with mirabegron solutions devoid of protein (178-TX-049). The Multistix® dipstick assay that was used in the toxicity studies and the pyrogallo red-molybdate protein detection assays yielded false positive readings for protein at mirabegron concentrations ≥ 0.25 mg/mL (no detection at ≤ 0.1 mg/mL) even though the solutions were devoid of protein. The BM Test® dipstick test was slightly positive for protein at mirabegron concentrations of 2.5 mg/mL but not at ≤ 1 mg/mL. The heat coagulation and sulfosalicylic acid tests did not detect protein in solutions of mirabegron up to 2 mg/mL.

Because of concerns for inaccurate clinical analysis of urine protein levels with marketed dipstick tests, protein quantification was assessed in human urine by five different dipstick methods with urine spiked with mirabegron alone or mirabegron with the two most abundant metabolites (M11 and M12) (178-TX-058). Since the dipstick methods rely on changes in color or refraction as endpoints, a control using turbidity as the detection method was also used (P-Modulator). Mirabegron and its metabolites did

not cause false positive protein readings when using the turbidity method (P-modulator) (Table below). Mirabegron spiked urine produced false positive readings for protein with all dipstick methods when urine was spiked at 0.1 to 1.0 mg/mL (no false detection at ≤ 0.05 mg/mL) (See table below). The addition of M11 and M12 had no effect except with the Combur[9] Test Strips were the minimal concentration of mirabegron needed for a false positive reading was reduced from 0.1 to 0.005 mg/mL however the false positive reading was weak until reaching a 0.5 mg/mL concentration of mirabegron. Mirabegron falsely exaggerated the level of protein in albumin spiked urine samples when detected by three dipstick methods at mirabegron concentrations ≥ 0.25 to 1 mg/mL.

Dipstick Proteinuria Analysis – Effect of Addition of Mirabegron (YM178) alone, Mirabegron + M11 and M12 or Enhanced Detection of Albumin in Presence of Mirabegron + M11 and M12				
		False Positive Protein Detection YM178 LOEL mg/mL (no protein added to urine)		Amplified Albumin Detection in Albumin Spiked Urine (YM178 LOEL mg/mL)
Proteinuria Product/Technique	Detection Method	YM178	YM178 + M11, M12	YM178 + M11, M12 & Albumin
Urifelt S (A Menari Diagnostices)	Refractometry	0.1	0.1	0.25
URISYS 2400 Cassette (Roche)	Refractometry	1.0	1.0	1.0
Combur[9] Test Strips (Roche)	Visual	0.1	0.005	-
Clinitck Atlas 10 (SIEMENS Inc.)	Refractometry	0.25	0.5	-
SIEMENS Multistix (SIEMENS Inc.)	Visual	0.25	0.1	0.25
P-Modulator (Roche)	Turbidity	None	None	No increase
Column 1: 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, or 2 mg/mL YM178 in human urine Column 2: 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, or 2 mg/mL YM178 + 1/10 M11 and M12 Column 3: Same as column 2 but also added 0.5 mg/mL of albumin to all test solutions				

False positive proteinuria findings in humans are not anticipated to be a substantial issue since proteinuria was observed in only 2.1-2.7% of the mirabegron subjects at 25 to 100 mg doses compared to 1.9% of the placebo subjects in the global 12-week Phase 2 and Phase 3 populations (Sponsor's Integrated Summary of Safety, Table 5.3.2). Additionally these in vitro studies suggest that there is minimal chance for mirabegron in urine to incorrectly inflate the protein levels. In humans administered a 160 mg dose of radiolabeled mirabegron, 25% of the dose was eliminated in urine unchanged (178-CL-007). The highest concentration of mirabegron in vitro that did not interfere with any dip stick urinalysis test was 0.05 mg/mL and the lowest concentration that interfered with protein analysis was 0.1 mg/mL. So assuming that 25% of a 50 mg mirabegron dose was eliminated in urine (12.5 mg), the maximal volume of urine necessary to interfere with the urinalysis tests would be between 125 mL and 250 mL (12.5 mg divided by 0.1 mg/mL or 0.05 mg/mL).

Alternatively the greatest amount of mirabegron was eliminated in humans during the first six hour following dosing (11.3% of dose eliminated in urine as unchanged mirabegron) (178-CL-007). This equates to 5.6 mg eliminated over the first six hours after a 50 mg dose. The maximal volume of urine necessary to interfere with the

urinalysis tests would be between 56 mL and 112 mL (5.6 mg divided by 0.1 mg/mL or 0.25 mg/mL). Because humans usually void much higher volumes over a six hour period, there does not appear to be a large potential for mirabegron in urine to elevate urine protein levels inappropriately.

11 Integrated Summary and Safety Evaluation

11.1 Pharmacology

Beta Adrenergic Receptor Selectivity
Mirabegron (YM178) a beta-3 adrenergic (β3-AR) agonist indicated for the treatment of overactive bladder, is a new molecular entity and first in its class for this indication. In vitro data suggests that mirabegron is primarily a β3-AR agonist with minimal β1-AR and essentially little to no β2-AR activity in animals and humans. However, animal models and a clinical study suggest that mirabegron has β1-AR activity in vivo. Humans administered a single 200 mg dose of mirabegron responded with elevated heart rate and systolic blood pressure, findings that were both repressed by co-administration with propanolol (β1/β2 antagonist) or bisoprolol (β1 antagonist) suggesting that mirabegron activates β1 receptors in humans (Study 178-CL-053).

