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APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

MEMORANDUM

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

DATE: July 3, 2012

TO: NDA 202-811; LINZESS (linaclotide) Capsules

FROM: Brian Strongin, R.Ph., MBA
Chief Project Management Staff
Division of Gastroenterology and Inborn Errors Products

SUBJECT: Non-Clinical Post-Marketing Requirements

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FDA Attendees:

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Donna Griebel, M.D.	Director, Division of Gastroenterology and Inborn Errors Products (DGIEP)
Andrew Mulberg, M.D.,	Deputy Director, DGIEP
Joyce Korvick, M.D.	Deputy Director for Safety, DGIEP
Ruyi He, M.D.	Medical Team Leader, DGIEP
Rob Fiorentino, M.D.	Medical Team Leader, DGIEP
Erica Wynn, M.D.	Medical Reviewer, DGIEP
Lara Dimick M.D.	Medical Reviewer, DGIEP
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Yuk-Chow Ng, Ph.D.	Pharmacology Reviewer
Melissa Tassinari, M.D.	Senior Clinical Analyst, Pediatric and Maternal Health Staff (PMHS)
Elizabeth Durmowicz, M.D.	Medical Officer, PMHS
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Brian Strongin, R Ph, M.B.A.	Chief, Project Management Staff, DGIEP

Background

NDA 202-811, sponsor Forest Laboratories, for LINZESS (linaclotide) Capsules, submitted and received August 9, 2011 provides for the treatment of IBS-C and chronic constipation. Sponsorship changed from Ironwood Pharmaceuticals to Forest Laboratories after the NDA was submitted. Linaclotide is an NME.

Linaclotide was lethal in two separate toxicology studies in juvenile mice. The mechanism for this lethality is unknown.

Linaclotide was lethal at 10 mcg/kg/day in neonatal mice after oral administration of 1 or 2 daily doses, starting on post partum day 7. Lethality was also observed in juvenile mice after a single oral administration on post partum day 14 (100 mcg/kg) and post partum day 21 (600 mcg/kg). The deaths were identified in mice with ages approximately equivalent to human infants and children age 1 to 23 months. There were no deaths in the control groups. There is currently no data for mice between ages of 21 days and 6 weeks. Linaclotide was not lethal in a study in juvenile mice age 6 weeks (approximately equivalent to humans age 12 to 16 years) at a dose of 20,000 mcg/kg/day for 28 days. The maximum recommended dose in adults is approximately 5 mcg/kg/day, based on a 60-kg body weight.

At today's teleconference, we discussed the following non-clinical post-marketing requirements related to the deaths in juvenile mouse studies:

1. A study to determine the mechanism of lethality

2. A study to obtain a more complete understanding of how sensitivity to linaclotide lethality changes with age in juvenile mice and to inform about the potential risk in older pediatric patients (i.e. older than 2 years).

Teleconference Discussion

The sponsor provided an overview of a position paper submitted to the NDA June 25, 2012. They contended that the totality of evidence supports exaggerated pharmacology manifested as an increased fluid secretion in juvenile mice. Mice are uniquely sensitive to increased fluid secretion in the gastrointestinal tract. The gastrointestinal tract is undeveloped in neonatal mice, which is the key difference between neonatal mice and human neonates. Mice have an underdeveloped capacity for water absorption. Neonatal mice have a higher level of intestinal GC-C receptors.

The FDA responded that this hypothesis was well presented and reasonable. Concern was expressed that there was no evidence in support of the sponsor's hypothesis, based on the Agency's review of the neonatal/juvenile mouse toxicity studies. The actual cause of lethality was not determined in the macroscopic and microscopic examinations, although it is clear from previous studies that the lethal dose levels were pharmacologically active in neonatal mice. There was no indication in the data about the exact cause of death, although the deaths were clearly drug related. GC-C receptors are found in other parts of the body, not just in the gastrointestinal tract, so it is possible that systemic effects were involved in the lethality in neonatal/juvenile mice. The active metabolite was detected in one plasma sample from neonatal mice, which is suggestive of systemic absorption.

The sponsor contended that a significant body of evidence in support of exaggerated pharmacology exists. They will submit more data to demonstrate this.

The FDA asked if the sponsor had ideas about further studies to investigate the mechanism of juvenile mouse deaths. The sponsor stated that they believe that the deaths were linked to a fluid shift into the intestine and will evaluate this in neonatal mice. Fluid secretion and its link to mortality will be a major endpoint in these studies and diarrhea and electrolyte shift will also be important. They added that intestinal weight is a possible endpoint. The FDA suggested that the sponsor investigate systemic exposure as well as intestinal fluid shift. The Agency is looking for assurance that the deaths were not due to unexpected systemic exposure, and stated that detection of drug or its active metabolite in plasma would evoke more concern and questions.

The sponsor stated that evidence exists that GC-C is expressed in other tissues, but there are very low levels outside the gastrointestinal tract and that GC-C outside the gastrointestinal tract has

little role in physiologic function. The FDA requested a study to investigate the mechanism of lethality in neonatal/juvenile mice. The sponsor responded that they will attempt to demonstrate an exaggerated pharmacological effect (i.e. excessive fluid secretion in the intestine), and a lack of systemic absorption as the first step. The agency stated that the next step is to generate data in age groups not explored, looking at lethality and general toxicity as mice mature.

The sponsor contended that they need to study mice with a more fully developed gastrointestinal tract. Neonatal mice are not representative of the human gastrointestinal tract and a dramatic maturation occurs after about 3 weeks of age, post-weaning.

The FDA stated that the sponsor's approach appears to be reasonable. Testing mice at age 4 weeks is a reasonable place to start. Doses up to 20,000 mcg/kg were tolerated at 6 weeks of age in mice. If deaths occur in 4 week-old mice, then 5-week old mice should be studied. If no deaths occur in 4-week old mice, then no more study is needed.

FDA suggested a high dose of 20,000 mcg/kg/day in 4-week old mice, since the same dose was used in 6-week old mice. The sponsor stated that a 20,000 mcg/kg dose is dramatically higher than the clinical dose. They asked if a smaller dose was acceptable, and proposed 500 mcg/kg/day (given for one week) as the high dose, which is 100-fold greater than the intended adult human dose.

The FDA stated that in the context of the existing lethality data, the use of 500 mcg/kg/day was not adequate, given that 100% mortality was seen at 600 mcg/kg in 21-day old mice. The Agency requested a higher dose in 28-day old mice (i.e. 1000 or 1200 mcg/kg as the high dose), to allow for evaluation of a shift in the lethality curve in 4-week old mice. The FDA and sponsor agreed that 1000 mcg/kg was reasonable for use as the high dose.

The sponsor stated that the first study will look at fluid shift as a mechanism of mortality. They will also look at other clinical parameters and evaluate the systemic exposure in neonatal mice.

They stated that their second study will look at 4-week old mice dosed for 7 days with 1000 mcg/kg as the high dose.

The discussion then turned to the sponsor's proposed pediatric plan. (b) (4)

The FDA responded that the requested animal data are needed before any human studies in pediatrics may proceed. The sponsor responded that they will submit a revised pediatric plan including nonclinical studies.

The call then concluded.

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/s/

BRIAN K STRONGIN
08/01/2012

ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW OF NDA 202,811 DATED April 10, 2012

Reviewer: Yuk-Chow Ng, Ph.D.

Date: 06/11/2012

1. In the Sponsor's proposed labeling, the EPC (Established Pharmacologic Class) term "GC-C agonist" was proposed for Linzess. After a thorough review of the literature, this reviewer recommended the term "guanylate cyclase-C receptor agonist" to be used as the EPC. Although the Sponsor's proposed EPC is scientifically valid, the term proposed by this reviewer provides more complete information. After reviewing the Agency's revisions of the labeling, the Sponsor proposed the EPC term "guanylate cyclase-C agonist", deleting the word "receptor". The Sponsor's argument is that the word "receptor" in the EPC term may be confusing because it suggests that Linzess is an agonist to a receptor for GC-C. This matter was discussed in a review team meeting on 5/24/2012. It was this reviewer's opinion that it is acceptable to delete the word "receptor" because the term "guanylate cyclase-C agonist" is also widely used in the literature and is scientifically valid as well. The review team concurred with the recommendation, and the EPC term for Linzess will be "guanylate cyclase-C agonist", as proposed by the Sponsor.

2. In neonatal/juvenile mice, oral toxicity studies revealed that young mice are particularly sensitive to linaclotide toxicity. The minimum lethal dose of linaclotide in a dose-ranging study in neonatal/juvenile mice was 50, 100, and 600 µg/kg/day when dosing was initiated on *post partum* days 7, 14, and 21, respectively. Thus, lethality was found to be highly age-dependent. In a repeated-dose oral toxicity study in neonatal/juvenile mice, increased mortality was observed in the 10 µg/kg/day group after 1 or 2 doses of linaclotide. This minimum lethal dose in neonatal mice (10 µg/kg/day) is approximately 2 times the maximum recommended human dose, based on a µg/kg comparison. The cause of death in the pups without signs of gavage-related injury could not be determined due to the absence of clinical signs, or macroscopic and microscopic lesions. The Sponsor hypothesized that the increased sensitivity of neonatal/ juvenile mice to linaclotide may be related to the increased expression of intestinal guanylate cyclase-C in young animals, or possibly to other factors such as those related to an immature GI system. However, data from the studies showed that there was no bodyweight loss in the neonatal mice that died within 24 hours of dosing, which is inconsistent with the possibility of significant fluid loss due to an exaggerated pharmacologic effect of linaclotide. Furthermore, toxicokinetic data showed that there was no consistent detection of linaclotide or the active metabolite in plasma from groups in which lethality was observed. Therefore, based on the limited information available in the toxicity studies, it is

not possible to determine the cause of the increased lethality of linaclotide in neonatal/juvenile mice. This information is critical in evaluating the safety of conducting future pediatric clinical studies. The review team discussed this issue and came to the conclusion that the Sponsor should conduct, as a PMR (post-marketing requirement), nonclinical studies to elucidate the mechanism(s) of linaclotide lethality in neonatal/juvenile mice. The Sponsor should submit their proposed study protocols to obtain the Agency's concurrence before initiating the studies.

_____ _____
Yuk-Chow Ng, Ph.D. Date
Pharmacologist
Division of Gastroenterology and Inborn Errors Products

_____ _____
David B. Joseph, Ph.D. Date
Pharmacology Team Leader
Division of Gastroenterology and Inborn Errors Products

cc:
NDA 202,811
DGIEP
DGIEP/PM
DGIEP/Dr. Joseph
DGIEP/Dr. Ng
R/D Init.: D. Joseph 6/11/12

APPENDIX/ATTACHMENTS
None

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/s/

YUK-CHOW NG
06/11/2012

DAVID B JOSEPH
06/11/2012
I concur.

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

FROM: David B. Joseph
Pharmacology Team Leader

DATE: April 17, 2012

SUBJECT: NDA 202,811 (SD # 1 dated August 9, 2011)

Sponsor: Ironwood Pharmaceuticals, Inc.

Drug Product: LINZESS (linaclotide) capsules

Comments:

1. Linzess (linaclotide) is a synthetic 14-amino acid peptide that was developed for the treatment of chronic constipation and irritable bowel syndrome with constipation (IBS-C). Linaclotide is structurally related to the guanylin peptide family, which consists of three endogenous peptides (guanylin, uroguanylin, and lymphoguanylin) and one exogenous peptide toxin (bacterial enterotoxin ST_a). These small cysteine-rich peptides bind to and activate GC-C (guanylate cyclase C), a cell-surface receptor with intrinsic guanylate cyclase activity. Similar to guanylin peptides, linaclotide stimulates production of cGMP through direct activation of the GC-C receptor. The resulting increase in intracellular cGMP produces changes in cellular function. Linaclotide and its active metabolite act locally in the intestine to increase secretion of chloride and bicarbonate ions into the intestinal lumen, resulting in increased intestinal fluid secretion and accelerated transit of intestinal contents. When tested in a colorectal distension model in rats, orally administered linaclotide produced relief of inflammation- and stress-induced colorectal hyperalgesia.

2. The absorption of linaclotide and the active metabolite is extremely limited. The achievement of quantifiable plasma levels of either of these peptides (LLQ = 0.5-3.0 ng/ml) in animals required oral linaclotide dose levels at least 500 times the proposed maximum human dose, based on a µg/kg comparison. Thus, systemic exposure was achieved in the general toxicology studies, reproductive and developmental studies, and the carcinogenicity studies. However, neither linaclotide nor its active metabolite was detected in human plasma after administration of the recommended dose levels.

3. The nonclinical dataset did not identify any safety issues that would impact the approvability of linaclotide. The most notable finding in the nonclinical data was the potent lethality in neonatal/juvenile mice. In a dose ranging study, the minimum

lethal doses were 50, 100, and 600 µg/kg/day PO when dosing was initiated on *post partum* days 7, 14, and 21, respectively. All deaths occurred within 24 hours after the first daily dose. In a 9-week oral toxicity study in neonatal/juvenile mice with dosing initiated on *post partum* day 7, the minimum lethal dose was determined to be 10 µg/kg/day in mice less than 9 days of age. The incidence of drug-related deaths at 10 µg/kg/day was 5/40. However, no signs of toxicity were observed in mice which survived beyond 9 days *post partum* (i.e. more than two days of dosing). The original high-dose level of 30 µg/kg/day was removed from this study due to a high incidence of deaths after a single dose. The lethality of linaclotide in neonatal/ juvenile mice is in marked contrast to the toxicity data from adult mice, where a NOAEL of 20,000 µg/kg/day was observed in a 26-week oral toxicity study. Mortality was observed at doses ≥ 40,000 µg/kg/day in pregnant mice, and at doses ≥ 80,000 µg/kg/day in repeat-dose toxicity studies in adult mice. The totality of the mouse toxicity data indicates that linaclotide-induced lethality was highly age-dependent.

4. It should be noted that the minimum lethal dose in 7-day old mice (10 µg/kg) is approximately 2 times the maximum recommended clinical dose in adults, based on a bodyweight comparison. Therefore, I concur with the recommendation of the Pharmacology/Toxicology reviewer, Dr. Yuk-Chow Ng, to include the information about lethality in neonatal/juvenile mice in the labeling, given the potential for off-label use in infants or very young pediatric patients with chronic constipation. The Sponsor provided hypotheses to explain the potent lethality of linaclotide in neonatal/juvenile mice (see "Brief Discussion of Nonclinical Findings" in Dr. Ng's review). However, given the limited information available from these studies, it is not possible to determine the mechanism of lethality. (b) (4)

Recommendations:

There are no nonclinical issues which preclude the approval of Linzess. I concur with Dr. Ng's recommendation for approval, and his recommendations for labeling revisions.

_____ _____

David B. Joseph, Ph.D. Date
Pharmacology Team Leader
Division of Gastroenterology and Inborn Errors Products

Reference ID: 3117940

cc:
NDA 202,811
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/s/

DAVID B JOSEPH
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 REVIEW AND EVALUATION

Application number:	202,811
Supporting document/s:	001
Applicant's letter date:	08/09/2011
CDER stamp date:	08/09/2011
Product:	LINZESS (linaclotide) capsules
Indication:	Treatment of irritable bowel syndrome with constipation and treatment of the signs and symptoms of chronic constipation
Applicant:	Ironwood Pharmaceuticals, Inc.
Review Division:	Gastroenterology and Inborn Errors Products
Reviewers:	Niraj Mehta, Ph.D. and Yuk-Chow Ng, Ph.D.
Supervisor/Team Leader:	David B. Joseph, Ph.D.
Division Director:	Donna Griebel, M.D.
Project Manager:	Brian K. Strongin

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of NDA 202,811 are owned by Ironwood Pharmaceuticals or are data for which Ironwood Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 202,811 that Ironwood Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 202,811.

Reference ID: 3114166

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1 Executive Summary

1.1 Introduction

Linaclotide is a synthetic 14-amino acid peptide that is structurally related to the guanylin peptide family, which consists of three endogenous peptides, including guanylin, uroguanylin, and lymphoguanylin, and one exogenous peptide toxin, the bacterial enterotoxin ST_a. Linaclotide contains 3 disulfide bonds, instead of the 2 disulfide bonds found in the endogenous guanylin peptides, which increase the stability of linaclotide. Receptor binding and receptor function studies showed that guanylate cyclase C (GC-C), a cell surface receptor, is the molecular target for linaclotide in the GI tract. Linaclotide binds to GC-C and stimulates the production of cGMP (cyclic guanosine 3', 5'-monophosphate) in the intestinal epithelium, which in turn stimulates secretion of chloride, bicarbonate, and fluid. Oral administration of linaclotide in mice and rats stimulates gastrointestinal transit and intestinal secretion. When tested in a colorectal distension model in rats, orally administered linaclotide produced relief of inflammation- and stress-induced colorectal hyperalgesia. MM-419447, an active metabolite of linaclotide, is a 13-amino acid peptide formed through hydrolytic cleavage by carboxypeptidase A in the intestine. The pharmacological activity of MM-419447 in the *in vitro and in vivo* assays is equivalent to that of linaclotide. The proposed indications for linaclotide are for treatment of irritable bowel syndrome with constipation, and treatment of chronic constipation.

1.2 Brief Discussion of Nonclinical Findings

Linaclotide binds to the guanylate cyclase-C (GC-C) receptor, stimulates the production of cGMP and increases the secretion of chloride and bicarbonate ions into the intestinal lumen, resulting in increased intestinal fluid and accelerated transit. Extracellular cGMP decreases the mechano-sensitivity of splanchnic high-threshold colonic pain afferents. In animal models, linaclotide was shown to reduce inflammation- and stress-induced colorectal hyperalgesia. MM-419447 is an active metabolite of linaclotide that is formed in the intestine. The oral bioavailability of both linaclotide and MM-419447 is extremely limited. The pharmacology and pharmacokinetic data suggest that linaclotide and MM-419447 interact with the GC-C receptors lining the intestinal tract and elicit their pharmacological effects through local actions in the intestinal mucosa.

Oral administration of linaclotide was generally well tolerated in rats, mice, and monkeys. In a 13-week oral toxicity study in rats, the NOAEL (no observed adverse effect level) was 50,000 μg/kg/day. In a 26-week oral toxicity study in mice, the NOAEL was 20,000 μg/kg/day, and in a 39-week oral toxicity study in Cynomolgus monkeys, the NOAEL was 5000 μg/kg/day.

Oral toxicity studies in neonatal/juvenile mice revealed that young mice are particularly sensitive to linaclotide toxicity. In a dose range-finding study in neonatal/juvenile mice, linaclotide was administered at doses of 0.1, 10, 50, 100, and 600 μg/kg/day for up to 5 days starting on *post partum* day 7, 14, or 21. Linaclotide-related adverse clinical signs

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(decreased activity, cold to touch, and lying on side) and mortality were observed within 24 hours after the first dose. Lethality was found to be highly age-dependent; the minimum lethal dose of linaclotide in neonatal/juvenile mice was 50, 100, and 600 µg/kg/day when dosing was initiated on *post partum* days 7, 14, and 21, respectively. No drug-related changes were observed at necropsy in pups from any study group. By contrast, in a dose range-finding study in juvenile rabbits, single or repeated (5 days) doses of up to 40,000 µg/kg/day linaclotide was well tolerated when dosing was initiated on *post partum* day 14.

In a repeated-dose oral toxicity study in neonatal/juvenile mice, animals were treated with 1, 3, or 10 µg/kg/day from *post partum* day 7 to *post partum* day 70. Increased mortality was observed in the 10 µg/kg/day group after 1 or 2 doses of linaclotide. However, after *post partum* day 9 the 10 µg/kg/day dose was well tolerated for the remainder of the treatment period. Gavage-related injury was determined to be the cause of death in some pups in linaclotide-treated groups. However, the cause of death in pups without signs of gavage-related injury could not be determined due to the absence of clinical signs, macroscopic lesions, or microscopic lesions. These deaths were considered to be treatment-related, and the incidence of drug-related deaths at 10 µg/kg/day was determined to be 5/40. There were no toxicologically significant drug-related changes in the surviving animals at necropsy. Based on mortality, the NOAEL in mice less than 9 days of age was considered to be 3 µg/kg/day. The minimum lethal dose in neonatal mice (10 µg/kg/day) is approximately 2 times the maximum recommended human dose, based on a µg/kg comparison.

The Sponsor hypothesized that the increased sensitivity of neonatal/juvenile mice to linaclotide may be related to the increased expression of intestinal GC-C receptors in young animals (Al-Majali et al, Lab Animal Sci., 49: 254-259, 1999; Cohen et al., Pediatr Res., 20: 555-560, 1986), or possibly to other factors such as those related to an immature GI system (Walthall et al., Birth Defects Research (Part B), 74:132-156, 2005; Heller, Arch Dis. Child, 26: 195-204, 1951). It is noteworthy that doses as low as 2.5 µg/kg were shown to be pharmacologically active in a study in suckling mice. However, based on the limited information available in the toxicity studies in neonatal/juvenile mice, it is not possible to determine whether the drug-related deaths were due to exaggerated pharmacological activity (i.e. excessive intestinal fluid secretion and loss of electrolytes). The relevance of the data from neonatal/juvenile mice to the potential for lethality or other adverse events in infants or young pediatric patients is unknown. It is noted that the levels of expression of the receptor for heat-stable enterotoxin (i.e. GC-C) in the small and large intestine of children is age dependent; a greater number of receptors are present in infants and the number decreases with increasing age (Cohen et al., Gastroenterology, 94: 367-373, 1988).

Oral administration of up to 100,000 µg/kg/day in rats and 40,000 µg/kg/day in rabbits produced no maternal toxicity and no effects on embryo-fetal development. In mice, oral dose levels ≥ 40,000 µg/kg/day produced severe maternal toxicity including death, reduced fetal weights, effects on fetal morphology, and reduced gravid uterine weight. However, an oral dose of 5000 µg/kg/day did not produce maternal toxicity or any

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adverse effects on embryo-fetal development in mice. This dose is approximately 1000 times the maximum recommended human dose, based on a µg/kg comparison.

Linaclotide was negative in the Ames test and *in vitro* chromosomal aberration assay in human peripheral blood lymphocytes, and was not tumorigenic in rats or mice.

1.3 Recommendations

1.3.1 Approvability

The application is recommended for approval.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Established Pharmacologic Class

Linzess (linaclotide) is a first-in-class NME. The Sponsor has proposed the term "GC-C agonist" as the established pharmacologic class for Linzess. This proposal is evaluated below.

Linaclotide is a synthetic 14-amino acid peptide that is structurally related to the guanylin peptide family. This family of peptides consists of three endogenous peptides, including guanylin, uroguanylin, and lymphoguanylin, and one exogenous peptide toxin, the bacterial enterotoxin ST_a (Chinkers and Garbers, Annu. Rev. Biochem., 60: 553, 1991; Forte, Pharmacology & Therapeutics, 104: 137, 2004). A comparison of the core regions of linaclotide and the endogenous peptides shows a high degree of amino acid sequence identity with uroguanylin and guanylin (Bryant et al., Life Sciences, 86:760, 2010). These small cysteine-rich peptides activate cell-surface receptors that have intrinsic guanylate cyclase activity, and they modulate cellular function via the intracellular second messenger, cyclic GMP (Chinkers and Garbers, Annu. Rev. Biochem., 60: 553, 1991; Fan et al., Endocrinology, 138: 4636, 1997; Forte et al., Annu. Rev. Physiol., 62:673, 2000; Potter, Pharmacology & Therapeutics, 130: 71, 2011).

The receptor activated by linaclotide and the guanylin peptides is a subtype of a family of guanylate cyclases, referred to as receptor guanylate cyclases or membrane guanylate cyclases (Chinkers and Garbers, Annu. Rev. Biochem. 60: 553, 1991; Wong and Garbers, J. Clin. Invest., 90: 299, 1992; Forte et al., Annu. Rev. Physiol., 62:673, 2000). The receptor guanylate cyclases have five domains, consisting of the extracellular ligand-binding domain, the single membrane-spanning domain, the kinase homology domain, the dimerization domain, and the highly conserved catalytic guanylate cyclase domain. In humans, there are now at least 5 known receptor guanylate cyclases, which include the ANP (atrial natriuretic peptide) and BNP (B-type natriuretic peptide) activated guanylate cyclase A (GC-A), the CNP (C-type natriuretic

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peptide) activated guanylate cyclase B (GC-B), and the guanylin peptide activated guanylate cyclase C (GC-C). GC-E and GC-F are the other known human receptor guanylate cyclases, but no ligands have been identified that activate these subtypes (Potter, Pharmacology & Therapeutics, 130: 71, 2011).

Receptor guanylate cyclase-C is the intestinal receptor for guanylin and uroguanylin that is responsible for the stimulation of Cl^- and HCO_3^- secretion into the intestinal lumen (Forte, Pharmacology & Therapeutics, 104: 137, 2004; Vaandrager, Mol. Cell. Biochem., 230:73, 2002). Guanylin and uroguanylin are produced within the intestinal mucosa and serve in a paracrine fashion in regulating intestinal fluid and electrolyte secretion. Pharmacology studies conducted by the Sponsor and others demonstrated that linaclotide increases intracellular concentrations of the second messenger cGMP through activation of the GC-C receptor located on the apical surface of epithelial cells throughout the intestine, as observed with the naturally occurring GC-C ligands (Bryant et al., Life Sciences, 86:760, 2010).

Thus, based on its mechanism of action, linaclotide fits the definition of a receptor agonist because it binds to and activates the GC-C receptor and produces biological responses, mimicking the actions of the endogenous ligands. The terms GC-C agonist and GC-C receptor agonist have been used in the literature to describe the guanylin peptides and linaclotide (Wensel and Luthin, The Annals of Pharmacotherapy, 45:1535, 2011; Camilleri, Aliment Pharmacol. Ther., 31:35, 2010; Bryant et al., Life Sciences, 86:760, 2010; Forte, Pharmacology & Therapeutics, 104: 137, 2004; Shailubhai, Curr. Opin. Drug Discov. Devel., 5:261, 2002; Bharucha and Linden, Neurogastroenterol. Motil., 22:227, 2010).

In summary, after a thorough review of the literature, this reviewer recommends that, based on the mechanism of action, the established pharmacologic class for linaclotide should be, “guanylate cyclase-C receptor agonist”. This term is scientifically valid, and accurately communicates the concept of a specific ligand-receptor interaction between linaclotide and the GC-C receptor. The term “guanylate cyclase-C receptor agonist” is fundamentally similar to the Sponsor’s proposed established pharmacologic class, “GC-C agonist”, but provides more complete information.

Sponsor’s Proposed Version:

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

(b) (4)

Evaluation:

Reference ID: 3114166

The established pharmacologic class for Linzess should be changed to "guanylate cyclase-C receptor agonist" (see explanation above).

Recommended Version:

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE
LINZESS is a guanylate cyclase-C receptor agonist indicated for:
- Treatment of irritable bowel syndrome with constipation (IBS-C) (1.1)
- Treatment of chronic constipation (CC) (1.2)

Sponsor's Proposed Version:

8.1 Pregnancy

(b) (4)

Evaluation:

Because maternal and fetal toxicities were observed in mice, albeit at high multiples of the maximum recommended human dose, the Pregnancy Category should be "C". (b) (4) For drugs that are systemically absorbed and cleared by the kidney or liver, animal to human dose comparisons based on AUC or mg/m^2 are usually appropriate. However, absorption of linaclotide has not been detected in humans at the proposed clinical doses, whereas systemic exposure in animals was shown to occur at the doses used in the embryo-fetal developmental studies. (b) (4)

(b) (4). However, it is appropriate to state the maximum recommended human dose expressed in mcg/kg, to provide a context for understanding the animal study results.

Recommended Version:

8.1 Pregnancy

Pregnancy Category C

The potential for linaclotide to cause teratogenic effects was studied in rats, rabbits, and mice. Oral administration of up to 100,000 mcg/kg/day in rats and 40,000 mcg/kg/day in rabbits produced no maternal toxicity and no effects on embryo-fetal development. In mice, oral dose levels ≥ 40,000 mcg/kg/day produced severe maternal toxicity including death, reduced fetal weights, effects on fetal morphology, and reduced gravid uterine weight. However, an oral dose of 5000 mcg/kg/day did not produce maternal toxicity or any adverse effects on embryo-fetal development in mice. The maximum recommended human dose is approximately 5 mcg/kg/day, based on a 60-kg bodyweight. Systemic exposure to linaclotide was achieved at the tested dose levels in animals, whereas no evidence of absorption was found in humans. Therefore, animal and human doses should not be compared directly for evaluating relative exposure. There are no adequate and well-controlled studies in pregnant women. Linzess should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Sponsor's Proposed Version:

12.1 Mechanism of Action

(b) (4). Both linaclotide and its active metabolite bind to the GC-C receptor and act locally on the luminal surface of the intestinal epithelium. Activation of the GC-C (b) (4) results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation in intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, through activation of the CFTR ion channel, resulting in increased intestinal fluid and accelerated transit. (b) (4) In animal models, linaclotide has been shown to both accelerate GI transit and reduce intestinal pain.

Evaluation:

Pharmacology studies performed by the Sponsor provided evidence that linaclotide is a guanylate cyclase-C (GC-C) receptor agonist. Receptor binding and receptor function

studies showed that GC-C is the molecular target for linaclotide in the GI tract. Linaclotide binds with selectivity and high affinity (low nanomolar range) to GC-C. Linaclotide stimulates the production of cGMP (cyclic guanosine 3', 5'-monophosphate) in human colonic epithelial cells and in the intestine of mice and rats. Stimulation of cGMP production in the intestine results in increased secretion of chloride, bicarbonate, and fluid. Oral administration of linaclotide in mice and rats stimulates gastrointestinal transit and stimulates intestinal secretion. Extracellular cGMP decreases the mechano-sensitivity of splanchnic high-threshold colonic pain afferents. In animal models, linaclotide was shown to reduce inflammation- and stress-induced colorectal hyperalgesia.

The Sponsor provided no direct evidence that linaclotide stimulates CFTR (cystic fibrosis transmembrane conductance regulator) channel activity in intestinal epithelial cells. However, data in the literature provide ample evidence that the guanylin peptides, including the heat-stable enterotoxin (ST_a), increase cGMP production through GC-C activation, thereby stimulating chloride and bicarbonate secretion through CFTR-dependent mechanisms. However, CFTR-independent mechanisms have also been postulated to play a role, albeit minor, in the actions of the guanylin peptides and ST_a. For example, it has been postulated that CIC-2 (voltage-gated chloride channel) may be activated by the peptides (Forte et al., Annu Rev Physiol., 62:673-695, 2000; Joo et al., Am. J. Physiol., 274: G633-644, 1998). Also, cGMP is known to inhibit the Na^+/H^+ exchanger thereby decreasing sodium and chloride absorption (Basu et al., Mol. Cell Biochem., 334: 67-80, 2010; Vaandrager et al., Gastroenterology 118: 108-114, 2000).

The terms (b) (4) and (b) (4) are descriptive terms that are relative and subjective, and therefore should be omitted from the label. Other recommended changes to improve the clarity of this section are shown below.

Recommended Version:

Linaclotide is a guanylate cyclase-C receptor agonist. Both linaclotide and its active metabolite bind to the GC-C receptor and act locally on the luminal surface of the intestinal epithelium. Activation of the GC-C receptor results in an increase in both intracellular and extracellular concentrations of cyclic guanosine 3', 5' monophosphate (cGMP). Elevation in intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, mainly through activation of the CFTR ion channel, resulting in increased intestinal fluid and accelerated transit. In animal models, linaclotide has been shown to both accelerate GI transit and reduce intestinal pain. The linaclotide-induced reduction in visceral pain is thought to be mediated by increased extracellular cGMP, which was shown to decrease the activity of pain-sensing nerves.

Sponsor's Proposed Version:

13.1 Carcinogenesis, Mutagenesis, and Impairment of Fertility

Carcinogenesis

(b) (4)

Mutagenesis
Linaclotide was not genotoxic in an *in vitro* bacterial reverse mutation (Ames) assay or in the *in vitro* chromosomal aberration assay in cultured human peripheral blood lymphocytes.

Impairment of Fertility
Linaclotide had no effect on fertility or reproductive function in male and female rats at oral doses of up to (b) (4)

Evaluation:

(b) (4)
. Absorption of linaclotide has not been detected in humans at the proposed clinical doses. In contrast, based on the known oral bioavailability of linaclotide in rats and mice, systemic exposure was expected to have occurred at the mid- and high-dose levels in the carcinogenicity studies, and at all dose levels in the fertility study in rats. (b) (4) However, it is appropriate to state the maximum recommended human dose expressed in mcg/kg, to provide a context for understanding the animal study results.

Only the highest doses tested in the carcinogenicity studies need to be stated.

Recommended Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis
In 2-year carcinogenicity studies, linaclotide was not tumorigenic in rats at doses up to 3500 mcg/kg/day or in mice at doses up to 6000 mcg/kg/day. The maximum recommended human dose is approximately 5 mcg/kg/day based on a 60-kg bodyweight. Systemic exposure to linaclotide was achieved at the tested dose levels in animals, whereas no evidence of absorption was found in humans. Therefore, animal and human doses should not be compared directly for evaluating relative exposure.

Mutagenesis

Linaclotide was not genotoxic in an *in vitro* bacterial reverse mutation (Ames) assay or in the *in vitro* chromosomal aberration assay in cultured human peripheral blood lymphocytes.

Impairment of Fertility
Linaclotide had no effect on fertility or reproductive function in male and female rats at oral doses up to 100,000 mcg/kg/day.

13.2 Animal Toxicology and/or Pharmacology

Evaluation: (b) (4) However, the potent lethality of linaclotide in neonatal/juvenile mice raises a safety concern about the potential use of this drug in infants, given that chronic constipation has been observed in pediatric patients as young as 6 months. Therefore, the lethality data in neonatal/juvenile mice should be presented in section 13.2. A summary of all juvenile animal toxicity studies is provided below.

Oral toxicity of linaclotide was evaluated in neonatal/juvenile mice and rabbits. In the dose range-finding study in neonatal/juvenile mice, linaclotide was administered at doses of 0.1, 10, 50, 100 or 600 mcg/kg/day for up to 5 days starting on *post partum* day 7, 14 or 21. Linaclotide-related adverse clinical effects and mortality were observed within 24 hours after the first dose. No drug-related changes were observed at necropsy in pups from any of these groups. The toxicities were found to be highly age-dependent; the maximum tolerated doses of linaclotide in neonatal/juvenile mice were 10, 50, and 100 mcg/kg/day when treatment was initiated on *post partum* days 7, 14, and 21, respectively. At higher dose levels, most or all of the animals died. In the dose range-finding study in juvenile rabbits (post partum day 14 at treatment initiation), single or repeated (5 days) doses of up to 40,000 mcg/kg/day linaclotide was well tolerated.

In a repeated-dose oral toxicity study in neonatal/juvenile mice, animals were treated with 1, 3, or 10 mcg/kg/day from *post partum* day 7 to *post partum* day 70. Increased mortality was observed in the 10 mcg/kg/day group after 1 or 2 doses of linaclotide. However, after *post partum* day 9 the 10 µg/kg/day dose was well tolerated for the remainder of the treatment period. The cause of death of some of the pups was attributed to gavage errors. However, the cause for other deaths could not be determined based on clinical signs or macroscopic and microscopic examinations, and was considered to be treatment-related. Thus, the incidence of drug-related deaths at 10 mcg/kg/day was determined to be 5/40. There were no toxicologically significant drug-related changes in the surviving animals at necropsy. Based on mortality, the NOAEL in mice less than 9 days of age was considered to be 3 mcg/kg/day. The minimum lethal dose in neonatal mice (10 mcg/kg/day) is approximately 2 times the maximum recommended human dose, based on a mcg/kg comparison. Because the 10 mcg/kg/day dose was well tolerated after *post partum* day 9, the NOAEL in juvenile mice is considered to be at least 10 mcg/kg/day.

Reference ID: 3114166

Recommended Version:

13.2 Animal Toxicology and/or Pharmacology

Linaclotide was lethal at 10 mcg/kg/day PO in neonatal mice after administration of 1 or 2 daily doses, starting on post partum day 7. Lethality was also observed in juvenile mice after a single oral administration on post partum day 14 (100 mcg/kg) and post partum day 21 (600 mcg/kg). The maximum recommended dose in adults is approximately 5 mcg/kg/day, based on a 60-kg bodyweight. The relevance of the animal data to the potential for lethality or other adverse events in infants or young pediatric patients is unknown.

2 Drug Information

2.1 Drug

CAS Registry Number – 851199-59-2

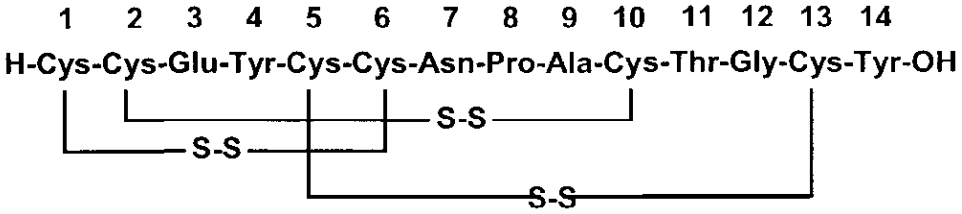
Generic Name - Linaclotide

Code Name – MD-1100 Acetate; MM-416775

Chemical Name -
L-cysteinyl-L-cysteinyl-L-glutamyl-L-tyrosyl-L-cysteinyl-L-cysteinyl-L-asparaginyl-L-prolyl-L-alanyl-L-cysteinyl-L-threonyl-L-glycyl-L-cysteinyl-L-tyrosine, tricyclic

Molecular Formula/Molecular Weight – $C_{59}H_{79}N_{15}O_{21}S_6 \cdot (C_2H_4O_2)_x \cdot (H_2O)_y$/1526.8 (anhydrous free base)

Structure or Biochemical Description



Pharmacologic Class – guanylate cyclase-C receptor agonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 63,290 (MD-1100 acetate for the treatment of constipation-predominant irritable bowel syndrome, constipation, [redacted] (b) (4))
Microbia, Inc., Massachusetts

Reference ID: 3114166

2.3 Drug Formulation

Linaclotide beads are encapsulated into size 3 hard gelatin capsules to produce the finished dosage form, linaclotide capsules 145 µg.

The components and composition of linaclotide capsule, 145 µg, are shown in the tables below, taken from the Sponsor's report.

Table 3.2.P.1.1–1. Composition of Linaclotide (b) (4)

Component	*Function*	*Quality Standard*	*Theoretical Weight (mg/capsule)*	*Theoretical Weight (% w/w)*
Linaclotide	Active Ingredient	In House Standard[a]	0.145[b]	0.26
Calcium chloride dihydrate	(b) (4)	USP	(b) (4)	
L-leucine		USP		
Hypromellose		USP		
Microcrystalline cellulose		NF		
(b) (4		USP		
		NF		
Total (Linaclotide (b) (4)			56.36	100.00

Table 3.2.P.1.1–2. Components and Composition of Linaclotide Capsules, 145 ug

Component	*Function*	*Quality Standard*	*Theoretical Weight (mg/capsule)*	*Theoretical Weight (% w/w)*
Empty Hard Gelatin Capsule, Size 3	Capsule Shell	In-house standard[a, b]	(b) (4)	
Linaclotide Beads (b) (4)	Bead	In-process controls[d]		
Total Weight			104	100.0

Linaclotide Capsules, 290 µg are immediate release capsules supplied as a locked, size 2, white to off-white opaque hard gelatin capsule with a gray imprint "FL 290"on the cap.

The components and composition of linaclotide capsule, 290 µg, are shown in the tables below, taken from the Sponsor's report.

Table 3.2.P.1.1–1. Composition of Linaclotide (b) (4)

Component	*Function*	*Quality Standard*	*Theoretical Weight (mg/capsule)*	*Theoretical Weight (% w/w)*
Linaclotide	Active Ingredient	In-house standard[a]	0.29[b]	0.26
Calcium chloride dihydrate	(b) (4)	USP	(b) (4)	
L-leucine		USP		
Hypromellose		USP		
Microcrystalline cellulose		NF		
(b) (4)		USP		
		NF		
Total (Linaclotide (b) (4)			112.70	100.00

Table 3.2.P.1.1–2. Components and Quantitative Composition of Linaclotide Capsules, 290 ug

Component	*Function*	*Quality Standard*	*Theoretical Weight (mg/capsule)*	*Theoretical Weight (% w/w)*
Empty Gelatin Capsule, Size 2	Capsule Shell	In-house standard[a, b]	(b) (4)	
Linaclotide Beads (b) (4)	Bead	In-process controls[d]		
Total Weight			173	100.0

2.4 Comments on Novel Excipients

There are no novel excipients used in the manufacture of Linaclotide Capsules.

2.5 Comments on Impurities/Degradants of Concern

Two (b) (4) degradation products, (b) (4) (b) (4) are present at levels (b) (4) the ICH qualification threshold in the drug product. The Sponsor conducted studies to qualify the impurities. (b) (4) are considered to be qualified at the proposed limits of (b) (4) and (b) (4), respectively (see Integrated Summary for details).

(b) (4) The proposed acceptance criterion for total (b) (4) content in the (b) (4) is (b) (4) The (b) (4) were present in the linaclotide lots used in the repeat-dose toxicity studies, the Ames test, and the chromosomal aberration test. The results support an acceptance limit (b) (4) for (b) (4) in linaclotide (see Integrated Summary for details).

2.6 Proposed Clinical Population and Dosing Regimen

Patients with irritable bowel syndrome with constipation: 290 mcg orally once daily
Patients with chronic constipation: 145 (b) (4) mcg orally once daily

2.7 Regulatory Background

IND 63,290 was submitted by Microbia, Inc., Massachusetts on 9/30/2004 for MD-1100 acetate (linaclotide) for the treatment of constipation-predominant irritable bowel syndrome, constipation, (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

THE FOLLOWING PHARMACOLOGY STUDIES WERE REVIEWED (starting on page 23)

Test Article: Linaclotide					
Type of Study/Description	***GLP***	***Test System***	***Method of Administration***	***Testing Facility***	***Study Report Number***
IN VITRO PHARMACOLOGY-Binding to GC-C Receptor					
Relative binding affinities of linaclotide and MM-419447 [a] to the GC-C receptor	No	Human T84 cells	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-060-PHR-01]
Effect of pH on the relative binding affinities of linaclotide, MM-419447 [a], ST [b], STc [c] guanylin, and uroguanylin to the GC-C receptor	No	Human T84 cells	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-067-PHR-01]
Effect of linaclotide on GC-C receptor binding using mucosal epithelial cells from the small intestine of rats	No	Sprague-Dawley rat intestinal mucosal epithelial cells	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-024-PHR-01] [d]
Relative binding affinity of linaclotide to the GC-C receptor	No	Sprague-Dawley rat intestinal mucosal epithelial cells	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-059-PHR-01]
Relative binding affinity of linaclotide and MM-419447 [a] to the GC-C receptor	No	Sprague-Dawley rat intestinal brush-border membranes	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-071-PHR-01]

Test Article: Linaclotide					
Type of Study/Description	***GLP***	***Test System***	***Method of Administration***	***Testing Facility***	***Study Report Number***
Effect of linaclotide on GC-C receptor binding using mucosal epithelial cells from the small intestine of KO and WT mice	No	GC-C KO or WT C57/BL6 mouse intestinal mucosal epithelial cell extracts	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-026-PHR-01] [e]
Relative binding affinity of linaclotide for GC-C KO and WT to the GC-C receptor	No	GC-C KO or WT C57/BL6 mouse intestinal mucosal cell extracts	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-058-PHR-01]
IN VITRO PHARMACOLOGY-cGMP Production					
Pharmacological activity of linaclotide and MM-419447 [a] (cGMP production in T84 cells)	No	Human T84 cells	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-001-PHR-01]
Effect of linaclotide and STc [c] on cGMP efflux	No	Caco-2 cells	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-053-PHR-01]
Inhibition of intracellular cGMP efflux by three transporter inhibitors after GC-C receptor stimulation	No	Caco-2 monolayers	In vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-081-PHR-01]

Test Article: Linaclotide					
Type of Study/Description	***GLP***	***Test System***	***Method of Administration***	***Testing Facility***	***Study Report Number***
IN VIVO PHARMACOLOGY-Effects on Secretion					
Effect of linaclotide and tegaserod maleate on mouse GI secretion	No	7-9 day old CD-1 suckling mice	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-015-PHR-01]
Effect of linaclotide and MM-419447[a] on mouse GI secretion	No	7-9-day old CD-1 suckling mice	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-018-PHR-01][f]
Effect of linaclotide on secretion and cGMP production in rats	No	Sprague-Dawley rats (female)	Intra-intestinal injection	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-024-PHR-01][d]
Effects of linaclotide and MM-419447 [a] on GI secretion in rats	No	Sprague-Dawley rats (female)	Intra-intestinal injection	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-085-PHR-01]
Effect of linaclotide on secretion and cGMP production in GC-C KO and WT mice	No	GC-C KO or WT C57/BL6 mice (female)	Intra-intestinal injection	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-026-PHR-01][e]
IN VIVO PHARMACOLOGY-Effects on GI Transit and Stool Consistency					
Effect of linaclotide and tegaserod maleate on mouse GI transit	No	CD-1 mice (male and female)	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-016-PHR-01]
Effect of linaclotide and tegaserod maleate on mouse GI transit, acute versus subacute	No	CD-1 mice (female)	Single oral dose or repeated oral dosing for 5 days	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-017-PHR-01]
Effect of linaclotide and tegaserod maleate on rat GI transit	No	Sprague-Dawley rats (male and female)	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-013-PHR-01]

Test Article: Linaclotide					
Type of Study/Description	*GLP*	*Test System*	*Method of Administration*	*Testing Facility*	*Study Report Number*
Effect of linaclotide and MM-419447[a] on rat GI transit	No	Sprague-Dawley rats (female)	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-018-PHR-01] [f]
Effect of linaclotide on mouse GI transit	No	GC-C KO and WT C57/BL6 mice (male and female)	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-025-PHR-01]
Effect of linaclotide on stool consistency in rodents	No	Sprague-Dawley rats and CD-1 mice (male and female)	Single dose by oral gavage Twice-daily oral dosing or single oral dose for 5 days	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-032-PHR-01]
IN VIVO PHARMACOLOGY-Effects on Visceral Pain					
Effect of linaclotide on stress-induced visceral hyperalgesia in the rat (water-avoidance-stress model)	No	Wistar rats (male)	Single dose by oral gavage	(b) (4)	[MDP-103-022-PHR-01]
Effect of linaclotide on inflammation and stress-induced colorectal hyperalgesia in the rat (TNBS and partial restraint stress model)	No	Wistar rats (male and female)	Single dose by oral gavage		[MDP-103-014-PHR-01]
Effect of linaclotide on inflammation-induced rectal hyperalgesia to distension in the rat (TNBS model)	No	Wistar rats (male)	Single dose by oral gavage		[MDP-103-030-PHR-01]

Test Article: Linaclotide					
Type of Study/Description	*GLP*	*Test System*	*Method of Administration*	*Testing Facility*	*Study Report Number*
Effect of linaclotide on inflammation-induced visceral hypersensitivity in the mouse (TNBS model)	No	GC-C KO and WT C57/BL6 mice (male)	Oral gavage dose at two time points (baseline and post-TNBS-induction)	(b) (4)	[MDP-103-023-PHR-01]
Effect of (b) (4) (cGMP) on stress-induced visceral hypersensitivity	No	Wistar rats (female)	Single dose by oral gavage		[MDP-103-083-PHR-03]
Effect of (b) (4) (cGMP) on inflammation-induced visceral hypersensitivity (TNBS model)	No	Wistar rats (male)	Single dose by oral gavage		[MDP-103-084-PHR-02]
Effect of (b) (4) (cGMP) on pelvic visceral afferents (TNBS model)	No	Sprague-Dawley rats (female)	Ex vivo		[MDP-103-082-PHR-03]
Effect of (b) (4) (cGMP) on colonic afferent mechanosensitivity (TNBS model)	No	C57/BL6 mice (male)	In vitro		[MDP-103-080-PHR-02]

Test Article: Linaclotide					
Type of Study/Description	*GLP*	*Test System*	*Method of Administration*	*Testing Facility*	*Study Report Number*
SECONDARY PHARMACOLOGY					
Linaclotide selectivity assay for receptor and ion channel binding and activity	No	50 different mammalian receptors and ion channels	In vitro	(b) (4)	[MDP-103-019-PHR-01]
Effect of linaclotide on rat GI transit (postoperative ileus model)	No	Sprague-Dawley rats (female)	Oral gavage dose at two time points (immediately following surgery and after 1 hour recovery)	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-027-PHR-01]
Effect of linaclotide on rat GI transit (opiate-induced constipation model)	No	Sprague-Dawley rats (female)	Single dose by oral gavage	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-043-PHR-01]
Effect of linaclotide on stress-induced increased colonic paracellular permeability in the rat	No	Wistar rats (female)	Single dose by oral gavage	(b) (4)	[MDP-103-031-PHR-01]

THE FOLLOWING PHARMACOKINETIC STUDIES WERE REVIEWED (starting on page 34)

Test Article: Linaclotide				
Study Description	*Species or Test System*	*Method of Administration*	*Testing Facility*	*Study Report Number*
Absorption				
Single-dose pharmacokinetics	CD-1 Mouse	PO – 8 mg/kg IV – 8 mg/kg	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-003-PKR-02]
Single-dose pharmacokinetics	Sprague-Dawley Rat	PO – 10 mg/kg IV – 10 mg/kg	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-006-PKR-02]
Single-dose pharmacokinetics of linaclotide and MM-419447[a]	Sprague-Dawley Rat	PO – 10 mg/kg IV – 10 mg/kg	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-010-PKR-02]
Single-dose pharmacokinetics after dosing with MM-419447[a]	Sprague-Dawley Rat	PO – 10 mg/kg IV – 10 mg/kg	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-011-PKR-02]
Systemic and portal vein concentrations of linaclotide and MM-419447[a] after dosing with linaclotide	Sprague-Dawley Rat	PO – 10 mg/kg	(b) (4)	[MDP-103-087-PKR-01]
Distribution				
Bioanalysis of brain, liver, and adipose tissues for linaclotide and MM-419447[a] from mice dosed with linaclotide for up to 26 weeks	CD-1 Mouse	PO – 5, 20, and 100/80[b] mg/kg/day for 26-weeks	(b) (4)	[MDP-103-065-BAR-02]

Test Article: Linaclotide				
Study Description	***Species or Test System***	***Method of Administration***	***Testing Facility***	***Study Report Number***
Metabolism				
Metabolic stability of linaclotide and MM-419447[a] in the presence of conditions known to degrade peptides	Proteolytic Enzymes Simulated Gastric Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-049-IAR-01]
Metabolic stability under various reducing conditions	Proteolytic Enzymes in the Presence and Absence of a Thiol Reducing Agent	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-038-IAR-01]
Reduction by thiols	Thiol Reducing Agents Deproteinized Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-037-IAR-01]
Linaclotide and MM-419447[a] metabolic stability in fluid from mouse ligated intestinal loops	CD-1 Mouse Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-052-IAR-01]
Linaclotide and MM-419447[a] metabolic stability in fluid from rat ligated intestinal loops	Sprague-Dawley Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-035-IAR-01]
Effects of temperature and thiol concentration on metabolic stability in fluid from rat ligated intestinal loops	Sprague-Dawley Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-036-IAR-01]
Metabolism and digestion pathway in fluid from rat ligated intestinal loops	Sprague-Dawley Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-039-IAR-01]
Complete digestion of linaclotide and its initial metabolic products to single amino acids	Protein-Concentrated Sprague-Dawley Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-040-IAR-01]

Test Article: Linaclotide				
Study Description	***Species or Test System***	***Method of Administration***	***Testing Facility***	***Study Report Number***
Cytochrome P450 induction	Human Hepatocytes	In Vitro	(b) (4)	[MDP-103-088-IAR-01]
Cytochrome P450 inhibition	Human Intestinal Microsomes	In Vitro		[MDP-103-066-IAR-01]
Cytochrome P450 inhibition	Human Liver Microsomes	In Vitro		(b) (4)21]
Metabolic stability in the rat intestine	Sprague-Dawley Rats with Ligated Intestinal Loops	Intra-Intestinal Injection (625-5000 ng)	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-034-AMR-01]
Excretion				
Bioanalysis of stools from linaclotide-dosed rats for excreted linaclotide and MM-419447[a]	Sprague-Dawley Rat	PO – 10 mg/kg	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-033-BAR-02]
Other Pharmacokinetic Studies				
Single-dose pharmacokinetics of linaclotide and MM-419447[a] in normal and functionally nephrectomized rats[c]	Sprague-Dawley Rat (Normal and Functionally Nephrectomized)	IV – 1 mg/kg	(b) (4)	[MNP-103-042-PKR-02]
Pharmacokinetic Studies With Drug Substance Impurities				
Metabolic stability of linaclotide multimers in fluid from rat ligated intestinal loops	Sprague-Dawley Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	[MDP-103-055-IAR-01]

Test Article: Linaclotide				
Study Description	***Species or Test System***	***Method of Administration***	***Testing Facility***	***Study Report Number***
Pharmacokinetic Studies With Drug Product Degradants				
Single-dose pharmacokinetics of (b) (4)	Sprague-Dawley Rat	PO – 10 mg/kg IV – 10 mg/kg	**In-Life** Ironwood Pharmaceuticals (Cambridge, MA) **Bioanalysis** (b) (4)	(b) (4)
Single-dose pharmacokinetics of (b) (4)	Sprague-Dawley Rat	PO – 10 mg/kg IV – 10 mg/kg	**In-Life** Ironwood Pharmaceuticals (Cambridge, MA) **Bioanalysis** (b) (4)	(b) (4)
Metabolic stability of (b) (4) and (b) (4) in simulated gastric fluid	Simulated Gastric Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	(b) (4)
Metabolic stability of (b) (4) and (b) (4) in fluid from rat ligated intestinal loops	Sprague-Dawley Rat Intestinal Fluid	In Vitro	Ironwood Pharmaceuticals (Cambridge, MA)	(b) (4)

Nonclinical Study	**Testing Laboratory**	**Report #**	**Lot #**	**Page**
SAFETY PHARMACOLOGY				
Effects of hERG channel current in HEK293 cells	(b) (4)	MNP-103-024-SPR-01	--	32
Cardiovascular and respiratory study in anesthetized dogs using intravenous administration	(b) (4)	MNP-103-025-SPR-01	--	33
PHARMACOLOGIC PROFILES OF (b) (4) IMPURITIES				
Pharmacological activity of linaclotide (b) (4) formed during (b) (4)	Ironwood	(b) (4)	--	31
PHARMACOLOGIC PROFILES OF (b) (4) DEGRADANTS				
Relative binding affinities of the (b) (4) degradant (b) (4) to the GC-C receptor	Ironwood	(b) (4)	--	32
Pharmacological activity of the (b) (4) degradants (b) (4)	Ironwood	(b) (4)	--	32
GENERAL TOXICOLOGY				
Single-dose oral gavage toxicity and toxicokinetic study with MD-1100 acetate in rats with a 14-day recovery period	(b) (4)	7480-104	PPL-MD11000401	49
Single-dose intravenous injection toxicity and toxicokinetic study with MD-1100 acetate in rats with a 14-day recovery period	(b) (4)	7480-103	PPL-MD11000401	49
Up- and-down toxicity study with MM-416775 in Cynomolgus monkeys	(b) (4)	7480-113	--	52
Escalating dose-range finding intravenous injection study with MD-1100 acetate in Cynomolgus monkeys	(b) (4)	7480-115	PPL-MD11000401	53

14-day oral gavage toxicity and toxicokinetic study with MD-1100 acetate in rats with a 14-day recovery period	(b) (4)	7480-106	PPL-MD11000401	57
14-day nasogastric toxicity and toxicokinetic study with MD-1100 acetate in Cynomolgus monkeys with a 14-day recovery period	(b) (4)	7480-102	PPL-MD11000401	60
13-week oral toxicity study of MD-1100 acetate in mice followed by a 2-week recovery period	(b) (4)	MNP-103-007	PPL-MD11000601B1	67
13-week oral toxicity study of MD-1100 acetate in rats followed by a 2-week recovery period	(b) (4)	MNP-103-005	PPL-MD11000501	80
A 13-week toxicity study of MD-1100 acetate administered by oral (nasogastric) intubation to Cynomolgus monkeys, with a 2-week recovery period	(b) (4)	MNP-103-006	PPL-MD11000501	87
26-week oral toxicity study of MD-1100 acetate in mice followed by 4-week recovery period	(b) (4)	MNP-103-027	PPL-MD11000601D	100
39-week toxicity study of MD-1100 acetate administered by oral (nasogastric) intubation to Cynomolgus monkeys with s 4-week recovery period	(b) (4)	MNP-103-028	PPL-MD11000601C, PPL-MD11000603A, and PPL-MD11000603C	115
A 4-week oral toxicity study of linaclotide (and degradant impurities) in mice with a 2-week recovery period	(b) (4)	MNP-103-061	PPL-MD11000903B; X12054A1; Y06063A1	128
GENETIC TOXICOLOGY				
Effect of MD-1100 acetate on the Salmonella-Escherichia coli/Mammalian-microsome reverse mutation assay with a confirmatory assay	(b) (4)	MNP-103-022-GTR-01	PPL-MD11000401	137
Bacterial reverse mutation assay (effects of the impurities)	(b) (4)	MNP-103-068-GTR-01	X10025A1; Y06063A1	139
Effect of MD-1100 acetate on chromosomal aberrations in cultured human peripheral blood lymphocytes	(b) (4)	MNP-103-023-GTR-01	PPL-MD11000401	142
CARCINOGENICITY				
104-week oral carcinogenicity study of linaclotide acetate in rats	(b) (4)	MNP-103-029-CAR-01	PPL-MD11000602B	145
104-week oral carcinogenicity study of linaclotide acetate in mice	(b) (4)	MNP-103-030-CAR-01	PPL-MD11000602B; PPL-MD11000801	164
REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY				
An oral gavage fertility study of linaclotide acetate in the rat	(b) (4)	MNP-103-026-RTR-01	PPL-MD11000602A	188

A study of the effects of linaclotide acetate on embryo-fetal development in mice	(b) (4)	MNP-103-033-RTR-01	PPL-MD11000603C	193
MD-1100 acetate: An oral (gavage) embryo-fetal development study in the rat	(b) (4)	MNP-103-014-RTR-01	PPL-MD11000601A	208
MD-1100 acetate: An oral (gavage) embryo-fetal development study in the rabbit	(b) (4)	MNP-103-015-RTR-01	PPL-MD11000601A	219
Linaclotide acetate: An oral pre and postnatal study in the rat	(b) (4)	MNP-103-041-RTR-01	PPL MD11000604A, PPL-MD11000601D, PPL-MD11000601A	229
JUVENILE TOXICITY				
Linaclotide: An oral range-finding study in the juvenile mouse	(b) (4)	MNP-103-046	PPL-MD11000803	242
Linaclotide: An oral range-finding study in the juvenile rabbit	(b) (4)	MNP-103-TXR-01	PPL-MD11000803	244
Linaclotide: A 9-week oral gavage toxicity study in the juvenile mouse followed by a 4-week recovery period	(b) (4)	MNP-103-054-TXR-01	PPL-MD11000803	246

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Pharmacology/Toxicology Reviews of IND 63,290 by David B. Joseph, Ph.D. dated 1/5/2006, 6/5/2006, and 4/10/2007 are referenced.

4 Pharmacology

4.1 Primary Pharmacology

Linaclotide and MM-419447 Binding to Guanylate Cyclase-C (GC-C) Receptors on T84[1] Cells (Report # MDP-103-060-PHR-010)	
Materials and Methods	**Results and Conclusion**
The binding affinity of linaclotide and the primary active metabolite (MM-419447) to binding sites on cell-surface GC-C receptors on T84 cells was determined using a competitive-binding assay with a known GC-C agonist, heat-stable enterotoxin (pSTa). K_i and IC_{50} values were	Linaclotide and MM-419447 bound in a concentration-dependent manner and displayed high affinity (~2-3 nM) to GC-C receptors on T84 cells.

[1] Human colonic epithelial (T84) cells

determined based on the inhibition of radiolabeled pSTa ([^{125}I]MM-416251) binding.	

The Effect of pH on the Relative Binding Affinities of Linaclotide and Other Related Peptides to the Guanylate Cyclase-C Receptor on T84 Cells (Report # MDP-103-067-PHR-01)	
Materials and Methods	**Results and Conclusion**
The binding affinities of linaclotide, MM-419447, and the human GC-C receptor agonists STa, STc[2], guanylin, and uroguanylin for GC-C receptors were measured in a competitive-binding assay in T84 cells. K_i values were determined based on the inhibition of [^{125}I]pSTa binding under varying pH in the assay medium.	The binding affinity (K_i value) of linaclotide for the GC-C receptor at neutral pH was 3.1 nM. A change of pH did not significantly affect this value. MM-419447 or human STa and STc peptides bound to GC-C receptors independent of pH. The binding of guanylin or uroguanylin to GC-C receptors was pH-dependent. The binding of guanylin was reduced at an acidic pH compared to neutral and basic conditions, while uroguanylin showed the reverse trend. The Sponsor stated that these results were expected due to the difference in the number of disulfide bonds in linaclotide (2) and guanylin/uroguanylin (3).

***In vitro* Stimulation of Cyclic GMP Production by MD-1100 Acetate and MM-419447 in Human Colonic Epithelial Cells (Report # MDP-103-001-PHR-01)**	
Materials and Methods	**Results and Conclusion**
To study the ability of linaclotide (MD-1100 acetate) and MM-419447 to stimulate cGMP production in a concentration-dependent manner, human T84 cells were exposed to multiple concentrations of either linaclotide or MM-419447. cGMP production was measured using a competitive enzyme immunoassay.	Linaclotide and MM-419447 stimulated cGMP production in a concentration-dependent manner (range of 0.3 to 1,000 nM) in human T84 cells. The activity of MM-419447 was not statistically different from linaclotide. Therefore, linaclotide and MM-419447 have very similar pharmacological potencies.

Linaclotide and STc Peptide Induced Apical and Basolateral cGMP Efflux from a Caco-2 Cell Monolayer (Report # MDP-103-053-PHR-01)	
Materials and Methods	**Results and Conclusion**
To study the ability of linaclotide and STc to induce a cGMP influx from Caco-2 cells, linaclotide or STc was added to the apical side of a monolayer of Caco-2 cells, which endogenously express GC-C. After incubation, the apical and basolateral media were removed and cGMP was extracted from the Caco-2 cells.	Administration of linaclotide and STc produced a concentration-dependent increase in the intracellular cGMP levels and cGMP levels in both the apical and basolateral compartments. In general, the peptide STc showed a greater magnitude of increase in cGMP levels than linaclotide.

Active Transport of Cyclic Guanosine Monophosphate (cGMP) in Caco-2 Monolayers following Activation of the Guanylate Cyclase-C (GC-C) Receptor (Report # MDP-103-081-PHR-01)	
Materials and Methods	**Results and Conclusion**

[2] STc: Synthetic peptide comprised of the core sequence amino acids 6-19 of *E. coli* heat-stable enterotoxin derived from a human source

Reference ID: 3114166

To examine the GC-C receptor-stimulated efflux of cGMP using known active transport inhibitors, STc was added to the apical side of Caco-2 cell monolayers and incubated in the absence or presence of transport inhibitors. After incubation, the apical and basolateral media were removed and cGMP was extracted from the Caco-2 cells. The following transport inhibitors were tested: probenecid (inhibitor of the organic anion transporter and multidrug resistance-associated protein 4 and 5 (MRP4 and MRP5)), dipyridamole (MRP4 inhibitor), and sulfinpyrazone (MRP5 inhibitor).	STc significantly elevated intracellular cGMP concentrations in Caco-2 cell monolayers. The amount of cGMP in both the apical and basolateral compartments was also elevated. At higher concentrations (≥ 0.5 mM), probenecid caused a significant increase in intracellular cGMP compared to STc alone. Sulfinpyrazone did not affect intracellular cGMP accumulation. In contrast, dipyridamole increased the intracellular cGMP concentration, and this effect was associated with inhibition of apical and basolateral efflux of cGMP. Greater inhibition occurred in apical efflux. Thus, activation of the GC-C receptor resulted in the production of cGMP, which is actively transported out of cells by both apical and basolateral transporters.

Investigation of the Effects of cGMP on Colonic Sensory Afferents（Report # MDP-103-080-PHR-02)	
Materials and Methods	**Results and Conclusion**
To determine the effect of extracellular cGMP on sensory afferents in the mouse colonic wall, mice were treated with 2,4,6-trinitrobenzenesulfonic acid (TNBS) to induce visceral hypersensitivity (i.e., colitis). After 28 days (recovery), preparations of mouse colorectum with attached pelvic or splanchnic nerves were used to examine the mechano-sensitivity of colonic afferents, their responsiveness to cGMP, and the subsequent effect of cGMP on afferent mechano-sensitivity. cGMP was applied to the mucosal surface of the colon and any direct chemosensory effect was recorded.	In healthy control mice, cGMP significantly decreased the mechano-sensitivity of high-threshold colonic afferents (pain fibers) and increased the response of mucosal afferents (non-pain fibers). In mice recovering from TNBS-induced post-inflammatory hypersensitivity (demonstrated by increased mechano-sensitivity of pain fibers), cGMP decreased the mechano-sensitivity of high-threshold afferents in a concentration dependent manner. Therefore, cGMP decreased the mechano-sensitivity of splanchnic high-threshold colonic afferents in the absence (normal tissue) or presence of TNBS-induced visceral hypersensitivity. In the post-inflammatory state, cGMP no longer affected the response of mucosal afferents.

Effect of MD-1100 Acetate on Intestinal Secretion in Guanylate Cyclase-C Deficient Mice（Report # MDP-103-026-PHR-01)	
Materials and Methods	**Results and Conclusion**
To evaluate the role of GC-C in linaclotide-induced intestinal secretion and cGMP concentration, linaclotide was injected into intestinal loops in wild-type (WT) and GC-C knockout (KO) mice. To confirm that the effects of linaclotide are dependent on functional GC-C (the proposed receptor for linaclotide), the binding of linaclotide to extracts from intestinal epithelial cells from WT and GC-C KO mice was measured.	In WT mice, linaclotide bound specifically to the intestinal mucosa and increased cGMP levels and intestinal secretion. However, linaclotide produced no increase in either cGMP levels or intestinal secretion in GC-C KO mice. In addition, binding was reduced by ~ 90% in epithelial cell extracts from GC-C KO mice. Thus, the pharmacological effects of linaclotide in mice are mediated by functional GC-C.

Reference ID: 3114166

Binding Affinity of Linaclotide to Cell-Surface Receptors on Rat Intestinal Mucosa (Report # MDP-103-059-PHR-01)	
Materials and Methods	**Results and Conclusion**
The binding affinity of linaclotide for cell-surface GC-C receptors on intestinal mucosal cells from Sprague-Dawley rats was determined using a competitive binding assay with radiolabeled pSTa.	Linaclotide bound to GC-C receptors on rat intestinal mucosa with high affinity, as demonstrated by the inhibition of pSTa binding in a concentration-dependent manner (K_i = 4.3 nM).

Determination of the Relative Binding Affinities of Linaclotide and MM-419447 to Guanylate Cyclase-C Receptors on Rat Intestinal Brush Border Membranes (Report # MDP-103-071-PHR-01)	
Materials and Methods	**Results and Conclusion**
The binding affinities of linaclotide and MM-419447 for GC-C receptors on rat intestinal brush-border membranes were determined using a competitive binding assay with radiolabeled pSTa.	Linaclotide and MM-419447 bound to rat small intestinal brush-border membranes with high affinity. The K_i values for linaclotide and MM-419447 were 2.6 and 4.9 nM, respectively.

Linaclotide Binding to Intestinal Mucosal Cells and Brush-Border Membranes from Wild-Type and Guanylate Cyclase-C Knockout Mice (Report # MDP-103-058-PHR-01)	
Materials and Methods	**Results and Conclusion**
To investigate if the GC-C receptor is the molecular target of linaclotide, the binding of linaclotide to intestinal mucosal cell extract and brush-border membranes from WT and GC-C KO mice was investigated. Intestinal mucosal cell extracts from WT and GC-C KO mice were incubated with [^{125}I]linaclotide in the presence and absence of excess unlabeled linaclotide competitor. For binding to brush-border membranes, a range of linaclotide concentrations were incubated with [^{125}I]pSTa. K_i values were calculated for linaclotide binding to WT and GC-C KO mouse brush-border.	There was a 10-fold reduction in [^{125}I]linaclotide binding to intestinal mucosal cell extract from GC-C KO mice, compared to WT mice. The binding of [^{125}I]linaclotide to the WT intestinal mucosal cell extract was almost completely inhibited (95%) by the unlabeled competitor. [^{125}I]pSTa bound to GC-C KO brush border membranes, but at a reduced extent compared to WT membranes. Linaclotide competed with [^{125}I]pSTa for binding to intestinal brush border membranes from WT mice (K_i = 16.2 nM). The low level of [^{125}I]pSTa binding in GC-C KO intestinal brush-border membranes was inhibited by linaclotide in a concentration-dependent manner (K_i = 11.9 nM).

Effect of MD-1100 Acetate on Gastrointestinal Secretion in Rats (Report # MDP-103-024-PHR-01)	
Materials and Methods	**Results and Conclusion**
To measure linaclotide binding to the GC-C receptor, linaclotide and radiolabeled pSTa were incubated with intestinal epithelial cells from SD rats. To determine the effects of linaclotide on intestinal fluid secretion and cGMP levels, linaclotide (5 μg) was injected into ligated intestinal loops in SD rats and cGMP in the intestinal fluid was measured.	Linaclotide bound with high affinity to the rat GC-C receptors on intestinal epithelial cells (K_i = 4.5 nM). Injection of linaclotide into ligated intestinal loops increased luminal fluid secretion (~4.5-fold) and increased cGMP concentration (~17-fold).

Effect of MD-1100 Acetate and MM-419477 on Gastrointestinal Secretion in Rats (Report # MDP-103-085-PHR-01)	
Materials and Methods	**Results and Conclusion**

To investigate potential *in vivo* effects of linaclotide and MM-419447 on the transport of cGMP into the intestine and on fluid secretion in the intestine, linaclotide or MM-419447 (5 μg) was injected into ligated intestinal loops in SD rats and cGMP in the intestinal fluid was measured.	Injection of either linaclotide or MM-419477 resulted in a statistically significant increase in luminal fluid secretion and a significant increase in the amount of cGMP in different portions of the small intestine in rats.

Effect of cGMP on Colonic Afferents in Rats under Post-inflammatory Conditions (Report # MDP-103-082-PHR-03)	
Materials and Methods	**Results and Conclusion**
Control SD rats and rats with TNBS-induced colitis were intraduodenally administered with cGMP. The firing rates of colonic afferents in response to colorectal distension (CRD) was measured prior to and after cGMP administration.	At 3 mg/kg, cGMP had no significant effect upon afferent firing rates during colonic distension in control animals at intracolonic pressures of 10-50 mmHg, but did significantly reduce afferent firing rates (50%) at 60 mmHg. At a dose of 30 mg/kg, cGMP increased the basal afferent activity in control animals, compared to baseline (0.57 imp/s compared to 0.4 imp/s; $p<0.05$), but had no effect on afferent firing rates at all pressures tested. In animals with TNBS-induced colitis, 3 mg/kg cGMP decreased colonic afferent firing rates at distension pressures ranging from 30-60 mmHg when compared to baseline. Administration of 30 mg/kg cGMP significantly increased the baseline afferent firing rate (1.5 imp/s compared to 0.5 imp/s at baseline), but had no effect on afferent firing rates during colorectal distension at all pressures tested. Therefore, 3 mg/kg cGMP decreased colonic afferent firing in a rat model of colitis. This effect was not dose-dependent, given that afferent firing rates were unaffected at 30 mg/kg.

Effects of MD-1100 Acetate and Tegaserod Maleate (Zelnorm®) on Intestinal Secretion in Suckling Mice (Report # MDP-103-015-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide and tegaserod maleate on intestinal secretion, single oral doses (2 to 1,250 μg/kg) of linaclotide or tegaserod maleate were administered to groups of suckling CD-1 mice and fluid secretion into the intestines was measured.	Administration of linaclotide to mice increased intestinal secretion in a dose-dependent manner at doses ≥ 2.5 μg/kg. In contrast, there was no increase in secretion in the mice treated with tegaserod maleate.

Effects of MD-1100 Acetate and MM-419447 on Intestinal Secretion in Suckling Mice and Gastrointestinal Transit in Rats (Report # MDP-103-018-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide and MM-419447 on intestinal secretion in CD-1 suckling mice, single oral doses (2.5 to 10 μg/kg) of linaclotide or MM-419447 were administered to groups of suckling mice and intestinal fluid secretion was measured. To assess the effects of linaclotide and MM-419447 on GI transit, single oral doses	Administration of linaclotide or MM-419447 to mice resulted in a significant, dose-dependent increase in intestinal secretion at doses ≥ 2.5 μg/kg. In addition, the administration of linaclotide or MM-419447 to female rats resulted in a significant

(6.3 to 50 μg/kg) of linaclotide or MM-419447 were administered to female SD rats and intestinal transit was measured via charcoal ingestion (i.e., the distance the charcoal front travels along the small intestine in a given time period).	increase in GI transit at doses ≥ 6.3 μg/kg. There were no differences in transit values or intestinal secretion between linaclotide and MM-419447.

Effects of MD-1100 Acetate and Tegaserod Maleate (Zelnorm®) on Gastrointestinal Transit in Mice (Report # MDP-103-016-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide and tegaserod maleate on GI transit, single oral doses of linaclotide (6 to 3,000 μg/kg in females and 6 to 100 μg/kg in males) or tegaserod maleate (30 to 3,000 μg/kg in females) were administered to groups of mice and intestinal transit was measured via charcoal ingestion (i.e., the distance the charcoal front travels along the small intestine in a given time period).	Administration of linaclotide or MM-419447 to mice significantly increased GI transit at doses ≥ 25 μg/kg. In female mice treated with tegaserod maleate, only the 3,000 μg/kg group showed a significant increase in GI transit.

Effects of MD-1100 Acetate and Tegaserod Maleate (Zelnorm®) on Gastrointestinal Transit in CD (SD) Rats (Report # MDP-103-013-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide and tegaserod maleate on GI transit, single oral doses of linaclotide (1.3 to 20 μg/kg in males only) or tegaserod maleate (1,000 or 3,000 μg/kg in females only) were administered to groups of SD rats and intestinal transit was measured via charcoal ingestion (i.e., the distance the charcoal front travels along the small intestine in a given time period).	Compared to vehicle controls, administration of linaclotide at dose levels ≥ 5 μg/kg significantly increased GI transit in a dose-dependent manner in rats. Tegaserod maleate did not increase intestinal transit in female rats.

Effect of MD-1100 Acetate on Gastrointestinal Transit in Guanylate Cyclase-C Deficient Mice (Report # MDP-103-025-PHR-01)	
Materials and Methods	**Results and Conclusion**
To evaluate whether a functional GC-C receptor is required for a linaclotide-induced increase in GI transit, a single oral dose of linaclotide (100 μg/kg) was administered to either WT or GC-C KO mice and intestinal transit was measured via charcoal ingestion (i.e., the distance the charcoal front travels along the small intestine in a given time period).	Administration of linaclotide produced a significant increase in the GI transit rate in WT mice, but did not lead to an increase in GI transit in the GC-C KO mice. Thus, the GC-C receptor is necessary to mediate the effect of linaclotide on GI transit.

Effect of MD-1100 Acetate on TNBS-induced Visceral Hypersensitivity in WT and KO Guanylate Cyclase-C Deficient Mice (Report # MDP-103-023-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the role of GC-C in linaclotide-mediated reduction in inflammation-induced visceral hypersensitivity, WT and GC-C KO mice were treated with TNBS to induce colonic inflammation. Visceral hypersensitivity to CRD (colorectal distension) was examined via electromyography recordings, before and after TNBS-induced inflammation.	Under basal conditions, there was a significant difference in the basal sensitivity to CRD between WT and GC-C KO mice. Linaclotide (0.3 mg/kg) significantly reduced the sensitivity to specific distention volumes in both WT and GC-C KO mice. After TNBS-induced inflammation, both WT and GC-C KO mice have a similar visceral hypersensitivity to CRD. However, the

	administration of linaclotide (0.01 μg/kg) significantly reduced the hypersensitivity to CRD in WT mice, but not in GC-C KO mice. Thus, the linaclotide-mediated reduction in visceral pain was dependent on the presence of functional GC-C receptors.

Effect of Linaclotide on Stool Consistency in Mice and Rats (Report # MDP-103-032-PHR-01)	
Materials and Methods	**Results and Conclusion**
To determine the dose levels at which linaclotide produces an exaggerated pharmacological effect of altering stool consistency, the Sponsor conducted single dose, oral studies in CD-1 mice and SD rats, and a 5-day repeat-dose oral study in mice with linaclotide (50 to 200 mg/kg).	Acute doses of linaclotide increased the incidence of watery/non-formed stools in female mice and rats at doses ≥ 50 mg/kg, and male mice and rats at the 200 mg/kg dose. In the 5-day repeat-dose study, watery/non-formed stools were observed in the 200 mg/kg/day mice, however there were no adverse effects on body weight.

The Effects of Single and Repeated Exposure to MD-1100 Acetate and Tegaserod Maleate (Zelnorm®) on Gastrointestinal Transit in Mice (Report # MDP-103-017-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide and tegaserod maleate on GI transit, the Sponsor conducted a single dose oral study and a 5-day repeat-dose oral study in female CD-1 mice with linaclotide (60 or 250 μg/kg) or tegaserod maleate (1,000 μg/kg). Intestinal transit was measured using administration of activated charcoal.	Single- and repeat-dose studies showed that the administration of linaclotide resulted in a significant increase in GI transit for both doses tested. The magnitude of this effect was similar in the single- and repeat-dose studies. Therefore, repeated oral dosing of linaclotide did not sensitize nor desensitize female mice to the effects of this peptide. Mice treated with tegaserod maleate did not show a significant increase in intestinal transit.

Effect of MD-1100 Acetate in a Rat Model of Stress-Induced Visceral Hyperalgesia (Report # MDP-103-022-PHR-01)	
Materials and Methods	**Results and Conclusion**
The objective of this study was to assess the visceral anti-nociceptive effect of linaclotide under basal conditions, and the ability of linaclotide to reduce visceral hyperalgesia. Male Wistar rats were subjected to the water avoidance test as a model of stress-induced visceral hyperalgesia. The visceromotor response to CRD (colorectal distension) was measured using electromyography, prior to and after the WAS[3]. An oral dose of linaclotide (3 or 30 μg/kg) was given prior to WAS to assess the linaclotide effect under basal conditions. Linaclotide dosing was repeated after WAS at 3 and 10 μg/kg, to measure the drug effect on the visceromotor response during visceral hyperalgesia.	After WAS, the visceromotor response to CRD was increased, and the administration of 3 μg/kg linaclotide showed significant anti-hyperalgesic properties (i.e. reduction of the increased visceromotor response to CRD). Additionally, a reduced visceromotor response to CRD was noted after 10 μg/kg linaclotide. Therefore, 3 μg/kg linaclotide was anti-hyperalgesic after WAS, while the same dose did not affect basal visceral nociception in rats. However a 30 μg/kg dose showed a pro-nociceptive effect under basal conditions.

Effect of MD-1100 Acetate on Inflammation and Stress-Induced Colorectal Hyperalgesia to Distension in Rats (Report # MDP-103-014-PHR-01)

[3] WAS: water avoidance stress

Materials and Methods	Results and Conclusion
The objective of this study was to assess the anti-nociceptive effects of linaclotide on colorectal pain in two models of colorectal hyperalgesia. Male Wistar rats were subjected either to TNBS treatment (colonic inflammation) or restraint-induced stress, to induce visceral hyperalgesia. CRD (colorectal distension) was performed prior to and after TNBS treatment or restraint. In both models, rats were administered single oral doses of linaclotide (0.3 to 30 μg/kg) before CRD. Visceral pain was quantified via measurement of abdominal muscle contractions.	Linaclotide (0.3 μg/kg) significantly reduced the abdominal contractile response to CRD following TNBS-induced inflammation. Linaclotide (30 μg/kg) also significantly reduced the abdominal contractile response to CRD following partial restraint stress. Therefore, linaclotide reduced colorectal hyperalgesia in rat models of inflammation- and stress-induced visceral pain.

Effect of MD-1100 Acetate on TNBS-induced Rectal Hyperalgesia to Distension in Rats (Report # MDP-103-030-PHR-01)	
Materials and Methods	**Results and Conclusion**
A CRD (colorectal distension) procedure was conducted on Wistar rats prior to and after induction of colonic inflammation with TNBS treatment. To assess the effect of linaclotide on visceral pain in this model of inflammation-induced hyperalgesia, oral doses of linaclotide (0.01 to 3 μg/kg) were administered prior to the CRD procedure. Visceral pain was quantified via measurement of abdominal muscle contractions.	Under basal conditions, CRD was associated with an increase in abdominal contractions, and CRD-induced contractions were significantly increased after TNBS-administration. Linaclotide significantly reduced the abdominal response to CRD, indicative of anti-nociceptive activity, at doses of 0.01 to 0.3 μg/kg, but not at 3 μg/kg.

The Effect of cGMP on Stress-induced Visceral Pain in Rats （Report # MDP-103-083-PHR-03)	
Materials and Methods	**Results and Conclusion**
To assess the effect of cGMP on stress-induced colorectal hypersensitivity to distension, cGMP (0.03 to 30 mg/kg) was orally administered to female Wistar rats during partial restraint-induced stress, and before and after CRD. Visceral pain was quantified via measurement of abdominal muscle contractions.	Administration of 3 and 10 mg/kg cGMP significantly reduced stress-induced colorectal hypersensitivity to different levels of CRD. Therefore, at specific doses, cGMP reduced visceral pain in a rat model of stress-induced hypersensitivity.