Primary Pharmacology
The urinary bladder detrusor muscle responds to cholinergic stimulation by contracting while adrenergic stimulation decreases the detrusor contraction. Mirabegron is intended to alleviate overactive bladder symptoms by agonist activation of the β3-AR resulting in relaxation of the bladder detrusor muscle.

Animal models demonstrated that mirabegron can promote increases in bladder capacity and reduce urinary bladder contractions. Mirabegron was able to reduce pre-constricted isolated rat detrusor muscle, reduced distension induced bladder contractions in rats, reduced bladder contractions in a model of bladder outlet obstruction in rats, increase bladder capacity in water loaded rats and monkeys, and reduced intravesical pressure in dogs.

Absorption, Distribution, Metabolism, and Excretion
Absorption: Mirabegron was readily absorbed in rats, dogs, and humans. Bioavailability increased with dose in rats (23-76%), dogs (42-77%), and humans (24-45%). Absorption in rats occurred primarily from the small intestines and less from the colon and stomach. In vitro studies demonstrated that mirabegron binds to plasma proteins (73-79%) in mice, rats, and humans.

Distribution: Tissue distribution was assessed in albino mice after ^{14}C-mirabegron dosing. Compared to plasma levels, the highest levels of radioactivity were observed in the liver (20x), small intestine (14x), kidneys (11x), adrenals (7x), pituitary (7x), thyroid (6x), stomach (5x), cecum (4x), lungs (4x), pancreas (4x), large intestine (3x), spleen (3x), submaxillary glands (3x), bone marrow (3x), heart (2x), testes (2x), skin (1x), and fat (1x). The rank order of tissues with radioactivity less than the plasma level include the thymus, skeletal muscle, blood, eye, cerebellum, and cerebrum. Fifteen days after dosing, greater than 40% of the maximal radiolabel remained in the thyroid, kidney, testes, and bone marrow, suggestive of slow elimination from these organs. Accumulation of mirabegron and some of its metabolites in pigmented tissues is

predicted due to an apparent association with melanin in the eyes of pigmented rats. The half-life of drug related radioactivity was 157 days in the eyes of pigmented rats. Placental and lactational transfer was confirmed in rats dosed orally with ^{14}C-mirabegron.

Metabolism: In vitro studies suggest that mirabegron is primarily metabolized by CYP3A4 and to a lesser extent by CYP2D6. In plasma, mirabegron was hydrolyzed principally by the esterase butyrylcholinesterase. A total of 18 metabolites were identified in plasma, bile, urine, or feces of mice, rats, rabbits, monkeys, and humans. Eight of the metabolites were observed in human plasma and an additional two were observed in human urine, none of which were unique to humans. The metabolite exposure in humans was exceeded by that in at least one species in the chronic toxicity, carcinogenicity, and reproductive toxicity assays. All metabolites are considered qualified.

Elimination: In rats, mirabegron and metabolites were eliminated primarily in feces (73%) with less in urine (16%). Enterohepatic circulation was confirmed in rats. In monkeys radioactivity was excreted equally in urine and feces within 72 hrs of ^{14}C-mirabegron dosing. In humans receiving a 160 mg dose of ^{14}C-mirabegron, 55% and 34% of the radioactivity was recovered in urine and feces, respectively.

11.2 Toxicology

Summary of Toxicology

Toxicities observed in nonclinical studies were generally those expected of a β1/β3-AR agonist with the exception of hepatotoxicity, skin irritation and sensitization, and reproductive and developmental toxicity. Principle toxicities include, but are not limited to, hepatotoxicity, effects on body weight and metabolism, salivary gland toxicity, cardiovascular toxicity, and developmental effects.

Principle Nonclinical Toxicology Studies Conducted

The sponsor has conducted nonclinical safety pharmacology studies, local tolerance assessments, repeat dose toxicity studies in dogs (2–weeks), mice (2- and 13-weeks), rats (2-, 13-, and 26–weeks), monkeys (2-, 13-, and, 52 weeks), reproductive and developmental toxicity studies (fertility in male and female rats, embryo/fetal development in rats and rabbits, and pre- and postnatal development in rats), genotoxicity studies (AMES, in vitro chromosomal aberration, and in vivo micronuclei in rats), and two-year carcinogenicity studies in mice and rats. The sponsor also investigated in vivo mechanisms of developmental and cardiac toxicities.

The species used were generally acceptable. However, the following factors should be kept in mind. Weight loss at high doses or following prolonged exposure periods is an expected pharmacologic response in rodents but not expected in humans. Monkeys were not assessed at large multiples of the human exposure in the chronic toxicity study because of dose limiting ECG findings. Dogs were apparently not pursued as a model

species based on expected pharmacologic responses including severe adverse findings in the salivary glands after a few day of exposure, and adverse ECG findings.

Multiples of the clinical exposures in animals discussed below are derived by dividing the exposure in animals (AUC or Cmax) by the systemic exposure in fasted women at least 55 years old at the maximum recommended human dose (MRHD = 50 mg/day, AUC = 512 ng-hr/mL, Cmax = 66.3 ng/mL, Study 178-CL-072). With respect to age, sex, and fed state, this subpopulation was chosen because it was the subpopulation with the greatest steady state exposure (Studies 178-CL-072 and 178-CL-041).

11.2.1 Cardiac Toxicity

Pharmacology
General cardiac safety pharmacology was assessed in rats, rabbits, dogs, and monkeys; and mechanistic studies were conducted in rats, dogs, and rabbits to evaluate potential mechanisms related to increases in heart rate induced by mirabegron. To assess proarrhythmic risk of mirabegron and its five major metabolites, the sponsor conducted cardiac ion channel assays, hERG assays, isolated cardiac papillary muscle action potential assays, and canine ventricular wedge assays.

The in vitro studies suggest that there is little potential for mirabegron or its principle metabolites to impair cardiac ion channel activity, alter cardiac action potential, or prolong QT interval at clinically relevant exposures. Mechanistic studies in rats and dogs suggest that the increased heart rate at low doses of mirabegron is a compensatory response to vasodilating effects of β3-AR stimulation (reflex tachycardia). However, these studies also suggest that elevated heart rate at high doses of mirabegron is the result of direct chronotropic effects of off target β1-AR activation. Cardiac toxicities in pharmacology studies are discussed in the context of the toxicity assessment below.

Cardiovascular Toxicity
Potential Cardiac Findings near the Clinical Exposure:
Elevated heart rate was observed in rats after IV dosing at exposures <2x MRHD and after oral dosing in dogs at exposures ≥ 0.1x MRHD, rabbits at ≥ 9x MRHD, and monkeys at 12x MRHD. Systolic and mean **blood pressure was reduced** after oral dosing in dogs at 0.1x MRHD but not in monkeys at exposures up to 51x MRHD. PR interval was shortened after oral dosing in dogs at exposures ≥ 1x MRHD but prolonged in monkeys at exposures ≥ 11 x MRHD.

Severe Cardiac Findings at Super Therapeutic Exposures:
Ventricular tachycardia was observed after oral dosing in dogs (at the lethal dose, 20 mg/kg 29x MRHD), and monkeys at ≥ 37x MRHD. QRS was slightly prolonged in monkeys at exposures ≥ 17-23x MRHD. QTc was not clearly effected in dogs at exposures up to 32 MRHD (Matsunaga's correction) or monkeys at exposures up to 51x MRHD (Bazett's and Fridericia's correction). Clear drug related adverse cardiac histopathology was not reported in the toxicology studies.

Intravenous dosing generally resulted in similar but more severe cardiac findings in rats, dogs, and monkeys. For instance one male monkey at 3 mg/kg IV (13x MRHD based on Cmax) went into coma after the third dose although he later recovered. Additionally one monkey went into ventricular tachycardia and died within 15 minutes of administrating a 10 mg/kg IV dose (114x MRHD based on Cmax). Similarly intravenous dosing in dogs led to death at IV doses ≥ 10 mg/kg due to ventricular tachycardia that progressed to ventricular fibrillation within 5-10 minutes.

These findings suggest that mirabegron can reduce blood pressure in some species and increase heart rate at near clinical exposure levels, and promote tachycardia and death at extremely high exposures which is expected pharmacology for a mixed β-adrenergic agonist. These studies in conjunction with the in vitro assays also suggest that there is little potential for QTc prolongation in animals.

11.2.2 CNS Toxicity

Adverse signs suggestive of CNS toxicity which were observed at or near clinical exposures include lacrimation and prone position at ≥ 0.2x MRHD and salvation at ≥ 2x MRHD in rats: and prone position with slight hyperthermia (≥ 2x MRHD) in mice.

Adverse signs of CNS toxicity which were observed at or near the lethal exposures include the following:

- Rats - deep respiration, reduced grip strength, closed eyes, and decreased body and abdominal tone, clonic convulsions, mydriasis, and tachypnea (45-160x MRHD)
- Mice - decreased alertness, spontaneous movement, and muscle tone (29x MRHD), and colonic convulsions (63-72x MRHD)
- Rabbits - hyperpnea, tremor, dyspnea, and tonic convulsions (> 21x MRHD)
- Monkeys - ptosis, decreased motor activity, and staggering (8x MRHD), and vomiting and ventricular tachycardia (29-34 MRHD).

Since exposure to the brain was low in rats and monkeys and the findings in animals were reversible at sublethal doses and without clear adverse histopathology in the toxicology studies, there does not appear to be a significant toxicological concern for mirabegron-induced CNS toxicity at the clinical exposure level.

11.2.3 Hepatotoxicity

Hepatotoxicity was observed in rodents and dogs but not monkeys. The liver was the organ with the greatest exposure to radiolabeled mirabegron with levels reaching 20 times the plasma concentration in rats. Similarly in monkeys the liver was the organ with the greatest concentration of radioactivity when assessed seven days after dosing with ^{14}C-mirabegron.

Hepatic findings in mice were likely only of toxicological relevance at the lethal dose.

Minimal to mild increase in hepatic glycogen content accompanied by increased food consumption and body weight was observed at exposures ≥ 10x MRHD in the 13-week study. Although minimal hepatocyte hypertrophy was observed in both sexes at the lethal exposure (48x MRHD) in mice after 13 weeks, liver weights and LFTs were not affected and findings of hepatocyte hypertrophy were not dose-dependent in the 2-year mouse carcinogenicity study at the highest dose administered (21x MRHD).