Anti-nociceptive Effect of cGMP on Basal Sensitivity and Post-inflammatory Colorectal Hypersensitivity to Distension in Rats （Report # MDP-103-084-PHR-02)	
Materials and Methods	**Results and Conclusion**
To assess the anti-nociceptive properties of cGMP on both basal and post-inflammatory colorectal hypersensitivity, male Wistar rats were subjected to CRD under basal conditions and at 5 days after intrarectal administration of TNBS. Oral doses of cGMP (3, 30 or 50 mg/kg) were administered before both CRD procedures.	Administration of TNBS significantly increased the abdominal response to CRD relative to baseline. Administration of cGMP (3 mg/kg) significantly reduced the abdominal response under basal conditions and with post-inflammatory hypersensitivity. Thus, only 3 mg/kg cGMP significantly reduced TNBS-induced hypersensitivity in rats, whereas higher doses had no effect.

4.2 Secondary Pharmacology

Effects of MD-1100 Acetate in a Panel of *In Vitro* Receptor Binding Assays（Report # MDP-103-

019-PHR-01)	
Materials and Methods	**Results and Conclusion**
To evaluate the selectivity of linaclotide for the GC-C receptor and to identify potential interactions of linaclotide with other receptors and ion channels, linaclotide binding affinity and activity were measured against a panel of 50 diverse mammalian receptors and ion channels.	Linaclotide (10 μM) showed no significant binding or activity against various mammalian receptors and ion channels. 10 μM linaclotide is >1,000-times the *in vitro* pharmacologically active concentration of linaclotide for the GC-C receptor, thus the results suggest that linaclotide is highly selective for the GC-C receptor.

Effect of MD-1100 Acetate on Postoperative Ileus in Rats （Report # MDP-103-027-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide on GI transit in a rat model of postoperative ileus, female SD rats underwent abdominal surgery followed by administration of single oral doses of linaclotide (10 μg/kg). Intestinal transit was measured via charcoal ingestion (i.e., the distance the charcoal front travels along the small intestine in a given time period).	Linaclotide administration resulted in a significant increase in intestinal transit following abdominal surgery. Therefore, linaclotide reversed the surgically-induced delay in transit in a rat model of post-operative ileus.

Effect of Linaclotide on Opiate-Induced Delayed GI Transit in Rats （Report # MDP-103-043-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effects of linaclotide on morphine-induced slowing of GI transit, female SD rats were administered morphine (ip injection) followed by administration of single oral doses of linaclotide (13 to 50 μg/kg). Intestinal transit was measured via charcoal ingestion (i.e., the distance the charcoal front travels along the small intestine in a given time period).	Linaclotide administration resulted in a dose-dependent reversal of the morphine-induced delay in GI transit.

Influence of MD-1100 Acetate on Basal (Sham) and Stress-induced Increased Colonic Paracellular Permeability in Rats （Report # MDP-103-031-PHR-01)	
Materials and Methods	**Results and Conclusion**
To assess the effect of linaclotide on stress-induced increases in paracellular permeability, female Wistar rats were surgically implanted with a catheter inserted at the ceco-colonic junction. The colonic paracellular permeability was evaluated by measuring urine levels of a radiolabeled compound (^{51}Cr-EDTA) infused into the colon. The urine measurements were conducted prior to and after a partial restraint stress session. Rats were administered single oral doses of linaclotide (0.3 to 10 μg/kg) after stress induction.	Twenty-four hours after the partial restraint stress session, rats showed a significant increase in urinary excretion of the radiolabeled compound. Oral administration of linaclotide did not modulate the stress-induced change in excretion of radiolabeled compound. Therefore, linaclotide did not change colonic paracellular permeability in rats.

Pharmacological Activity of Linaclotide (b) (4) (b) (4)	
Materials and Methods	**Results and Conclusion**
Linaclotide (b) (4) are similar to those formed (b) (4). Linaclotide was incubated in 20 mM sodium phosphate buffer to generate (b) (4),	In the cells incubated with the (b) (4), the cGMP concentrations increased by 0.44 and 0.82 nM, while cells incubated with linaclotide showed a 16.8 and

which were isolated from unchanged linaclotide and purified using multiple rounds of size-exclusion chromatography and high-resolution mass spectrometry. The purified solution was tested for GC-C agonist activity by incubating with T84 cells, and testing cell lysates for the concentration of cGMP.	15.1 nM increase. The minimal increase in cGMP levels associated with the (b) (4) indicates that the (b) (4) are not proficient GC-C receptor agonists and do not share the pharmacological activity of linaclotide.

Determination of the Binding Affinities of Linaclotide (b) (4) Degradants (b) (4) and (b) (4) to the Guanylate Cyclase-C Receptor on T84 Cells	
Materials and Methods	**Results and Conclusion**
The binding affinities of the linaclotide (b) (4) degradants, (b) (4), for the GC-C receptor on T84 cells were measured in a competitive-binding assay in T84 cells. K_i values were determined based on the inhibition of radiolabeled pSTa binding. (b) (4) were used in this study. Linaclotide was also tested as a comparator.	(b) (4) and linaclotide competitively inhibited the specific binding of radiolabeled pSTa to cell-surface GC-C receptors on T84 cells. The inhibition constants (K_i) were not statistically different [K_i = 3.9 nM (linaclotide), 1.4 nM (b) (4) and 5.2 nM (b) (4)]. Therefore, the (b) (4) degradants bind to GC-C receptors with affinities similar to that of linaclotide.

***In Vitro* Pharmacological Guanylate Cyclase-C Agonist Activity of Linaclotide (b) (4) Degradants (b) (4) and (b) (4) in T84 Cells**	
Materials and Methods	**Results and Conclusion**
The *in vitro* pharmacological activity of the linaclotide-related (b) (4) degradants, (b) (4), (b) (4), was evaluated by testing (b) (4) and (b) (4) for GC-C receptor agonist activity. Linaclotide was also tested as a comparator.	(b) (4) and linaclotide produced an increase in intracellular cGMP levels. Thus, both of the degradants are GC-C agonists. Although there were no statistically significant differences in EC_{50} values, the EC_{50} for (b) (4) was (b) (4) lower than linaclotide.

4.3 Safety Pharmacology

The following study was previously reviewed by David B. Joseph, Ph.D. (Pharmacology/ Toxicology Review of IND 63,290 dated June 5, 2006).

Effects on hERG Channel Current in HEK293 Cells (Study #MNP-103-024-SPR-01)

Methods: The human embryonic kidney cell line HEK293 was stably transfected with hERG cDNA. Whole cell recordings were performed using a patch clamp amplifier. Phase 1 of the study was a concentration range-finding experiment. The effects of MD-1100 acetate (10 μM) on the activation of hERG current and steady state hERG current were measured. The voltage was clamped at –80 mV. hERG channel current was activated by increasing the voltage to +20 mV for 1 sec, followed by a gradual repolarization to –80 mV. Each recording ended with an application of a supramaximal concentration of the reference substance (500 nM E-4031) to assess the contribution of endogenous currents. The remaining unblocked current was subtracted from the data. Three cells per condition were tested. In Phase 2 of the study, MD-1100 acetate was tested at 100 μM (n = 3). The cells were depolarized to +20 mV and a steady-state current was maintained for 20 sec before application of the test article or positive control

compound. Peak current was measured until a new steady state was achieved or until 12 min of exposure time had elapsed. The vehicle for MD-1100 acetate was HEPES-buffered physiological saline, which was tested as a control article.

Results/Conclusions: MD-1100 acetate (10 and 100 μM) had no effect on hERG channel current (mean inhibition of hERG channel current < 4%). The positive control compound terfenadine (60 nM) inhibited hERG channel current by 83%.

The following study was previously reviewed by David B. Joseph, Ph.D. (See Pharmacology/Toxicology Review of IND 63,290 dated June 5, 2006).

Cardiovascular and Respiratory Study in Anesthetized Dogs Using Intravenous Administration (Study #MNP-103-025-SPR-01)

Methods: Beagle dogs (4 males and 4 females, about 6-12 months old, 8.8-13.2 kg) were used. The dogs were anesthetized by continuous intravenous (iv) infusion of propofol. A cannula filled with heparin/saline was placed in the femoral artery for measurement of blood pressure. A similar cannula was placed in the femoral vein for removal of blood samples. A catheter tip transducer was inserted in the left ventricle via the left carotid artery for measurement of ventricular pressure. At the end of the surgical procedures, the anesthetic dose was altered as necessary and the dogs were allowed to breathe spontaneously. Respiratory parameters were monitored through an endotracheal tube connected to a pneumotachograph and a pressure transducer. The study groups were then treated with escalating doses of MD-1100 acetate or vehicle during a single session (2 dogs/sex/group). The drug-treated group was given 0.2, 1, and 5 mg/kg iv MD-1100 acetate. Each dose was administered by slow injection over 15 min. The control group was treated with 0.1, 0.5, and 2.5 ml/kg iv phosphate buffered saline (the vehicle used for the test article). The dose volumes used for the vehicle control group were identical to those used in the drug-treated group. The test and control articles were administered via the jugular vein at intervals of at least 25 min. Cardiovascular parameters, including lead II ECG, were measured prior to drug administration (-20 and -10 min), at 2, 10, and 20 min after dosing with 0.2 and 1 mg/kg, and at 2, 10, 20, 30, 60, and 90 min after dosing with 5 mg/kg. Respiratory parameters were also measured at these time-points. Blood was collected at 0 (end of injection), 15, 30, 60, and 90 min after administration of 5 mg/kg for measurement of plasma drug concentrations. Analysis of the dosing solution used revealed that the drug concentration was 89.2% of the target concentration of 2 mg/ml.

Results: The following cardiovascular parameters were reported as the combined mean for males and females: systolic pressure, diastolic pressure, mean arterial pressure, heart rate, dP/dt, femoral artery blood flow, resistance, RR, QRS, PR, QT, QTc (Fridericia's formula), R amplitude, P amplitude, and T amplitude. No effects were observed. The following respiratory parameters were reported as the combined mean for males and females: peak inspiratory flow, peak expiratory flow, tidal volume, minute volume, and respiratory rate. There were no effects. The plasma kinetic parameters

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determined for the 5 mg/kg dose level are shown in the following table. The C_{max} and AUC values were slightly higher in the males.

Dose (mg/kg)	Sex	$t_{1/2}$ (min)	C_{max} (µg/ml)	$AUC_{15\text{-}90\ min}$ (µg•min/ml)
5	Male	27.3 ± 0.2	30.8 ± 2.0	1374 ± 314
5	Female	30.4 ± 0.1	24.3 ± 1.8	994 ± 81

Values are the mean ± S.D. from 2 animals.

Conclusions: Cardiovascular parameters (including ECG) and respiratory parameters were not affected. The true dose levels used in this study were approximately 0.18, 0.89, and 4.5 mg/kg iv, based on the result of the dose formulation analysis.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1 Brief Summary

Oral bioavailability of linaclotide in rats and mice is extremely limited, ranging from 0.08% to 0.20%. MM-419447, the active metabolite, was detected following IV and oral administration of linaclotide. The oral bioavailability of this metabolite in rats also is extremely low, as observed for the unchanged drug. Linaclotide and MM-419447 have no detectable accumulation in brain, liver, or adipose tissues after oral administration. The degradation pathway of linaclotide in the intestine involves formation of the active metabolite MM-419447 by enzymatic removal of the C-terminal tyrosine of linaclotide. Under reducing conditions, the disulfide bonds of linaclotide and MM-419447 are reduced, resulting in the loss of tertiary structure and pharmacologic activity. The loss of tertiary structure is rapidly followed by proteolysis to small peptides and amino acids. Both linaclotide and MM-419447 are mainly cleared in the kidneys. However, even in nephrectomized rats, the concentration of linaclotide and MM-419447 decreased with time, suggesting the presence of non-renal clearance pathways. The detection of both active peptides in the bile of normal rats shortly after intravenous dosing indicated that biliary clearance is an additional route of elimination. Neither linaclotide nor MM-419447 is an inducer or inhibitor of the hepatic cytochrome P450 enzymes. In addition, linaclotide is not an inhibitor of the intestinal cytochrome P450 enzymes. Oral bioavailability of two linaclotide (b) (4) degradants, (b) (4) and (b) (4) is also very low. These degradants were also rapidly metabolized in the intestinal fluid.

5.1.2 Methods of Analysis

[see under individual study reviews]

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5.1.3 Absorption

The first four studies presented below were reviewed by Dr. David Joseph (Pharmacology/Toxicology review of IND 63,290 dated 6/5/2006). Because the Sponsor has re-analyzed the studies, modified reviews of the study are presented below. It is noted that the conclusions of the studies remain the same.

Oral Bioavailability Study of MD-1100 Acetate in Mice Using LC/MS (Study No.: MDP-103-003)

Methods: Female CD-1 mice (approximately 25 g, age 7-8 weeks) were treated with 8 mg/kg MD-1100 acetate by either intravenous or oral administration (9 mice for IV administration and 6 mice for oral administration). The dose volume was 8 ml/kg for both routes of administration. The vehicle was 20 mM Tris-HCl, pH 7.5. Blood samples were collected at 2, 5, 20, 40, 60, 90, 180, 240, and 360 min following IV administration, and at 20, 40, 60, 90, 180, and 240 min following oral administration (3 mice/time-point). Serum drug concentration was measured using a LC/MS method. The lower limit of detection (LOD) was 1.83 ng/ml. Values that were lower than the LOD were set to 0 ng/ml for all calculations.

Results: The calculated plasma kinetic parameters are shown in the following table taken from the study report.

Table 5. Calculated Pharmacokinetic Parameters for Linaclotide in CD-1 Mice Following an 8 mg/kg Dose

INTRAVENOUS					
PK Parameter	$AUC_{0\text{-}inf}$ (ng·h/mL)	C_0 (ng/mL)	Cl_s (mL/kg/h)	V_d (mL/kg)	$t_{1/2}$ (h)
Value	8,170	43,200	979	854	0.605
ORAL					
PK Parameter	AUC_{all} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	F (%)	
Value	5.85	8.80	0.33	0.0716	
SE	5.53	7.87	NA	NA	

SE = Standard Error
NA = Not Applicable

Using the oral and intravenous AUC values, the absolute oral bioavailability of linaclotide was calculated to be 0.0716%.

Conclusions: Oral bioavailability of linaclotide in female mice was calculated to be 0.07%, and is therefore extremely limited.

Oral Bioavailability Study of MD-1100 acetate in Rats Using LC/MS (Study No.: MDP-103-010)

Methods: Female CD rats (about 188 g, age not stated) were treated with 10 mg/kg MD-1100 acetate by either intravenous or oral administration (12 rats/group). The dose volume was 1.6 ml/kg for both routes of administration. The vehicle was 20 mM Tris-HCl, pH 7.5. Blood samples were collected at 2, 5, 20, 40, 60, 90, 180, 240, 360, and 1440 min following IV administration, and at 5, 20, 40, 60, 90, 180, 240, 360, and 1440 min following oral administration (6 rats/time-point). Plasma concentration of MD-1100 acetate and its metabolite MM-419447 (the parent peptide without the carboxy-terminal tyrosine) was measured using a LC/MS method. The lower limit of detection (LOD) for MD-1100 acetate and MM-419447 was 0.153 ng/ml and 0.682 ng/ml, respectively. Values that were lower than the LOD were assumed to be equal to the LOD for the purpose of estimating the AUC value.

Results: The plasma kinetic parameters are shown in the following table taken from the study report.

Table 5. PK Parameters for Linaclotide and MM-419447 after Oral and Intravenous Administration of Linaclotide to Sprague-Dawley Rats

	Intravenous	Oral			F
	AUC_{all} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC_{all} (ng·h/mL)	
Linaclotide	18,800	19.0	0.08	≤ 19.8	≤ 0.105%
MM-419447	≤ 81.4	1.66	1	≤ 4.91	NA
Total Active Peptide	≤ 18,900	---	---	≤ 24.7	≤ 0.131%

NA = Not applicable

Using the oral and IV AUC values, the absolute oral bioavailability of linaclotide was calculated to be ≤ 0.105%. Systemic exposure to total active peptide (linaclotide and MM-419447) was estimated by including the AUC values for MM-419447 in the %F calculation and the bioavailability was ≤0.131%.

MD-1100 acetate was detected up to the 6 hr time-point after IV administration, and up to 4 hr in the orally-treated rats. The elimination of drug from plasma was extremely rapid during the first 20 min after IV administration, followed by a slower beta phase. The elimination of metabolite MM-419447 from plasma following IV administration was slower than that observed for the unchanged drug, based on the appearance of the concentration vs. time curve. The AUC value for MM-419447 in intravenously-treated rats exceeded the value in orally-treated rats by about 16-fold. However, the $AUC_{MM-419447}/AUC_{drug}$ ratio was markedly higher in orally-treated rats (0.248 for oral administration and 0.004 for IV administration).

Conclusions: Oral bioavailability of linaclotide in female rats was calculated to be ≤ 0.105%, and therefore is extremely low. MM-419447 was detected following IV and oral administration of MD-1100 acetate. The $AUC_{MM-419447}/AUC_{drug}$ ratio was markedly

higher in orally-treated rats, suggesting a higher proportion of the drug was converted to the metabolite following oral administration.

Oral Bioavailability Study of MD-1100 Acetate in Rats Using a Radioimmunoassay (Study No.: MDP-103-006)

Methods: Female CD rats (about 153 g, age not stated) were treated with 10 mg/kg MD-1100 acetate using either IV or oral administration (6 rats/group). The dose volume was 2 ml/kg for both routes of administration. The vehicle was 20 mM Tris-HCl, pH 7.5. Blood samples were collected at 2, 5, 20, 40, 60, 90, 180, 240, and 360 min following IV administration, and at 5, 20, 40, 60, 90, 180, 240, and 360 min following oral administration (3 rats/time-point). Plasma drug concentration was measured using a radioimmunoassay method. The lower limit of detection (LOD) was 1 nM (1.5 ng/ml). Values that were lower than the LOD were assumed to be equal to the LOD for the purpose of estimating the AUC value. The maximum observed plasma concentration (C_{max}) after oral dosing and the time to the maximum observed plasma concentration (T_{max}) were determined using the oral concentration-time curve.

Results: The plasma kinetic parameters are shown in the following table taken from the study report.

Table 4. Pharmacokinetic Values for Linaclotide after Oral and IV Dosing of Linaclotide to CD Rats

C_{max} (ng/mL)	T_{max} (h)	$AUC_{i.v.(all)}$ ng-hr/mL	$AUC_{p.o.(all)}$ ng-hr/mL
13.4	0.67	23,700	≤ 48.33

Using the oral and IV AUC values, the absolute oral bioavailability of linaclotide was calculated to be ≤ 0.20%. The $t_{1/2}$ values were not reported. However, the elimination of drug from plasma was extremely rapid during the first 20 min after IV administration, followed by a slower beta phase.

Conclusions: Oral bioavailability in female rats was calculated to be ≤ 0.20%, and therefore is extremely low. The results of this study are similar to those obtained in the bioavailability study in rats using a LC/MS method for the measurement of plasma drug concentration (reviewed above).

Oral Bioavailability Study of MM-419447 in Rats Using LC/MS (Study No.: MDP-103-011)

Methods: Female CD rats (about 157 g, age not stated) were treated with 10 mg/kg MM-419447 using either IV or oral administration (12 rats/group). MM-419447 is a

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metabolite of MD-1100 acetate, and differs from the parent peptide by the absence of the carboxy-terminal tyrosine. The dose volume was 1.9 ml/kg for both routes of administration. The vehicle was 20 mM Tris-HCl, pH 7.5. Blood samples were collected at 2, 5, 20, 40, 60, 90, 180, 240, and 360 min following IV administration, and at 5, 20, 40, 60, 90, 180, 240, and 360 min following oral administration (6 rats/time-point). Plasma concentration of MM-419447 was measured using a LC/MS method. The lower limit of detection was 1.06 ng/ml. Values that were lower than the LOD were assumed to be equal to the LOD for the purpose of estimating the AUC value.

Results: MM-419447 was detected at all time points following IV administration. In orally-treated rats, the peptide was detectable for up to 240 min. At 180 min post-dose in intravenously-treated rats, approximately 10% of the initial concentration (measured at 2 min) remained in plasma. The AUC values are shown in the following table taken from the study report.

Table 5. Oral and Intravenous Pharmacokinetics Parameters Following Oral Dosing with MM-419447

Intravenous	Oral		
AUC_{all} (ng·h/mL)	AUC_{all} (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)
36,100	≤ 29.7	27.0	0.33

The oral profile demonstrates the minimal systemic circulation of MM-419447 following oral administration. Using the oral and intravenous AUC_{all} values, the absolute oral bioavailability of MM-419447 was calculated to be ≤ 0.08%.

The elimination of MM-419447 from plasma following intravenous administration was somewhat slower than that observed for the unchanged drug in other PK studies in rats, based on the appearance of the concentration vs. time curve. No other plasma kinetic parameters were reported.

Conclusions: The oral bioavailability of the metabolite MM-419447 in female rats was extremely low (≤0.08%), similar to that observed for the unchanged drug.

Systemic and Portal Vein Concentrations of Linaclotide and MM-419447 Following Oral Administration of Linaclotide to Female Rats [Study No.: MDP-103-087]

Methods: Female Sprague-Dawley rats were equipped with surgically implanted jugular vein cannula or portal vein cannula (4 rats/group) for blood collection. The rats were treated orally with 10 mg/kg linaclotide. Blood samples were collected via the jugular vein or the portal vein prior to dosing and at 5, 20, and 40 minutes, and 1, 1.5, 3,

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4.5, 6, and 24 hours following dosing. Plasma was extracted from the samples and analyzed for linaclotide and its primary metabolite, MM-419447, using LC/MS/MS.

Results: In Sprague-Dawley rats, systemic exposure to linaclotide and MM-419447 was minimal after oral dosing. The majority of plasma samples, whether taken from the jugular or portal vein, had concentrations below the quantitation limits for linaclotide (1 ng/ml) and MM-419447 (2 ng/ml). In the systemic circulation (jugular vein), the maximum observed linaclotide plasma concentration (C_{max}) in any animal was 2.97 ng/ml, and the time of the maximum observed plasma concentration (T_{max}) was 5 min. The corresponding highest individual portal vein C_{max} for linaclotide was 5.95 ng/ml and the T_{max} was 5 min. For MM-419447, the highest individual systemic C_{max} was 2.28 ng/ml and the highest individual portal vein C_{max} was 5.09 ng/ml. The T_{max} in both cases was 5 min. By 40 min, both systemic and portal vein plasma concentrations mostly were below the lower limit of quantitation for linaclotide and MM-419447.

Conclusions: Following a 10 mg/kg oral dose of linaclotide to female Sprague-Dawley rats, concentrations of linaclotide and its active primary metabolite, MM-419447, were low (≤ 6 ng/mL) in plasma samples from either the jugular vein or portal vein, indicating both peptides are poorly absorbed and hepatic exposure to linaclotide and MM-419447 is minimal.

5.1.4 Distribution

Analysis of Brain, Liver, and Adipose Tissue for the Presence of Linaclotide and MM-419447 in Mice Dosed Daily with Linaclotide for up to 26 weeks (Study No.: MDP-103-065)

Methods: Male and female CD-1 mice were treated orally with 0 (control), 5, 20, or 100/80 mg/kg/day of linaclotide for up to 26 weeks. Each group consisted of 112 animals (56 male and 56 female), except for the control group which had 22 animals (11 male and 11 female). Immediately following the toxicokinetic blood collection on days 1, 90, and 180, the animals were sacrificed, and brain, liver, and adipose tissue was collected. The samples were analyzed by a quantitative LC-MS/MS method. Approximately one-third of the collected tissue samples were analyzed for linaclotide and its active metabolite, MM-419447 (a total of 74 brain, 74 liver, and 77 perirenal adipose samples). The lower limits of quantitation (LLOQs) for linaclotide were 50 ng/g for brain and adipose tissue and 150 ng/g for liver; MM-419447 had an LLOQ of 150 ng/g in the brain and adipose tissue samples and was not quantifiable in the liver samples. Following the quantitative analysis, qualitative LC-MS/MS analysis was performed to confirm or refute the initial findings.

Results: Initial analyses of the brain and perirenal adipose tissue samples appeared to show the presence of linaclotide and MM-419447 in a small number of the samples. Linaclotide was not detected in any of the liver samples, and due to technical issues MM-419447 could not be measured in the liver. Following the initial analyses, all the brain and perirenal adipose tissue extracts with sufficient volume were re-analyzed for

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the presence of linaclotide and MM-419447. Qualitative analysis confirmed that neither linaclotide nor MM-419447 was present in the brain or perirenal adipose tissue samples. The false positives were speculated to be due to cross-contamination or interferences by unspecified MS/MS peaks.

Conclusions: There was no detectable accumulation of linaclotide in brain, liver, or adipose tissues over the course of 180 days of daily dosing with linaclotide in mice. Similarly, in the tissues that could be analyzed for the active primary metabolite (brain and adipose), there was no detectable accumulation of MM-419447.

5.1.5 Metabolism

In vitro metabolism and P450 studies

In Vitro Metabolism

1. *IN VITRO* PROTEOLYTIC STABILITY OF LINACLOTIDE AND ITS PRIMARY METABOLITE MM-419447 [MDP-103-049-IAR-01]	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
Linaclotide and MM-419447 were each incubated at 37oC in simulated gastric fluid (SGF) designed to mimic the low-pH conditions in the stomach, and in a series of buffer solutions, each containing a protein-hydrolyzing enzyme commonly found in the mammalian gastrointestinal tract (carboxypeptidase A, pepsin, chymotrypsin, trypsin, and aminopeptidase). Sampling was conducted at 0, 1, and 3 h. The proteases were deactivated, and the samples were analyzed using LC/MS.	Linaclotide was not degraded in the SGF, and of the five proteases tested only carboxypeptidase A (CPD-A) was able to hydrolyze linaclotide. MM-419447 was the only metabolite of linaclotide detected. It lacks the C-terminal tyrosine residue of linaclotide and is not a substrate for CPD-A. Thus, neither linaclotide nor MM-419447 was degraded *IN VITRO* by simulated gastric fluid or the peptide-digesting enzymes under non-reducing conditions, except carboxypeptidase A.

2. SUSCEPTIBILITY OF OXIDIZED AND REDUCED LINACLOTIDE TO PROTEASE DIGESTION [MDP-103-IAR-01]	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
Linaclotide was incubated in the presence and absence of a chemical reducing agent, dithiothreitol (DTT). DTT treatment was shown to reduce all 3 disulfide bonds of the peptide. Proteases tested included chymotrypsin, CPD-A, and pancreatin. After a 60 minute incubation at 37oC, the reaction mixtures were analyzed by LCMS/MS.	CPD-A removes the C-terminal tyrosine of the reduced or oxidized linaclotide, generating the active metabolite MM-419447. However, linaclotide was cleaved by chymotrypsin and extensively proteolyzed by pancreatin only under reducing conditions. The results suggest that in the environment found in the rat intestine, reduced linaclotide can be readily digested by intestinal enzymes.

3. EFFECTS OF THIOLS ON LINACLOTIDE [MDP-103-037-IAR-01]	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
Linaclotide was incubated with DTT, glutathione (GSH), or components of the glutaredoxin system, which includes glutathione reductase, glutaredoxin, and NADPH. The role of enzymes in the reduction of linaclotide in intestinal fluid was investigated by	Linaclotide was sensitive to 0.5 mM DTT treatment, which reduced all 3 disulfide bonds. GSH also reduced linaclotide, but not as completely as DTT. In the presence of the components of the glutaredoxin system, linaclotide can be completely

Reference ID: 3114166

treating linaclotide with rat intestinal fluid (RIF) from which proteins 10 kDa or larger were removed. Rat intestinal fluid (RIF) was obtained by surgically ligating loops in the small intestine of rats and injecting 0.2 ml of a Krebs-Ringer solution at pH 7.0. The luminal contents were then removed and frozen. The number of reduced disulfide bonds was determined and the resulting mixture was analyzed by LC-TOF/MS.	reduced. The thiols in the deproteinized ligated loop fluid did not reduce linaclotide, suggesting the reduction and digestion of linaclotide in the intestine is an enzyme-mediated process.

4. Kinetics of Linaclotide and MM-419447 Metabolism in Mouse Intestinal Fluid [MDP-103-052-IAR-01]	
Experimental Design and Methods	**Results and Conclusions**
Mouse intestinal fluid (MIF) was obtained by surgically ligating loops in the small intestine of mice and injecting 0.2 ml of a Krebs-Ringer solution at pH 7.0. The luminal contents were then removed and frozen. Linaclotide was incubated at 37oC in the presence of the MIF for up to 280 minutes. Linaclotide and MM-419447 were extracted and quantified by LC/MS/MS.	Linaclotide was rapidly metabolized and degraded in the MIF, with a half-life of 3.0 minutes. The primary active metabolite was MM-419447, and it was also rapidly degraded.

5. Kinetics of Linaclotide and MM-419447 Digestion in Rat Intestinal Fluid [MDP-103-035-IAR-01]	
Experimental Design and Methods	**Results and Conclusions**
Rat intestinal fluid (RIF) was prepared as described above. Linaclotide was incubated at 37oC in the presence of the RIF for up to 280 minutes. Linaclotide and MM-419447 were extracted and quantified by LC/MS/MS or radioimmunoassay (RIA)	Linaclotide was rapidly metabolized and degraded in the RIF, with a half-life of 3.3 minutes. The primary active metabolite was MM-419447, and it was also rapidly degraded.

6. Characterization of the Fluid Isolated from Rat Small Intestinal Ligated Loops [MDP-103-036-IAR-01]	
Experimental Design and Methods	**Results and Conclusions**
Linaclotide was incubated in dilutions of the RIF for different reaction times and at different temperatures to characterize its degradation *IN VITRO*. The reaction was stopped by protein precipitation, and linaclotide was extracted and measured by radioimmunoassay. Total thiol in the intestinal fluid was measured using a colorimetric reaction to characterize the disulfide reducing activity present in intestinal loop fluid. Free thiol was measured after protein removal.	Linaclotide degradation kinetics in the intestinal fluid was temperature sensitive, consistent with an enzymatic process. Rat small intestinal fluid contained high levels of free thiols, ranging from 0.2 mM to 2 mM. In rat small intestine, the high thiol concentrations, along with required enzyme(s), reduce the protective disulfide bonds of linaclotide, rendering it susceptible to protease digestion.

7. The Linaclotide Metabolism and Digestion Pathway in Rat Intestinal Fluid [MDP-103-039-IAR-01]	
Experimental Design and Methods	**Results and Conclusions**
Linaclotide and the isotopically labeled [13C1]-alanine-linaclotide were incubated with fluid	Incubation of linaclotide with diluted RIF resulted in the reduction of one disulfide bond, the loss of the

obtained from rat small intestinal ligated loops. The fluid was diluted to slow down the digestive degradation and potentially trap peptide intermediates. The reactions were stopped and the reaction intermediates were profiled and identified by LC-TOF/MS. To detect reduced disulfide species, the peptides were alkylated with iodoacetamide. For detection of linaclotide fragments, the reaction products were reduced completely with DTT followed by iodoacetamide alkylation.	C-terminal tyrosine to form MM-419447, or both. Peptide fragments of linaclotide containing 3-13 amino acids were first detected after 15 minutes of incubation and increased over the first few hours. The fragments were completely degraded after 19 hours. Reduced linaclotide or the reduced active metabolite had no pharmacological activity in the cGMP accumulation assay in human T84 cells. Thus, the degradation pathway of linaclotide in the ligated intestinal loop fluid involves formation of the metabolite MM-419447, reduction of the disulfide bonds in both linaclotide and MM-419447, and subsequent proteolytic degradation.

8. Complete Digestion of Linaclotide to Single Amino Acids in Rat Intestinal Fluid [MDP-103-040-IAR-01]	
Experimental Design and Methods	**Results and Conclusions**
Linaclotide, MM-419447, and two inactive degradation products of chymotrypsin cleavage of reduced linaclotide, MM-421202 and MM-421522, were incubated with fluid from rat small intestinal ligated loops whose proteins were concentrated using an ultrafiltration centrifugation device, and in the presence of 2 mM reduced glutathione, and 1 mM NADPH. Individual amino acids were measured by LC/MS/MS and ultra-performance liquid chromatography.	Incubation of linaclotide in rat intestinal fluid in the presence of 2 mM GSH and 1 mM NADPH resulted in degradation of linaclotide within 1.5 h. Overnight incubation resulted in complete digestion to individual amino acids. Similar results were obtained with the linaclotide metabolite MM-419447, and the chymotrypsin proteolysis products MM-421202 and MM-421522.

9. Stability of Linaclotide in Rat Small Intestine Ligated Loops [MDP-103-034-AMR-01]	
Experimental Design and Methods	**Results and Conclusions**
The metabolic stability of linaclotide was studied by injecting the peptide directly into ligated small intestinal loops of rats. In the first experiment, linaclotide (625-5000 ng) was injected into loops in the jejunum. The rats were sacrificed after 90 minutes, and the loops were removed and their contents frozen for analysis. In the second experiment, 2,500 ng of linaclotide was injected into intestinal loops at the duodenal, jejunal, or ileal portions of the small intestine. At 2, 5, 15, 45, and 90 minutes, the rats were sacrificed, the loops were removed, and the luminal contents collected and frozen for analysis. The amount of linaclotide was determined by RIA.	The injected linaclotide in ligated intestinal loops was below the limit of detection (1.5 ng/ml) after 90 minutes of incubation in the intestinal loops. There was no linaclotide detected within 5 minutes of introduction into the rat duodenum and middle segment of jejunum, and the half-life was determined to be less than 1 min. The degradation of linaclotide occurs more slowly in the ileum, with a half-life of 36 minutes.

Induction and Inhibition of Cytochrome P450

***In vitro* Evaluation of Linaclotide and MM-419447 as Inducers of Cytochrome P450 Expression in Human Hepatocytes [MDP-103-088-IAR-01]**	
Experimental Design and Methods	**Results and Conclusions**
Three preparations of cultured human hepatocytes from 3 separate livers were treated once daily for 3	Treatment of cultured human hepatocytes with up to 50 ng/ml of linaclotide or MM-419447 caused

consecutive days with vehicle (DMSO, 0.1% v/v), linaclotide, MM-419447, or one of 3 known human CYP inducers (omeprazole - an AhR activator and CYP1A2 inducer; phenobarbital - a CAR activator and CYP2B6 inducer; and rifampin - a PXR agonist and inducer of CYP3A4). Linaclotide and MM-419447 were tested at concentrations of 0.25, 2.5, and 50 ng/ml in the CYP1A2, CYP2B6, and CYP3A4/5 assays. Additional concentrations of each test article (625, 1250, and 5000 ng/mL) were tested in the CYP3A4/5 assay due to the presence of CYP3A4 in the intestine, where the highest concentrations of linaclotide and MM-419447 are possible. After treatment, the cells were harvested to isolate microsomes for the analysis of phenacetin *O*-dealkylation (marker for CYP1A2), bupropion hydroxylation (marker for CYP2B6), and testosterone 6β-hydroxylation (marker for CYP3A4/5) by LC/MS/MS.	little or no change in CYP1A2 and CYP2B6 activity (< 2.0-fold increase and < 3% of the effect caused by the appropriate positive control). Likewise, treatment of cultured human hepatocytes with up to 5000 ng/ml linaclotide or MM-419447 caused little or no significant change in CYP3A4/5 activity. Neither linaclotide nor its primary metabolite MM-419447 induces CYP1A2, 2B6, or 3A4/5 *IN VITRO* at any of the concentrations tested (up to 50 ng/mL for CYP1A2 and CYP2B6, and up to 5000 ng/mL for CYP3A4/5).

***IN VITRO* ASSESSMENT OF HUMAN INTESTINAL CYTOCHROME P450 INHIBITION POTENTIAL OF LINACLOTIDE [MDP-103-066-IAR-01]**	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
CYP450-specific probe substrates (tolbutamide as substrate for CYP2C9 and midazolam and testosterone as substrates for CYP3A4) were incubated with pooled human intestinal microsomes in the presence or absence of standard inhibitors or linaclotide (0, 0.1625, 0.325, 0.65, 1.3, 2.6, and 5.2 μg/ml). In addition, linaclotide (at concentrations noted above) was pre-incubated for 30 minutes with pooled human intestinal microsomes before the addition of the CYP450-specific probe substrate to assess potential time-dependent inhibition. The effects of standard inhibitors and linaclotide on the rate of production of the relevant probe substrate metabolites were evaluated.	Linaclotide at concentrations up to 5.2 μg/ml did not significantly inhibit CYP2C9 and CYP3A4. Following a 30-minute pre-incubation with NADPH, linaclotide showed no significant inhibition of CYP2C9 and CYP3A4, indicating that linaclotide is not a time-dependent (mechanism-based) inhibitor of these isoforms. The results showed that linaclotide, at concentrations up to 5.2 μg/ml, does not inhibit the CYP isoforms tested, and is therefore unlikely to produce drug-drug interactions related to inhibition of CYP2C9 and CYP3A4 metabolism.

***IN VITRO* ASSESSMENT OF HUMAN LIVER CYTOCHROME P450 INHIBITION POTENTIAL OF LINACLOTIDE [(b) (4) 21]**	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
Linaclotide and MM-419447 were incubated separately at final incubation concentrations of 0, 0.05, 0.5, 5, 15 and 50 ng/ml with pooled human liver microsomes (0.025, 0.1, or 0.2 mg/ml protein) in the presence of NADPH, phosphate buffer, and CYP450-selective chemical substrates. The effects of standard inhibitors and linaclotide on the rate of production of the relevant probe substrate metabolites were evaluated. Reactions were terminated by the addition of methanol with internal standard. Proteins were removed by filtration and the samples were analyzed by LC/MS/MS.	Linaclotide and MM-419447 caused negligible direct inhibition of cytochrome P450 enzymes, including CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4. At the highest concentration tested (50 ng/mL), the greatest inhibitory effect was observed with CYP2C9, which was inhibited 12.0% and 20.5% by linaclotide and MM-419447, respectively. The IC50 was greater than 50 ng/ml for all CYP450 enzymes tested. The data indicated that linaclotide and MM-419447 are not inhibitors of the liver cytochrome P450 enzymes tested at concentrations up to 50 ng/ml.

5.1.6 Excretion

Fecal Recovery of Linaclotide and the Active Metabolite MM-419447 in Rats Following Oral Dosing of Linaclotide Acetate (Study No.: MDP-103-033)

Methods: Three female CD rats (approximately 182 g) were treated orally with 10 mg/kg linaclotide acetate. Feces were collected over 55 hours. The feces were extracted and the amount of recovered linaclotide was determined by radioimmunoassay (RIA). LC/MS/MS was used to confirm the amount of both linaclotide and the active metabolite MM-419447 in a subset of the rat feces.

Results: The total amount of linaclotide recovered from the feces of 3 rats over a 55 hour period after oral dosing (10 mg/kg), as measured by RIA, was 0.04% of the oral dose (see table below taken from the study report). The RIA was unable to detect MM-419447.

Table 2. Recovery of Linaclotide from Rat Feces After Linaclotide Oral Dosing, RIA Assay Method

Rat	Linaclotide Dose (mg)	Linaclotide recovered in feces (ng)	% oral dose
1	1.817	857.7	0.05
2	1.817	388.8	0.02
3	1.817	1002	0.06
Average	1.817	749.5	0.04
Standard Deviation		320.6	0.02

These results were confirmed in Rat # 3 by LC/MS/MS. Furthermore, 0.95% of the oral linaclotide was found to be in the form of the active metabolite MM-419447 (see table below taken from the study report).

Table 3. Recovery of Linaclotide and MM-419447 from Rat 3 Feces After Oral Dosing of Linaclotide by LC/MS/MS

Rat	Linaclotide Dose (mg)	Linaclotide recovered in Feces (ng)	% of Oral Dose	MM-419447 recovered in Feces (ng)	% of Oral Dose
3	1.817	453.5	0.03	17242.5	0.95

In all the rats tested, the majority of fecal excretion of linaclotide and MM-419447 occurred in the first 24 hours after dosing.

Conclusions: Only a small amount of linaclotide (< 1%) was recovered in feces after oral dosing in the form of linaclotide or the active metabolite. Thus, fecal excretion does not play a significant role in the elimination of linaclotide and its active metabolite MM-419447.

5.1.7 Pharmacokinetic Drug Interactions

No studies were submitted.