In rats, slight elevations (< 2 fold) in ALT, ALP and cholesterol were observed in the 2-, 13-, and 26-week studies, with slightly elevated AST and decreased triglycerides also observed in the 13- and 26-week studies. The minimal dose necessary to elevate LFTs actually increased with continued exposure and ranged from 4-6x MRHD after two weeks of dosing, 10-44x MRHD after 13 weeks, and 17-55x MRHD after 26 weeks of dosing. Histological evaluation after 13 weeks revealed pigment deposition at exposures 44-50x MRHD, and hepatocyte swelling, fibrosis, and one rat with moderate centrilobular necrosis at the lethal dose (130-160x MRHD). The only hepatic finding after 26 weeks of dosing at exposures up to 55-59x MRHD was eosinophilic hepatocytes at exposures ≥ 12-17 MRHD. The significance of these findings is questionable since adverse hepatic histopathology was not observed in the 2-year rat carcinogenicity study at the highest doses tested in males (25x MRHD) or females (45x MRHD).

Although hepatotoxicity was not reported in the two-week dog toxicology study at the lethal dose of 20 mg/kg (16x MRHD), reversible hepatotoxicities were observed in a follow up study at the same dose but with higher exposures (25x MRHD) after three days of dosing. These findings include enlarged and yellow discolored livers with mild to moderate hepatocyte hypertrophy and hepatic vacuolation, slight glycogen accumulation, and mild deposition of lipid in peri- and centrilobular hepatocytes. Concurrent control group was not included but it appears that ALT, ALP, and AST may have been slightly elevated.

Hepatotoxicity was not observed in monkeys dosed for one year at exposures up to 8 times exposure at the MRHD.

In summary, since hepatotoxicity was limited to rodents and dogs, was reversible, and did not cause adverse histopathology except at or near the lethal dose with large multiples of the clinical exposure, the potential for hepatotoxicity at the maximum recommended dose in humans appears low.

11.2.4 Effects on Body Weight and Adipocytes

Indicative of increased lipolysis, lipid levels in brown and/or white adipocytes decreased in mice, rats, and potentially in monkeys after 2 to 13 weeks of dosing at or near the expected clinical exposure level. This was generally accompanied by increased food consumption and decreased body weight in rodents but not in monkeys. The findings in monkeys were not clearly treatment related due to the low incidence and lack of progression with continued exposure. In the two clinical studies were it was assessed,

body weight was not effected when dosing exceeding the MRHD for 12 weeks including a 10 week period at 200 mg/day (Studies 178-CL-003 and 178-CL-004).

11.2.5 Thymic Atrophy

Thymic atrophy of slight to minimal intensity was observed in mice at exposures ≥ 14x MRHD after 13 weeks, and in rats at exposures ≥ 50-156x MRHD after 2 and 13 weeks of dosing. However, thymic atrophy was not reported in the two-year carcinogenicity studies in mice or rats at exposures exceeding 20x MRHD. Additionally, thymic weight and atrophy were not clearly affected at the maximal dose in the 2-week dog toxicology study at exposures up to 16x MRHD, or at the maximal exposures in the 2-week (29-34x MRHD), 13-week (6-9x MRHD), or 52-week (8X MRDH) monkey toxicology studies.

Since thymus findings were only observed in rodents in subchronic toxicity studies at large multiples of the anticipated human exposure, thymic toxicities do not appear to be a safety concern for humans.

11.2.6 Reproductive Organ Findings

Mirabegron-induced toxicity to male or female reproductive tissues is not expected in humans. Clear adverse findings were not reported in reproductive tissues at the maximal dose in dogs after 2 weeks at 16x MRHD, in mice after 2 years at 21x MRHD, or in monkeys after 52 weeks at 8x MRHD.

Concern about potential reproductive toxicities in female rats (slight atrophy of the uterus at exposures ≥ 31-44x MRHD and decreased ovarian and uterine weights at exposures 44-156x MRHD) is of low concern because they occurred at high exposures in the 2- and 13-week studies, were not observed after 6 months or more of dosing at exposures up to 45x MRHD, and were reversible.

Distribution studies suggest that there may be potential for drug accumulation in the testes of rats. However, adverse testicular findings were not apparent in repeat-dose studies. Although reduced prostate weight at 136x MRHD and seminal vesicle weights at exposures ≥ 45x MRHD were observed after 2 and/or 13 weeks of dosing, they were secondary to reduced body weight, were reversible, and without histological correlates other than reduced seminal vesicle secretions at exposures ≥ 45x MRHD in the 2-week toxicology study. Also, male reproductive toxicities were not apparent in rats at the maximal doses in the 26-week study at 55x MRHD and 2-year carcinogenicity study at 25x MRHD.

11.2.7 Kidney

Questionable kidney pathology was reported in rats at very high doses but not at the maximal dose in dogs after 2 weeks of dosing at 16x MRHD; in mice after 2-weeks at exposures 98-123x MRHD, 13-weeks at exposures 48-51x MRHD, and 2 years at exposures 21-25x MRHD; or in monkeys after 2 week at exposures 29-34x MRHD, 13 weeks at exposures 6-9x MRHD, and 52 weeks at exposures 8x MRHD.

In rats, mirabegron decreased urine excretion (expected pharmacology) after a single dose at 1x MRHD and after 13 weeks of dosing at exposures ≥ 12x MRHD. Serum urea and creatinine were not affected in rats but potassium was slightly elevated (9-18%) after exposures for 2, 13, and 26 weeks at exposures ≥ 2-12x MRHD. Although slight to moderate lipoprotein (lipofuscin) deposition in the renal tubule epithelia was observed in rats after 13 weeks of dosing at exposures 50-130x MRHD and urinary bladder stones at 160x MRHD, adverse renal pathology was not reported after 26 weeks at 55x MRHD or 2 years of dosing at exposures up to 25-45x MRHD.