5.1.8 Other Pharmacokinetic Studies

Evaluation of the Pharmacokinetics and Excretion of Linaclotide Acetate in Renal Ligation Model in the Rat [MNP-103-042-PKR-021]

Methods: Male Sprague-Dawley rats were divided into 3 groups (10 rats/group): normal control (no surgery), renal-ligated, and bile duct-cannulated. The rats were treated intravenously with 1 mg/kg linaclotide. Urine samples were collected, when available, from control and bile duct cannulated animals for 2 hours prior to dosing, and from approximately 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 14, 14 to 16, 16 to 18, 18 to 20, 20 to 22, and 22 to 24 hours postdose. Blood samples were collected prior to dosing and at approximately 5, 15, and 30 minutes and 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose. Cohorts (5 rats/group) were bled at alternating time points. Bile samples (approximately 0.25 to 0.5 ml) were collected from bile duct cannulated animals via the implanted catheter prior to dosing and at approximately 0.5 1, 2, 4, 6, 8, 12, 18, and 24 hours postdose.

Results: The plasma pharmacokinetic parameters for the control and renal-ligated groups are shown in the table below (taken from the study report). In normal male rats, linaclotide was rapidly cleared following intravenous administration with a half-life of 1.15 hours and a systemic clearance of 627 ml/kg/hour. Some linaclotide was metabolized to MM-419447 in the plasma, and the elimination half-life of the metabolite was equivalent to linaclotide (1.20 hr).

Reference ID: 3114166

Table E1. Plasma Pharmacokinetic Parameters of Linaclotide and MM-419447 in Male Rats Normal Rat (Group 1)							
Linaclotide		C_0 (ng/mL)	AUC_{last} (hr*ng/mL)	$AUC_{0-\infty}$ (hr*ng/mL)	λ_z (1/hr)	Half-life (hr)	Cl_s (mL/hr/kg)
		2620	1590	1590	0.605	1.15	627
MM-419447	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	$AUC_{0-\infty}$ (hr*ng/mL)	λ_z (1/hr)	Half-life (hr)	Cl_s (mL/hr/kg)
	0.5	41.1	89.2	99.1	0.580	1.20	NC
NC – Not calculated							

Table E2. Plasma Pharmacokinetic Parameters of Linaclotide and MM-419447 in Male Rats After Renal Ligation (Group 2)							
Linaclotide		C_0 (ng/mL)	AUC_{last} (hr*ng/mL)	$AUC_{0-\infty}$ (hr*ng/mL)	λ_z (1/hr)	Half-life (hr)	Cl_s (mL/hr/kg)
		4800	27000	35100	0.062	11.14	28
MM-419447	T_{max} (hr)	C_{max} (ng/mL)	AUC_{last} (hr*ng/mL)	$AUC_{0-\infty}$ (hr*ng/mL)	λ_z (1/hr)	Half-life (hr)	Cl_s (mL/hr/kg)
	4.0	52.7	774	2480	0.0185	37.4	NC
NC – Not calculated							

Excretion of linaclotide and MM-419447 was observed in both urine and bile. The highest levels recovered were found during the first 2 hours post-administration. The average renal clearance of linaclotide was 39 ml/hr/kg and the mean fraction recovered unchanged from urine was 21%. In rats that had been functionally nephrectomized, clearance of linaclotide and MM-419447 was markedly slower, with a half-life of 11.14 hr and 37.4 hr, respectively. These results demonstrated that the kidney is a major organ of clearance for both peptides. The plasma concentrations of linaclotide and MM-419447 decreased with time in the nephrectomized rats. This suggests the presence of non-renal pathways of clearance, including biliary clearance, and was confirmed by the presence of linaclotide and MM-419447 in the bile shortly after dosing.

Conclusions: Linaclotide and MM-419447 were mainly cleared from plasma by the kidneys, but there was also some biliary clearance.

Pharmacokinetic Studies with Drug Substance Impurities

METABOLISM AND DEGRADATION OF LINACLOTIDE (b) (4) IN RAT INTESTINAL FLUID [MDP-103-055-IAR-01]	
EXPERIMENTAL DESIGN AND METHODS	RESULTS AND CONCLUSIONS
Linaclotide (b) (4) were generated by incubating 5 mg/mL linaclotide at 60°C for 4 hours in sodium phosphate buffer (pH 7.5), followed by quenching with sodium acetate. Rat intestinal fluid (RIF) was prepared by injecting PBS into ligated small intestinal loops of rats and	A time-dependent drop in peak area was observed for the (b) (4) species in the presence of RIF. At the end of the 60 min incubation, (b) (4) of the (b) (4), (b) (4) of the (b) (4), and (b) (4) of the (b) (4) (b) (4) had degraded. Linaclotide

removing the fluid contents after 30 minutes. The (b) (4)-enriched solution was incubated with the RIF at 37°C for 60 minutes, with samples taken at 0, 5, 30 and 60 minutes. The samples were analyzed by Fourier-Transform Mass Spectrometry (FTMS) and the peak area for each (b) (4) species at each time point was determined.	(b) (4) are rapidly degraded by rat intestinal fluid at a rate equal to or greater than linaclotide (b) (4) under identical conditions.

Pharmacokinetic Studies with Drug Product Degradants

ABSOLUTE ORAL BIOAVAILABILITY STUDY OF LINACLOTIDE (b) (4) DEGRADANT (b) (4) IN SPRAGUE-DAWLEY RATS USING LC/MS/MS (b) (4)	
Experimental Design and Methods	**RESULTS AND CONCLUSIONS**
Twelve female Sprague-Dawley rats, weighing 200-250 g each, were assigned to one of four groups (3 rats/group: two groups received 10 mg/kg (b) (4) intravenously and two groups received the same dose orally. Blood samples were drawn at 0, 1.8, 4.8, 20, 40, 60, 90, 180, 240, 360, 480, 1440 min from rotating groups so that one oral and one intravenous group were sampled per time point. Plasma was extracted from the samples and analyzed for (b) (4) using LC/MS/MS.	After intravenous dosing of (b) (4) ((b) (4) a linaclotide (b) (4) degradant, the C0 was 79,800 ng/mL, the AUC0-inf was 40,300 ng·h/mL, the volume of distribution was 279 ml/kg, and the terminal-phase half-life was 46.7 min. After oral dosing, systemic exposure to (b) (4) was minimal and was below the lower limit of quantitation of 2 ng/ml after 6 hours. The Cmax was 22.1 ng/mL and the Tmax was 20 min, while the AUC0-inf was 43.5 ng·h/mL with a terminal half-life of 1.58 h. Thus, systemic exposure to the linaclotide-related (b) (4) degradant (b) (4) in Sprague-Dawley rats after oral dosing with 10 mg/kg of (b) (4) was very low, with the absolute oral bioavailability calculated to be 0.11%. The distribution of systemically available (b) (4) into tissues is limited based on a small volume of distribution.

ABSOLUTE ORAL BIOAVAILABILITY STUDY OF THE LINACLOTIDE-RELATED (b) (4) DEGRADANT (b) (4) IN SPRAGUE-DAWLEY RATS USING LC/MS/MS (b) (4)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
Twelve jugular vein cannulated female Sprague-Dawley rats, weighing approximately 225-249 g, were assigned to one of four groups (3 rats/group). Two groups received 10 mg/kg of (b) (4) ((b) (4) intravenously while the other two groups received the same dose orally. Blood samples were drawn at 0, 1.8, 4.8, 20, 40, 60, 90, 180, 240, 360, 480, and 1440 min from rotating groups so that one oral and one intravenous group were sampled per time point. Plasma was extracted from the samples and the concentration of (b) (4) in each sample was quantified using LC/MS/MS.	After intravenous dosing of (b) (4) ((b) (4) a linaclotide (b) (4) degradant, the C0 was 39,300 ng/ml, the AUC0-inf was 17,900 ng·h/ml, the terminal-phase volume of distribution was 642 ml/kg, and the terminal-phase half-life was 60 min. After oral dosing, systemic exposure to (b) (4) was minimal. The Cmax was 22.1 ng/ml and the Tmax was 4.8 min, while the AUC0-inf was 9.58 ng·h/mL with a terminal half-life of 1.58 h. After 6 hours, the plasma concentrations were below the lower limit of quantitation of 2 ng/ml. Thus, systemic exposure to the linaclotide-related drug product degradant (b) (4) ((b) (4) in Sprague-Dawley rats after oral dosing with (b) (4) is minimal, with the absolute oral bioavailability calculated to be approximately 0.05%. The distribution of systemically available

	(b) (4) into tissues is limited based on a small volume of distribution.

STABILITY OF LINACLOTIDE (b) (4) DEGRADANTS (b) (4) AND (b) (4) IN SIMULATED GASTRIC FLUID (b) (4)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
This experiment was conducted by incubating 10 μg/mL of (b) (4) (b) (4) or 200 μg/mL of (b) (4) (b) (4) in simulated gastric fluid (SGF) at 37°C for two hours. The SGF was a buffer solution of 0.2% sodium chloride and 0.7% hydrochloric acid at pH 1. Linaclotide (10 μg/mL) was tested also as a positive control and reference compound. At designated time points (0, 5, 15, 45, 60, 90, and 120 min), an aliquot was removed from each incubation, and the samples were analyzed by LC/MS or LC/MS/MS.	Approximately 89% of (b) (4) and 104% of (b) (4) remained after a 2-hour incubation in SGF at 37°C, while 102% of linaclotide remained. The linaclotide (b) (4) degradants (b) (4) ((b) (4) and (b) (4) (b) (4) are stable in SGF at 37°C for at least 2 hours and have a similar stability profile as linaclotide.

IN VITRO METABOLISM AND DEGRADATION OF LINACLOTIDE (b) (4) IMPURITIES IN RAT INTESTINAL FLUID (b) (4)	
EXPERIMENTAL DESIGN AND METHODS	**RESULTS AND CONCLUSIONS**
The linaclotide (b) (4) degradants (b) (4) were incubated at 40 μg/mL in rat intestinal fluid (RIF) in duplicate for two hours at 37°C, and 50-μl aliquots were removed at predetermined time intervals: 0, 0.5, 1, 2, 3, 8, and 24 hours. RIF was prepared by injecting PBS into ligated small intestinal loops of Sprague-Dawley rats and removing the fluid contents after 30 minutes. Linaclotide and incubations containing no RIF were included as positive and negative controls, respectively. At the specified time points, the reactions were stopped and samples were analyzed by LC/MS. The peak area for each test article was determined at each time point and compared to the peak area at T0 to determine the percent remaining.	(b) (4) were both rapidly degraded *IN VITRO* in the presence of RIF. After two hours at 37°C, linaclotide and (b) (4) were completely degraded while trace amounts (~1.8%) of (b) (4) remained. All test articles were completely degraded by three hours. In the absence of RIF, all test articles were stable for the 24-hour incubation. Thus, the linaclotide (b) (4) degradants were degraded rapidly in the presence of rat intestinal fluid, as observed with linaclotide.

5.2 Toxicokinetics

See toxicity studies

6 General Toxicology

6.1 Single-Dose Toxicity

The following studies were previously reviewed by David B. Joseph, Ph.D. (see Pharmacology/Toxicology review of IND 63,290 dated June 5, 2006). Final study

reports were submitted in the NDA, however there were no changes in the data analysis.

Single-Dose Oral Gavage Toxicity and Toxicokinetic Study with MD-1100 Acetate in Rats with a 14-Day Recovery Period

Note: This study was submitted as a draft report.

Key Study Findings: MD-1100 acetate was not lethal at doses of up to 5 mg/kg po

Study #: 7480-104

Vol. 3, Pg. 223

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: April 9, 2004 (report dated January 28, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure

METHODS: Crl:CD®(SD)IGS BR rats (about 8 weeks old; males: 205-240 g; females: 162-191 g) were treated with a single oral administration of 0 (vehicle), 0.5, 2.5, or 5 mg/kg MD-1100 acetate (5 rats/sex/group). Toxicokinetic groups were treated with a single oral administration of 0.5, 2.5, or 5 mg/kg (9 rats/sex/group). The test and control articles were given by gavage using as dose volume of 5 ml/kg. The drug was administered as a solution. The vehicle was phosphate-buffered saline. Rats were sacrificed on day 16 (day of dosing was designated as day 1).

Observations and Times:

Mortality: twice daily on days 1-15

Clinical Signs: once daily on days 1-15

Bodyweight: weekly starting on day 1

Food Consumption: weekly

Gross Pathology: at sacrifice

Toxicokinetics: Blood samples were collected at 0.5, 1, 2, 4, 8, and 24 hr post-dose (3 rats/sex/ group/time-point). The method used for measurement of plasma drug concentration was previously validated, but no description of the method was provided.

RESULTS:

Mortality: None.

Clinical Signs: None.

Bodyweight: No effects on weight gain were observed.

Food Consumption: No effects.

Gross Pathology: No drug-related changes occurred.

Toxicokinetics: No drug was detected in plasma. The limit of quantification was 2 nM (3 ng/ml).

Conclusions: MD-1100 acetate was not lethal at doses of up to 5 mg/kg po. No signs of toxicity were observed at any dose level. The drug was not detected in plasma.

Single-Dose Intravenous Injection Toxicity and Toxicokinetic Study with MD-1100 Acetate in Rats with a 14-Day Recovery Period

Note: This study was submitted as a draft report.

Key Study Findings: not lethal at doses of up to 5 mg/kg iv

Study #: 7480-103

Vol. 4, Pg. 2

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: April 13, 2004 (report dated January 28, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure

Reference ID: 3114166

METHODS: Crl:CD®(SD)IGS BR rats (about 7 weeks old; males: 240-294 g; females: 163-207 g) were treated with a single intravenous administration of 0 (vehicle), 0.5, 2.5, or 5 mg/kg MD-1100 acetate (5 rats/sex/group). The toxicokinetic groups were treated with 0.5, 2.5, or 5 mg/kg (9 rats/sex/group). The vehicle was phosphate-buffered saline, and the dose volume was 2.5 ml/kg. The rats were sacrificed on day 16 (day of dosing was designated as day 1).

Observations and Times:

Mortality: twice daily on days 1-15

Clinical Signs: once daily on days 1-15

Bodyweight: weekly starting on day 1

Food Consumption: weekly

Gross Pathology: at sacrifice

Toxicokinetics: Blood samples were collected at 0.17, 0.5, 0.75, 1, 1.5, 2, 4, and 8 hr post-dose (3 rats/sex/group/time-point). The method used for measurement of plasma drug concentration was previously validated, but no description of the method was provided.

RESULTS:

Mortality: None.

Clinical Signs: None.

Bodyweight: Weight gain was increased by 31%, 108%, and 61% in the 0.5, 2.5, and 5 mg/kg females, respectively, during the week 1 (statistically significant in the 2.5 mg/kg group). The control females gained 13 ± 5.8 g during week 1. Weight gain was not affected in males.

Food Consumption: No effects.

Gross Pathology: One female in the 5 mg/kg group exhibited a distended urinary bladder with fluid in lumen, and an enlarged, dilated renal pelvis.

Toxicokinetics: The plasma kinetic parameters are shown in the table below (taken from the study report).

Reference ID: 3114166

Dose Group	Dose Level (mg/kg)	Gender	T_{max} (Hour)	C_{max} (nM)	AUC_{0-t} (nM·hr)	$AUC_{0-\infty}$ (nM·hr)	$t_{1/2}$ (Hour)
5	0.5	M	0.167	449	380	380	0.409
		F	0.167	548	376	382	0.328
6	2.5	M	0.167	3293	2321	2324	0.439
		F	0.167	2773	2190	2198	0.565
7	5	M	0.167	5760	4554	4561	0.441
		F	0.167	4860	4132	4145	0.489

Values were determined from 3 rats/sex/group/time-point.

The increases in AUC values were approximately proportional to the dose increment, relative to the low-dose. The increases in the male C_{max} values were slightly higher than the dose increment. This was not observed for the female C_{max} values. No consistent sex-related differences were observed.

Conclusions: MD-1100 acetate was not lethal at doses of up to 5 mg/kg iv. The only adverse effects occurred in one rat treated with 5 mg/kg. This animal exhibited a distended urinary bladder with fluid in lumen, and an enlarged, dilated renal pelvis.

Up-and-Down Toxicity Study with MM-416775 in Cynomolgus Monkeys

Key Study Findings: study was grossly deficient due to the use of only one animal per dose group; MM-416775 (MD-1100 acetate) was not lethal at oral doses of up to 5 mg/kg; drug was tolerated when administered for five days at 1.5 mg/kg/day po

Study # 7480-113

Vol. 3, Pg. 340

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: Not stated; report was dated March 5, 2004

GLP Compliance: This study was a non-GLP study.

QA Report: yes ( ) no (x)

Drug: Lot # and purity were not stated.

Reference ID: 3114166

METHODS: Male Cynomolgus monkeys (about 2-4 years of age, 2.2-4.2 kg) were used. In part 1 of the study, monkeys were treated with a single oral administration of 0.5, 1.5, 3, or 5 mg/kg MM-416775 (1 monkey/group). MM-416775 is the same drug as MD-1100 acetate. The groups in part 1 were observed for one day. In part 2 of the study, one male was treated with 1.5 mg/kg/day po for five days. The drug was administered via nasogastric intubation as a solution in phosphate-buffered saline. The dose volume was 3 ml/kg.

Observations and Times:

Mortality: twice daily on days 1-14

Clinical Signs: several times daily on days 1-14

Bodyweight: days 1 and 12 in part 2

RESULTS:

Mortality: None.

Clinical Signs: In part 1 of the study, non-formed feces occurred at 1.5 mg/kg and higher, starting as early as two hours post-dose. Slight abdominal distension was observed after administration of 1.5 and 3 mg/kg. Liquid feces was observed at 3 and 5 mg/kg. In part 2, liquid feces occurred on each day of dosing with 1.5 mg/kg. Non-formed feces occurred on days 3-5. Mild abdominal distension was observed on day 4 of treatment.

Bodyweight: The monkey treated with 1.5 mg/kg/day for five days gained 0.2 kg.

Conclusions: This study is of limited value due to the use of only one animal per dose group. MM-416775 (MD-1100 acetate) was not lethal at oral doses of up to 5 mg/kg. Laxative-type effects along with slight to moderate abdominal distension occurred at doses of 1.5 mg/kg and higher. No effects were observed at 0.5 mg/kg. The drug was tolerated when administered for five days at 1.5 mg/kg/day.

Escalating Dose-Range Finding Intravenous Injection Study with MD-1100 Acetate in Cynomolgus Monkeys

Note: This study was submitted as a draft report.

Key Study Findings: not lethal at doses of up to 15 mg/kg iv; RBC count was reduced after seven days of treatment with 15 mg/kg/day iv

Study # 7480-115

Reference ID: 3114166

Vol. 4, Pg. 136

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: April 20, 2004 (report dated February 21, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure

METHODS: Cynomolgus monkeys (approximately 2-3 years old; males: 2.2-2.6 kg; females: 2.2-2.4 kg) were used. In Phase 1 of the study, one male and one female were treated intravenously with 0.5, 2.5, 5, and 15 mg/kg on days 1, 4, 7, and 10, respectively. These animals were returned to the stock colony on day 13. In Phase 2, the monkeys were treated intravenously with 15 mg/kg/day for seven days (2 monkeys/sex). The Phase 2 animals were sacrificed on day 8. The vehicle was PBS (phosphate buffered saline), and the dose volume was 7.5 ml/kg.

Observations and Times:

Mortality: twice daily in Phase 1 and 2

Clinical Signs: multiple observations at 0.5-8 hr post-dose and twice daily on non-dosing days in Phase 1; multiple observations at 0.5-8 hr post-dose on days 1-7 in Phase 2

Bodyweight: prior to study initiation, on days 1, 4, 7, 10, and 13 in Phase 1, and on days 1, 7, and 8 in Phase 2

Ophthalmoscopy: prior to study initiation and on day 6 in all Phase 2 animals

Hematology: blood was collected prior to study initiation and at terminal sacrifice in the Phase 2 animals

Clinical Chemistry: blood was collected prior to study initiation and at terminal sacrifice in the Phase 2 animals

Urinalysis: urine was collected prior to study initiation and at terminal sacrifice in the Phase 2 animals

Gross Pathology: at sacrifice in the Phase 2 animals

Reference ID: 3114166

Histopathology: tissues with macroscopic lesions were examined in the Phase 2 animals

Toxicokinetics: Blood samples were collected from each animal at 0.17, 0.5, 0.75, 1, 1.5, 2, 4, and 8 hr post-dose on days 1 and 7 in Phase 2. The method used for measurement of plasma drug concentration was previously validated, but no description of the method was provided.

RESULTS:

Mortality: None.

Clinical Signs: In Phase 1 of the study, non-formed feces occurred in both animals one day after administration of 5 mg/kg iv, but not after treatment with next dose level (15 mg/kg). In Phase 2, liquid and non-formed feces were observed in each monkey treated with 15 mg/kg/day iv. Red skin on hind limbs were observed in 3/4 monkeys.

Bodyweight: The male and female in Phase 1 gained 0.2 kg and 0.1 kg, respectively, during the treatment period. Weight was unchanged in male monkeys in Phase 2, whereas the females gained 0.2 kg.

Ophthalmoscopy (Phase 2 only): No lesions were observed.

Hematology (Phase 2 only): The following changes are expressed relative to baseline values from day -7.

RBC: Reduced by 19% and 26% in the two males, and by 15% and 20% in the two females.

Hemoglobin: Decreased by 21% and 28% in the two males, and by 17% and 20% in the two females.

Hematocrit: Reduced by 19% and 28% in the two males, and by 14% and 20% in the two females.

Reticulocytes (absolute): Increased by 49% and 105% in the two males, and by 81% and 138% in the two females.

Clinical Chemistry (Phase 2 only): The following changes are expressed relative to baseline values from day -7.

ALT: Increased by 66% and 92% in the two males.

AST: Increased by 118% in one male.

Triglycerides: Reduced by 36% and 49% in the two males.

Reference ID: 3114166

Albumin: Decreased by 23% in one male and by 45% in one female.

A/G Ratio: Reduced by 55% in one female.

Phosphorus: Decreased by 36% in one male.

Urinalysis (Phase 2 only): No effects.

Gross Pathology (Phase 2 only): Three animals exhibited a dark area in skin on the hind limbs.

Histopathology (Phase 2 only): Skin lesions included an adventitial inflammatory reaction, hemorrhage, acute inflammation, and subcutaneous edema. Changes observed in the injection site included hemorrhage, subcutaneous edema, acute inflammation, and intimal proliferation.

Toxicokinetics (Phase 2 only): The plasma kinetic parameters are shown in the table below (taken from the study report).

Group	Dose Level (mg/kg/day)	Gender	C_{max} (nM)	T_{max} (Hours)	AUC_{0-t} (hr·nM)	$AUC_{0.167-8}$ (hr·nM)	$AUC_{0-\infty}$ (hr·nM)	$t_{1/2}$ (Hours)
				Day 1				
2	15	M	37000	0.167	52036	44709	53595	1.69
		F	32750	0.167	53794	47566	55040	1.50
				Day 7				
		M	33500	0.167	47934	41309	NA	1.65
		F	31950	0.167	48653	42411	NA	1.46

Values are the mean of 2 monkeys/sex.

No sex-related differences were observed. Plasma drug accumulation was not evident after seven days of administration.

Conclusions: MD-1100 acetate was not lethal at doses of up to 15 mg/kg iv. Laxative effects were observed at doses of 5 and 15 mg/kg. RBC count was reduced in all animals after seven days of treatment with 15 mg/kg/day, relative to baseline values.

6.2 Repeat-Dose Toxicity

The following studies were previously reviewed by David B. Joseph, Ph.D. (see Pharmacology/Toxicology review of IND 63,290 dated June 5, 2006). Final study reports were submitted in the NDA, however there were no changes in the data analysis.

14-Day Oral Gavage Toxicity and Toxicokinetic Study with MD-1100 Acetate in Rats with a 14-Day Recovery Period

Note: This study was submitted as a draft report.

Key Study Findings: NOAEL was 20 mg/kg/day, the highest dose tested; only trace levels of drug were present in plasma

Study # 7480-106

Vol. 5, Pg. 2

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: May 19, 2004 (report dated January 28, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure

METHODS: Crl:CD®(SD)IGS BR rats (approximately 7 weeks old; males: 211-257 g; females: 141-193 g) were treated orally with 0 (vehicle), 1, 5, or 20 mg/kg/day MD-1100 acetate for at least 14 days (10 rats/sex/group in the main study). These animals were sacrificed on day 16. The recovery groups were treated with the same dose levels for the same duration, and were sacrificed after a 14-day recovery period (5 rats/sex/group). The toxicokinetic groups were treated with 1, 5, or 20 mg/kg/day po (9 rats/sex/group). The drug was administered as a solution in phosphate-buffered saline. The test and control articles were given by gavage using a dose volume of 10 ml/kg. Dose selection was based on results of an acute oral toxicity study in rats (reviewed above), in which no signs of toxicity were observed at doses of up to 5 mg/kg, the highest dose tested.

Observations and Times:

Mortality: twice daily

Clinical Signs: once daily

Bodyweight: twice weekly starting on day 1

Food Consumption: once weekly

Ophthalmoscopy: prior to study initiation and on weeks 2 and 4

Hematology: blood samples were collected at the end of the main study and recovery phase (days 16 and 30, respectively)

Clinical Chemistry: blood samples were collected at the end of the main study and recovery phase (days 16 and 30, respectively)

Urinalysis: urine samples were collected at the end of the main study and recovery phase

Gross Pathology: at sacrifice

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroids/parathyroids, uterus; paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups in the main study and recovery phase, unless stated otherwise: adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur with marrow (articular surface), gross lesions (all groups), Harderian gland, heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland (females), optic nerves, ovaries, pancreas, Peyer's patch, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum (with bone marrow), stomach (forestomach, glandular), testes, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, vagina.

Adequate Battery: yes (x) no ( )
Peer Review: yes ( ) no (x)

Toxicokinetics: Blood was collected at 0.5, 1, 2, 4, 8, and 24 hr post-dose on days 1 and 14 (3 rats/sex/group/time-point). The method used for measurement of plasma drug concentration was previously validated, but no description of the method was provided.

RESULTS:

Mortality: None.

Reference ID: 3114166

Clinical Signs: Rough hair coat occurred in 1/30 rats in the 20 mg/kg/day group. No effects were observed in the recovery phase.

Bodyweight: Weight gain was not affected. The mean bodyweight in the control males and females was 234 ± 8.6 g and 166 ± 10.0 g, respectively, on day 1, and 327 ± 22.9 g and 207 ± 13.1 g, respectively, on day 15.

Food Consumption: Food intake was unaffected.

Ophthalmoscopy: No lesions were observed.

ECG: Not performed.

Hematology: No effects were observed in the main study or recovery phase. PT (prothrombin time) and APTT (activated partial thromboplastin time) were unaffected.

Clinical Chemistry: No changes were observed in the main study or recovery phase.

Urinalysis: The following parameters were reported: volume, specific gravity, clarity, color, pH, urobilinogen, glucose, ketones, protein, bilirubin, occult blood, sodium (mEq/l), sodium excretion (mEq/time), potassium (mEq/l), potassium excretion (mEq/time), chloride (mEq/l), chloride excretion (mEq/time), creatinine, RBC, WBC, epithelial cells, amorphous material, crystals, bacteria, casts, yeast, sperm, and mucus. No drug-related effects were observed.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported. No changes occurred in the main study groups. Absolute weight of lungs was increased by 13% and 14% in the 1 and 20 mg/kg/day recovery males, respectively.

Gross Pathology:

Main Study

Liver: Pale area occurred in the 20 mg/kg/day group (1/20 rats).

Stomach (glandular): Dark area was observed in the 20 mg/kg/day group (1/20 rats).

Recovery Phase

Seminal Vesicle: Small size was observed in the 20 mg/kg/day group (1/5 males).

Histopathology:

Main Study

Reference ID: 3114166

No treatment-related effects were observed.

Recovery Sacrifice

No treatment-related effects were observed.

Toxicokinetics: No drug was detected in plasma in the 1 and 5 mg/kg/day groups. On day 1, trace levels of drug (2.0-3.8 nM) were detected sporadically in the 20 mg/kg/day group at
0.5-2 hr. On day 14, drug was detected in only one high-dose animal (7.7 nM at 1 hr). The limit of quantification for the plasma drug assay was 2.0 nM (3 ng/ml).

Conclusions: The NOAEL was 20 mg/kg/day po. However, the actual dose administered to the 20 mg/kg/day group may have been slightly lower due to degradation of the dosing solution, which contained concentrations (determined from HPLC analysis) that were 12-23% lower than the intended concentration. The drug concentrations in the solutions used in the 1 and 5 mg/kg/day groups were nearly identical to the intended concentrations. Only the 20 mg/kg/day group had detectable plasma levels of drug. This group had only trace levels in plasma. No toxic effects were observed in this study. Therefore, higher dose levels should be used in future toxicology studies in rats, provided that this is technically feasible. The poor stability of drug in PBS at concentrations of 2 mg/ml and higher indicates the need for an alternative oral formulation if higher dose levels are to be used.

14-Day Nasogastric Toxicity and Toxicokinetic Study with MD-1100 Acetate in Cynomolgus Monkeys with a 14-Day Recovery Period

Note: This study was submitted as a draft report.

Key Study Findings: NOAEL was 0.5 mg/kg/day; liver was a target organ of toxicity a 2.5 and 5 mg/kg/day

Study # 7480-102

Vol. 7, Pg. 2

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: April 19, 2004 (report dated February 21, 2005)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure

Reference ID: 3114166

METHODS: Cynomolgus monkeys (approximately 2-4.5 years old; males: 2-3.5 kg; females: 2-2.8 kg) were treated orally with 0 (vehicle), 0.5, 2.5, or 5 mg/kg/day MD-1100 acetate for at least 14 days (3 monkeys/sex/group in the main study). These animals were sacrificed at the end of treatment. The recovery groups were treated with the same dose levels for the same duration, and were sacrificed after a 14-day recovery period (2 monkeys/sex/group). The test and control articles were given by nasogastric intubation using a dose volume of 3 ml/kg. The drug was administered as a solution in phosphate-buffered saline. The basis of dose selection was not stated.

Observations and Times:

Mortality: twice daily

Clinical Signs: multiple observations daily until no signs were present; examined for signs of intestinal pain or distress prior to study initiation and on days 1, 7, and 14

Bodyweight: daily in main study starting on day 1; weekly in recovery phase

Ophthalmoscopy: prior to study initiation and on day 12 (all groups) and 26 (recovery groups)

ECG: prior to study initiation, at 1-2 hr post dose on day 12, and on day 26

Hematology: blood samples were collected prior to study initiation and at the end of the main study and recovery phase

Clinical Chemistry: blood samples were collected prior to study initiation and at the end of the main study and recovery phase

Urinalysis: urine samples were collected prior to study initiation and at the end of the main study and recovery phase

Gross Pathology: at sacrifice

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver with gallbladder, lungs, ovaries, pituitary, prostate, salivary glands (mandibular), seminal vesicles, spleen, testes, thymus, thyroids/parathyroids, uterus; paired organs were weighed together

Histopathology: The following organs/tissues were examined in each animal: adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, femur with marrow (articular surface), gallbladder, gross lesions, heart, ileum, jejunum, kidneys, lacrimal gland, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland (females), optic nerves, ovaries, pancreas, Peyer's patch, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin,

Reference ID: 3114166

spinal cord (cervical, thoracic, lumbar), spleen, sternum (with bone marrow), stomach, testes, thymus, thyroids/parathyroids, tongue, trachea, urinary bladder, uterus, vagina.

Adequate Battery: yes (x) no ( )
Peer Review: yes ( ) no (x)

Toxicokinetics: Blood was collected from each monkey at 0.5, 1, 2, 4, 8, and 24 hr post-dose on days 1 and 14. The method used for measurement of plasma drug concentration was previously validated, but no description of the method was provided.

RESULTS:

Mortality: None.

Clinical Signs: The incidence of liquid feces was increased in the treatment groups (7/10, 6/10, and 9/10 monkeys in the 0.5, 2.5, and 5 mg/kg/day groups, respectively, compared to 1/10 control monkeys). Non-formed feces occurred in the 0, 0.5, 2.5, and 5 mg/kg/day groups (4/10, 9/10, 8/10, and 9/10 monkeys, respectively. No effects were observed in the recovery phase.

Bodyweight: The mean bodyweight in all study groups was unchanged during the main study. The mean weight of control males and females was 2.8 ± 0.57 kg and 2.3 ± 0.32 kg, respectively, on day 1, and 2.8 ± 0.53 kg and 2.3 ± 0.29 kg, respectively, on day 15. Slight increases (0.1-0.2 kg) in bodyweight occurred in all groups during the recovery phase.

Food Consumption: Not measured.
Ophthalmoscopy: No lesions were observed.

ECG: Heart rate, PR, QRS, QT, and QT_c were reported. There were no effects.

Hematology: No effects occurred in the main study or recovery phase. PT and APTT were unaffected.

Clinical Chemistry: In the main study, one male (# I55855) treated with 5 mg/kg/day exhibited a 2.8-fold increase in ALT and a 5.6-fold increase in AST, relative to the mean control value. One female (# I55870) in the 2.5 mg/kg/day group exhibited a 2.1-fold increase in ALT and a 3.7-fold increase in AST. The magnitude of increase in the individual ALT and AST levels relative to the individual baseline values was similar to those expressed relative to the mean control value. Globulin was reduced by 20%, 15%, and 12% in the 0.5, 2.5, and 5 mg/kg/day females, respectively, in the main study. A/G ratio was reduced by 28%, 23%, and 20% in the 0.5, 2.5, and 5 mg/kg/day females, respectively. No changes were observed in the recovery phase.

Urinalysis: The following parameters were reported: volume, specific gravity, clarity, color, pH, urobilinogen, glucose, ketones, protein, bilirubin, occult blood, sodium

Reference ID: 3114166

(mEq/l), sodium excretion (mEq/time), potassium (mEq/l), potassium excretion (mEq/time), chloride (mEq/l), chloride excretion (mEq/time), RBC, WBC, epithelial cells, amorphous material, crystals, bacteria, casts, yeast, sperm, and mucus. No treatment-related effects were observed.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Main Study

No effects.

Recovery Phase

Seminal Vesicle: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 5.2-, 4.4-, and 4.6-fold, respectively, in the 5 mg/kg/day males.

Thymus: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 2.2-, 2.5-, and 1.9-fold, respectively, in the 5 mg/kg/day females.

Gross Pathology:

Main Study

Lungs: Adhesion was observed in the 2.5 mg/kg/day group (1/6 monkeys).

Recovery Phase

Colon: Dark area occurred in the 0.5, 2.5, and 5 mg/kg/day groups (1/4 monkeys in each group).
Lungs: Adhesion was observed in the 2.5 mg/kg/day group (1/4 monkeys). Interlobar adhesion was observed in the 2.5 mg/kg/day group (1/4 monkeys).

Histopathology:

Main Study

Cecum: Submucosal inflammation was observed in the 5 mg/kg/day group (1/6 monkeys, # I55874). This change consisted of granuloma in the muscle layer. The authors stated that these lesions are often observed in monkeys and are generally associated with nematode parasites.

Colon: Submucosal inflammation was observed in the 5 mg/kg/day group (1/6 monkeys, # I55874). This change consisted of an abscess in the muscle layer. The authors stated that these lesions are often observed in monkeys and are generally associated with nematode parasites.

Reference ID: 3114166

Esophagus: Lymphocytic infiltrate occurred in the 0.5 and 5 mg/kg/day groups (1/6 monkeys in both groups).

Eye: Lymphocytic infiltrate in limbus occurred in the 5 mg/kg/day groups (1/6 monkeys).

Kidneys: Inflammation of pelvis occurred in the 5 mg/kg/day group (1/6 monkeys, # I55872). Regeneration of tubular epithelium was observed in the 5 mg/kg/day group (1/6 monkeys, # I55874).

Trachea: Inflammation was observed in the 0.5, 2.5, and 5 mg/kg/day groups (1/6, 2/6, and 2/6 monkeys, respectively).

Recovery Phase

Cecum: Multifocal hemorrhage in mucosa occurred in the 2.5 mg/kg/day group (1/4 monkeys, # I55850).

Colon: Multifocal hemorrhage in mucosa occurred in the 2.5 mg/kg/day group (1/4 monkeys, # I55850).

Epididymis: Lymphocytic infiltration was observed in the 2.5 mg/kg/day group (1/4 monkeys).

Esophagus: Lymphocytic infiltration was observed in the 2.5 mg/kg/day group (1/4 monkeys, # I55870).

Lungs: Fibrosis of pleura occurred in the 2.5 mg/kg/day group (1/4 monkeys).

Lymph Nodes (mesenteric): Hyperplasia was observed in the 2.5 mg/kg/day group (1/4 monkeys, # I55870).

Parathyroid: Cysts were present in the 2.5 and 5 mg/kg/day groups (1/4 monkeys in both groups).

Peyer's Patch (ileum): Lymphoid hyperplasia was observed in the 2.5 mg/kg/day group (1/4 monkeys, # I55870).

Toxicokinetics: Plasma kinetic parameters are shown in the following tables (taken from the study report.

Day 1

Reference ID: 3114166

Dose Group	Dose Level (mg/kg/day)	Gender		T_{max} (hr)	C_{max} (nM)	AUC_{0-t} (nM·hr)	AUC_{0-24hr} (nM·hr)
2	0.5	M	Mean	NA	NA	NA	NA
			SD	NA	NA	NA	NA
			N	0	0	0	0
		F	Mean	NA	NA	NA	NA
			SD	NA	NA	NA	NA
			N	0	0	0	0
3	2.5	M	Mean	1.33	5.17	12.9	17.1
			SD	0.58	1.21	6.6	7.8
			N	3	3	3	3
		F	Mean	1.00	3.75	7.45	10.6
			SD	NA	NA	NA	NA
			N	2	2	2	2
4	5	M	Mean	0.833	7.00	15.7	19.9
			SD	0.289	4.09	15.6	18.1
			N	3	3	3	3
		F	Mean	0.900	7.82	18.7	24.3
			SD	0.652	4.01	12.1	14.6
			N	5	5	4	4

Day 14

Dose Group	Dose Level (mg/kg/day)	Gender		T_{max} (hr)	C_{max} (nM)	AUC_{0-t} (nM·hr)	AUC_{0-24hr} (nM·hr)
2	0.5	M	Mean	NA	NA	NA	NA
			SD	NA	NA	NA	NA
			N	0	0	0	0
		F	Mean	NA	NA	NA	NA
			SD	NA	NA	NA	NA
			N	0	0	0	0
3	2.5	M	Mean	0.500	5.10	6.72	9.61
			SD	0	3.72	5.95	7.94
			N	3	3	3	3
		F	Mean	0.500	3.07	2.94	4.45
			SD	0	0.50	1.36	1.95
			N	3	3	3	3
4	5	M	Mean	1.00	4.53	8.57	11.9
			SD	0.61	3.16	12.20	15.4
			N	5	5	5	5
		F	Mean	0.600	7.03	11.1	14.3
			SD	0.224	3.89	11.5	13.3
			N	5	5	4	4

Only trace levels of drug were detected in plasma. No drug was detected at time-points later than 4 hr. The limit of quantification was 2.0 nM (3 ng/ml). No sex-related differences in plasma kinetic parameters or evidence of plasma drug accumulation were observed.

Conclusions: The NOAEL was 0.5 mg/kg/day po. One monkey in each of the 2.5 and 5 mg/kg/day groups exhibited increased ALT levels. No drug-related lesions were present in liver. However, liver is considered as a target organ of toxicity at 2.5 and 5 mg/kg/day, based on the increased ALT levels which occurred after only two weeks of treatment. One animal in the 5 mg/kg/day group exhibited submucosal inflammation in cecum and colon, and regeneration of renal tubular epithelium. A different animal in this group exhibited inflammation of the renal pelvis. Given the low incidence of the colonic and renal lesions and the absence of a clear dose-dependency, the relationship to drug treatment is uncertain. Liquid and/or non-formed stool occurred in all drug-treated groups, and was indicative of pharmacological activity. Only trace levels of drug were detected in plasma. Since the drug was well tolerated at all dose levels, higher doses should be used in future toxicity studies in monkeys.

The following studies were previously reviewed by David B. Joseph, Ph.D. (see Pharmacology/Toxicology review of IND 63,290 dated April 10, 2007).

13-Week Oral Toxicity Study of MD-1100 Acetate in Mice Followed by a 2-Week Recovery Period

Key Study Findings: A MTD cannot be estimated from this study due to drug-related deaths and target organ toxicity in the 100 and 200 mg/kg/day groups, and the absence of histopathologic evaluation of the 20 mg/kg/day group.

Study # MNP-103-007 (1220-004)

Amendment # 051, Vol. 4, Pg. 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: June 8, 2006 (report dated January 2, 2007)

GLP Compliance: A statement of compliance was included.