Since potential renal toxicities were only reported at high multiples of the clinical exposure in a single species without obvious effects on relevant renal biomarkers, these findings suggest that renal toxicity is not a significant safety concern for humans at the MRHD.

11.2.8 Ocular

Studies in pigmented and albino rats demonstrated that mirabegron and some of its metabolites have a long half-life in the eye due to their apparent association with melanin. The half-life of radioactivity in the eye of pigmented rats dosed with ^{4}C-mirabegron was 157 days. Radioactivity was distributed to the ciliary body, choroid, and conjunctiva of the eye at high levels while medium levels were observed in the iris and trace levels in the vitreous body. Similarly, the eye was the tissue with the third highest amount of radioactivity one week after oral administration of ^{14}C-mirabegron in monkeys.

Despite the potential for accumulation of drug in the eye, ocular toxicity was not apparent in repeat-dose studies in rats, dogs, and monkeys. Intraocular pressure was not assessed but clearly adverse histology and ophthalmoscopic findings were not observed in dogs after 2 weeks of dosing at exposures up to 15-16x MRHD, in rats after 26 weeks of dosing at exposures up to 55-59x MRHD or in monkeys for up to 52 weeks at to exposures up to 8x MRHD. Adverse dose related ocular histology was also not clearly observed in the 2-year rat and mouse carcinogenicity studies.

11.2.9 Salivary/Lacrimal Glands

Salivary gland secretion is stimulated by adrenergic and cholinergic agonists (10 and 11). Acetylcholine and alpha-1 adrenergic signaling primarily stimulate fluid secretion and beta adrenergic agonists promote release of protein storage granules and salivary proteins from acinar and ductal cells. Isoproterenol, a nonselective β-adrenergic agonist, induces salivary gland enlargement in rats via cell proliferation and hypertrophy theoretically via β1-AR and β2-AR stimulation (12). Enlargement of salivary glands was not reported in mirabegron treated mice, rats, dogs, or monkeys. In addition to salivation, lacrimation is expected as a result of off target agonism of the α_{1D} adrenergic and beta adrenergic receptors (6-9).

As expected pharmacology of a β1/β2 agonist, mirabegron promoted salivation and lacrimation in rats and promoted atrophy of the secretory cells in the salivary glands in mice and rats after 13 weeks, and dogs after 2 weeks at exposures near the clinical levels or the lowest dose evaluated. Findings in dogs after three days of dosing at exposures 25x MRHD (lethal dose) were especially adverse including hemorrhaging, atrophy, and necrosis of the salivary gland acinar and ductal cells. Salivation and lacrimation in rats increased with dose and duration of exposure beginning after at least 2 weeks of exposure. However, the significance of these findings is less concerning since adverse pathology was not reported in mice at exposures 21 to 25x MRHD or rats at 25 to 45x MRHD after two years of exposure, or in monkeys after a year of exposure at 8x MRHD. Additionally, the findings were generally recoverable or partially recoverable after drug withdrawal.

11.3 Principle Reproductive and Developmental Toxicity Findings

Reproductive and developmental toxicity studies assessed fertility in male and female rats, embryo/fetal development in rats and rabbits, and pre- and postnatal development in rats.

Effects on male and female fertility in rats are considered likely to be secondary to overt toxicity. No adverse effects on female fertility were observed in rats at exposures 22x MRHD. However, in female rats at exposures 96x MRHD, maternal body weight and food consumption decreased while the diestrus period was prolonged and the mean number of corporal lutea, implantations, and live fetuses were all slightly reduced. No adverse effects on male mating and fertility were observed in male rats exposed at up to 77x MRHD. However, at exposures that were lethal to 14/20 male rats (171x MRHD), only 3/6 of the surviving male rats were able to impregnate their non-mirabegron treated mate. The reduced male fertility is likely due to overt toxicity and unrelated to any drug-related effect on reproductive tissues.

Fetal exposure was confirmed in rats during gestation at levels slightly less than the maternal exposure. Effects on rat fetuses due to in utero exposure were not observed at exposures up to 6x MRHD. Adverse fetal findings at greater exposures include wavy ribs and slight decrease in ossification of the metatarsi at exposures ≥ 22x MRHD and sternebrae and vertebrae at 96x MRDH, along with decreased fetal weight and bone malformations at 96x MRHD. However, decreases in ossification and fetal weight were reversible and occurred only at exposures that caused maternal weight loss (≥ 22x MRHD) or death (96x MRHD). In rabbits, no adverse fetal effects were observed after in utero exposure to clinically relevant exposures and the only clear adverse effect observed at exposures 14x MRHD was a slight, 10-12%, decrease in fetal weight without other correlating fetal toxicities. At exposures 36x MRHD, post-implantation loss, fetal death, reduced fetal body weight, cardiomegaly, dilated aorta, fusion of the sternebrae, and a slight delay in ossification of some digits were observed. It is unclear if the malformed and delayed development of bone in rats and rabbits, and cardiomegaly and dilated aorta in rabbits are direct effects of mirabegron on the fetal tissues or if they are related to overt maternal toxicity and effects on maternal cardiac

function. Pregnancy in rabbits but not rats appeared to elevate maternal exposure to mirabegron roughly two fold. The effect of pregnancy on human pharmacokinetics is unknown.

Despite the demonstration of maternal transfer of ^{14}C-mirabegron to pups via lactation, in utero and lactational exposure to mirabegron had no discernible adverse developmental effects at exposures 6x MRHD. However, at 22x MRHD deaths of pups increased slightly during the first four days after birth and pup weight decreased 6-18% in the post weaning period which was recoverable in females only. In utero and lactational exposure did not affect behavior or fertility of the offspring.

Overall, mirabegron is not anticipated to significantly increase the risk of adverse reproductive and developmental outcomes in humans when used in accordance with the recommended dosing schedule.

11.4 Carcinogenicity

Nonclinical data suggest that mirabegron is neither a genotoxin nor a carcinogen. Mirabegron related neoplasms were not apparent after two years of daily oral dosing in mice who were exposed up to 21-25 times the MRHD or rats who were exposed up to 25-45 times the MRHD. Additionally mirabegron related neoplasms were not reported in monkeys dosed for one year at up to 8 times the MRHD.

In humans, the incidence of neoplasms (all types combined) in mirabegron treated subjects was elevated at the 100 mg dose (11/820) compared to the 50 mg dose (1/812) and to an active comparator group (4/812) during a one year study (178-CL-049). However, the sponsor discounts these findings because in their view mirabegron was not genotoxic or carcinogenicity to rodents, the duration of the clinical exposure was too short of a latency period, incidence did not increase with continued clinical exposure, tumor types were diverse in humans, and the incidence of malignancies was not different from that in the general population when adjusted for age.

A clinical oncology consult was requested to evaluate the significance of the neoplasms in humans. The clinical oncology consult concluded, that, “The increased number of neoplasms in the mirabegron 100 mg group is of concern” and “While a signal was not evident in the mirabegron 50 mg cohort, given the study size, the consultants cannot rule out an increased risk for the development and or detection of neoplasm.“

A limited amount of published data in animal and cell culture models suggests that sustained beta adrenergic stimulation may act to promote growth and metastasis of neoplasms (25-37). However, these nonclinical findings are not sufficient to form conclusions regarding the relevance to this theory to humans. Furthermore, the relevance of this theoretical mechanism of tumor promotion to mirabegron is unknown.

If mirabegron were a potent tumor promoter, natural background neoplasms in the rodent carcinogenicity studies would likely have been more aggressive and more easily detected resulting in an earlier onset and greater incidence of neoplasm. This was not

observed. From a nonclinical perspective it should be kept in mind that although rodent carcinogenicity studies are useful, they are not always predictive of findings in humans (38).

11.5 OVERALL CONCLUSION

Nonclinical data suggest that effects at exposures relative to the clinical dose proposed for marketing may be limited to expected pharmacology including, decreased frequency of urination, decreased blood pressure, increased heart rate, and potential increases in salivation and lacrimation. At exposures well in excess of the clinical exposure the following toxicities may be predicted in humans: acute CNS toxicity, hepatotoxicity, impairment of cardiac function including ventricular tachycardia, and reproductive and developmental toxicities. Nonclinical data suggest that mirabegron is not genotoxic or carcinogenic.

Overall the nonclinical program supports approval of this product in proposed population and indication and a maximum daily dose of 50 mg.

5 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

Appendix B – Executive Carcinogenesis Assessment Committee Meeting Minutes

EXECUTIVE CAC MEETING MINUTES

Date of Meeting: March 15, 2011

Committee: David Jacobson Kram, Ph.D., DABT, OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
Al DeFelice, Ph.D., DCaRP, Alternate Member
Lynnda Reid, Ph.D., DRUP Supervisor
Eric Andreasen, Ph.D., DRUP Presenting Reviewer

Author of Minutes: Eric Andreasen

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

IND 69,416
Drug Name: Mirabegron (YM178)
Sponsor: Astellas Pharma Global Development, Inc.

Background:
YM178 is a new molecular entity that is being developed by the Sponsor for treatment of overactive bladder. YM178 is a β3 adrenergic agonist with some cross activity with the β1 adrenergic receptor. The proposed mechanism of action of YM178 is to relax the contraction of the detrusor muscle in the urinary bladder. YM178 has been investigated in 41 clinical studies to date with nearly 6,000 subjects receiving YM178 in Phase 2 or Phase 3 studies.

Two Year Mouse Carcinogenicity Study
Five to six week old B6C3F1 mice were dosed daily with YM178 in 0.5% methylcellulose by gavage for two years at 0, 25, 50 or 100 mg/kg. There were 70 animals per sex and dose group. Appropriate multiples of the maximal clinical exposure were achieved in both males (4, 9 and 25) and females (5, 9 and 21) based upon AUC and a 50 mg clinical dose.

Survival was not affected by YM178. Survival was between 73% and 89% in all groups independent of sex. At the terminal sacrifice, body weight gains were reduced 8%, 19% and 21% in low to high dose males and 22%, 28% and 31% in the low to high dose females compared to controls. However, this is the expected pharmacology of a β3 adrenergic agonist (induces lipolysis in white and brown fat). Food intake was dramatically increased in all YM178 groups beginning during the first week of dosing throughout the dosing period in both males and females. This suggests that the MTD was not exceeded.

The only statistically significant finding was an increased incidence of hepatocellular adenomas and hepatocellular adenomas or carcinomas, combined, in females in the low dose group (16% adenomas) compared to the control group (1% adenomas) ($p = 0.0017$, FDA statistical reviewer). The incidence was inversely related to dose and no statistically significant increased incidences

of hepatocellular adenomas or adenomas or carcinomas (combined) were seen in male mice or in rats of either sex.