QA Report: yes ( ) no (x)

Drug: PPL-MD11000601B1; 93.0% peptide; 98.96% peptide purity

METHODS: Crl:CD1®(Icr) mice (approximately 8 weeks old; males: 28.0-36.0 g; females: 23.0-28.1 g) were treated orally with 0 (vehicle), 20, 100, or 200 mg/kg/day MD-1100 acetate for 13 weeks (10 mice/sex/group in main study). The recovery groups were treated orally with 0 (vehicle) or 200 mg/kg/day and were sacrificed at two weeks after the end of treatment (10 mice/ sex/group). Additional groups were treated orally with 0 (vehicle), 20, 100, or 200 mg/kg/day for measurement of toxicokinetic parameters (20 mice/sex/group). The main study and recovery groups were sacrificed on days 92 and 106, respectively. Animals that died during days 1-7 were replaced. The test and control articles were administered by gavage using a dose volume of 10 ml/kg. The vehicle was 0.5% methylcellulose. Drug suspensions were prepared with adjustment for the peptide content (93.0%). The authors stated that dose selection was based on available data from previous studies, but no details were provided. The following parameters were recorded:

Observations and Times:

Mortality: twice daily

Clinical Signs: weekly

Bodyweight: weekly

Reference ID: 3114166

Food Consumption: weekly

Ophthalmoscopy: prior to study initiation and at the end of the main study and recovery phase

Hematology: Blood samples were collected from the first half of the surviving main study and recovery animals on days 92 and 106, respectively.

Clinical Chemistry: Blood samples were collected from the second half of the surviving main study and recovery animals on days 92 and 106, respectively.

Gross Pathology: performed for main study and recovery animals that were found dead or sacrificed *in extremis*, and for animals sacrificed at the end of the main study (day 92) or recovery phase (day 106)

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary gland (mandibular), spleen, testes, thymus, thyroid/parathyroid, uterus (with cervix); paired organs were weighed together

Histopathology: The following organs/tissues were examined in the 0, 100, and 200 mg/kg/day groups, including some of the animals that were found dead or sacrificed *in extremis*: adrenals, aorta, bone with marrow (femur and sternum), brain (cerebrum, midbrain, cerebellum, medulla/pons), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes (with optic nerve), gall bladder, gross lesions (all groups), Harderian glands, heart (including 20 mg/kg/day females), ileum, jejunum, joint (tibiofemoral), kidneys, lacrimal glands, larynx, liver, lungs, lymph nodes (mandibular, mesenteric), mammary gland (females only), ovaries, pancreas, Peyer's patch, pharynx, pituitary, prostate, rectum, salivary gland (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, stomach (glandular, non-glandular), testes, thymus, thyroid/parathyroid, tissue masses with regional lymph node (all groups), tongue, trachea, ureter, urinary bladder, uterus, vagina.

Adequate Battery: yes (x) no ( )
Peer Review: yes ( ) no (x)

Toxicokinetics: Blood samples were collected at 0.5, 1, 2, 4, 8, and 24 hr post-dose on days 1, 27, and 85 (3 mice/sex/group/time-point). Plasma concentrations of MD-1100 acetate and its metabolite MM-419447 were measured using a validated LC/MS/MS method. The lower limit of quantification was 15 ng/ml for MD-1100 acetate and 30 ng/ml for MM-419447.

RESULTS:

Mortality: The incidence of death is shown in the tables below.

Reference ID: 3114166

Mortality in Main Study and Recovery Groups After Day 7		
Dose (mg/kg/day)	**Deaths**	**Day(s)**
0	1/20 males	74
20	none	
100	3/10 males 5/10 females	68, 88, 88 83, 88, 88, 89, 89
200	6/20 males 6/20 females	9, 84, 87, 88, 88, 89 9, 9, 37, 54, 82, 88

Mortality in Toxicokinetic Groups After Day 7		
Dose (mg/kg/day)	**Deaths**	**Days**
0	2/20 females	8, 27*
20	2/20 males 2/20 females	8, 29 31, 31
100	1/20 males 3/20 females	83 71, 74, 83
200	1/20 males 2/20 females	67 9, 31

*Animal died after blood collection.

Animals Replaced After Death on Days 1-7 (Main Study, Recovery, and Toxicokinetic Groups)	
Dose (mg/kg/day)	**Deaths**
0	1 female
20	2 females
100	2 males 7 females
200	4 males 17 females

In most cases, animals were found dead, with only a few animals sacrificed *in extremis*. Animals that died during days 1-7 were replaced. The authors considered all deaths in the 100 and 200 mg/kg/day groups as treatment-related. The deaths in the 20 mg/kg/day toxicokinetic group were not discussed by the authors. None of the deaths were attributed to gavage-related injury.

Clinical Signs: The 100 and 200 mg/kg/day groups exhibited decreased activity, tremors, convulsions, absence of or reduced feces, partial or complete closure of eyelids, brown or yellow discoloration of hair (mostly in the anogenital region), and cold skin. The 200 mg/kg/day group also exhibited slow breathing. No signs were observed in the 20 mg/kg/day group. Animals that died prematurely exhibited breathing anomalies (i.e. shallow, rapid, or difficult), loss of righting reflex, leaning, and moribund condition.

Bodyweight: The effects on weight gain at the end of the main study are shown for the main study and toxicokinetic groups in the tables below.

Dose (mg/kg/day)	**Change in Bodyweight in Main Study Groups on Day 91 (g)**	
	Males	**Females**
0	+7.38	+5.49
20	+6.39	+5.91
100	+7.10	+5.50
200	+6.62	+6.63

Dose (mg/kg/day)	**Change in Bodyweight in Toxicokinetic Groups on Day 84 (g)**	
	Males	**Females**
0	+4.76	+5.08
20	+4.98	+5.20
100	+6.50	+5.54
200	+6.16	+5.99

Weight gain in the main study males was reduced by 13%, 4%, and 10% at 20, 100, and 200 mg/kg/day, respectively. In contrast, the same dose levels were associated with increased weight gain (5-36%) in the toxicokinetic males. Thus, the totality of the bodyweight data indicates that MD-1100 acetate had no effect on weight gain in males. Drug administration was associated with weight gain increases of 0-21% in the main study females, and 2-18% in the toxicokinetic females. Given the highly variable bodyweight data in males, the increased weight gain in females is not considered to be drug-related. Bodyweight data from the main study and toxicokinetic groups are shown in the figures below (taken from the study report).

Reference ID: 3114166

Figure 1 Mean Body Weight Values – MALE

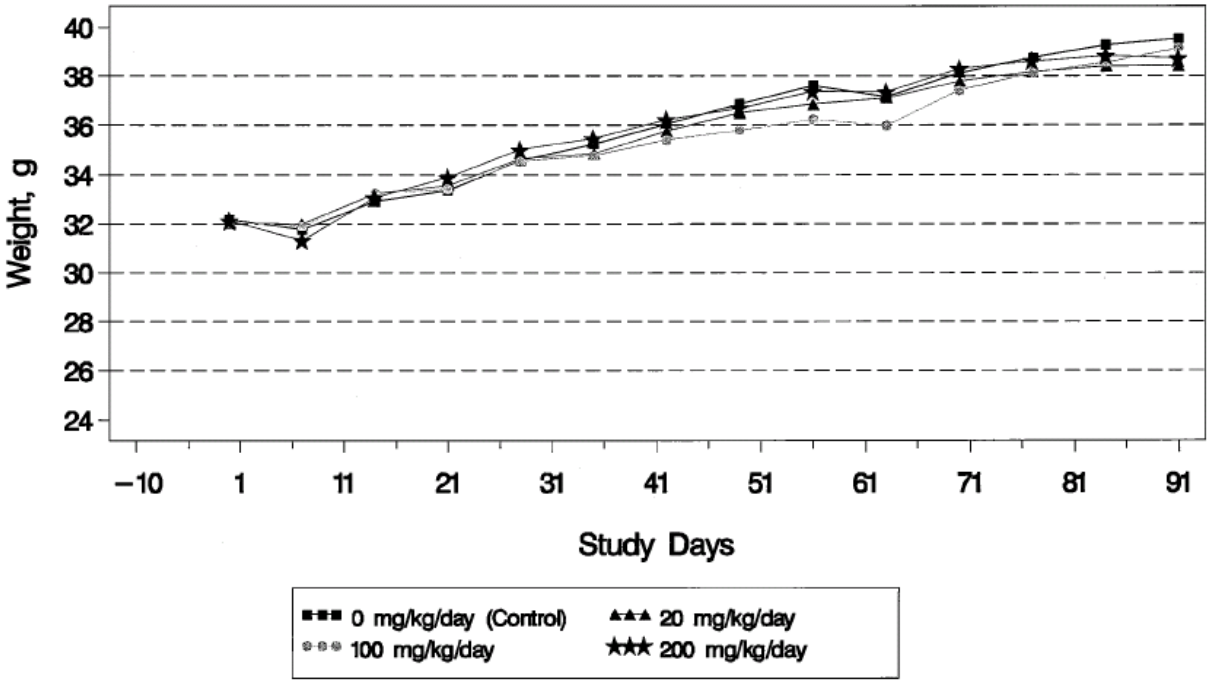


Figure 1A Mean Body Weight Values – FEMALE

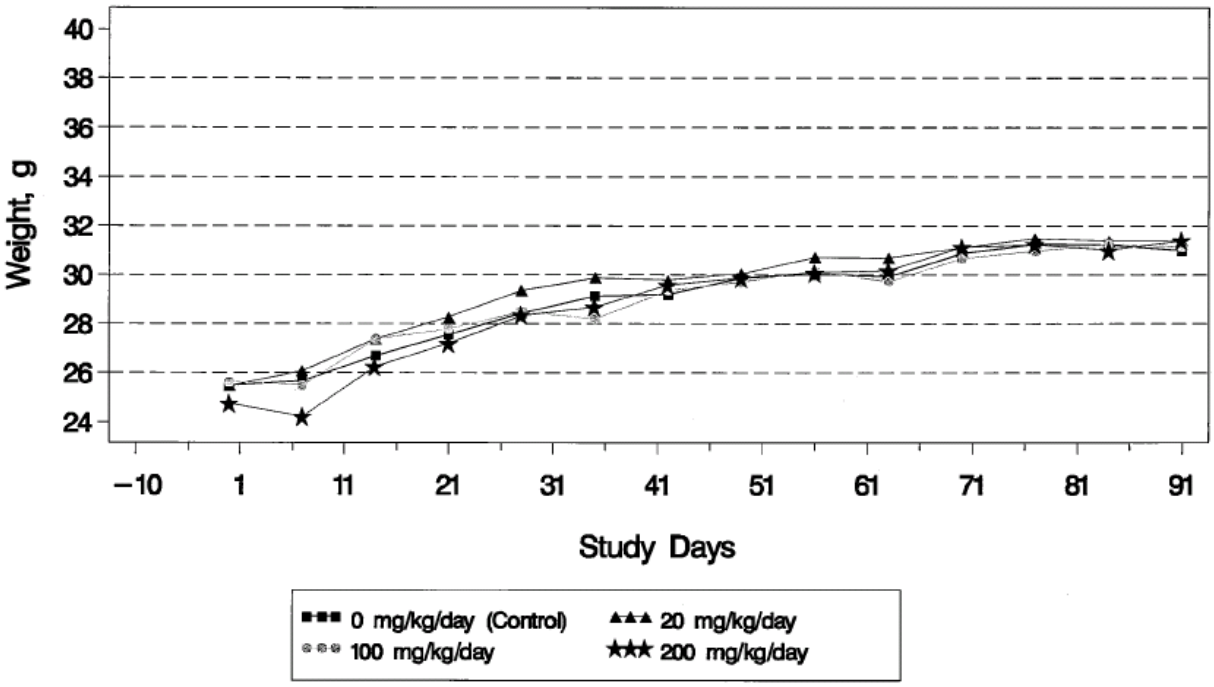


Figure 1D

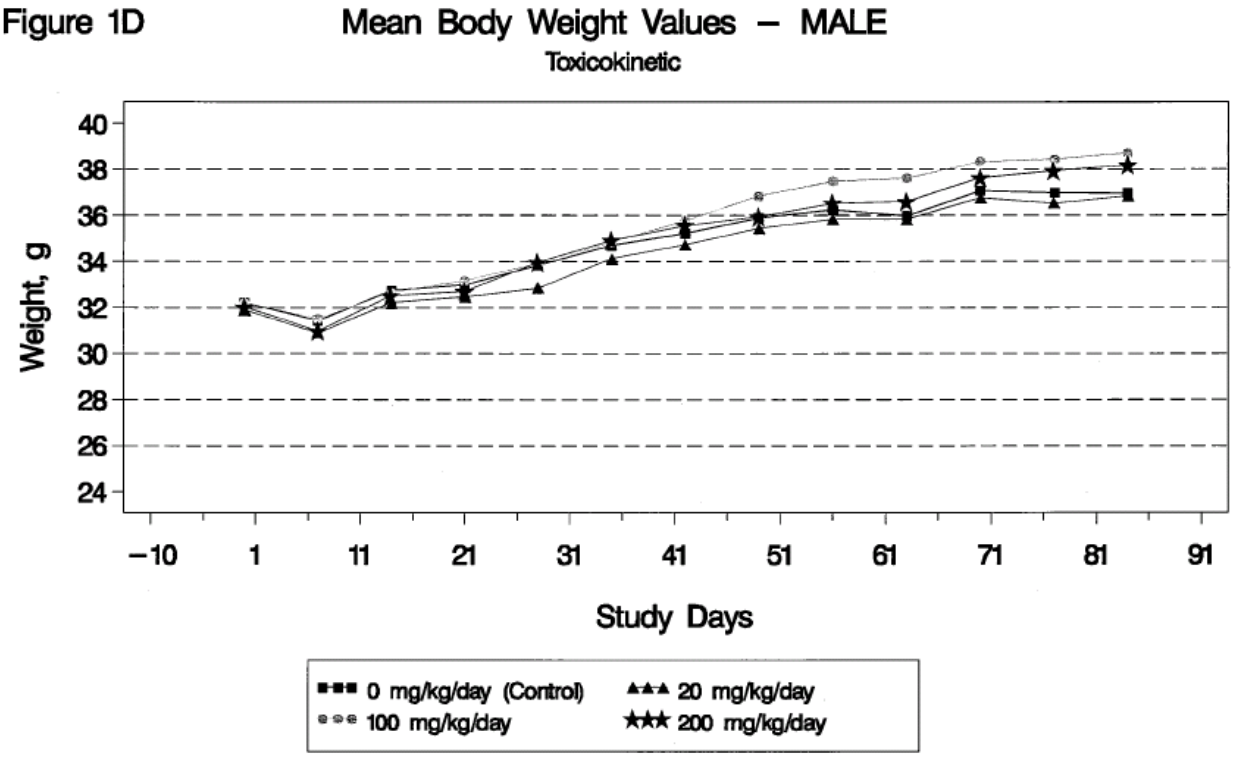
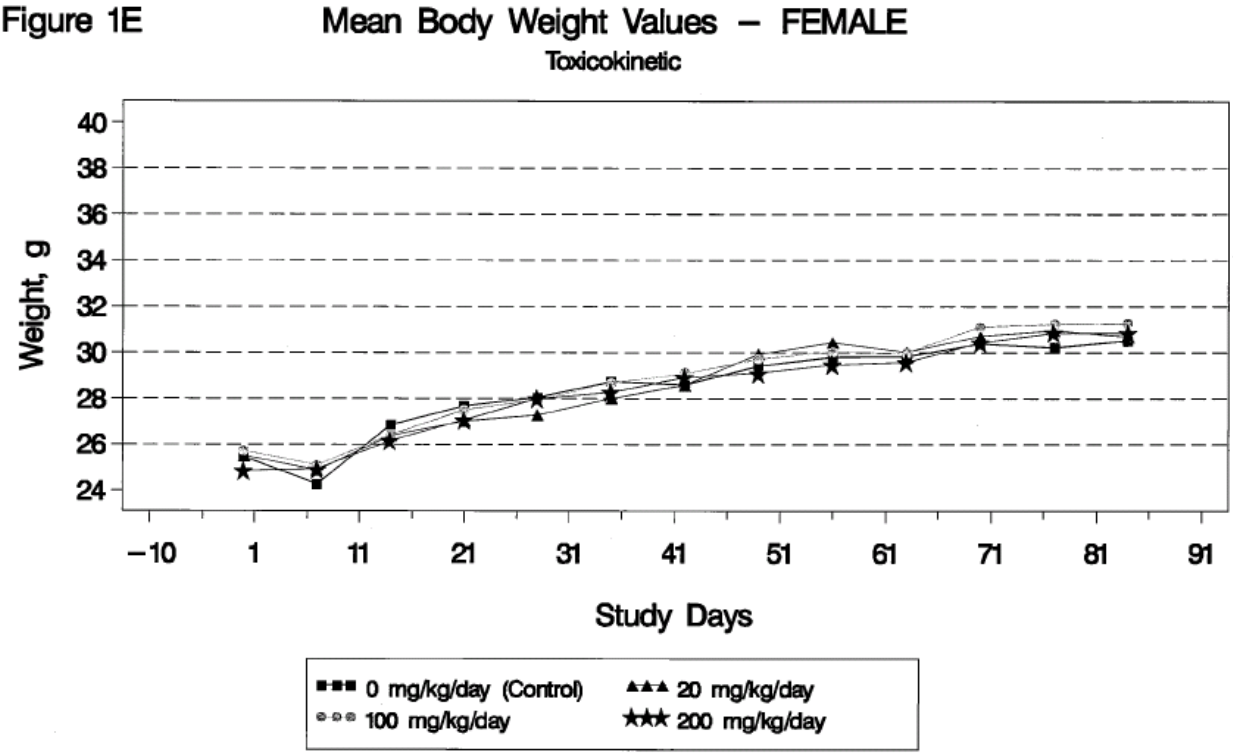


Figure 1E



For the main study, the mean bodyweight (± S.D.) of the control males and females was 32.2 ± 2.0 g and 25.5 ± 1.3 g, respectively, at study initiation, and 39.5 ± 2.4 g and 31.0 ± 2.1 g, respectively, on day 91.

Food Consumption: Food intake was decreased by 10-13% in the 200 mg/kg/day females during weeks 1 and 2.

Ophthalmoscopy: No drug-related lesions were observed.

ECG: Not performed.

Hematology: The reported hematology values are from measurements in the first half of mice of both sexes in each dose group (3-5/sex/group). The results are shown in the table below.

Parameter	Dose (mg/kg/day)	Change	Week
RBC	200^{f}	10% decrease (ns)	13
Hemoglobin	200^{f} 200^{m}, 200^{f}	5% decrease (ns) 8%, 6% decrease	13 15 (recovery)
Hematocrit	200^{m}	6% decrease	15 (recovery)
MCH	200^{f}	5% increase	13
Reticulocyte count	200^{m}	40% increase	15 (recovery)
WBC	20^{m}, 100^{m}, 200^{m} 200^{m} 200^{f}	61%, 99%, 42% increase (ns) 59% increase (ns) 35% decrease (ns)	13 15 (recovery) 15 (recovery)
Neutrophils	20^{m}, 100^{m}, 200^{m} 200^{m} 200^{f}	42%, 139%, 19% increase 59% increase (ns) 28% decrease (ns)	13 15 (recovery) 15 (recovery)
Lymphocytes	20^{m}, 100^{m}, 200^{m} 200^{m} 200^{f}	73%, 88%, 50% increase (ns) 58% increase (ns) 35% decrease (ns)	13 15 (recovery) 15 (recovery)
Monocytes	100^{m}, 200^{m} 200^{m} 200^{f}	3.2-, 2.3-fold increase (ns) 4.1-fold increase (ns) 62% decrease (ns)	13 15 (recovery) 15

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			(recovery)
Eosinophils	200[m] 200[f]	69% increase (ns) 51% decrease (ns)	15 (recovery) 15 (recovery)
Large unstained cells	200[f] 200[m] 200[f]	176% increase (ns) 107% increase (ns) 45% decrease (ns)	13 15 (recovery) 15 (recovery)

ns: not significant
m: males
f: females

At the end of the main study, neutrophils and lymphocytes were increased in males at all dose levels, although the effects were not dose-dependent. Monocytes were increased in the 100 and 200 mg/kg/day males. The increase in neutrophils, lymphocytes, and monocytes was also observed in the 200 mg/kg/day recovery males. RBC count was slightly reduced in the 200 mg/kg/day females at the end of the main study. Coagulation parameters were not measured.

Clinical Chemistry: The reported clinical chemistry values are from measurements in the second half of mice of both sexes in each dose group (2-6/sex/group). The results are shown in the table below.

Parameter	**Dose (mg/kg/day)**	**Change**	**Week**
Urea Nitrogen	200[f] 200[m]	26% decrease 89% increase (ns)	13 15 (recovery)
Glucose	200[f]	31% increase	13

ns: not significant
m: males
f: females

Urinalysis: No effects were observed.
Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Main Study

Adrenals: Absolute weight was reduced by 17% and 22% in the 20 and 200 mg/kg/day females, respectively. Organ/bodyweight ratio was decreased by 24% in the 200 mg/kg/day females. Organ/brain weight ratio was reduced by 18% in the 200 mg/kg/day females.

Epididymides: Absolute weight was reduced by 19-20% in the 20, 100, and 200 mg/kg/day males. Organ/bodyweight ratio was decreased by 15-17% in the 20, 100, and 200 mg/kg/day males. Organ/brain weight ratio was reduced by 15% in the 200 mg/kg/day males.

Lungs: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 13%, 19%, and 17%, respectively, in the 200 mg/kg/day males.

Pituitary: Absolute weight was reduced by 18%, 13%, and 13% in the 20, 100, and 200 mg/kg/day females, respectively. Organ/bodyweight ratio was decreased by 16%, 15%, and 19% in the 20, 100, and 200 mg/kg/day females, respectively. Organ/brain weight ratio was reduced by 18%, 16%, and 15% in the 20, 100, and 200 mg/kg/day females, respectively.

Prostate: Absolute weight was reduced by 43% and 56% in the 100 and 200 mg/kg/day males, respectively. Organ/bodyweight ratio was decreased by 44% and 58% in the 100 and
200 mg/kg/day males, respectively. Organ/brain weight ratio was reduced by 38% and 57% in the 100 and 200 mg/kg/day males, respectively.

Thymus: Absolute weight was reduced by 18% in the 20 and 100 mg/kg/day females. Organ/bodyweight ratio was decreased by 21%, 22%, and 18% in the 20, 100, and 200 mg/kg/day females, respectively. Organ/brain weight ratio was reduced by 19-20% in the 20 and 100 mg/kg/day females.

Thyroid: Absolute weight and organ/brain weight ratio were increased by 17% and 20%, respectively, in the 200 mg/kg/day females.

Uterus (with cervix): Absolute weight was reduced by 21% and 16% in the 100 and 200 mg/kg/day females, respectively. Organ/bodyweight ratio was decreased by 21% and 19% in the 100 and 200 mg/kg/day females, respectively. Organ/brain weight ratio was reduced by 21% and 15% in the 100 and 200 mg/kg/day females, respectively.

Recovery Phase

Testes: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 20%, 22%, and 18%, respectively, in the 200 mg/kg/day males.

Thymus: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 38%, 43%, and 43%, respectively, in the 200 mg/kg/day males.

Thyroid: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 21%, 15%, and 25%, respectively, in the 200 mg/kg/day males.

Gross Pathology:

Reference ID: 3114166

Main Study

Heart: Tan discoloration occurred in a 200 mg/kg/day female that survived until study termination.

Stomach (glandular): Red discoloration was observed in a 200 mg/kg/day female that died before study termination.

Recovery Phase

No treatment-related effects were observed.

Histopathology:

Premature Deaths

Histopathologic evaluation of the dead animals was incomplete, since many of these animals were not examined. The number of animals examined out of the total number of deaths was as follows: 1/4 mice in the control group, 0/6 mice in the 20 mg/kg/day group, 10/21 mice in the 100 mg/kg/day group, and 23/36 mice in the 200 mg/kg/day group. Observations from the examination of dead animals are listed below.

Adrenals: Subcapsular cell hyperplasia occurred in the 200 mg/kg/day group (1/23 mice).

Cecum: Marked ulceration occurred in the 200 mg/kg/day group (2/23 mice).

Epididymides: Lymphohistiocytic inflammation, sperm granuloma, single cell necrosis in tubules, and tubular hyperplasia were observed in the 200 mg/kg/day males. For each of these lesions, the incidence was 1/9 males.

Heart: Degeneration of myocardium was observed in the 200 mg/kg/day group (1/23 mice). Lymphohistiocytic inflammation occurred in the 200 mg/kg/day group (2/23 mice). Mineralization of myocardium was observed in the 200 mg/kg/day group (5/23 mice). Necrosis of myocardium occurred in the 200 mg/kg/day group (2/23 mice).

Kidneys: Mineralization was observed in the 100 and 200 mg/kg/day groups (1/10 and 1/23 mice, respectively). Proximal tubular necrosis occurred in the 200 mg/kg/day group (4/23 mice). Tubular epithelial hypertrophy was observed in the 200 mg/kg/day group (4/23 mice). Tubular protein accumulation occurred in the 200 mg/kg/day group (1/23 mice).

Liver: Inflammation with multifocal necrosis occurred in the 200 mg/kg/day group (1/23 mice).

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Lungs: Hemorrhage was observed in the 200 mg/kg/day group (1/23 mice). Apoptosis in bronchiolar epithelium occurred in the 200 mg/kg/day group (1/23 mice). Arterial thrombosis was observed in the 200 mg/kg/day group (1/23 mice).

Nerve (sciatic): Nerve fiber degeneration occurred in the 200 mg/kg/day group (1/23 mice).

Ovaries: Decreased corpora lutea was observed in the 200 mg/kg/day group (1/14 females).

Parathyroids: Lymphocytic infiltration occurred in the 200 mg/kg/day group (1/23 mice).

Peyers Patches: Lymphoid necrosis was observed in the 100 mg/kg/day group (1/10 mice).

Pharynx: Muscle degeneration occurred in the 200 mg/kg/day group (1/23 mice). Lymphocytic infiltration was observed in the 100 mg/kg/day group (1/10 mice). Neutrophilic inflammation occurred in the 200 mg/kg/day group (1/23 mice).

Pituitary: Vacuolar degeneration in pars distalis was observed in the 200 mg/kg/day group (1/23 mice).

Spleen: Lymphoid depletion occurred in the 100 and 200 mg/kg/day groups (2/10 and 1/23 mice, respectively). Lymphoid necrosis was observed in the 100 mg/kg/day group (3/10 mice).

Stomach (glandular): Ulcers occurred in the 100 and 200 mg/kg/day groups (1/10 and 5/23 mice, respectively). Hemorrhage was observed in the 200 mg/kg/day group (1/23 mice). Apoptosis occurred in the 200 mg/kg/day group (1/23 mice).

Testes: Necrosis of seminiferous tubules was observed in the 200 mg/kg/day group (1/9 males).

Thymus: Atrophy of cortex occurred in the 200 mg/kg/day group (1/23 mice). Lymphoid necrosis occurred in the 100 mg/kg/day group (1/10 mice). Mineralization was observed in the 200 mg/kg/day group (2/23 mice).

Main Study Terminal Sacrifice

Histopathologic evaluation was performed in the 100 and 200 mg/kg/day groups. No examination was performed in the 20 mg/kg/day group, with the exception of heart in the 20 mg/kg/day females. Given that drug-related deaths and tissue lesions occurred in the 100 and 200 mg/kg/day groups, the histopathology data is considered as incomplete due to the absence of observations from the 20 mg/kg/day group. Lesions in the 100 and 200 mg/kg/day groups are described below.

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Eyes: Fold/rosette was observed in the 200 mg/kg/day group (3/14 mice).

Gallbladder: Epithelial hyperplasia occurred in the 200 mg/kg/day group (1/14 mice). Neutrophilic inflammation was observed in the 200 mg/kg/day group (1/14 mice).

Heart: Mineralization occurred in the 100 and 200 mg/kg/day groups (1/12 and 1/14 mice, respectively).

Kidneys: Mineralization occurred in the 100 and 200 mg/kg/day groups (2/12 and 2/14 mice, respectively).

Liver: Vacuolization was observed in the 200 mg/kg/day group (2/14 mice).

Lymph Node (mandibular): Hemorrhage occurred in the 100 and 200 mg/kg/day groups (1/12 and 1/14 mice, respectively).

Nerve (sciatic): Nerve fiber degeneration occurred in the 100 mg/kg/day group (1/12 mice).

Ovaries: Decreased corpora lutea was observed in the 200 mg/kg/day group (1/8 females).

Parathyroids: Lymphocytic infiltration occurred in the 200 mg/kg/day group (1/14 mice).

Peyers Patches: Lymphoid necrosis was observed in the 200 mg/kg/day group (1/14 mice).

Pituitary: Hemorrhage was observed in the 200 mg/kg/day group (1/14 mice).

Skeletal Muscle: Lymphohistiocytic inflammation occurred in the 200 mg/kg/day group (1/14 mice).

Skin: Neutrophilic inflammation was observed in the 200 mg/kg/day group (1/14 mice).

Spleen: Lymphoid depletion occurred in the 200 mg/kg/day group (1/14 mice).

Stomach (glandular): Inflammation with ulcer was observed in the 200 mg/kg/day group (1/14 mice).

Thymus: Atrophy of cortex and lymphoid hyperplasia occurred in the 200 mg/kg/day group (1/14 mice).

Uterus (with cervix): Dilatation was observed in the 200 mg/kg/day group (2/14 mice).

Recovery Phase

Reference ID: 3114166

Examination of recovery groups was limited to females. Lymphohistiocytic inflammation in heart occurred in the 200 mg/kg/day group (2/6 females).

Toxicokinetics: Plasma levels of MD-1100 acetate and its metabolite MM-419447 were measured. The lower limit of quantification was 15 ng/ml for MD-1100 acetate and 30 ng/ml for MM-419447. Toxicokinetic parameters for MD-1100 acetate are shown in the table below (taken from the study report).

Toxicokinetic Parameters for MD-1100 Acetate

Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-2} (ng•hr/mL)	AUC_{0-t} (ng•hr/mL)
		Day 1			
200	M	39.8	1.00	49.4	86.0
	F	50.0	0.500	51.2	51.2
		Day 27			
200	M	38.7	0.500	38.4	25.7
		Day 85			
200	M	27.9	0.500	39.0	39.0
	F	28.7	2.00	24.0	24.0

Values were determined from 3 mice/sex/time-point.

Parameters are shown only for the 200 mg/kg/day group, since this was the only group which had toxicokinetic data that was sufficient for analysis. MD-1100 acetate was sporadically detected in the 100 mg/kg/day group, and was undetected in the 20 mg/kg/day group. The drug was undetectable at 2-4 hr after dosing. The data indicates that MD-1100 acetate did not accumulate in plasma after repeated dosing. MM-419447 was not detected.

Conclusions: Drug-related deaths occurred in the 100 and 200 mg/kg/day groups. Deaths also occurred in the 0 and 20 mg/kg/day groups (4 and 6 mice, respectively). However, histopathologic evaluation was not performed on any of the dead animals in the 20 mg/kg/day group, nor did the authors comment on these deaths. All dead animals in the 20 mg/kg/day group were toxicokinetic animals. The protocol stated that a necropsy would not be performed on toxicokinetic animals that died before study termination. Thus, the relationship of mortality at 20 mg/kg/day to treatment with MD-1100 acetate is uncertain. No deaths at any dose level were attributed to gavage-related injury. Target organs of toxicity in dead animals included kidneys, spleen, and glandular stomach (100 and 200 mg/kg/day). Cecum and heart were also target organs of toxicity in dead animals in the 200 mg/kg/day group. Among the surviving animals,

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lesions were observed in eyes (200 mg/kg/day), heart (100 and 200 mg/kg/day), kidneys (100 and 200 mg/kg/day), liver (200 mg/kg/day), and several other organs. Histopathologic examination was not performed in the 20 mg/kg/day group, despite the incidence of drug-related deaths and target organ toxicity in the 100 and 200 mg/kg/day groups. Clinical signs in the 100 and 200 mg/kg/day groups included decreased activity, tremors, convulsions, absence of or reduced feces, partial or complete closure of eyelids, and cold skin. A MTD (maximum tolerated dose) cannot be estimated from this study due to mortality and target organ toxicity in the 100 and 200 mg/kg/day groups, and the absence of histopathologic evaluation of the 20 mg/kg/day group.

The following studies were previously reviewed by David B. Joseph, Ph.D. (see Pharmacology/Toxicology review of IND 63,290 dated January 14, 2007).

13-Week Oral Toxicity Study of MD-1100 Acetate in Rats Followed by a 2-Week Recovery Period

Key Study Findings: a single death at 100 mg/kg/day was possibly drug related; NOAEL was 50 mg/kg/day in males and 100 mg/kg/day in females; no target organs of toxicity were identified

Study # MNP-103-005 (1220-003)

Amendment # 044, Vol. 1, Pg. 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: March 31, 2006 (report dated November 2, 2006)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000501; 93.1% peptide, 96.13% peptide purity

METHODS: Crl:CD®(SD) rats (age 8 weeks; males: 227-263 g; females: 173-200 g) were treated orally with 0 (vehicle), 10, 50, or 100 mg/kg/day MD-1100 acetate for 91 days (10 rats/sex/group in the main study). These animals were sacrificed at the end of treatment (day 92). The recovery groups were treated with 0 or 100 mg/kg/day for the same duration, and were sacrificed after a 14-day recovery period (6 rats/sex/group). The test and control articles were administered orally via gavage using a dose volume of 10 ml/kg. The drug was administered as a suspension in 0.5% methylcellulose. Drug suspensions were prepared with adjustment for the

peptide content (93.1%). Dose selection was based on results of the 2-week oral toxicity study in rats, in which MD-1100 acetate was well tolerated at all dose levels (1, 5, and 20 mg/kg/day).

Observations and Times:

Mortality: twice daily

Clinical Signs: twice daily

Bodyweight: weekly starting on day -1

Food Consumption: weekly

Ophthalmoscopy: prior to study initiation and on week 13

Hematology: blood samples were collected on day 92 and at the end of the recovery phase (day 106)

Clinical Chemistry: blood samples were collected on day 92 and at the end of the recovery phase (day 106)

Urinalysis: urine samples were collected on day 92 and at the end of the recovery phase (day 106)

Gross Pathology: at sacrifice

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, salivary glands (mandibular + sublingual), spleen, testes, thymus, thyroid/parathyroids, uterus (with cervix); paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups, except where stated otherwise**:** adrenals, aorta, bone (femur and sternum with marrow), brain (cerebrum, midbrain, cerebellum, and medulla/pons), cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gross lesions (all groups), Harderian gland, heart, ileum, jejunum, joint (tibiofemoral), kidneys, lacrimal gland, larynx, liver, lungs, lymph nodes (mandibular, mesenteric), masses with regional lymph node (all groups), optic nerve, ovaries, pancreas, parathyroids, Peyer's patch, pharynx, pituitary, prostate, rectum, salivary glands (mandibular, parotid, sublingual), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, lumbar, thoracic), spleen, stomach (glandular and non-glandular), testes, thymus, thyroid, tongue, trachea, ureter, urinary bladder, uterus, vagina.

Adequate Battery: yes (x) no ( )
Peer Review: yes (x) no ( )

Reference ID: 3114166

Toxicokinetics: Blood was collected at 0.5, 1, 2, 4, 8, and 24 hr post-dose on days 1, 27, and 85 (3 rats/sex/group/time-point). Plasma concentrations of MD-1100 acetate and its metabolite MM-419447 were measured using a HPLC and MS/MS method. The lower limit of quantification was 7.5 ng/ml for both the unchanged drug and the metabolite.

RESULTS:

Mortality: One male in the 100 mg/kg/day group was found dead on day 85. No gross abnormalities were observed at necropsy. A cause of death was not determined from microscopic examination. The authors considered this death as unrelated to treatment.

Clinical Signs: No treatment-related signs were observed.

Bodyweight: Weight gain in the 10, 50, and 100 mg/kg/day males was increased by 12%, 9%, and 6%, respectively, at the end of the main study. The mean (± S.D.) weight of control males and females was 251 ± 10 g and 184 ± 6 g, respectively, on day 1, and 512 ± 40 g and 307 ± 20 g, respectively, on week 13. Weight gain was unaffected during the recovery period.

Food Consumption: No treatment-related effects were observed.

Ophthalmoscopy: No drug-related lesions were observed.

ECG: Not performed.

Hematology: The results are shown in the table below.

Parameter	Dose (mg/kg/day)	Change	Week
Reticulocyte count	10^m, 50^m, 100^m 100^m	17%, 19%, 16% decrease (ns) 8% decrease (ns)	13 15 (recovery)
WBC	100^m	32% increase	15 (recovery)
Neutrophils	100^m	41% increase (ns)	15 (recovery)
Lymphocytes	100^m	29% increase (ns)	15 (recovery)
Monocytes	100^m	54% increase (ns)	15 (recovery)
Eosinophils	100^m 100^f	58% increase 40% decrease (ns)	15 (recovery) 15 (recovery)
Large unstained cells	100^m	68% increase	15 (recovery)

ns: not significant
m: males
f: females

Reductions in reticulocyte count were observed in males at all dose levels of MD-1100 acetate. Increases in neutrophils, lymphocytes, monocytes, eosinophils, and large unstained cells were observed in the 100 mg/kg/day males at the end of recovery.

APTT (activated partial thromboplastin time) and PT (prothrombin time) were unaffected.

Clinical Chemistry: Potassium was increased by 9-12% in the 10, 50, and 100 mg/kg/day males on week 13 (not significant). One male in the 100 mg/kg/day group exhibited a 106% increase in ALT on week 13 (relative to the mean control value). Two females in the 50 mg/kg/day group exhibited ALT and AST increases of 2-3-fold on week 13.

Urinalysis: No effects were observed.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Main Study

Ovaries: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio in the 10 and 100 mg/kg/day females were decreased by 12-15%.

Salivary Glands: Absolute weight and organ/brain weight ratio in the 10, 50, and 100 mg/kg/day males were increased by 11-14%. Organ/bodyweight ratio in the 100 mg/kg/day males was increased by 13%.

Thymus: Absolute weight and organ/brain weight ratio in the 50 and 100 mg/kg/day males were increased by 20-24%. Organ/bodyweight ratio in the 50 and 100 mg/kg/day males was increased by 16% and 22%, respectively. Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 10%, 11%, and 13%, respectively, in the 100 mg/kg/day females.

Uterus: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 29-31% in the 50 mg/kg/day females. Absolute weight and organ/bodyweight ratio were increased by 11-12% in the 100 mg/kg/day females.

Recovery Phase

Adrenals: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 16%, 21%, and 14%, respectively, in the 100 mg/kg/day females.

Heart: Absolute weight and organ/bodyweight ratio were decreased by 11% and 14%, respectively, in the 100 mg/kg/day males.

Liver: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 26%, 18%, and 28%, respectively, in the 100 mg/kg/day females.

Ovaries: Organ/bodyweight ratio was decreased by 13% in the 100 mg/kg/day females.

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Pituitary: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 24%, 16%, and 26%, respectively, in the 100 mg/kg/day females.

Thymus: Absolute weight and organ/brain weight ratio were increased by 15% and 17%, respectively, in the 100 mg/kg/day females.

Thyroid/Parathyroid: Absolute weight and organ/brain weight ratio were increased by 12% and 16%, respectively, in the 100 mg/kg/day males. Absolute weight and organ/brain weight ratio were increased by 15% and 18%, respectively, in the 100 mg/kg/day females.

Gross Pathology:

Premature Deaths (one high-dose male)

No abnormalities were observed.

Main Study

No treatment-related effects were observed.

Recovery Phase

No treatment-related effects were observed.

Histopathology:

Premature Deaths (one high-dose male)

Liver: Focal necrosis of minimal severity was observed.

Main Study

No treatment-related effects were observed.

Recovery Phase

No data was provided.

Toxicokinetics: The results from measurement of MD-1100 acetate and its active metabolite MM-419447 are shown in the tables below (taken from the study report).

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Toxicokinetic Parameters for MD-1100 Acetate in Rat Plasma

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-4} (ng·hr/mL)
			Day 1			
3	50	M	9.36	0.500	6.88	11.3
		F	6.97	0.500	9.14	11.6
4	100	M	24.0	0.500	28.7	36.0
		F	300	0.500	174	174
			Day 27			
3	50	M	4.51	1.00	NC	NC
		F	6.48	1.00	8.32	11.4
4	100	M	18.8	0.500	21.0	24.3
		F	15.9	0.500	19.5	23.7
			Day 85			
3	50	M	4.52	0.500	2.92	4.23
		F	15.2	0.500	9.16	12.3
4	100	M	23.2	0.500	27.7	38.8
		F	33.5	0.500	43.5	72.3

Values were determined from 3 rats/sex/group/time-point.

Toxicokinetic Parameters for MM-419447 in Rat Plasma

Dose Group	Dose Level (mg/kg/day)	Sex	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-t} (ng·hr/mL)	AUC_{0-4} (ng·hr/mL)	AUC_{0-4} Metabolite to Parent Ratio
				Day 1			
3	50	M	8.02	0.500	NC	NC	NA
		F	9.15	0.500	25.6	15.4	1.32
4	100	M	19.4	0.500	21.5	24.4	0.678
		F	18.2	0.500	12.2	18.4	0.106
				Day 27			
3	50	M	7.89	1.00	11.1	15.6	NA
		F	11.0	0.500	7.63	11.8	1.04
4	100	M	21.0	0.500	38.3	38.3	1.58
		F	20.5	0.500	20.7	25.1	1.06
				Day 85			
3	50	M	NA	NA	NA	NA	NA
		F	19.7	0.500	11.4	14.4	1.17
4	100	M	18.3	0.500	22.1	29.2	0.752
		F	31.0	0.500	32.9	48.2	0.667

Values were determined from 3 rats/sex/group/time-point.