Two Year Rat Carcinogenicity Study
Six to seven week old CDF F344/DuCrl rats were dosed daily with YM178 in 0.5% methylcellulose by gavage for two years. Male rats were dosed with YM178 at 0, 12.5, 25 or 50 mg/kg and females were dosed with YM178 at 0, 25, 50 or 100 mg/kg. Appropriate multiples of the maximal anticipated clinical exposure were achieved for both males (7, 12 and 25) and females (11, 24 and 45) based upon AUC and a 50 mg clinical dose. There were 60 animals per sex and dose group.

Survival was acceptable in all groups but there was a statistically significant decrease in survival in females in the high does group (40%) compared to the control group (73%) in pairwise comparisons. In addition to the decreased survival of females in the high dose group, the time to death appeared dose dependent, suggesting that the MTD was exceeded in this group.

The incidence of endometrial stromal polyps (uterus and cervix data combined) was numerically increased in the low dose group (22%, 12 times the clinical AUC) compared to the control (12%). The incidences of endometrial stromal polyps and of endometrial stromal polyps or endometrial stromal sarcomas, combined, were not statistically significant by CDER criteria.

Executive CAC Recommendations and Conclusions:

Mouse:
- The Committee agreed that the mouse carcinogenicity study was adequate. Concurrence of the protocol by the FDA on 8/17/2004 was noted.

- The Committee concluded that there was no clear evidence that YM178 caused an increased incidence of neoplasms in mice.

Rats:
- The Committee agreed that the rat carcinogenicity study was adequate. Concurrence of the protocol by the FDA on 8/17/2004 was noted.

- The Committee concurred that there were no drug-related neoplasms.

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

cc:\
/Lynda Reid, DRUP
/Eric Andreasen, DRUP
/Freshnie Deguia, DRUP
/ASeifried, OND IO

Reference ID: 2919082

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/s/

ADELE S SEIFRIED
03/16/2011

DAVID JACOBSON KRAM
03/16/2011

Appendix C - References

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/s/

ERIC A ANDREASEN
04/11/2012

LYNNDA L REID
04/12/2012
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 202-611

NDA Number: 202-611	**Applicant: Astellas Pharma US, Inc.**	**Stamp Date: 8-29-11**
Drug Name: Mirabegron	**NDA Type: 505(b)(1)**	

Nonclinical Filing Status Recommendation

The nonclinical body of data in the NDA appears adequate to support filing of the NDA. Requests for further nonclinical investigations are not anticipated at this time.

Currently, there are no nonclinical requests for Advisory Committee input, issues that may warrant postmarketing safety activity, need for consultant review, or need for nonclinical inspections.

Brief Summary of the Nonclinical Program

Beta Adrenergic Receptor Selectivity
Mirabegron (YM178) is a beta 3 adrenergic agonist and is the first in its class. In vitro data suggests that YM178 is primarily a β3 agonist with minimal β1 and essentially no β2 activity in animals and humans. However, animal models and a clinical study suggest that YM178 has clinically relevant β1-AR activity in vivo. Humans administered a single 200 mg dose of YM178 responded with elevated heart rate and systolic blood pressure findings that were both repressed by co-administration with propanolol (β1/β2 antagonist) or bisoprolol (β1 antagonist) suggesting that YM178 activates β1 receptors in humans (Study 178-CL-053).

Principle Nonclinical Studies Conducted
The Sponsor has conducted nonclinical safety studies, local tolerance assessments, in vitro metabolism, ADME, repeat dose toxicity studies (dogs 2 weeks, mice 13 weeks, rats 26 weeks, monkeys 52 weeks), reproductive and developmental toxicity studies (fertility in male and female rats, embryo/fetal development in rats and rabbits, and pre- and postnatal development in rats), genotoxicity studies (AMES, in vitro chromosomal aberration and in vivo micronuclei in rats) and two year carcinogenicity studies (mice and rats). Since the end of phase two meeting the Sponsor investigated in vivo metabolism (mice, rats, monkeys and rabbits), carcinogenicity (2-years mice and rats), and mechanisms of developmental and cardiac toxicities.

Safety Pharmacology
CNS – rat, dogs and monkeys
Cardiovascular – dogs, rabbits, rats, monkeys and in vitro studies
Respiratory - monkey

ADME
In vitro metabolism – rat, dog, monkey and human
Extensive metabolite assessment at steady state – mice, rats, rabbits, monkeys and humans
ADME – rats (pigmented and albino) and monkeys
Placental transfer – rats
Transfer to milk - rats

Toxicology - Multiple Oral Doses
Dogs – 2 weeks
Mice – 2 and 13 weeks
Rats – 2, 13 and 26 weeks
Monkeys – 2, 13 and 52 weeks

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 202-611

Reproductive and Developmental Toxicology
Fertility and early embryonic development – rats (male and female)
Embryo-fetal development – rats and rabbits
Pre- postnatal development - rats

Carcinogenicity
Mouse – 2 years
Rat – 2 years

Genotoxicity
In vitro – Reverse mutation assay in bacteria
- Chromosomal aberration assay in human lymphocytes
In vivo – Bone marrow micronuclei in rats

Other Toxicology Studies
Local tolerance – dermal irritation in rabbits
- eye irritation in rabbits
- intravascular irritation in rabbits
Dermal sensitization – guinea pigs

Toxicology
Toxicities observed in nonclinical studies were generally those expected of a β1/β3-AR agonist and include, but are not limited to, hepatotoxicity, effects on body weight and metabolism, cardiovascular toxicity and reproductive/developmental effects. The cardiovascular and developmental toxicities remain the most concerning toxicities. The Sponsor has implied that these toxicities are not applicable to humans since they were believed to be mediated through the β1-AR in animals and YM178 does not appreciably bind or activate the human β1-AR in vitro. However, their clinical data suggests that YM178 does activate the β1-ARs in humans and therefore these cardiovascular and reproductive findings should be applicable to humans.