The data indicates that both the unchanged drug and its active metabolite were present in plasma on each day of measurement in the 50 and 100 mg/kg/day groups, whereas neither peptide was detectable in the 10 mg/kg/day group. The lower limit of quantification was 7.5 ng/ml for both the unchanged drug and the metabolite. MD-1100 acetate and MM-419447 were generally undetectable at time-points beyond 2-4 hr. The AUC and C_{max} values were increased with dose level. AUC values for MD-1100 acetate and MM-419447 were similar.

Conclusions: For the 100 mg/kg/day male that was found dead, the cause of death was not determined from necropsy or microscopic examination, although focal necrosis (minimal) in liver was observed. This death should be considered as possibly drug related, given that it occurred at the highest dose. Target organs of toxicity were not identified in animals sacrificed at study termination. The NOAEL (no observed adverse effect level) in males is considered to be 50 mg/kg/day, based on the single death at 100 mg/kg/day. The NOAEL in females was

100 mg/kg/day.

A 13-Week Toxicity Study of MD-1100 Acetate Administered by Oral (Nasogastric) Intubation to Cynomolgus Monkeys, with a 2-Week Recovery Period

Key Study Findings: a NOAEL could not be determined due to deficiencies in the study methods, although the drug was tolerated at all doses; kidney was a target organ of toxicity at
50 mg/kg/day; substantial reduction in weight of testes occurred at all doses without corresponding gross or microscopic lesions

Study # MNP-103-006 (ZKS00006)

Amendment # 044, Vol. 3, Pg. 1

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: April 10, 2006 (report dated November 8, 2006)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000501; 93.1% peptide, 96.13% peptide purity

METHODS: Cynomolgus monkeys (males age 2.7-3.5 years, 1.9-3.5 kg; females age 2.8-5.1 years, 1.8-3.5 kg) were treated orally with 0 (vehicle), 5, 10, or 50 mg/kg/day MD-1100 acetate for 91 days (4 monkeys/sex/group in the main study). These animals were sacrificed at the end of treatment (day 93). The recovery groups were treated with 0 or 50 mg/kg/day for the same duration, and were sacrificed after a 14-day recovery period (3 monkeys/sex/group). The test and control articles were given by nasogastric intubation using a dose volume of 3 ml/kg. The drug was administered as a suspension in 0.5% methylcellulose. Drug suspensions were prepared with adjustment for the peptide content (93.1%). Dose selection was based on results of the 2-week oral toxicity study in Cynomolgus monkeys, in which MD-1100 acetate was well tolerated at all dose levels (0.5, 2.5, and 5 mg/kg/day).

Observations and Times:

Mortality: twice daily

Clinical Signs: twice daily

Bodyweight: weekly starting on day -1

Food Consumption: daily

Ophthalmoscopy: prior to study initiation and on week 13
ECG: prior to study initiation, and on week 13 (time of measurement relative to dosing was not stated)

Hematology: blood samples were collected prior to study initiation, week 4, week 13, and at the end of the recovery phase (week 15)

Clinical Chemistry: blood samples were collected prior to study initiation, week 4, week 13, and at the end of the recovery phase (week 15)

Urinalysis: urine samples were collected prior to study initiation, week 4, week 13, and at the end of the recovery phase (week 15)

Gross Pathology: at sacrifice

Organ Weights: adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid/parathyroids; paired organs were weighed together

Histopathology: The following organs/tissues were examined in the control and high-dose groups, except where stated otherwise: adrenals, aorta, bone (femoral head, sternum with marrow, 7th rib), brain, cecum, cervix, colon, duodenum, epididymides, esophagus, eyes, gallbladder, gross lesions (all groups), heart, ileum, jejunum, kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), optic nerves, ovaries, pancreas, Peyer's patch, pituitary, prostate, rectum, salivary glands (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin/mammary gland, spinal cord (thoracic), spleen, stomach, testes, thymus, thyroid/parathyroids, tongue, trachea, urinary bladder, uterus, vagina.

Adequate Battery: yes (x) no ( )
Peer Review: yes ( ) no (x)

Toxicokinetics: Blood was collected from each monkey prior to dosing and at 0.5, 1, 2, 4, 8, and 24 hr post-dose on day 1 and weeks 4 and 13. The method used for measurement of plasma concentrations of MD-1100 acetate and its active metabolite, MM-419447, was not stated. The lower limit of quantification was 3 ng/ml for both MD-1100 acetate and MM-419447.

RESULTS:

Mortality: One male in the 10 mg/kg/day group was sacrificed in moribund condition on day 32. Multiple abnormalities and lesions were observed in the gross and microscopic

Reference ID: 3114166

examination. The observed changes included emaciation, dehydration, red watery contents in cecum and colon, vacuolization and necrosis of renal proximal tubules, atrophy and vacuolization in gastric epithelium, mucosal degeneration in lower GI tract (cecum-rectum), desquamation of esophageal epithelium with colonization by bacteria and fungi, lymphoid depletion in germinal centers of lymph nodes and spleen, severe diffuse atrophy in thymic cortex, and sternal bone marrow hypoplasia. The lesions in lymphoid tissues and bone marrow along with the bacterial/fungal infection of the esophagus were suggestive of a substantial immunologic compromise. The authors considered the death as unlikely related to the test article, since none of the observed lesions occurred in the 50 mg/kg/day group, or in the other 10 mg/kg/day animals.

Clinical Signs: The incidence of watery feces was increased in a dose-dependent manner at 5, 10, and 50 mg/kg/day. This event was considered as a pharmacological effect rather than an adverse effect. Watery feces did not occur during the recovery phase. The 10 mg/kg/day male that was sacrificed in moribund condition exhibited hunched posture (days 29-32), reduced food consumption, and weight loss.

Bodyweight: Weight gain in the 50 mg/kg/day females was reduced by 39% at the end of the main study. The mean weight (± S.D.) of control males and females was 2.64 ± 0.50 kg and 2.43 ± 0.60 kg, respectively, on day 1, and 3.06 ± 0.65 kg and 2.74 ± 0.70 kg, respectively, on week 14. Weight gain was unaffected during the recovery period.

Food Consumption: Although food consumption was measured daily, the study report contains no data. The authors indicated that food intake was not affected, except in the 10 mg/kg/day male that was sacrificed in moribund condition.

Ophthalmoscopy: No drug-related lesions were observed.

ECG: The authors stated that ECGs were qualitatively within normal limits, and that no arrhythmias were found. However, no data was provided.

Hematology: Group mean values were calculated using combined data from males and females. Mean values for either gender were not provided. Unstained cell count was increased by 23% in the 50 mg/kg/day group on week 13 (not significant). RBC count, hemoglobin, and hematocrit were reduced by 9% in the 50 mg/kg/day recovery group. PT and APTT were unaffected.

Clinical Chemistry: Group mean values were calculated using combined data from males and females. Mean values for either gender were not provided. CO_2 concentration in the
50 mg/kg/day group was increased by 10% on week 4.

Urinalysis: A strong positive result in the occult blood test occurred in the 10 mg/kg/day male that was sacrificed in moribund condition (week 4). Since no erythrocytes were observed in microscopic examination of urine, the positive occult blood test result is considered as indicative of hemoglobinuria. One female (#3503) in the 10 mg/kg/day

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group and one male (#4001) in the 50 mg/kg/day group exhibited hematuria (i.e. strong positive result in the occult blood test along with presence of erythrocytes) on week 13. Hemoglobinuria occurred in two females (#4501 and 4502) in the 50 mg/kg/day group on week 4.

Organ Weights: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reported.

Main Study

Adrenals: Absolute weight and organ/brain weight ratio in the 50 mg/kg/day males was reduced by 14% and 15%, respectively. Organ/brain weight ratio was decreased by 14% in the 50 mg/kg/day females.

Brain: Organ/bodyweight ratio in the 5, 10, and 50 mg/kg/day males was increased by 15%, 14%, and 20%, respectively.

Epididymides: Absolute weight and organ/brain weight ratio in the 50 mg/kg/day males was reduced by 25% and 23%, respectively.

Heart: Absolute weight in the 5, 10, and 50 mg/kg/day males was reduced by 20%, 14%, and 21%, respectively. Organ/brain weight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 16%, 16%, and 18%, respectively. Organ/brain weight ratio in the 5 and 50 mg/kg/day females was decreased by 12% and 14%, respectively.

Kidneys: Absolute weight in the 5, 10, and 50 mg/kg/day males was reduced by 20%, 13%, and 25%, respectively. Organ/brain weight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 16%, 13%, and 21%, respectively.

Liver: Absolute weight in the 5, 10, and 50 mg/kg/day males was reduced by 19%, 15%, and 18%, respectively. Organ/brain weight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 16%, 18%, and 16%, respectively.

Lungs: Absolute weight in the 5, 10, and 50 mg/kg/day males was reduced by 22%, 12%, and 27%, respectively. Organ/bodyweight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 20%, 15%, and 24%, respectively. Organ/brain weight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 20%, 15%, and 24%, respectively.

Spleen: Absolute weight in the 50 mg/kg/day males was reduced by 25%. Absolute weight in the 5, 10, and 50 mg/kg/day females was decreased by 43%, 20%, and 15%, respectively. Organ/bodyweight ratio in the 5, 10, and 50 mg/kg/day females was reduced by 28%, 17%, and 17%, respectively. Organ/brain weight ratio in the 5, 10, and 50 mg/kg/day females was reduced by 38%, 17%, and 23%, respectively.

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Testes: Absolute weight in the 5, 10, and 50 mg/kg/day males was reduced by 45%, 39%, and 51%, respectively. Organ/bodyweight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 29%, 31%, and 35%, respectively. Organ/brain weight ratio in the 5, 10, and 50 mg/kg/day males was decreased by 41%, 40%, and 49%, respectively.

Thymus: Organ/bodyweight ratio in the 10 and 50 mg/kg/day males was increased by 45% and 38%, respectively. Organ/brain weight ratio in the 10 and 50 mg/kg/day males was increased by 29% and 20%, respectively.

Thyroid: Organ/brain weight ratio in the 50 mg/kg/day males was decreased by 24%.

Recovery Phase

Adrenals: Organ/bodyweight ratio was increased by 22% in the 50 mg/kg/day females.

Brain: Organ/bodyweight ratio was increased by 22% in the 50 mg/kg/day females.

Heart: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were reduced by 12%, 14%, and 12%, respectively, in the 50 mg/kg/day males.

Kidneys: Absolute weight and organ/brain weight ratio were decreased by 21% and 26%, respectively, in the 50 mg/kg/day females.

Liver: Absolute weight and organ/brain weight ratio were decreased by 17% and 22%, respectively, in the 50 mg/kg/day females.

Lungs: Absolute weight and organ/brain weight ratio were decreased by 19% and 25%, respectively, in the 50 mg/kg/day females.

Ovaries: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were increased by 40%, 66%, and 31%, respectively, in the 50 mg/kg/day females.

Spleen: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were decreased by 24%, 29%, and 25%, respectively, in the 50 mg/kg/day males. Organ/brain weight ratio was reduced by 19% in the 50 mg/kg/day females.

Testes: Absolute weight, organ/bodyweight ratio, organ/brain weigh ratio were increased by 28%, 24%, and 25%, respectively, in the 50 mg/kg/day males.

Thymus: Absolute weight, organ/bodyweight ratio, and organ/brain weight ratio were decreased by 38%, 28%, and 42%, respectively, in the 50 mg/kg/day females.

Thyroid: Absolute weight and organ/brain weight ratio were decreased by 14% in the 50 mg/kg/day females.

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Gross Pathology:

Premature Death (10 mg/kg/day male)

Cecum + Colon: Red watery contents were present.

Esophagus: Raised tan coalescing plaques in mucosa of distal segment and red discoloration of mucosa were observed.

Thymus: Size reduction was observed.

Main Study

No drug-related changes were observed.

Recovery Phase

No drug-related changes were observed.

Histopathology:

Premature Death (10 mg/kg/day male)

Bone Marrow (sternum): Mild diffuse hypoplasia was observed.

Cecum, Colon, Rectum: Altered epithelium was observed in all segments of the lower GI tract. This effect was characterized by decreased numbers of mucous cells and in some areas, flattening of epithelium.

Duodenum: Diffuse degeneration (moderate) occurred. This effect was characterized by shortened and fused villi.

Esophagus: Desquamation with plaques containing numerous bacterial colonies and fungi was observed.

Eyes: Cataract was observed in one eye.

Heart: Focal inflammation with lymphohistiocytic infiltration was observed.

Ileum: Diffuse degeneration (marked) was observed. This effect was characterized by shortened and fused villi.

Jejunum: Diffuse degeneration (mild) was observed. This effect was characterized by shortened and fused villi. Dilatation of lacteals also occurred.

Kidneys: Necrosis and marked vacuolization of proximal tubules were observed.

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Liver: Lymphohistiocytic inflammation was observed.

Lungs: Lymphohistiocytic inflammation was observed.

Lymph Nodes (mandibular, mesenteric): Marked lymphoid depletion in germinal centers occurred. The germinal centers were populated by cells that appeared to be histiocytes and by an amorphous substance similar to amyloid.

Pancreas: Diffuse zymogen depletion was observed. The authors proposed that this effect was secondary to reduced food intake.

Spleen: Lymphoid depletion in germinal centers occurred. The germinal centers were populated by cells that appeared to be histiocytes and by an amorphous substance similar to amyloid.

Stomach: Vacuolization of epithelium and atrophy were observed. Atrophy was more severe in the cardiac and pyloric regions.

Thymus: Vacuolization of epithelium and severe atrophy of cortex were observed.

Main Study (control and 50 mg/kg/day groups)

Cecum: Submucosal inflammation occurred in the 50 mg/kg/day group (1/8 monkeys).

Duodenum: Focal mucosal hemorrhage was observed in the 50 mg/kg/day group (1/8 monkeys).

Epididymides: Lymphohistiocytic inflammation occurred in the 50 mg/kg/day males (1/4 monkeys). Hemorrhage was observed in the 50 mg/kg/day males (1/4 monkeys).

Eyes: Cataracts were observed in the 10 and 50 mg/kg/day groups (1/8 monkeys in each group).

Ileum: Hemorrhage at the ileo-cecal junction occurred in the 50 mg/kg/day group (1/8 monkeys).

Kidneys: Parenchymal hypoplasia occurred in the same 50 mg/kg/day male (#4001) that exhibited hematuria on week 13. This lesion was characterized by sclerotic glomeruli with fibrosis of surrounding tissue, and small areas of tubular of hypoplasia. Fibrosis was observed in the same 50 mg/kg/day female (#4501) that exhibited hemoglobinuria on week 4.

Lungs: A cyst was observed in the 50 mg/kg/day group (1/8 monkeys).

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Lymph Nodes: Edema in mesenteric lymph nodes occurred in the 50 mg/kg/day group (1/8 monkeys).

Skeletal Muscle: Lymphohistiocytic inflammation was observed in the 50 mg/kg/day group (1/8 monkeys).

Spleen: Hyperplasia of follicle was observed in the 50 mg/kg/day group (1/8 monkeys).

Thyroid: Mineralization occurred in the 50 mg/kg/day group (1/8 monkeys).

Recovery Phase

No observations were reported.

Toxicokinetics: The results from measurement of MD-1100 acetate and its active metabolite MM-419447 are shown in the tables below (taken from the study report).

Summary of Toxicokinetic Parameters for MD-1100 Acetate in Male and Female Cynomolgus Monkey Plasma: Day 1

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-4} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)
2	5	M	Mean	8.78	1.00	18.4	24.9	24.9	NA	NA
			SD	6.47	0.71	NA	NA	NA	NA	NA
			N	4	4	2	2	2	0	0
		F	Mean	12.8	0.500	19.2	25.1	25.1	NA	NA
			SD	7.8	0	NA	NA	NA	NA	NA
			N	3	3	2	2	2	0	0
3	10	M	Mean	11.7	1.00	25.6	27.6	34.9	52.6	1.57
			SD	7.8	0.71	12.0	10.4	12.2	NA	NA
			N	4	4	4	4	4	1	1
		F	Mean	9.81	0.875	14.6	18.6	18.6	NA	NA
			SD	3.88	0.750	2.6	4.1	4.1	NA	NA
			N	4	4	3	3	3	0	0
4	50	M	Mean	41.9	1.57	132	110	168	158	1.82
			SD	18.0	1.24	48	27	60	NA	NA
			N	7	7	7	7	7	1	2
		F	Mean	88.5	0.786	250	207	284	265	1.51
			SD	37.5	0.267	82	64	90	79	0.23
			N	7	7	7	7	7	7	7

Summary of Toxicokinetic Parameters for MD-1100 Acetate in Male and Female Cynomolgus Monkey Plasma: Week 4

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-4} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)
2	5	M	Mean	10.5	0.500	19.3	22.6	25.7	NA	NA
			SD	7.3	0	NA	NA	NA	NA	NA
			N	4	4	2	2	2	0	0
		F	Mean	5.23	0.625	9.66	13.5	13.5	NA	NA
			SD	2.26	0.250	NA	NA	NA	NA	NA
			N	4	4	2	2	2	0	0
3	10	M	Mean	17.8	0.625	47.2	41.8	62.0	NA	2.65
			SD	13.0	0.250	42.3	26.8	54.7	NA	0.39
			N	4	4	4	4	4	0	3
		F	Mean	8.56	0.625	18.5	21.4	24.1	NA	NA
			SD	2.23	0.250	10.1	7.6	12.2	NA	NA
			N	4	4	3	3	3	0	0
4	50	M	Mean	56.6	1.36	174	137	214	NA	1.49
			SD	26.8	1.28	86	61	117	NA	0.13
			N	7	7	7	7	7	0	4
		F	Mean	107	0.643	228	200	260	NA	1.53
			SD	67	0.244	118	88	130	NA	0.38
			N	7	7	7	7	7	0	6

Summary of Toxicokinetic Parameters for MD-1100 Acetate in Male and Female Cynomolgus Monkey Plasma: Week 13

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-t} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)
2	5	M	Mean	7.14	0.625	12.2	16.1	16.1	NA	NA
			SD	3.44	0.250	NA	NA	NA	NA	NA
			N	4	4	2	2	2	0	0
		F	Mean	12.5	0.500	19.3	26.1	26.1	NA	NA
			SD	5.9	0	6.4	8.0	8.0	NA	NA
			N	4	4	3	3	3	0	0
3	10	M	Mean	22.2	0.500	38.6	41.8	48.1	NA	1.72
			SD	17.6	0	40.2	37.5	48.5	NA	NA
			N	3	3	3	3	3	0	1
		F	Mean	26.9	0.642	64.4	54.4	89.7	NA	3.37
			SD	10.5	0.283	75.2	40.5	110.7	NA	NA
			N	4	4	4	4	4	0	1
4	50	M	Mean	54.1	0.714	148	131	173	NA	1.60
			SD	16.7	0.267	84	57	96	NA	0.26
			N	7	7	7	7	7	0	6
		F	Mean	71.1	0.655	168	148	191	NA	1.47
			SD	30.5	0.265	83	60	90	NA	0.17
			N	7	7	7	7	7	0	6

Summary of Toxicokinetic Parameters for MM-419447 in Male and Female Cynomolgus Monkey Plasma: Day 1

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-4} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AUC_{0-4} Metabolite to Parent Ratio
2	5	M	Mean	7.15	2.25	23.0	23.0	33.5	NA	NA	1.03
			SD	3.11	1.26	6.5	6.5	8.7	NA	NA	NA
			N	4	4	3	3	3	0	0	2
		F	Mean	25.9	1.25	78.4	78.4	95.3	164	1.68	3.28
			SD	38.3	0.87	NA	NA	NA	NA	NA	NA
			N	4	4	2	2	2	1	1	2
3	10	M	Mean	17.5	2.75	62.0	47.1	99.3	NA	NA	1.71
			SD	7.5	1.50	41.4	20.2	68.8	NA	NA	0.53
			N	4	4	4	4	4	0	0	4
		F	Mean	17.1	2.00	45.0	45.0	60.2	NA	NA	2.88
			SD	10.3	1.41	28.2	28.2	32.1	NA	NA	2.00
			N	4	4	4	4	4	0	0	3
4	50	M	Mean	111	3.14	378	249	464	NA	NA	2.09
			SD	127	1.07	301	240	323	NA	NA	1.65
			N	7	7	7	7	7	0	0	7
		F	Mean	81.6	1.71	286	204	357	126	2.02	1.14
			SD	33.8	0.49	125	69	149	NA	NA	0.68
			N	7	7	7	7	7	1	1	7

Summary of Toxicokinetic Parameters for MM-419447 in Male and Female Cynomolgus Monkey Plasma: Week 4

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-4} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AUC_{0-4} Metabolite to Parent Ratio
2	5	M	Mean	15.6	2.25	46.8	46.8	67.1	NA	NA	1.74
			SD	4.6	1.26	15.0	15.0	18.7	NA	NA	NA
			N	4	4	4	4	4	0	0	2
		F	Mean	22.5	2.00	55.0	55.0	76.5	NA	NA	5.05
			SD	13.7	1.41	25.7	25.7	31.1	NA	NA	NA
			N	4	4	4	4	4	0	0	2
3	10	M	Mean	51.7	3.00	531	160	595	NA	9.51	3.36
			SD	47.1	1.15	766	173	724	NA	NA	1.57
			N	4	4	4	4	4	0	1	4
		F	Mean	23.2	2.00	109	65.1	133	NA	2.84	3.01
			SD	5.1	1.41	54	21.8	64	NA	NA	0.18
			N	4	4	4	4	4	0	1	3
4	50	M	Mean	115	2.14	463	286	513	NA	1.76	2.26
			SD	59	0.90	255	152	263	NA	NA	1.08
			N	7	7	7	7	7	0	1	7
		F	Mean	112	1.36	370	277	433	NA	1.85	1.66
			SD	59	0.63	231	149	255	NA	0.29	1.08
			N	7	7	7	7	7	0	3	7

Summary of Toxicokinetic Parameters for MM-419447 in Male and Female Cynomolgus Monkey Plasma: Week 13

Dose Group	Dose Level (mg/kg)	Sex		C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	AUC_{0-4} (ng•hr/mL)	AUC_{0-24} (ng•hr/mL)	$AUC_{0-\infty}$ (ng•hr/mL)	$t_{1/2}$ (hr)	AUC_{0-4} Metabolite to Parent Ratio
2	5	M	Mean	16.2	2.30	60.8	41.5	87.7	NA	NA	2.06
			SD	3.7	1.25	25.9	11.2	36.5	NA	NA	NA
			N	4	4	4	4	4	0	0	2
		F	Mean	32.8	1.53	115	99.9	138	NA	NA	5.56
			SD	27.5	0.61	82	57.0	84	NA	NA	NA
			N	4	4	3	3	3	0	0	2
3	10	M	Mean	52.3	1.75	181	121	227	NA	1.98	5.23
			SD	28.5	0.65	84	58	89	NA	NA	4.29
			N	3	3	3	3	3	0	1	3
		F	Mean	57.1	2.03	216	146	306	NA	1.54	4.16
			SD	25.5	1.39	103	57	195	NA	NA	4.02
			N	4	4	4	4	4	0	1	4
4	50	M	Mean	146	1.71	510	354	677	NA	NA	2.80
			SD	72	0.49	304	172	342	NA	NA	0.99
			N	7	7	7	7	7	0	0	7
		F	Mean	126	1.71	477	322	601	NA	1.78	2.32
			SD	76	1.11	414	216	583	NA	NA	1.28
			N	7	7	7	7	7	0	2	7

The data indicates that both the unchanged drug and its active metabolite were present in plasma on each day of measurement. The AUC and C_{max} values for MD-1100 acetate were increased with dose level, although the increases were variable with respect to the proportional dose increment (i.e. lower, equal to, and greater than the dose increment). The AUC and C_{max} values for the active metabolite (MM-419447) exceeded those of MD-1100 acetate. In addition, a progressive increase in AUC values for MM-419447 was observed on weeks 4 and 13, relative to the day 1 values. MD-1100 acetate was not detected at time-points after 8 hr. MM-419447 was occasionally detected as late as 24 hr post-dose. No consistent sex-related differences in toxicokinetic parameters for the unchanged drug or active metabolite were observed.

Conclusions: The single death that occurred in the 10 mg/kg/day group was probably unrelated to drug treatment. The absence of histopathologic examination of the low- and intermediate-dose groups was a deficiency in this study. All tissues from these groups should be examined microscopically, and the results should be submitted. The increased incidence of watery feces at 5, 10, and 50 mg/kg/day was considered as a pharmacological effect rather than an adverse effect. Weight gain in the 50 mg/kg/day females was reduced by 39%. Hematuria and hemoglobinuria was observed at 10 and 50 mg/kg/day. These urine abnormalities were associated with kidney lesions in the 50 mg/kg/day group (10 mg/kg/day group was not examined). Therefore, kidney is considered to be a target organ of toxicity in the 50 mg/kg/day group. Absolute and

relative weight of testes was substantially decreased in all treatment groups. However, no abnormalities in testes were reported in gross or microscopic examination. In the 50 mg/kg/day group, lesions occurred at low incidence (1/8 monkeys) in multiple organs. The relationship of these lesions to drug treatment is uncertain, particularly in the absence of information from the 5 and 10 mg/kg/day groups. No limiting toxicity was apparent at any dose level. A NOAEL cannot be determined due to deficiencies in the study methods, although the drug was tolerated at all doses. Group mean values for the hematology and clinical chemistry data were calculated using combined data from males and females. The Sponsor should be advised that mean values should be reported separately for males and females in future toxicology studies.

Study title: 26-Week Oral Toxicity Study of MD-1100 Acetate in Mice Followed by a 4-Week Recovery Period	
Study no.:	MNP-103-027
Study report location:	(Original amendment) 8/9/11: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 8, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MD1100 Acetate, PPL-MD11000601D, 90.1% relative to total peptide content

Key Study Findings

Mice were treated orally with 5, 20, or 100/80 mg/kg/day linaclotide (MD-1100 acetate) for 26 weeks. The 100 mg/kg/day dose was reduced to 80 mg/kg/day due to the mortality observed in both sexes during the first week of dosing. A total of 2, 1, 3, and 8 animals in the 0, 5, 20 and 100/80 mg/kg/day main study groups, respectively, were found dead or euthanized after week 2 of dosing. There were no significant test article-related effects observed in clinical observations, body weight, food consumption, ophthalmoscopic evaluation, clinical pathology parameters, or macroscopic or microscopic evaluations. There were significant dose-dependent decreases in absolute and relative thyroid/parathyroid gland weights, however there were no correlated changes noted in the microscopic findings. No changes in organ weights were found in the 100/80 mg/kg/day group after the 4-week recovery period. Systemic exposure to linaclotide was highly variable with a high amount of intragroup variability, but in general linaclotide exposure increased with dose, and females had higher exposure levels than did the males. Exposure to the metabolite MM-419447 was below the limit of quantitation for almost all the mice in the 5 and 20 mg/kg/day groups, and the $AUC_{0\text{-}24\ hr}$ ratio between MM-419447 and linaclotide was small at the 100/80 mg/kg/day dose. Based on mortality, the no-observed-adverse-effect-level (NOAEL) was 20 mg/kg/day.

Reference ID: 3114166

Methods	
Doses:	5, 20, and 100/80 mg/kg/day (100 mg/kg/day reduced to 80 mg/kg/day on day 6)
Frequency of dosing:	Daily
Route of administration:	Oral
Dose volume:	10 mL/kg
Formulation/Vehicle:	0.5% methylcellulose in deionized water
Species/Strain:	Crl:CD1®(ICR)
Number/Sex/Group:	16 for main study, 10 for recovery (control and high dose)
Age:	~6 weeks
Weight:	Males (20 to 35 g) and females (18 to 25 g)
Satellite groups:	Yes (see below)
Unique study design:	None
Deviation from study protocol:	Minor deviations that did not affect the quality or integrity of the study.

Table 1: Study Design (26-week Oral Toxicity Study in Mice)

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
Main Study			
1	0 (Control)	26[a]	26[a]
2	5	16	16
3	20	16	16
4	100/80[b]	26[a]	26[a]
Toxicokinetic			
5	0 (Control)	11	11
6	5	56	56
7	20	56	56
8	100/80[b]	56	56

[a]The last 10 animals/sex were designated as recovery animals.
[b]The animals in these groups received the test article at a dose level of 100 for the first 5 days. Based on mortality, the dose was reduced to 80 mg/kg/day beginning on Day 6.

Observations and Results

Mortality

Animals were observed twice daily. During the first seven days of dosing, many animals, especially in the 20 and 100 mg/kg/day group, were found dead in the main study and toxicokinetic (TK) groups (see table below). The cause of death in most animals was not investigated; however one 20 mg/kg/day female had macroscopic evidence of gavage injury. Based on the deaths noted in the 100 mg/kg/day group, the high dose was reduced to 80 mg/kg/day on day 6. All the animals found dead through day 7 were replaced and the tissues were retained for possible future examination.

Table 2: Animals Found Dead (Days 1-7) in a 26-week Oral Toxicity Study in Mice

	Main Study Group Animals Found Dead (M/F)	Toxicokinetic Group Animals Found Dead (M/F)
0 mg/kg/day	0/0	0/0
5 mg/kg/day	0/1	0/5
20 mg/kg/day	1/2	2/5
100/80 mg/kg/day	2/9	11/10

A total of 2, 1, 3, and 8 animals in the 0, 5, 20 and 100/80 mg/kg/day main study groups, respectively, were found dead or euthanized after week 2 of dosing (see table below). Decreased activity, hunched posture, cold skin and/or difficulty in breathing were noted in all animals that died during the study period. After microscopic examination, the cause of death of 3 females in the 20 mg/kg/day group, and 3 males and 1 female in the 100/80 mg/kg/day group appeared to be gavage-related. The cause of death of one 100/80 mg/kg/day female, which died during the recovery period, was severe chronic glomerular and tubular disease (glomerulonephrosis). However, a cause of death was not determined for the other mortalities. Microscopic examination of the other animals that were found dead or euthanized showed the following: moderate diffuse autolysis in the urinary bladder of 2/3 and 4/4[4] females in the 20 and 100 mg/kg/day groups, respectively; mild/moderate autolysis in the spleen in 3/3 and 3/4 females in the 20 and 100/80 mg/kg/day groups, respectively; mild/moderate diffuse lymphoid atrophy in 3/3 males in the 100/80 mg/kg/day group.

Table 3: Mortality in the 26-week Oral Toxicity Study in Mice

Mortality-Beginning Week 2 (Animals not replaced)			
0 mg/kg/day	5 mg/kg/day	20 mg/kg/day	100/80 mg/kg/day
Number of Animals (M/F)	Number of Animals (M/F)	Number of Animals (M/F)	Number of Animals (M/F)
1/1-MS, 0/0-TK	1/0-MS, 0/0-TK	0/3-MS, 1/1-TK	3/5-MS, 5/10-TK
MS – Main Study; TK - Toxicokinetics			

Clinical Signs

[4] Although 5 females in the 100/80 mg/kg/day group were found dead or euthanized, only 4 were microscopically examined because one female died during the 4-week recovery period.

A detailed clinical examination of each main study animal was performed weekly. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, bizarre behavior, and palpation of tissue masses.

On weeks 25-26, a scabbed area on the skin was observed in 4-5/23 males in the 100/80 mg/kg/day group compared to 0-1/26 control males. During weeks 27 and 28 of the recovery phase, missing portion of the tail was noted in 2 females in the 100/80 mg/kg/day group compared to 0 control females.

Body Weights

Body weights for all main study animals were measured and recorded prior to randomization and weekly during the study. Body weight changes are summarized in the table below.

Table 4: Body Weight Changes in the 26-week Oral Toxicity Study in Mice

Dose (mg/kg)	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)	% Control Group Weight Gain
	Males			
0	30.2 ± 1.29	41.9 ± 2.42	11.7	100%
5	30.4 ± 1.24	42.2 ± 3.14	11.8	101%
20	30.3 ± 1.90	43.0 ± 2.97	12.7	109%
100/80	30.2 ± 1.40	40.8 ± 2.43	10.6	91%
	Females			
0	24.9 ± 1.09	33.0 ± 2.42	8.1	100%
5	23.8 ± 1.20	33.8 ± 3.03	10.0	123%
20	25.4 ± 1.52	34.6 ± 1.95	9.2	114%
100/80	24.5 ± 1.84	34.3 ± 2.90	9.8	121%

Body weight values are the mean ± S.D.

No test article-related effects were noted on body weight or body weight gain.

Feed Consumption

Food consumption was measured and recorded for main study animals weekly during the study. In general, there were no major test article-related changes in food consumption. During week 11, males in the 20 mg/kg/day group had a significant ($p<0.05$) increase (17%) in food consumption compared to control males, however the increase was due to a high amount of intergroup variability in the 20 mg/kg/day males. During week 14, there was a significant ($p<0.01$) decrease in food consumption (17%)

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in the 20 mg/kg/day males and females, and a decrease of 15% in the 100/80 mg/kg/day males, compared to the control values. During week 14, there was a 10% decrease in food consumption in the 100/80 mg/kg/day females compared to control females.

Ophthalmoscopy

No test article-related changes in ophthalmic findings were observed.

ECG

Not recorded.

Hematology

Blood samples (from vena cava) were collected at the terminal necropsy from 6 to 8 animals/sex/group and at recovery necropsy from 4 animals/sex/group. The following tables list the parameters that were measured and a summary of the data.

Table 5: Hematology Parameters Measured in the 26-week Oral Toxicity Study in Mice

Leukocytes	Erythrocytes	Hemoglobin
Hematocrit	Mean corpuscular hemoglobin	Mean corpuscular volume
Mean corpuscular hemoglobin concentration	Absolute reticulocytes	Platelet count
Blood cell morphology	Neutrophils	Eosinophils
Lymphocytes	Monocytes	Basophils
Large unstained cells	Prothrombin time (PT)	Activated partial thromboplastin time (APTT)

Table 6: Percent Change in Hematology Parameters in the 26-week Oral Toxicity Study in Mice

Parameter	Dose (mg/kg/day)	Change at termination compared to control
Leukocytes	5 (males) 100/80 (females)	↓ 29% ↑ 36%
Platelets	5 (male) 5, 20, 100/80 (female)	↓ 12% ↓ 29%, ↓ 24%, ↓ 31%
Absolute reticulocytes	20 (female)	↑ 19%
Neutrophils	5, 20, 100/80 (male) 5, 100/80 (female)	↓ 20%, ↑ 18%, ↑ 13% ↑ 23%, ↑ 62%
Lymphocytes	5, 100/80 (male) 100/80 (female)	↓ 31%, ↓ 14% ↑ 29%
Monocytes	5, 100/80 (male)	↓ 56%, ↓ 19%
Eosinophils	5, 100/80 (male)	↓ 34%, ↓16%

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	5, 100/80 (female)	↑ 111%, ↑ 36%
Basophils	5, 100/80 (male) 5, 20, 100/80 (female)	↓47%, ↓ 26% ↑ 157%, ↑ 143%, ↑ 86%
Large unstained cells	5 (male) 5, 20, 100/80 (female)	↓ 54% ↓ 17%, ↑ 77%, ↑ 20%

The effects noted in the table above occurred mostly in the 5 mg/kg/day group, and were not dose-dependent or statistically significant. Thus the hematology changes are not considered to be test article-related. Some of the changes noted above were also observed after the recovery period. Specifically, neutrophils were significantly ($p<0.05$) increased by 197% in the 100/80 mg/kg/day recovery males compared to the control males. The high-dose recovery males also showed increases in lymphocytes (57%), large unstained cells (92%), eosinophils (213%), monocytes (237%), and basophils (400%). The 100/80 mg/kg/day recovery females showed increases in leukocytes (16%), lymphocytes (24%), basophils (42%), and large unstained cells (54%). However, the changes observed after the 4-week recovery period were mostly the result of a small sample size (n=4/sex) and a high amount of intergroup variability. There were no test-article related changes to APTT and PT.

Clinical Chemistry

Blood samples (from vena cava) were collected at the terminal necropsy from 7 to 8 animals/sex/group and at recovery necropsy from 4 to 5 animals/sex/group. The following tables list the parameters that were measured and a summary of the data.

Table 7: Clinical Chemistry Parameters Measured in the 26-week Oral Toxicity Study in Mice

Alkaline phosphatase	Total bilirubin	Total cholesterol
Aspartate aminotransferase	Creatinine	Calcium
Alanine aminotransferase	Total protein	Phosphorus
Gamma glutamyl transferase	Albumin	Globulin
Albumin/globulin ratio	Glucose	Electrolytes (sodium, potassium, chloride)
Sorbitol dehydrongenase	Urea nitrogen	

Table 8: Percent Change in Clinical Chemistry Parameters in the 26-week Oral Toxicity Study in Mice

Parameter	**Dose (mg/kg/day)**	**Change at termination compared to control**
Aspartate aminotransferase	20, 100/80 (male) 5, 20, 100/80 (female)	↓ 23%, ↑ 59% ↓ 15%, ↓ 26%, ↓ 58%
Alkaline phosphatase	20, 100/80 (male) 20, 100/80 (female)	↓ 11%, ↓ 12% ↑ 18%, ↑ 26%
Alanine aminotransferase	5, 20, 100/80 (male) 20, 100/80 (female)	↓ 15%, ↓ 26%, ↑ 156% ↑ 40%, ↓ 58%
Total cholesterol	5, 20 (male)	↑ 23%, ↑ 16%

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	20 (female)	↑ 18%
Total bilirubin	20 (female)	↑ 15%
Urea nitrogen	20, 100/80 (female)	↓ 37%, ↓ 23%

The effects noted in the table above are not considered to be test article-related, given the high amount of intergroup variability and absence of dose-dependency or statistical significance. Some of the changes were still observed after the recovery period. Specifically, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels increased by 53% and 183%, respectively, in the 100/80 mg/kg/day males. However, the changes observed after the 4-week recovery period were mostly the result of a small sample size (n=5/sex) and a high amount of intergroup variability. In females, AST, ALT, and urea nitrogen remained decreased by 27%, 21%, and 24%, respectively, after the 4-week recovery period.

Urinalysis

Urine was collected before terminal necropsy. The table below lists the parameters that were measured.

Table 9: Urinalysis Parameters Measured in the 26-week Oral Toxicity Study in Mice

Volume	Specific gravity	pH
Color and appearance	Protein	Glucose
Bilirubin	Ketones	Occult blood
Urobilinogen	Microscopy of spun deposit	

There was a dose-dependent increase in urine volume of 31%, 59%, and 96% in the 5, 20, and 100/80 mg/kg/day males respectively, compared to control males. In females, the urine volume increased by 36%, 13%, and 51% in the 5, 20, and 100/80 mg/kg/day groups, respectively, compared to controls. In contrast, after the 4-week recovery period, urine volume was decreased by 45% and 37% in the 100/80 mg/kg/day males and females, respectively, compared to control animals.

Gross Pathology

The animals were examined carefully for external abnormalities including palpable masses. The abdominal, thoracic, and cranial cavities were examined for abnormalities. The Sponsor's table below lists the tissues preserved for macroscopic examination.

Table 10: Organs Weighed and Collected at Necropsy for Macroscopic and Microscopic Examination in the 26-week Oral Toxicity Study in Mice

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Term[a]	Rec[b]
Adrenal gland	X	X	X	
Aorta		X	X	
Bone with bone marrow, femur		X	X	
Bone with bone marrow, sternum		X	X	
Bone marrow smear[c]		X		
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	X	
Epididymis	X	X	X	
Esophagus		X	X	
Eye (with optic nerve)		X	X	
Gallbladder		X	X	
Heart	X	X	X	
Joint, tibiofemoral		X	X	
Kidney	X	X	X	
Lacrimal gland, exorbital		X	X	
Large intestine, cecum		X	X	
Large intestine, colon		X	X	
Large intestine, rectum		X	X	
Larynx		X	X	
Liver	X	X	X	
Lung with bronchi	X	X	X	

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination	
			Term[a]	Rec[b]
Lymph node, mandibular		X	X	
Lymph node, mesenteric		X	X	
Mammary gland (process females only)		X	X	
Nerve, sciatic		X	X	
Ovary	X	X	X	
Oviducts		X	X	
Pancreas		X	X	
Peyer's patch		X	X	
Pituitary	X	X	X	
Prostate	X	X	X	
Salivary gland, mandibular[d]	X	X	X	
Salivary gland, parotid		X	X	
Salivary gland, sublingual		X	X	
Seminal vesicles		X	X	
Skeletal muscle, biceps femoris		X	X	
Skin		X	X	
Small intestine, duodenum		X	X	
Small intestine, ileum		X	X	
Small intestine, jejunum		X	X	
Spinal cord, cervical		X	X	
Spinal cord, lumbar		X	X	
Spinal cord, thoracic		X	X	
Spleen	X	X	X	
Stomach, glandular		X	X	
Stomach, nonglandular		X	X	
Target Organs[e]		X	X	X
Testis	X	X	X	
Thymus	X	X	X	
Thyroid gland (with parathyroid)[f]	X	X	X	
Tongue		X	X	
Trachea		X	X	

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination Term[a]	Rec[b]
Ureters		X	X	
Urinary bladder		X	X	
Uterus with cervix	X	X	X	
Vagina		X	X	
Gross lesions		X	X	X
Tissue masses with regional lymph node[g]		X	X	X

[a] *Terminal (Groups 1-4)*
[b] *Recovery*
[c] *Bone marrow smears will be prepared only for animals necropsied at scheduled intervals. Evaluation will be performed at the discretion of the Study Director and/or Sponsor (additional cost).*
[d] *The combined weight of the right mandibular/sublingual salivary gland will be obtained.*
[e] *Target organs (and target organ gross lesions) will be designated by the Study Director, Pathologist and/or Sponsor based on experimental findings (additional cost).*
[f] *Parathyroids cannot always be identified macroscopically. They will be examined if in the plane of section and in all cases where they are noted as grossly enlarged.*
[g] *A regional lymph node drains the region where a tissue mass is located. A regional lymph node may not always be identified when a mass is present.*

There were no test article-related macroscopic changes.

Ovaries: Cysts were noted in 5/16 females in the 5 mg/kg/day main study group compared to 2/15 main study control females.

Organ Weights

Body weight and the organ weights identified in the table above were recorded for all animals at scheduled necropsies and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs will be weighed together. Organs were not weighed for animals that died prematurely or euthanized *in extremis*. The organ weight changes as compared to control are summarized below. '*' and '**' denote values that are statistically different from the control group; $p \leq 0.05$ and $p \leq 0.01$, respectively. Unless otherwise noted, the organ weight changes noted above were not observed in the 100/80 mg/kg/day groups following the 28-day recovery period.

<u>Adrenal Glands:</u> Absolute weight was decreased by 23%, 23%, and 15% in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 21%, 26%, and 12% in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 23%, 22%, and 14% in the 5, 20, and 100/80 mg/kg/day males, respectively.

Absolute weight was increased by 30%, 30%, and 40% in the 5, 20, and 100/80 mg/kg/day females, respectively. Organ/body weight ratio was increased by 31%, 26%, and 38% in the 5, 20, and 100/80 mg/kg/day females, respectively. Organ/brain weight

ratio was increased by 24%, 31%, and 36% in the 5, 20, and 100/80 mg/kg/day females, respectively.

Absolute weight, organ/body weight ratio, and organ/brain weight ratio were decreased by 12%, 19%, and 11% in the 100/80 mg/kg/day recovery females, respectively.

Epididymides: Absolute weight was decreased by 15%* in both the 5 and 100/80 mg/kg/day males. Organ/body weight ratio was decreased by 15%*, 12%, and 13% in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 15%* and 17%* in the 5 and 100/80 mg/kg/day males, respectively.

Organ/body weight ratio and organ/brain weight ratio were increased by 13% in the 100/80 mg/kg/day recovery males.

Ovaries: Absolute weight was decreased by 21% and increased by 18% in the 5 and 100/80 mg/kg/day females, respectively. Organ/body weight ratio was decreased by 18% and 22% in the 5 and 20 mg/kg/day females respectively, and increased by 14% in the 100/80 mg/kg/day females. Organ/brain weight ratio was decreased by 21% and 13% in the 5 and 20 mg/kg/day females respectively, and increased by 15% in the 100/80 mg/kg/day females.

Absolute weight and organ/brain weight ratio were decreased by 17% and 22% in the 100/80 mg/kg/day recovery females, respectively.

Pituitary gland: Absolute weight was decreased by 14%, 14%, and 17% in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 14%, 14%, and 15% in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 14%, 12%, and 18% in the 5, 20, and 100/80 mg/kg/day males, respectively.

Absolute weight was decreased by 16%* in the 100/80 mg/kg/day females. Organ/body weight ratio was decreased by 16% and 18%* in the 20 and 100/80 mg/kg/day females, respectively. Organ/brain weight ratio was decreased by 12% and 17%* in the 20 and 100/80 mg/kg/day females, respectively.

Prostate: Absolute weight was decreased by 16% and 12% in the 20 and 100/80 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 18% in the 20 mg/kg/day males. Organ/brain weight ratio was decreased by 14% and 13% in the 20 and 100/80 mg/kg/day males, respectively.

Absolute weight, organ/body weight ratio, and organ/brain weight ratio were decreased by 23%, 18%, and 17% in the 100/80 mg/kg/day recovery males, respectively.

Salivary glands: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were decreased by 18%, 20%*, and 18%, respectively, in the 100/80 mg/kg/day females.

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Spleen: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were increased by 16%, 21%, and 14%, respectively, in the 100/80 mg/kg/day males. Organ/body weight ratio was decreased by 12% in the 20 mg/kg/day females.

Thymus: Absolute weight was increased by 28% and 24% in the 5 and 100/80 mg/kg/day males, respectively. Organ/body weight ratio was increased by 29% and 27% in the 5 and 100/80 mg/kg/day males, respectively. Organ/brain weight ratio was increased by 30% and 25% in the 5 and 100/80 mg/kg/day males, respectively.

Absolute weight was decreased by 18%, 26%, and 16% in the 5, 20, and 100/80 mg/kg/day females, respectively. Organ/body weight ratio was decreased by 20%, 31%, and 16% in the 5, 20, and 100/80 mg/kg/day females, respectively. Organ/brain weight ratio was decreased by 22%, 27%, and 15% in the 5, 20, and 100/80 mg/kg/day females, respectively

Thyroid/parathyroid gland: Absolute weight was decreased by 21%*, 22%*, and 27%** in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 21%*, 23%*, and 24%** in the 5, 20, and 100/80 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 21%*, 21%*, and 28%** in the 5, 20, and 100/80 mg/kg/day males, respectively.

Absolute weight was decreased by 30%**, 30%**, and 31%** in the 5, 20, and 100/80 mg/kg/day females, respectively. Organ/body weight ratio was decreased by 30%**, 35%**, and 31%** in the 5, 20, and 100/80 mg/kg/day females, respectively. Organ/brain weight ratio was decreased by 32%** in the 5, 20, and 100/80 mg/kg/day females.

Uterus with cervix: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were increased by 22%, 22%, and 20%, respectively, in the 100/80 mg/kg/day females.

Absolute weight, organ/body weight ratio, and organ/brain weight ratio were decreased by 19%, 23%, and 17% in the 100/80 mg/kg/day recovery females, respectively.

Histopathology

The tissues collected for microscopic examination are listed in the table above (Table 13)

Adequate Battery

Yes.

Peer Review

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Yes.

Histological Findings

There were no test article-related microscopic findings. The incidence of specific findings were generally comparable between control and test-article treated groups. There was a neoplastic lesion, bronchiolar-alveolar adenoma, noted in a 5 mg/kg/day male. This lesion is considered as incidental due to its singular occurrence in a low dose animal. The microscopic changes noted below were not observed in animals following a 28-day recovery period.

Kidneys: Multifocal medullary mineralization was observed in 4/14 males in the 100/80 mg/kg/day group compared to 1/16 control males. Multifocal cortical lymphocytic infiltration was observed in 5/15, 4/16, and 6/14 males in the 5, 20, and 100/80 mg/kg/day groups respectively, compared to 3/16 control males.

Liver: Focal lymphohistiocytic inflammation was observed in 3/14 males in the 100/80 mg/kg/day group compared to 1/16 control males. Multifocal lymphohistiocytic inflammation was observed in 3/16, 4/13, and 5/14 females in the 5, 20, and 100/80 mg/kg/day groups respectively, compared to 2/15 control females.

Lung with bronchi: Focal acute hemorrhage was noted in 2/14 males in the 100/80 mg/kg/day group compared to 0/16 control males. A primary benign papillary bronchiolar-alveolar adenoma was noted in 1/14 males in the 100/80 mg/kg/day group, compared to 0/16 control males.

Lymph node (mesenteric): Diffuse lymphoid atrophy was observed in 2/12 females in the 100/80 mg/kg/day group compared to 0/13 control females.

Salivary gland (mandibular): Focal lymphocytic infiltration was noted in 2/16, 2/13, and 3/14 females in the 5, 20, and 100/80 mg/kg/day groups, respectively, compared to 1/15 control females.

Spleen: Multifocal increased hematopoiesis was noted in 2/14 males in the 100/80 mg/kg/day group compared to 0/16 control males.

Stomach: Focal mucosal hyperplasia was noted in 7/31, 7/29, and 12/28 mice in the 5, 20, and 100/80 mg/kg/day groups, respectively, compared to 6/30 mice in the control group.

Thyroid glands: Focal collicular cyst was noted in 2/13 and 3/14 females in the 20 and 100/80 mg/kg/day groups, respectively, compared to 0/15 control females.

Urinary bladder: Focal submucosal lymphocytic infiltration was observed in 3/14 females in the 100/80 mg/kg/day group compared to 0/15 control females.

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Special Evaluation

None.

Toxicokinetics

Blood samples from 3 animals/sex were collected from TK animals via the vena cava for determination of the plasma concentrations of the test article and metabolite MM-419447. The samples were collected 0.5 hours post-dose from the control group and 0.5, 1, 2, 4, 8, and 24 hours post-dose from the treated groups, on days 1, 90, and 180. Plasma concentrations of the test-article were below the limits of quantitation (0.5 ng/mL) in the control group. Linaclotide plasma concentrations increased rapidly, reaching maximal concentration generally within 0.5 to 1 hour after dosing. For most animals in the 5 and 20 mg/kg/day dose groups, plasma drug levels declined rapidly after the C_{max} was achieved, with drug levels below the limit of quantitation within 2 to 4 hours after dosing. In the 100/80 mg/kg/day group, after day 1 (100 mg/kg/day dose), the plasma concentrations of linaclotide were quantifiable 24 hours after dosing. On days 90 and 180 (80 mg/kg/day dose), the plasma concentrations of linaclotide were quantifiable only up to 8 hours after dosing. Thus, there were no signs of drug accumulation. C_{max} and $AUC_{0\text{-}24\ hr}$ values were highly variable between days of blood collection and dose groups. With the exception of the 5 mg/kg/day females on day 90, plasma exposure of linaclotide increased in the 5, 20, and 100/80 mg/kg/day dose groups. Dose proportionality was difficult calculate due to the variability of the exposure data, especially in the 100/80 mg/kg/day group. An analysis of the individual animal data showed high intragroup variability in the 5 and 100/80 mg/kg/day females on day 90 and in the 100/80 mg/kg/day males on day 180. In general, females had higher plasma exposure levels than males with the exception of the 20 mg/kg/day males on day 90 and the 100/80 mg/kg/day males on day 180. The results are presented in the Sponsor's table below.

Plasma concentrations of MM-419447 were below the limit of quantitation (5 ng/mL) in the 5 and 20 mg/kg/day dose group (plasma exposure could only be quantitated on days 1 and 180 in one animal each day). The highest average C_{max} and $AUC_{0\text{-}24\ hr}$ of MM-419447 were noted on day 1 (100 mg/kg/day dose of linaclotide). There were no significant differences in plasma exposure of MM-419447 between males and females in the 100/80 mg/kg/day group. Accumulation of MM-419447 was not observed, and dose proportionality was not analyzed due to the lack of exposure in the 5 and 20 mg/kg/day groups. The results are presented in the Sponsor's table below.

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Table 11: Toxicokinetic Parameters of Linaclotide Analyzed in a 26-week Oral Toxicity Study in Mice

Dose (mg/kg/day)	Day	Gender	t_{max} (h)	C_{max} (ng/mL)	C_{max}/D	AUC_t (ng h/mL)	AUC_{0-2} (ng h/mL)	AUC_{0-24} (ng h/mL)
5	1	Female	1.0	3.017	0.603	1.495	3.003	3.003
		Male	1.0	1.150	0.230	0.566	1.141	1.141
	90	Female	1.0	24.367	4.873	6.499	18.682	18.682
		Male	0.5	0.988	0.198	0.553	0.670	0.670
	180	Female	0.5	0.481	0.096	0.553	0.553	0.913
		Male	0.5	1.003	0.201	0.564	0.690	0.690
20	1	Female	1.0	7.710	0.386	9.724	7.764	11.484
		Male	1.0	6.780	0.339	7.492	7.492	8.376
	90	Female	0.5	3.577	0.179	4.305	4.305	4.954
		Male	0.5	2.807	0.140	6.942	3.956	8.202
	180	Female	4.0	19.350	0.968	25.138	5.090	63.838
		Male	0.5	5.913	0.296	5.382	5.382	7.148
100/80	1	Female	2.0	86.900	0.869	432.580	97.525	432.580
		Male	0.5	28.200	0.282	62.082	33.645	99.276
	90♦	Female	1.0	648.793	6.488	553.717	499.790	758.517
		Male	0.5	16.800	0.168	18.673	12.100	23.289
	180♦	Female	2.0	16.840	0.168	44.761	24.654	48.625
		Male	1.0	196.980	1.970	162.214	156.795	163.366

Notes: NC = not calculated, ♦ = dose was 80 mg/kg/day, NA = not applicable

Table 12: Toxicokinetic Parameters of MM-419447 (metabolite) Analyzed in a 26-week Oral Toxicity Study in Mice

Dose (mg/kg/day)	Day	Gender	t_{max} (h)	C_{max} (ng/mL)	C_{max}/D	AUC_t (ng h/mL)	AUC_{0-2} (ng h/mL)	AUC_{0-24} (ng h/mL)
5	1	Female	NC	0.000	0.000	0.000	0	0.000
		Male	NC	0.000	0.000	0.000	0	0.000
	90	Female	NC	0.000	0.000	0.000	0	0.000
		Male	NC	0.000	0.000	0.000	0	0.000
	180	Female	NC	0.000	0.000	0.000	0	0.000
		Male	NC	0.000	0.000	0.000	0	0.000
20	1	Female	1	1.697	0.085	0.424	1.273	1.273
		Male	NC	0.000	0.000	0.000	0	0.000
	90	Female	NC	0.000	0.000	0.000	0	0.000
		Male	NC	0.000	0.000	0.000	0	0.000
	180	Female	NC	0.000	0.000	0.000	0	0.000
		Male	0.5	2.077	0.104	0.519	1.039	1.039
100/80	1	Female	0.5	14.467	0.145	7.716	8.681	8.681
		Male	0.5	13.977	0.140	10.308	10.308	12.151
	90♦	Female	0.5	4.467	0.045	1.117	2.234	2.234
		Male	NC	0.000	0.000	0.000	0	0.000
	180♦	Female	0.5	7.480	0.075	4.177	5.050	5.050
		Male	0.5	5.067	0.051	3.218	4.586	4.586

Notes: NC = not calculated, ♦ = dose was 80 mg/kg/day, NA = not applicable

Dosing Solution Analysis

The individual results for homogeneity verification analysis were between 99.6% and 113.7% of the target. For concentration verification analysis, results were between 89.5% and 113.7% of the target. All samples from weeks 1, 2, 7, 13, and 26 met the acceptance criteria for precision, accuracy and homogeneity.

Study title: 39-Week Toxicity Study of MD-1100 Acetate Administered by Oral (nasogastric) Intubation to Cynomolgus Monkeys with a 4-Week Recovery Period	
Study no.:	MNP-103-028
Study report location:	(Original amendment) 8/9/11: 4.2.3.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 14, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MD1100 Acetate, lot #sPPL-MD11000601C, PPL-MD11000603A, and PPL-MD11000603C, and 90.1%, 93.2%, and 92.6% respectively, relative to total peptide content.

Key Study Findings

Cynomolgus monkeys were treated orally with 5, 10, or 50 mg/kg/day linaclotide (MD-1100 acetate) for 39 weeks. There were two test article-related deaths (i.e., animals sacrificed in moribund condition); a 10 mg/kg/day male and a 50 mg/kg/day female. Clinical signs in both animals included severe watery feces, hunched appearance, low/no food consumption, lethargy, and decreased activity. In addition, at sacrifice, the 10 mg/kg/day male and 50 mg/kg/day female had a 24% and 14% decrease in body weight, respectively, compared to their starting body weights. Microscopic examination of the 10 mg/kg/male and 50 mg/kg/day female showed that there were treatment-related effects which included the following: necrosis/degeneration in the cecal, colonic, and rectal mucosa; lymphoid depletion in the mandibular lymph node, and spleen; thymic cortical atrophy; pancreatic zymogen depletion. The main clinical sign noted in the other test article-treated animals was watery feces. Body weight gain was decreased at all doses with the exception of the 5 mg/kg/day females. After 39 weeks of dosing, the 10 and 50 mg/kg/day females had a 37% and 48% decrease in body weight gain, respectively. There were no significant test article-related effects on food consumption, ophthalmoscopic evaluation, ECG, hematology, clinical chemistry, organ weights, or macroscopic or microscopic evaluations. Toxicokinetic analysis showed that the $AUC_{0\text{-}24\ hr}$ values for linaclotide and its metabolite, MM-419447, were increased with

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dose. Based on the mortality and decrease in body weight gain, the NOAEL was 5 mg/kg/day.

Methods	
Doses:	5, 10, and 50 mg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (nasogastric intubation)
Dose volume:	3 mL/kg
Formulation/Vehicle:	0.5% methylcellulose in deionized water
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	20
Age:	Males (2.7 to 4.9 years) and females (2.4 to 4.2 years)
Weight:	Males (2.5 to 3.9 kg) and females (2.3 to 3.3 kg)
Satellite groups:	Yes (see below)
Unique study design:	None
Deviation from study protocol:	Minor deviations occurred that did not affect the quality or integrity of the study.

Table 13: Study Design (39-week Oral Toxicity Study in Monkeys)

Group No.	Number of Males/Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	Number Necropsied: Day 274	Day 302/303**
1	6/6	0 (control)	3	0	4/4	2/2
2	4/4	5	3	1.67	4/4	-
3	4/4	10	3	3.33	3/4*	-
4	6/6	50	3	16.7	4/3*	2/2

*One Group 3 male (3001) was euthanized on Day 76, and one Group 4 female (4506) was euthanized on Day 106.
**Set A is Day 303, Set B is Day 302

Observations and Results

Mortality

Animals were observed twice daily. One 10 mg/kg/day male and one 50 mg/kg/day female were euthanized in moribund condition on days 76 and 106, respectively. Clinical signs in both animals included severe watery feces, hunched appearance, sunken eyes (10 mg/kg/day male only), low/no food consumption, lethargy, and decreased activity. In addition, at sacrifice, the 10 mg/kg/day male and 50 mg/kg/day female had a 24% and 14% decrease in body weight, respectively, compared to their starting body weights[5].

[5] Both animals were treated with subcutaneous and/or intravenous fluids along with high fiber supplements to reduce the watery feces and high calorie supplements to increase body weight, and the 10 mg/kg/day male was given sucralfate for approximately 5 weeks as a treatment for the watery feces.

Analysis of hematology parameters showed that, when compared to pre-study values, the 10 mg/kg/day male had a 138% increase in white blood cells, an 18% decrease in lymphocytes, and a 67% increase in unclassified cells at the time of sacrifice. The 10 mg/kg/day male and 50 mg/kg/day female had a 52% and 36% increase in reticulocytes, an 83% and 90% increase in platelets, a 118% and 94% increase in monocytes, a 138% and 45% increase in basophils, a 41% and 30% decrease in eosinophils, and a 286% and 38% increase in segmented neutrophils respectively, when compared to pre-study values. There was evidence of Dohle bodies in the neutrophils of the 10 mg/kg/day male. Analysis of coagulation parameters showed that, when compared to pre-study values, the 10 mg/kg/day male and 50 mg/kg/day female had a 73% and 49% increase in fibrinogen, respectively, and the 10 mg/kg/day male had a 32% increase in activated partial thromboplastin time (APTT) at the time of sacrifice.

Analysis of clinical chemistry parameters showed the following changes from pre-study values at the time of sacrifice for the 10 mg/kg/day male and 50 mg/kg/day female: a 42% and 70% decrease in alanine aminotransferase (ALT); a 35% and 35% decrease in aspartate aminotransferase (AST); a 55% and 19% decrease in gamma glutamyl transferase (GGT); a 59% and 48% decrease in lactate dehydrogenase levels; a 49% and 85% increase in phosphorus levels; and a 52% and 32% increase in globulin levels, respectively. In addition, in the 10 mg/kg/day male, there was a 100% increase in blood urea nitrogen, a 48% decrease in carbon dioxide, a 31% decrease in glucose, a 32% decrease in cholesterol, a 52% increase in triglycerides, a 43% decrease in albumin, and a 60% decrease in albumin/globulin ratio. In the 50 mg/kg/day female, there was 100% increase in the creatinine level, a 22% decrease in potassium levels, a 22% increase in cholesterol levels, and a 159% increase in triglycerides.

Histopathologic evaluation showed that the 10 mg/kg/day male and 50 mg/kg/day female had minimal to moderate degeneration/necrosis of the mucosal glands and surface epithelium in the cecum, colon, and rectum. Foci of neutrophils appeared in degenerating glands within the cecum, colon, and rectum of the 10 mg/kg/day male and in the cecum of the 50 mg/kg/day female. Mucosal and/or submucosal hemorrhage was observed in the cecum and colon of the 10 mg/kg/day male and mucosal congestion was observed in the cecum of the 50 mg/kg/day female. Additionally, the 10 mg/kg/day male had a bilateral increase in the adrenals, minimal to mild lymphoid depletion in the lymph node (tracheobronchial) and spleen, severe cortical atrophy in the thymus (correlated with decrease in thymus weight), decreased size and discoloration of the pancreas, and discoloration of the mandibular salivary gland. The 50 mg/kg/day female had an increase in size of the mesenteric lymph node and a decreased size of the thymus.

Clinical Signs

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A detailed clinical examination of each main study animal was performed daily. All test article-treated animals had an increase in the incidence of watery feces. During the recovery period, the occurrence of watery feces was decreased. On day 59, 2/5 females in the 50 mg/kg/day had abnormal colored feces compared to 0/6 control females. One 5 mg/kg/day male and one 50 mg/kg/day female were observed to have tremors on days 48 and 49 and days 279 to 286, respectively. Hunched appearance and rectal prolapse were noted in a few test article-treated animals. Additional clinical signs observed in all animals included bruising, reddened area, and alopecia.

Body Weights

Body weights for all main study animals were measured and recorded prior to randomization and weekly during the study. Body weight changes are summarized in the table below.

Table 14: Body Weight Changes in the 39-week Oral Toxicity Study in Monkeys

Dose (mg/kg)	Initial Body Weight (kg)	Final Body Weight (kg)	Body Weight Gain (kg)	% Control Group Weight Gain
	Males			
0	3.17 ± 0.31	4.48 ± 0.63	1.31	100%
5	3.35 ± 0.39	4.25 ± 0.47	0.9	69%
10	2.83 ± 0.25	3.93 ± 0.40	1.1	85%
50	3.12 ± 0.43	4.23 ± 0.39	1.11	85%
	Females			
0	2.62 ± 0.16	3.25 ± 0.19	0.63	100%
5	2.53 ± 0.40	3.30 ± 0.35	0.77	122%
10	2.48 ± 0.17	2.88 ± 0.24	0.40	63%
50	2.65 ± 0.40	2.98 ± 0.92	0.33	52%

With the exception of the 5 mg/kg/day females, body weight gain was decreased in all test article treated animals when compared to the control group. The decreases in body weight gain were not statistically significant. During the recovery period, the 50 mg/kg/day males and females gained more weight than the control animals.

Feed Consumption

Food consumption was evaluated by observing the number of biscuits remaining from the previous days feed ration, and a notation of low food consumption was made when less than approximately half of the rations are consumed. No significant test article-related changes in food consumption were observed.

Ophthalmoscopy

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No test article-related changes were observed.

ECG

ECG measurements were taken prior to study initiation and during weeks 20 and 39 (approximately 2 hours post-dose). The Sponsor did not provide raw data related to standard ECG measurements. However, the report stated that all ECGs evaluated in this study were considered normal and no arrhythmias were found.

Hematology

Blood samples were collected within one week prior to the initiation of dosing and during weeks 13, 26, and 39, and from remaining animals near the end of the recovery period. The following tables list the parameters that were measured and a summary of the data.

Table 15: Hematology Parameters Measured in the 39-week Oral Toxicity Study in Monkeys

Leukocytes	Red blood cells	Hemoglobin
Hematocrit	Mean corpuscular hemoglobin	Mean corpuscular volume
Mean corpuscular hemoglobin concentration	Absolute reticulocytes	Platelet count
White blood cells	Neutrophils	Eosinophils
Lymphocytes	Monocytes	Basophils
Unclassified cells	Prothrombin time (PT)	Activated partial thromboplastin time (APTT)
Fibrinogen		

Table 16: Percent Change in Hematology Parameters in the 39-week Oral Toxicity Study in Monkeys

Parameter	Dose (mg/kg/day)	Change at termination compared to control
Absolute reticulocytes	10, 50 (male)	↓ 14%, ↓ 29%
White blood cells	10, 50 (male)	↑ 40%, ↑ 30%
Eosinophils	10 (male) 5, 10 (female)	↑ 66% ↑ 51%, ↑ 126%
Lymphocytes	10, 50 (male) 10 (female)	↑ 55%, ↑ 44% ↑ 18%
Monocytes	10, 50 (male) 10 (female)	↑ 46%, ↑ 32% ↑ 63%
Basophils	10, 50 (male) 10 (female)	↑ 26%, ↑ 50% ↑ 39%
Unstained cells	10, 50 (male)	↑ 47%, ↑ 15%

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	10 (female)	↑ 41%

The changes noted above were not dose-dependent or statistically significant, thus the hematology changes are not considered to be test article-related. The high-dose females had no change in any parameter. After the recovery period, hematology parameters in the 50 mg/kg/day males were similar to the control males. The changes observed in the 10 mg/kg/day females were mainly due to a high amount of intergroup variability. There were no test-article related changes in APTT and PT.

Clinical Chemistry

Blood samples were collected within one week prior to the initiation of dosing and during weeks 13, 26, and 39, and from remaining animals near the end of the recovery period. The following tables list the parameters that were measured and a summary of the data.

Table 17: Clinical Chemistry Parameters Measured in the 39-week Oral Toxicity Study in Monkeys

Alkaline phosphatase	Total bilirubin	Total cholesterol
Aspartate aminotransferase	Creatinine	Calcium
Alanine aminotransferase	Total protein	Phosphorus
Gamma glutamyl transferase	Albumin	Globulin
Albumin/globulin ratio	Glucose	Electrolytes (sodium, potassium, chloride)
Carbon dioxide	Urea nitrogen	
Lactate dehydrogenase		

Table 18: Percent Change in Clinical Chemistry Parameters in the 39-week Oral Toxicity Study in Monkeys

Parameter	**Dose (mg/kg/day)**	**Change at termination compared to control**
Alanine aminotransferase	5 (female)	↓ 24%
Albumin	50 (female)	↑ 18%
Alkaline phosphatase	50 (male) 50 (female)	↑ 19% ↓ 33%
Aspartate aminotransferase	10, 50 (female)	↓ 16%, ↓ 20%
Creatinine	10 (female)	↑ 16%
Gamma glutamyl transferase	10, 50 (male)	↑ 35%, ↑ 24%
Glucose	10 (male)	↑ 14%
Lactate dehydrogenase	5 (male) 50 (female)	↓ 45% ↓ 40%
Potassium	50 (female)	↓ 18%
Total bilirubin	5, 50 (male) 10, 50 (female)	↓ 21%*, ↓ 27%* ↓ 29%, ↓ 20%
Triglycerides	10 (male)	↓ 40%

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Urea nitrogen	50 (female)	↑ 39%

* Statistically significant: ($p \leq 0.05$)

The effects noted in the table above are not considered to be test article-related, given the absence of dose-dependency or statistical significance (with the exception of total bilirubin in the 5 and 50 mg/kg/day males). Some of the changes were still observed after the recovery period. Specifically, gamma glutamyl transferase levels increased by 37% in the 50 mg/kg/day males, and aspartate aminotransferase, alkaline phosphatase and urea nitrogen were decreased by 16%, 18%, and 29%, respectively, in the 50 mg/kg/day females. However, the changes observed after the 4-week recovery period were likely due to the small sample size (n=1-2/sex).

Urinalysis

Urine was collected before terminal necropsy. The table below lists the parameters that were measured.

Table 19: Urinalysis Parameters Measured in the 39-week Oral Toxicity Study in Monkeys

pH	Specific gravity	Microscopics
Color/character	Protein	Glucose
Bilirubin	Ketones	Occult blood

During week 40 (prior to scheduled necropsy), the specific gravity increased by 48% and 31% in the 10 mg/kg/day males and females, respectively, and decreased by 25% in the 50 mg/kg/day males and females, compared to controls. The Sponsor did not provide a summary of quantitative data associated with the other urinalysis parameters (individual data was submitted). There were no test article-related changes in urinalysis parameters.

Gross Pathology

All animals were subjected to a complete gross necropsy examination, which included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. The Sponsor's table below lists the tissues preserved for macroscopic and microscopic examination.

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Table 20: Organs Collected at Necropsy for Macroscopic and Microscopic Examination in the 39-week Oral Toxicity Study in Monkeys

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Peyer's Patches[b]	Adrenals
Large Intestine	Pituitary
Cecum	Thyroid/Parathyroids[a]
Colon	Skin/Musculoskeletal
Rectum	Skin/Mammary Gland
Pancreas	Bone (femoral head)
Liver	Bone (7th rib)
Gallbladder	Skeletal Muscle (psoas and diaphragm)
Respiratory	Nervous/Special Sense
Trachea	Eyes with Optic Nerve
Lung	Sciatic Nerve
Lymphoid/Hematopoietic	Brain
Bone Marrow (sternum)	Spinal Cord (thoracic)
Thymus	Other
Spleen	Animal Number Tattoo
Lymph Nodes	Gross Lesions
Mandibular	
Mesenteric	

[a] The occasional absence of the parathyroid gland from the routine tissue section will not require a recut of the section.
[b] The occasional absence of Peyer's patches in the sections of small intestine will not require a recut of the section

There were no test article-related macroscopic changes.

Colon: Minimal to mild discoloration (red) was noted in 4/8, 2/7, and 2/7 animals in the 5, 10, and 50 mg/kg/day groups respectively, compared to 0/8 control animals. One male in the 50 mg/kg/day recovery group had minimal discoloration, compared to 0 control group males.

Ileocecal valve: Minimal to mild discoloration (red) was noted in 1/8, 2/7, and 3/7 animals in the 5, 10, and 50 mg/kg/day groups, respectively, compared to 0/8 control animals.

Organ Weights

The organs listed in the Sponsor's table below were weighed and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together. Organs were not weighed for animals found dead or euthanized prior to necropsy. The organ weight changes as compared to control are summarized below. '*' denotes value that is statistically different from the control group; $p \leq 0.05$.

Table 21: Organs Weighed after Necropsy in the 39-week Oral Toxicity Study in Monkeys

Organs Weighed	
Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary	Spleen
Testes	Thymus
Thyroid with parathyroids	

Adrenal: Absolute weight was increased by 16% and 15% in the 5 and 50 mg/kg/day males, respectively. Organ/body weight ratio was increased by 20% in the 5 and 50 mg/kg/day males. Organ/brain weight ratio was increased by 15% in the 5 mg/kg/day males.

Organ/body weight ratio was increased by 17% in the 10 mg/kg/day females.

Epididymides: Absolute weight was decreased by 43%, 62%, and 44% in the 5, 10, and 50 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 37%, 67%, and 37% in the in the 5, 10 and 50 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 44%, 74%*, and 48% in the 5, 10 and 50 mg/kg/day males, respectively.

Heart: Absolute weight was decreased by 15% and 13% in the 10 and 50 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 11%, 23% and 18% in the 5, 10, and 50 mg/kg/day males, respectively.

Liver: Absolute weight and organ/brain weight ratio were decreased by 16% in the 10 mg/kg/day females.

Ovaries: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were increased by 17%, 13%, and 20%, respectively, in the 5 mg/kg/day females..

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Pituitary gland: Absolute weight, organ/body weight ratio, and organ/brain weight ratio were increased by 21%, 35%, and 13%, respectively, in the 50 mg/kg/day males.

Spleen: Organ/body weight ratio was increased by 21% in the 50 mg/kg/day females.

Testes: Absolute weight was decreased by 45%, 90%*, and 67% in the 5, 10, and 50 mg/kg/day males, respectively. Organ/body weight ratio was decreased by 38%, 88%, and 62% in the in the 5, 10 and 50 mg/kg/day males, respectively. Organ/brain weight ratio was decreased by 46%, 91%*, and 70% in the 5, 10 and 50 mg/kg/day males, respectively. The robust decreases of the testes weight in the treatment groups are the result of a high amount of variability in the control group.

Thymus: Absolute weight was increased by 40%, 175%*, and 166%* in the 5, 10 and 50 mg/kg/day males, respectively. Organ/body weight ratio was increased by 36%, 193%*, and 174%* in the 5, 10 and 50 mg/kg/day males, respectively. Organ/brain weight ratio was increased by 36%, 151%*, and 155% in the 5, 10 and 50 mg/kg/day males, respectively.

Thyroid: Absolute weight was decreased by 14% in the 5 and 10 mg/kg/day males. Organ/brain weight ratio was decreased by 20%, 21%, and 18% in the 5, 10, and 50 mg/kg/day males, respectively.

Histopathology

The tissues collected for microscopic examination are listed in the above table (Table 24)

Adequate Battery

Yes.

Peer Review

Yes.

Histological Findings

Test article-related microscopic findings were only observed in the 10 mg/kg/day male and 50 mg/kg/day female sacrificed in moribund condition. The findings are described under the “Mortality” section above. Briefly, the test article-related effects in the two moribund animals included: necrosis/degeneration in the cecal, colonic, and rectal mucosa; lymphoid depletion in the mandibular lymph node and spleen; thymic cortical atrophy; pancreatic zymogen depletion. In addition, single incidences of mucosal glandular degeneration of minimal severity occurred in cecum and colon in a 50 mg/kg/day female (recovery group) and in the rectum of a 50 mg/kg/day male (recovery group).

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Special Evaluation

None.

Toxicokinetics

Blood samples (venipuncture from any vein) were collected from animals for determination of the plasma concentrations of the test article and metabolite MM-419447. The samples were collected pre-dose and 0.5, 1, 2, 4, 8, and 24 hours post-dose on day 1 and during weeks 13 and 32. Plasma concentrations of linaclotide were below the limits of quantitation (0.5 ng/mL) in the control group and prior to dosing in the test article treated groups. Linaclotide plasma concentrations increased rapidly, reaching maximal concentration generally within 1 to 3 hours after dosing, and declined rapidly thereafter. The mean terminal half-life was 1.8 to 4.6 hours. At 24 hours after dosing, plasma concentrations of the test article were mostly below the limit of quantitation.

The C_{max} and $AUC_{0\text{-}24\ hr}$ values for linaclotide increased with dose, and were generally higher on day 222 (week 32) compared to days 1 and 89 (week 13). Females tended to have a higher plasma exposure than males. The results are presented in the Sponsor's table below.

Plasma concentrations of MM-419447 were below the limits of quantitation (3 ng/mL) in the control group. Plasma levels of MM-419447 increased between 2 and 8 hours (mean t_{max}), but decreased rapidly after 8 hours to levels below the limit of quantitation in a majority of the animals, especially in the 5 mg/kg/day group. Therefore, the terminal half-life in the 10 and 50 mg/kg/day groups ranged from 1.6 to 6.6 hours. In addition, plasma concentrations of MM-419447 increased with dose and increased in a dose-dependent manner between the 10 and 50 mg/kg/day groups. C_{max} and $AUC_{0\text{-}24\ hr}$ values were higher on day 89 than on days 1 or 222. There were no significant differences in plasma exposure to MM-419447 between males and females in the 5 and 10 mg/kg/day group, but females did have slightly higher plasma exposure levels in the 50 mg/kg/day group (specifically days 89 and 222). Accumulation of MM-419447 was not observed. The results are presented in the Sponsor's table below.

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Table 22: Mean Toxicokinetic Parameters of Linaclotide in the 39-week Oral Toxicity Study in Monkeys

Dose (mg/kg/day)	Day	Gender	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (ng/mL)	AUC_t (ng h/mL)	AUC_{0-24} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)
5	1	Female	4.32 (1.53)	1.50 (0.577)	4.27 (0.985)	20.7 (1.65)	32.0 (5.02)	30.2 (6.89)
		Male	3.54 (1.36)	2.50 (1.00)	3.80 (1.40)	19.7 (5.89)	29.1 (8.01)	23.5 (5.69)
	89	Female	4.64 (1.86)	1.63 (1.60)	7.08 (4.14)	36.2 (23.1)	44.0 (18.2)	NA
		Male	1.83 (0.717)	0.875 (0.750)	5.88 (2.47)	13.5 (2.86)	16.7 (3.92)	NA
	222	Female	2.85 (0.37)	1.50 (0.58)	6.0 (3.2)	29 (18)	39 (24)	NA
		Male	3.23 (1.05)	1.75 (0.500)	8.00 (4.92)	35.5 (22.4)	49.8 (32.2)	NA
10	1	Female	2.83 (1.92)	2.00 (1.41)	11.5 (3.93)	50.8 (10.5)	67.9 (19.5)	54.8 (10.1)
		Male	4.27 (2.63)	3.00 (1.15)	8.71 (3.93)	57.1 (31.5)	72.8 (23.7)	80.0 (26.1)
	89	Female	2.07 (1.09)	0.625 (0.250)	18.2 (7.26)	52.9 (16.9)	61.6 (13.6)	NA
		Male	2.90 (1.52)	2.17 (1.76)	20.2 (24.2)	48.5 (28.3)	71.4 (36.0)	NA
	222	Female	2.41 (1.06)	0.750 (0.289)	21.7 (14.9)	75.8 (40.2)	94.8 (50.5)	NA
		Male	3.98 (1.60)	1.67 (0.577)	10.4 (6.41)	57.2 (36.5)	81.4 (50.6)	NA
50	1	Female	2.75 (1.17)	1.33 (1.33)	48.9 (25.2)	182 (49.4)	236 (68.5)	235 (39.9)
		Male	2.97 (0.665)	1.50 (1.34)	46.0 (23.9)	242 (178)	294 (164)	302 (169)
	89	Female	4.29 (3.00)	1.25 (1.37)	80.3 (50.7)	309 (219)	342 (208)	NA
		Male	2.14 (0.685)	1.08 (0.736)	70.2 (46.5)	196 (82.6)	239 (98.2)	NA
	222	Female	3.77 (2.53)	1.90 (1.34)	109 (76.9)	618 (355)	656 (355)	NA
		Male	3.42 (1.26)	2.00 (1.64)	47.9 (14.7)	229 (60.5)	292 (48.1)	NA

NA = not applicable; standard deviation presented in parentheses

Table 23: Mean Toxicokinetic Parameters of MM-419447 (metabolite) in the 39-week Oral Toxicity Study in Monkeys

Dose (mg/kg/day)	Day	Gender	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (ng/mL)	AUC_t (ng h/mL)	AUC_{0-24} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)
5	1	Female	NC	8.00 (NC)	1.06 (2.11)	6.06 (12.1)	14.5 (29.0)	NC
		Male	NC	3.00 (1.41)	2.10 (2.50)	3.94 (5.81)	8.12 (10.5)	NC
	89	Female	NC	3.50 (1.00)	10.6 (2.12)	45.0 (14.3)	72.6 (28.6)	NA
		Male	NC	3.50 (1.00)	16.2 (7.89)	47.7 (19.9)	75.7 (27.0)	NA
	222	Female	NC	4.00 (NC)	2.60 (5.20)	12.0 (24.0)	20.0 (40.1)	NA
		Male	NC	4.00 (NC)	3.58 (7.15)	3.58 (7.15)	10.7 (21.5)	NA
10	1	Female	NC	2.50 (1.00)	10.5 (5.59)	26.4 (11.3)	39.6 (11.9)	NC
		Male	NC	3.50 (1.00)	5.62 (3.45)	24.7 (20.2)	49.0 (32.8)	NC
	89	Female	3.88 (0.841)	1.75 (0.500)	19.6 (15.4)	89.1 (87.3)	133 (139)	NA
		Male	2.31 (NC)	4.17 (3.75)	26.1 (16.7)	118 (94.3)	255 (210)	NA
	222	Female	3.04 (1.46)	2.25 (1.26)	28.9 (9.03)	142 (50.7)	208 (86.7)	NA
		Male	4.85 (0.665)	2.67 (1.15)	16.2 (10.2)	82.8 (54.8)	146 (88.1)	NA
50	1	Female	2.48 (2.16)	2.83 (1.33)	38.8 (34.1)	120 (53.8)	171 (61.3)	142 (76.6)
		Male	3.73 (1.42)	2.83 (1.33)	38.7 (28.1)	176 (124)	266 (172)	233 (102)
	89	Female	3.34 (1.52)	2.67 (1.03)	171 (118)	950 (848)	1140 (874)	NA
		Male	1.58 (0.436)	2.33 (1.37)	134 (35.1)	497 (245)	666 (364)	NA
	222	Female	4.31 (3.10)	3.20 (1.10)	113 (41.4)	690 (374)	1020 (655)	NA
		Male	6.63 (2.56)	3.33 (1.03)	82.0 (35.5)	471 (196)	599 (116)	NA

NA = not applicable; NC = not calculated; standard deviation presented in parentheses

Dosing Solution Analysis

The Sponsor stated that all test article dosing solutions were acceptable. The individual results for homogeneity verification analysis were between 108.3% and 113.7% of the target concentration. For concentration verification analysis, the results were between 96.9% and 114.8% of the target. All of the week 1 samples met the acceptance criteria

for precision and homogeneity, and the 5 mg/kg/day samples met acceptance criteria for accuracy, but the 10 and 50 mg/kg/day groups failed with mean percent recoveries of 110.8% and 110.2%. The week 4 samples met the acceptance criteria for precision, but failed for accuracy with mean percent recoveries of 111.5%, 111.4%, and 114.5% for the 5, 10, and 50 mg/kg/day groups, respectively. Week 13 and 20 samples all met the acceptance criteria for precision and accuracy, and the week 39 samples all met the acceptance criteria for homogeneity, precision, and accuracy. The Sponsor investigated the week 1 and 4 results, and discovered that the recoveries were artificially inflated due to the purity of the test article being used for standard preparation. Week 1 and 4 results were recalculated and were found acceptable

Study title: A 4-Week Oral Toxicity Study of Linaclotide (and degradant impurities) in Mice with a 2-Week Recovery Period	
Study no.:	MNP-103-061 (804984)
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/12/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide, PPL-MD11000903B, 98.78-99.13% Linaclotide spiked with 0.2% (b) (4) (Lot X12054A1) and 0.5% (b) (4) (Lot Y06063A1), Lot 903 Blend, 97.94%

Key Study Findings

This study was conducted to support the qualification of the impurities (b) (4) (b) (4) degradant of linaclotide) and (b) (4) (b) (4) degradant of linaclotide). The study groups included a vehicle control, 20 mg/kg/day linaclotide, and 20 mg/kg/day linaclotide spiked with 40 µg/kg/day (b) (4) and 80 µg/kg/day (b) (4) There were higher incidences of oily fur and dehydration in males treated with linaclotide or linaclotide spiked with impurities ((b) (4) and (b) (4) compared to the control group. Body weights and bodyweight gains were higher in the females treated with linaclotide or spiked linaclotide. Microscopic lesions generally occurred at very low frequencies in groups treated with linaclotide or spiked linaclotide. There were no significant differences in the adverse effects of linaclotide or linaclotide spiked with (b) (4) and (b) (4) after 28 days of oral administration in mice.