Carcinogenicity
In humans, the incidence of neoplasms (all types combined) in YM178 treated subjects was elevated compared to an active comparator group during a one year study. However, the Sponsor discounts these findings because in their view YM178 was not genotoxic or carcinogenicity to rodents, the duration of the clinical exposure was too short of a latency period, incidence did not increase with continued clinical exposure, tumor types were diverse in humans, and the incidence of malignancies was not different from that in the general population when adjusted for age.

The Sponsor's nonclinical data suggest that YM178 is neither a genotoxin nor a carcinogen. YM178 related neoplasms were not apparent in the two-year rat and mouse carcinogenicity studies. However, there is a theoretical potential for YM178 to act as a tumor promoter. A potential mechanism for YM178 related tumor promotion is by beta adrenergic enhancement of Hif1α dependent expression of VEGF leading to increased vascularization of neoplasms (1-4). This is supported by a limited body of cell culture and in vivo data in animals suggesting that beta agonists including norepinephrine (which activates all beta receptors) and stress itself may act as tumor promoters. Stress induced tumor formation in mice was inhibited with exposure to β-antagonist propanolol in vivo (5). The β1/β2 antagonist propanolol inhibited the norepinephrine stimulated invasion of cancer cells in vitro (6). Epinephrine enhanced and propanolol partially reversed the incidence of pulmonary carcinomas that were induced by a tobacco carcinogen in hamsters suggesting that beta adrenergic signaling can promote tumor growth in

vivo (7). Similarly, propanolol inhibited the norepinephrine dependent increase in the incidence of metastases in a mouse model (8).

On **initial** overview of the NDA application for RTF:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		See the list above containing the principle nonclinical studies submitted to support marketing. Juvenile studies were not conducted since this population is not intended for marketing under this NDA. The ExCAC meeting date for the 2-year rat and mouse carcinogenicity results was March 15, 2011.
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).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		Sponsor, "*The core battery studies were conducted in compliance with the standards for nonclinical studies on drug safety (Good Laboratory Practice: GLP), in accordance with the Guidance for Industry Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals (ICH M3(R2)) and in accordance with the safety pharmacology study guideline (ICH S7A).*" Sponsor, "*All pivotal toxicity studies were conducted in compliance with GLP, in*

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA 202-611

	Content Parameter	Yes	No	Comment
				accordance with drug toxicity study method guidelines or guidelines of the ICH."
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		All requested studies have been submitted.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Dose multiples in the proposed label are described in relation to exposure (AUC) or maximal concentration (Cmax) where appropriate. Warning for impaired measurement of protein in urine by dipstick methods is under review. The Sponsor conducted experiments with several urinary protein detection methods with YM178 solutions devoid of protein and found that **YM178 directly caused false positives in the Multistix® dipstick assay** that was used in the toxicity studies but negative findings were found with some of the other detection methods (Study 178-TX-049). YM178 spiked in human urine at high concentrations also caused false positive readings for protein by five different dipstick methods (178-TX-058). Although further review is necessary, false positive proteinuria findings in humans are not anticipated to be a substantial issue since proteinuria was observed in only 1.9% of the subjects in the global Phase 2 and Phase 3 studies (Sponsor's Integrated Summary of Safety, Table 5.3.1).
10	Have any impurity – etc. issues been addressed?	X		The Sponsor addressed impurities. Drug substance impurities have been identified that were above the drug substance quantification limit (b) (4) but all but (b) (4) were below the reporting threshold of (b) (4) The specification for each drug related impurity was NMT (b) (4) and NMT (b) (4) for all impurities combined. Specification for (b) (4) was set at NMT (b) (4) because of levels observed pilot, production and stability batches. The Sponsor accepted current guidance for the specifications for genotoxic impurities. The specifications for the drug product are NMT (b) (4) for each drug related impurity and NMT (b) (4) of all drug related impurities combined.

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	Content Parameter	Yes	No	Comment
11	Has the applicant addressed any abuse potential issues in the submission?	X		Sponsor Module 2-7-4, *"Based upon pharmacology, beta 3-ARs are not amongst the central nervous system (CNS) receptors known to mediate abuse-related effects. Evaluation of the clinical data in the mirabegron program suggests that mirabegron is unlikely to demonstrate abuse potential. Among the 5863 patients who received at least one dose of mirabegron in phase 2/3 studies, there were no reported AE suggesting a risk of abuse liability. In both the nonclinical and clinical mirabegron studies, there is no evidence of withdrawal or rebound. There were no AE with the PT of drug withdrawal syndrome or withdrawal syndrome among the 5863 patients who received at least one dose of mirabegron in phase 2/3 studies."*
12	If this NDA is to support a Rx to OTC switch, have all relevant studies been submitted?	NA		Not applicable. This application is only for prescription use of their product.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE?

YES

Recommended Comment to Sponsor:

The necessity for a warning in labeling sections 5 and 7 for inaccurate overestimation of protein in urine by dipstick methods is under review. Labeling may indicate that high concentrations of mirabegron in urine may incorrectly yield elevated protein levels by dipstick methods. If clinically indicated, secondary follow-up methods for protein detection in urine will be recommended for labeling to ensure that proteinuria assessments are accurate.

References

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/s/

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10/26/2011

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10/26/2011