Methods	
Doses:	0 (vehicle control), 20 mg/kg/day linaclotide, and 20 mg/kg/day linaclotide spiked with 40 µg/kg/day (b) (4) and 80 µg/kg/day (b) (4)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	Linaclotide in 0.5% methylcellulose / 0.5% methylcellulose
Species/Strain:	Mice/Crl:CD1(1CR)
Number/Sex/Group:	16/sex/group main study group and 10/sex/group recovery group
Age:	6 weeks of age
Weight:	Males 24 g to 33 g and females 20 g to 26 g
Satellite groups:	13/sex/group for toxicokinetic study
Unique study design:	N/A
Deviation from study protocol:	N/A

Observations and Results

Mortality

Animals were observed twice daily. There were no deaths.

Clinical Signs

Animals were observed twice daily. The clinical signs were limited to high incidences of oily fur and dehydration in males treated with 20 mg/kg/day linaclotide or spiked 20 mg/kg/day linaclotide, compared to the control group. The results are shown in the table below (taken from the study report).

Text Table 5 Linaclotide or Spiked Linaclotide-Related Clinical Observations – Day 1 to Day 29

Dose (mg/kg/day):	0		20		20[a]	
Sex:	M	F	M	F	M	F
Dehydration	2 (4)	-	6 (28)	-	11 (34)	-
Oily fur	1 (3)	-	8 (20)	-	8 (21)	-

a spiked linaclotide
A dash (-) indicates absence of finding in group
Data are expressed as the number of animals affected (total number of occurrences/group)

The clinical signs continued to be noted during the recovery period, although at a lower incidence. The results are shown in the table below (taken from the study report).

Text Table 6 Linaclotide or Spiked Linaclotide-Related Clinical Observations – Day 30 to Day 43

Dose (mg/kg/day):	0		20		20[a]	
Sex:	M	F	M	F	M	F
Dehydration	1 (2)	-	2 (4)	-	2 (4)	-
Oily fur	1 (2)	-	3 (5)	-	4 (7)	-

There were no other drug-related clinical signs.

Body Weights

Body weights were measured on the day of randomization and weekly starting on the last week of the pretreatment period and continuing throughout the treatment and recovery periods. In addition, animals were weighed (fasted) before scheduled necropsy.

Mean body weight was increased by 6% in the linaclotide-treated females on week 2, and by 9% in the spiked linaclotide-treated females on week 1, compared to the control females (statistically significant in both groups). This trend continued until the end of the dosing period, although the differences were not statistically significant. The changes in body weights correlated with a statistically significant higher mean body weight gain in linaclotide on week 2 and spiked linaclotide treated females on week 1, 43% and 47%, respectively. However, there were no differences in the body weight or bodyweight gain between the linaclotide and spiked linaclotide groups. The data are presented in the graphs below (taken from the study report).

Figure 1 **Group Mean Body Weights - Males**

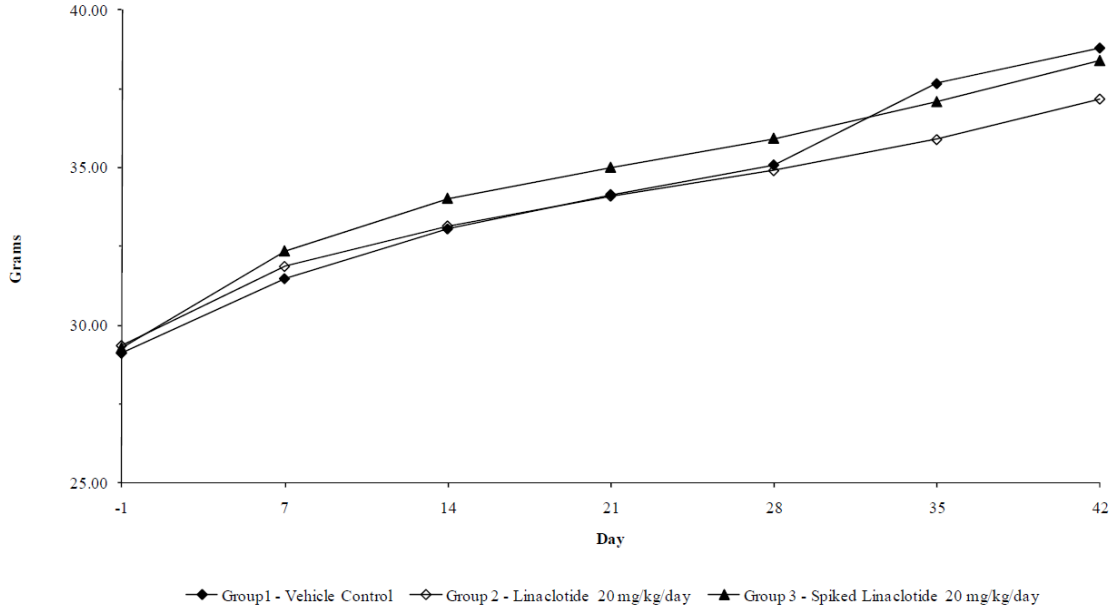
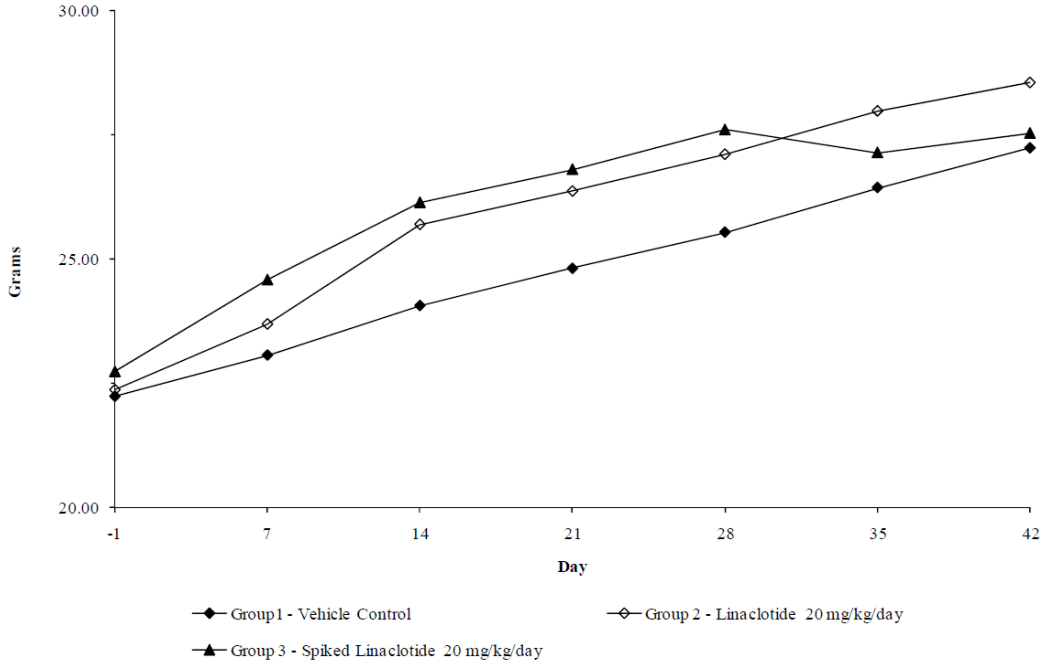


Figure 2 **Group Mean Body Weights - Females**



During the recovery period, there were no differences in mean body weights; however, mean absolute bodyweight gains were lower, 50% and 52%, respectively, for females receiving 20 mg/kg/day linaclotide or spiked 20 mg/kg/day linaclotide.

Feed Consumption

Food consumption was measured weekly starting the last week of the pretreatment period and throughout the treatment and recovery periods.

Statistically significant increases in food consumption (9 to 12%) were observed in females treated with spiked linaclotide 20 mg/kg/day from day -7 to day 8, compared to controls.

Ophthalmoscopy

Funduscopic and biomicroscopic examinations were performed once prior to the start of treatment (all animals) and during week 4 (excluding toxicokinetic animals). There were no linaclotide- or spiked linaclotide-related ocular changes.

ECG – Not performed

Hematology

Blood samples were collected from all main study and recovery animals at necropsy on day 29 and 43, respectively. The hematologic parameters included red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, platelet count, red cell distribution width, mean platelet volume, white blood cell count (total, absolute and percent differential), blood cell morphology, and reticulocyte count (absolute and percent).

There were no meaningful significant changes in any of the hematologic parameters examined between the control group, the 20 mg/kg/day linaclotide group, and the spiked 20 mg/kg/day linaclotide group.

Clinical Chemistry

Blood samples were collected from all main study and recovery animals at necropsy on day 29 and 43, respectively. The parameters measured included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, urea nitrogen, creatinine, calcium, phosphorus, total protein, albumin, globulin, albumin/globulin ratio, glucose, cholesterol, sodium, potassium, and chloride.

There were no meaningful significant changes in any of the clinical chemistry parameters examined between the control group, the 20 mg/kg/day linaclotide group, and the spiked 20 mg/kg/day linaclotide group.

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Urinalysis – Not performed

Gross Pathology

Necropsies were conducted at sacrifice (days 29 and 43). There were no linaclotide or spiked linaclotide-related gross pathological changes.

Organ Weights

The following organs were collected on the day of sacrifice (days 29 and 43): adrenal, glands, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary, prostate, spleen, salivary gland, thymus, thyroid lobes (with parathyroids), and uterus. Paired organs were weighed together and organ weight ratios relative to body and brain weights were calculated.

A statistically significant increase (9%) in absolute kidney weight was observed in the spiked 20 mg/kg/day linaclotide female group. The change was small in magnitude, occurred in a single gender, and was not accompanied by histopathology findings. There were no other significant linaclotide- or spiked linaclotide-related organ weight changes, compared to the control group, as measured by either absolute or relative (to brain or to terminal bodyweight) weights.

Histopathology

The following tissues and organs were examined (table taken from the study report):

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Tissue	Weight	Collect	Microscopic Evaluation	Comment
Bone marrow smear	-	X	-	Bone marrow smears (2) will be collected from the femur at scheduled necropsies only (for possible examination).
Bone marrow, femur	-	X	X	Collect with bone, femur, decalcified before sectioning.
Bone marrow, sternum	-	X	X	Collect with bone, sternum, decalcified before sectioning.
Bone, femur	-	X	X	Collect distal end to include femoral tibial joint, decalcified before sectioning.
Bone, sternum	-	X	X	Decalcified before sectioning.
Brain	X	X	X	Forebrain, midbrain, cerebellum, and medulla oblongata
Cervix	-	X	X	Collect with uterus.
Epididymides	-	X	X	Fixed in modified Davidson's fluid (euthanized animals only). Paired examination.
Esophagus	-	X	X	-
Eyes	-	X	X	Paired examination; Fixed in Davidson's fixative (euthanized animals only).
Gallbladder	-	X	X	-
Glands, adrenal	X	X	X	Paired weight and examination.
Glands, lacrimal	-	X	X	Paired examination
Gland, mammary	-	X	X	Collect with inguinal skin; Process females only.
Gland, parathyroid	-	X	X	Collect with thyroid; Examine only if present in the routine section of thyroid.
Gland, pituitary	X	X	X	-
Gland, prostate	X	X	X	-
Gland, salivary[a]	X	X	X	Mandibular/sublingual; Only 1 required for examination.
Glands, seminal vesicle	-	X	X	Paired examination
Glands, thyroid	X	X	X	Paired weight and examination; weight includes parathyroid.
Gross lesions/masses	-	X	X	-
Heart	X	X	X	-
Kidneys	X	X	X	Paired weight and examination
Large intestine, cecum	-	X	X	-
Large intestine, colon	-	X	X	-
Large intestine, rectum	-	X	X	-
Larynx	-	X	X	One level examined.
Liver	X	X	X	Sample of 2 lobes
Lung	X	X	X	Infuse with 10% neutral buffered formalin after weighing. All lobes retained. Sample of 2 lobes examined.
Lymph node, mandibular	-	X	X	Only 1 required for examination.
Lymph node, mesenteric	-	X	X	-
Muscle, skeletal	-	X	X	From thigh
Nerves, optic	-	X	X	Fixed in Davidson's fixative (euthanized animals only); Examine only if present in the routine section of the eye.
Nerve, sciatic	-	X	X	Only 1 required for examination.

Tissue	Weight	Collect	Microscopic Evaluation	Comment
Ovaries with oviducts	X	X	X	Paired weight and examination
Pancreas	-	X	X	-
Skin	-	X	X	Inguinal; Collect with mammary gland.
Small intestine, duodenum	-	X	X	-
Small intestine, jejunum	-	X	X	-
Small intestine, ileum	-	X	X	-
Spinal cord	-	X	X	Cervical, thoracic, lumbar.
Spleen	X	X	X	-
Stomach	-	X	X	Glandular and nonglandular regions.
Testes	X	X	X	Paired weight and examination; Fixed in Modified Davidson's fixative (euthanized animals only).
Thymus	X	X	X	-
Tongue	-	X	X	-
Trachea	-	X	X	-
Urinary bladder	-	X	X	-
Uterus	X	X	X	Horns and body
Vagina	-	X	X	-

Adequate Battery - Yes

Peer Review - No

Histological Findings

Main Study

Esophagus: Focal muscular regeneration (1/16 M) and diffuse chronic inflammation (1/16 M) were observed in the spiked 20 mg/kg/day linaclotide group.

Prostate: Focal lymphocytic inflammation (1/16 M) was observed in the spiked 20 mg/kg/day linaclotide group.

Salivary gland: Multifocal lymphocytic infiltration (1/16 F) was observed in the spiked 20 mg/kg/day linaclotide group.

Thyroid: Focal follicular cyst (1/16 M) and multiple follicular cyst (1/16 M) was observed in the 20 mg/kg/day linaclotide group.

Larynx: Diffuse chronic inflammation (1/16 M) was observed in 20 mg/kg/day linaclotide group.

Liver: Multifocal subacute inflammation (1/16 M) and focal lymphocytic infiltration (1/16 M) were observed in the spiked 20 mg/kg/day linaclotide group.

Lung: Focal acute hemorrhage was observed in the 20 mg/kg/day linaclotide group (2/16 M) and in the spiked 20 mg/kg/day linaclotide group (1/16 M). Multifocal perivascular lymphocytic infiltration (1/16 F) was observed in the 20 mg/kg/day linaclotide group.

Lymph node, mandibular: Diffuse lymphocytic hyperplasia (1/14M) and focal acute hemorrhage (1/13 F) were observed in the spiked 20 mg/kg/day linaclotide group.

Lymph node, mesenteric: Diffuse lymphoid hyperplasia (1/16 F) was observed in the 20 mg/kg/day linaclotide group.

Ovaries: Unilateral paraovarian cyst (2/16 F) was observed in the 20 mg/kg/day linaclotide group, and focal luteal cyst (1/16 F) was observed in the spiked 20 mg/kg/day linaclotide group.

Oviducts: Focal mineralization (1/16 F) was observed in the 20 mg/kg/day linaclotide group.

Spleen: Diffuse lymphoid depletion was observed in the 20 mg/kg/day linaclotide group (1/16 F) and in the spiked 20 mg/kg/day linaclotide group (1/16 F).

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Stomach: Focal lymphohistiocytic inflammation (1/16 M) was observed in the spiked 20 mg/kg/day linaclotide group.

Urinary bladder: Multiple submuscosal lymphocytic infiltration (1/16 F) was observed in the 20 mg/kg/day linaclotide group.

Recovery group
There were no significant microscopic findings.

The microscopic lesions generally occurred at very low frequencies. The relation of these lesions to drug treatment is uncertain because only a single dose was examined, and dose-dependent effects cannot be discerned. There were no apparent differences in the toxicity profile of linaclotide and linaclotide spiked with (b) (4) and (b) (4) Thus, these impurities do not appear to have significant toxicity in mice at the doses tested.

Special Evaluation – N/A

Toxicokinetics

Blood samples were collected on days 1 and 28 from 3 animals/sex at 0.5 hour after dosing. The samples were analyzed for linaclotide and the active metabolite, MM-419447. On day 1, the average concentration of linaclotide was 4.63 ng/ml in the 20 mg/kg/day linaclotide mice, and there was no measurable amount of MM-419447 (LLQQ was 5 ng/ml). In the spiked 20 mg/kg/day linaclotide mice, the average concentration of linaclotide was 4.09 ng/ml, and there was no measurable amount of MM-419447. On day 28, in the 20 mg/kg/day linaclotide mice, the average concentration of linaclotide was 5.15 ng/ml, and there was no measurable amount of MM-419447 in 5 of the 6 animals. In the spiked 20 mg/kg/day linaclotide mice, the average concentration of linaclotide was 4.09 ng/ml, and there was no measurable amount of MM-419447.

Thus, systemic exposure of linaclotide was very low, and the active metabolite, MM-419447, was undetectable in mice treated with 20 mg/kg/day linaclotide or spiked 20 mg/kg/day linaclotide. The impurities, (b) (4) and (b) (4) do not appear to alter the systemic exposure of linaclotide or the activate metabolite MM-419447.

Dosing Solution Analysis

The study samples had mean concentrations within the acceptance criteria of ±15% of their theoretical concentrations, with the exception of (b) (4) in the group 3 samples prepared for week 1 (mean: 142%; mean of location: 142% (top), 141% (middle), 143% (bottom)) and (b) (4) in the group 3 samples prepared for week 4 (mean: 135%). In the homogeneity test, the relative standard deviation (RSD) of the

concentrations for each group was ≤ 5%. Acceptance criteria with respect to homogeneity were met. Therefore, the dose formulations were within concentration specification, with the exception of (b) (4) in the group 3 samples prepared for week 1 and week 4. Homogeneity testing showed that the formulation technique used produces homogenous preparations.

7 Genetic Toxicology

The following studies were previously reviewed by David B. Joseph, Ph.D. (see Pharmacology/Toxicology review of IND 63,290 dated January 5, 2006).

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

Effect of MD-1100 Acetate on the *Salmonella-Escherichia coli*/Mammalian-Microsome Reverse Mutation Assay with a Confirmatory Assay

Key Findings: negative

Study # 7480-117 (25809-0-409OECD)

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Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: March 4, 2004 (final report dated September 1, 2004)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure

Vehicle: phosphate buffered saline

Methods: The mutagenic potential of MD-1100 acetate was evaluated using the Ames test (Ames et al., Mutation Res, 31, 1975). The histidine-dependent *Salmonella typhimurium* strains TA-98, TA-100, TA-1535, and TA-1537, as well as the tryptophan-dependent *E. coli* WP2*uvr*A strain were tested. A dose range-finding assay was performed on strains TA-100 and WP2*uvr*A using dose levels of 6.67, 10, 33.3, 66.7, 100, 333, 667, 1000, 3330, and 5000 μg/plate MD-1100 acetate, in the absence or presence of S9 liver fraction (1 plate/dose). The plate incorporation method was used for this assay. The plates were incubated for 52 ± 4 hr at 37 ± 2°C. Toxicity was

evaluated based on appearance of the background bacterial lawn and the revertant colony count. In the mutagenicity assay, all bacterial strains were incubated with 33.3, 100, 333, 1000, 3330, and 5000 μg/plate MD-1100 acetate, in the absence or presence of S9 liver fraction (3 plates/dose). The mutagenicity assay was performed twice using the plate incorporation method, and the same incubation conditions as described above. The number of revertant colonies was counted by an automated colony counter or by hand. The positive control compounds that were used in the main assay are listed in the following table.

Tester Strain	- S9	+ S9
TA-98	2-nitrofluorene,1 μg/plate	benzo[a]pyrene, 2.5 μg/plate
TA-100	sodium azide, 2 μg/plate	2-aminoanthracene, 2.5 μg/plate
TA-1535	sodium azide, 2 μg/plate	2-aminoanthracene, 2.5 μg/plate
TA-1537	ICR-191, 2 μg/plate	2-aminoanthracene, 2.5 μg/plate
E. coli WP2*uvr*A	4-nitroquinoline-N-oxide,1 μg/plate	2-aminoanthracene, 25 μg/plate

The vehicle used for the positive control compounds was not stated. The S9 liver fraction was prepared from male Sprague Dawley rats treated with 500 mg/kg i.p. Aroclor 1254 (S9 was purchased from a commercial vendor). PBS, the solvent used for MD-1100 acetate, was added to the negative control cultures. The criteria for a valid assay were the following: demonstration of the *rfa* mutation (crystal violet sensitivity) in strains TA-98, TA-100, TA-1535, and TA-1537; demonstration of the pKM101 plasmid presence (ampicillin resistance) in strains TA-98 and TA-100; number of spontaneous revertants was within the historical control range; cell density in cultures was at least 0.5 x 10^9/ml prior to use in the assay; positive control compounds produced an increase of at least 3-fold in the number of revertants, compared to the vehicle control plates; a minimum of at least three non-toxic doses were available for evaluation of mutagenicity. The criterion for a positive result in the TA-98, TA-100, and WP2*uvr*A strains was a dose-dependent increase of at least 2-fold in the revertant colony count. The criterion for a positive response in the TA-1535 and TA-1537 strains was a dose-dependent increase of at least 3-fold in the revertant colony count.

Results:

Study validity: The study methods used and the criteria for a positive result were adequate. All criteria for a valid study were fulfilled.

Study outcome: MD-1100 acetate produced no toxicity in the dose range-finding and mutagenicity assays. The drug tested negative in the range-finding and mutagenicity assays.

Conclusions: MD-1100 acetate was not mutagenic under the study conditions.

Addendum: The table below taken from the Sponsor's report has been added to summarize the results of the study.

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	Dose/Plate	TA98		TA100		TA1535		TA1537		WP2*uvr*A		Back-ground Lawn[a]
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Microsomes: Rat Liver												
Vehicle Control		36	8	98	11	9	1	6	3	21	7	N
Test Article	33.3 µg	36	6	109	19	9	4	5	4	18	7	N
	100 µg	28	3	112	7	11	3	10	2	22	5	N
	333 µg	24	4	113	2	14	2	7	1	20	5	N
	1000 µg	26	6	105	16	13	1	8	3	23	5	N
	3330 µg	25	7	107	6	11	2	5	1	18	5	N
	5000 µg	28	12	98	12	11	5	4	2	17	2	N
Positive Control[b]		381	93	1030	94	133	1	162	11	245	16	N
Microsomes: None												
Vehicle Control		11	3	97	12	16	2	8	5	18	4	N
Test Article	33.3 µg	16	4	97	17	26	7	7	1	15	8	N
	100 µg	22	2	89	12	17	1	7	1	19	3	N
	333 µg	12	3	84	10	14	4	10	1	16	2	N
	1000 µg	20	3	82	15	24	2	8	2	16	4	N
	3330 µg	18	2	72	7	23	6	11	2	20	7	N
	5000 µg	15	5	91	9	23	3	11	1	14	3	N
Positive Control[c]		335	8	1183	134	833	13	1184	83	393	32	N

[a] Background Lawn Evaluation Codes:
N = normal R = reduced O = obscured A = absent P = precipitate

[b] TA98	benzo[a]pyrene	2.5 µg/plate	[c] TA98	2-nitrofluorene	1.0 µg/plate
TA100	2-aminoanthracene	2.5 µg/plate	TA100	sodium azide	2.0 µg/plate
TA1535	2-aminoanthracene	2.5 µg/plate	TA1535	sodium azide	2.0 µg/plate
TA1537	2-aminoanthracene	2.5 µg/plate	TA1537	ICR-191	2.0 µg/plate
WP2*uvr*A	2-aminoanthracene	25.0 µg/plate	WP2*uvr*A	4-nitroquinoline-N-oxide	1.0 µg/plate

Study title: Bacterial Reverse Mutation Assay (Effects of the Impurities)	
Study no.:	MNP-103-068 (AD12MN-MP.503.BTL)
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/10/2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	(b) (4) X10025A1, and 89.2% peptide content (b) (4) Y06063A1, and 91.5% peptide content

Key Study Findings: This study was conducted to support the qualification of the impurities (b) (4) ((b) (4) degradant of linaclotide) and (b) (4) (b) (4)-

(b) (4) degradant of linaclotide). (b) (4) and (b) (4) were negative in the bacterial reverse mutation assay.

Methods	
Strains:	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537, and *Escherichia coli* WP2 *uvr*A
Concentrations in definitive study:	50, 150, 500, 1500, and 5000 μg per plate
Basis of concentration selection:	Negative findings in the initial toxicity/ mutation assay; neither precipitate nor background lawn toxicity was observed at the maximum dose tested (5000 μg/plate).
Negative control:	50 mM sodium phosphate buffer, pH 6.5
Positive control:	In the absence of metabolic activation: sodium azide (SA, 1.0 µg/plate) for TA1535 and TA100, 2-nitrofluorene (2NF, 1.0 µg/plate) for TA98, 9-aminoacridine (9AAD, 75 µg/plate) for TA1537, and methyl methanesulfonate (MMF, 1000 μg/plate) for WP2 *uvr*A. In the presence of metabolic activation: 2-aminoanthracene (AA, 1.0 μg/plate) for TA98, TA1535, and TA1537; 2.0 μg/plate AA for TA100; 10 μg/plate AA for WP2 *uvr*A
Formulation/Vehicle:	(b) (4) and (b) (4) were dissolved in 50 mM sodium phosphate buffer, pH 6.5 / 50 mM sodium phosphate buffer, pH 6.5
Incubation & sampling time:	The plate incorporation method was used. Aroclor 1254-induced rat liver S9 (10%) was used as the metabolic activation system. The plates (triplicate in the confirmation assay) were incubated for approximately 48 to 72 hours at 37±2°C. The condition of the bacterial background lawn was evaluated for evidence of test article toxicity, and precipitate was evaluated after the incubation period by visual examination. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted either entirely by automated colony counter or entirely by hand unless the plate exhibited toxicity.

Study Validity: Selection of bacterial tester strains was appropriate. The positive control compounds produced the expected responses. Dose selection for the plate incorporation method was adequate based upon use of the limit dose (i.e., 5000 µg/plate). Neither precipitate nor background lawn toxicity was observed at the maximum dose of 5000 µg/plate. The S9 concentration (10%) was within acceptable limits.

Results:

In the initial toxicity-mutation assays, the dose levels used were 1.5, 5.0, 15, 50, 150, 500, 1500, and 5000 µg per plate. No positive mutagenic responses were observed with any of the tester strains in the presence or absence of S9 activation for either (b) (4) or (b) (4) However, a reduction in revertant counts was observed at 5000 µg per plate with tester strain TA1537 in the presence of S9 activation for both (b) (4) and (b) (4)

In the confirmatory mutagenicity assays, no positive mutagenic responses were observed with any of the tester strains in the presence or absence of S9 activation for either (b) (4) or (b) (4) Results of the confirmatory mutagenicity assay are summarized in the tables below.

		Strain				
Article	**Dose**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2*uvr*A**
		-S9				
(b) (4)	5000 µg	0.9	1.1	0.8	0.3	0.6
	1500 µg	0.9	1.1	1.0	0.6	0.8
	500 µg	1.1	1.1	1.8	0.7	0.8
	150 µg	0.6	1.1	1.3	1.2	0.7
	50 µg	0.8	1.0	1.6	1.1	0.8
		+S9				
	5000 µg	0.8	0.9	1.0	0.7	0.8
	1500 µg	0.8	0.9	0.9	0.8	1.0
	500 µg	1.0	1.0	0.8	0.7	0.6
	150 µg	1.0	1.0	1.1	0.6	1.0
	50 µg	1.1	1.0	1.1	0.7	1.1
		-S9				
2NF	1 µg	10.1				
SA	1 µg		4.3			
SA	1 µg			27.2		
9AAD	75 µg				49.2	
MMS	1000 µg					4.5
				+S9		
2AA	1.0 µg	7.9				
2AA	2.0 µg		4.6			
2AA	1.0 µg			9.2		

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2AA	1.0 µg				4.7	
2AA	10.0 µg					7.7

Values are the treatment/negative control ratios of revertants.

		Strain				
		-S9				
Article	**Dose**	**TA98**	**TA100**	**TA1535**	**TA1537**	**WP2*uvr*A**
(b) (4)	5000 µg	1.3	1.0	1.1	1.0	0.7
	1500 µg	1.3	1.2	0.7	1.0	0.6
	500 µg	1.1	1.0	1.0	1.4	0.9
	150 µg	1.3	1.2	0.8	1.4	0.9
	50 µg	1.0	0.9	1.1	1.1	0.7
		+S9				
	5000 µg	1.4	1.0	1.1	1.0	1.0
	1500 µg	1.6	1.1	0.9	0.8	0.9
	500µg	1.7	1.2	1.2	1.0	1.0
	150 µg	1.3	1.1	1.0	1.1	1.0
	50µg	1.5	1.1	1.2	0.7	1.2
		-S9				
2NF	1 µg	12.9				
SA	1 µg		4.2			
SA	1 µg			19.8		
9AAD	75 µg				101.1	
MMS	1000 µg					4.5
		+S9				
2AA	1.0 µg	15.5				
2AA	2.0 µg		6.1			
2AA	1.0 µg			12.9		
2AA	1.0 µg				6.9	
2AA	10.0 µg					11.6

Values are the treatment/negative control ratios of revertants.

In summary, (b) (4) and (b) (4) were negative in the bacterial reverse mutation assay, either in the presence or absence of metabolic activation.

7.2 *In Vitro* Assays in Mammalian Cells

Effect of MD-1100 Acetate on Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

Note: This study was submitted as a draft report.

Key Findings: negative

Study #: 7480-118 (25809-0-449OECD)

Vol. 9, Pg. 34

Conducting Laboratory and Location: (b) (4)

Date of Study Initiation: February 25, 2004 (report dated December 3, 2004)

GLP Compliance: A statement of compliance was included.

QA Report: yes (x) no ( )

Drug: lot # PPL-MD11000401; 99.08% pure
Vehicle: phosphate buffered saline

Methods: Peripheral blood lymphocytes were obtained from healthy human donors. Lymphocyte cultures were initiated by adding 0.6 ml of blood to 9.4 ml RPMI 1640 medium supplemented with 25 mM HEPES, 20% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 2% phytohemagglutinin M. The cultures were incubated at 37°C in 5% CO_2/air mixture for two days. No preliminary toxicity assay was performed. In the initial assay, lymphocytes were treated with 6.78, 9.69, 13.8, 19.8, 28.2, 40.4, 57.6, 82.4, 118, 168, 240, 343, 490, 700, or 1000 μg/ml MD-1100 acetate for 3 hr in the absence or presence of S9 liver fraction (duplicate cultures). The control cultures included a negative control (culture medium) and a vehicle control (duplicate cultures). Dose selection was limited by the poor stability of MD-1100 acetate at concentrations above 10 mg/ml, thus limiting the high dose to 1000 μg/ml. Toxicity was evaluated by determination of the mitotic index. Cells that were treated with 343, 490, 700, or 1000 μg/ml, with and without S9, were selected for assessment of chromosomal aberrations. In the confirmatory assay, lymphocytes were incubated with 50, 100, 200, 300, 400, 500, 650, 800, or 1000 μg/ml MD-1100 acetate for 19 hr in the absence of S9 liver fraction, or with 300, 400, 500, 650, 800, or 1000 μg/ml for 3 hr in the presence of S9 (duplicate cultures). Cells that were treated with 500, 650, 800, or 1000 μg/ml in the 19-hour treatment assay without S9 were selected for assessment of chromosomal aberrations. In the 3-hour treatment assay with S9, cells that were treated with 500, 650, 800, or 1000 μg/ml were selected for assessment of chromosomal aberrations. Mitomycin C was used as a positive control in the absence of S9 (1 μg/ml in the 3-hour treatment, 0.3 μg/ml in the 19-hour treatment, duplicate cultures). Cyclophosphamide (25 μg/ml) was used as a positive control in the presence of S9 in the 3-hour treatment assay (duplicate cultures). The incubation procedures for the 3-hour and 19-hour treatments are described in the following table.

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Without S9			
1. 3 hr incubation with test article or control articles	**2.** Cells were washed and resuspended in fresh medium; incubation was continued for 17 hr	**3.** 0.1 μg/ml Colcemid® was added to medium and incubation continued for 2 hr	**4.** Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations
1. 19 hr incubation with test article or control articles	**2.** Cells were washed and resuspended in medium containing 0.1 μg/ml Colcemid®; incubation was continued for 2 hr	**3.** Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations	
With S9			
1. 3 hr incubation with test article or control articles	**2.** Cells were washed and resuspended in fresh medium; incubation was continued for 17 hr	**3.** 0.1 μg/ml Colcemid® was added to medium and incubation continued for 2 hr	**4.** Cells were harvested, fixed, mounted on slides, stained with 5% Giemsa solution, and analyzed for chromosomal aberrations

The mitotic index was calculated based on the number of mitotic cells per 1000 cells in each culture. Cells in metaphase (100/culture) were examined for chromosomal aberrations. The incidence of polyploid cells and endoreduplication per 100 metaphase cells was also determined. The S9 liver fraction was prepared from rats treated with 500 mg/kg i.p. Aroclor 1254 (S9 was purchased from a commercial vendor). The assay was considered as valid based on the following criteria: the incidence of cells with chromosomal aberrations in the negative and vehicle control cultures is less than 5%; the proportion of cells with aberrations in the positive control cultures is increased in a statistically significant manner ($p \leq 0.01$), relative to the vehicle control cultures; if the results are negative and no significant reduction (approximately 50% or more) in mitotic index occurs, the assay must include the highest applicable dose or a dose exceeding the solubility limit; at least three dose levels must be suitable for analysis. The criterion for a positive response was the following: a statistically significant increase ($p \leq 0.01$) in the number of cells with chromosomal aberrations at one or more dose levels, with evidence of a dose-response. The results were considered as negative if no significant

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increase in the number of cells with chromosomal aberrations occurred at any dose level, or equivocal if cells with aberrations were increased in a non-dose-dependent manner.

Results:

Study validity: The study methods used and the criteria for a positive result were adequate. The criteria for a valid study were fulfilled.

Study outcome: No toxicity was observed in the 3-hr treatment assays in the presence of S9. Treatment of cultures with 1000 μg/ml MD-1100 acetate for 3 hr in the absence of S9 produced a 16% reduction in mitotic index, relative to the vehicle control cultures. Mitotic index was unaffected at lower dose levels in the 3-hr treatment assay without S9. Furthermore, no evidence of toxicity was observed in the 19-hr treatment assay without S9, using similar dose levels.
MD-1100 acetate produced no significant increase in chromosomal aberrations.

Conclusions: MD-1100 acetate was not clastogenic under the study conditions.

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

None

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Study title: 104-Week Oral Carcinogenicity Study of Linaclotide Acetate in Rats	
Study no.:	MNP-103-029
Study report location:	Ironwood Pharmaceuticals, Inc.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MD1100 Acetate, PPL-MD11000602B, 94.7% relative to total peptide content MD1100 Acetate, PPL-MD11000801, 94.1% relative to total peptide content
CAC concurrence:	Yes (meeting minutes dated January 20, 2012)

Key Study Findings

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Dose levels of 0 (two vehicle control groups), 300, 1,000 and 3,500 μg/kg/day were tested in males and females. In general, there were no linaclotide-related effects on survival in males or females. Females in control group 1 had a statistically significant decrease in survival compared to control group 2. The test article-treated males had similar or increased survival rates when compared to control groups 1 and 2. However, the test article-treated females had lower survival rates when compared control group 2. The 300 μg/kg/day females had a statistically significant decrease in survival rate compared to control groups 1 and 2. There were no significant linaclotide-related clinical observations in animals that died before the end of the dosing period or in animals that were sacrificed at the end of the dosing period. The clinical observations that were noted occurred with low incidence, and in a low number of animals. The observed clinical signs were not interpreted as linaclotide-related since most were seen in the control groups, and did not occur in a dose-dependent manner.

Mean body weights of females in control group 2 were consistently higher compared to control group 1, however the mean body weights of males in control groups 1 and 2 were similar. There were minor decreases in mean body weight gains in the 300, 1,000 and 3,500 μg/kg/day females compared to the value from combined control groups, however the decreased mean body weight gain in females was not considered to be drug-related due to the relatively small magnitude of change. The mean food consumption in control group 2 females was consistently higher than control group 1, and there were sporadic increases in food consumption in the 300, 1,000, and 3,500 μg/kg/day males, however there were no linaclotide-related effects on mean food consumption in males or females. There were minor changes in hematology parameters in the 1,000 μg/kg/day males and females, however there were no dose-dependent increases noted. Therefore, there were no drug-related effects on the hematology parameters evaluated.

The macroscopic changes observed in the study were not dose-dependent and only noted in a small number of animals, therefore there were no drug-related macroscopic findings. The microscopic examination showed minor non-neoplastic changes in linaclotide-treated animals. In males, minimal/mild focal cortical hyperplasia in the adrenal glands was noted in 27% of the males in the 3,500 μg/kg/day group sacrificed at the end of the dosing period compared to 12% of the control males sacrificed at the end of the dosing period. The other minor non-neoplastic changes observed were unrelated to dose, and occurred in only a few animals. The neoplastic changes included benign pituitary adenomas of the pars distalis in the pituitary gland, which were observed in 71% of the males in the 3,500 μg/kg/day group compared to 53% of the males in the control group. According to the Fisher exact test, the incidence of adenomas in the 3,500 μg/kg/day males was statistically significant when compared to the control groups. The Sponsor stated that the percent incidence of benign pituitary adenomas of the pars distalis ranges from 32.9 to 75.0% in historical control neoplastic data from 2-year carcinogenicity studies using male Sprague Dawley rats. Only the 1,000 μg/kg/day females had a higher percent incidence (80.0%) of benign pituitary adenomas than the historical control data.

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In addition, benign interstitial cell (Leydig cell) adenomas of the testes was observed in 4/70 males in the 3,500 μg/kg/day group compared to 0/140 control males. According to the FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012, there was a statistically significant (p=0.0049) dose response relationship in the incidence of the benign Leydig cell adenomas in male rats. However, according to the sponsor, the tumor incidence in the current study was within the range of historical control neoplastic data from 2-year carcinogenicity studies using Sprague Dawley rats (0.0-5.7%). In females, benign granular cell tumor in uterus with cervix was observed in 4/70 females in the 1,000 μg/kg/day group compared to 1/140 control females. According to the FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012, pair-wise comparisons of treated groups and the combined controls indicated there was a statistically significant (p=0.0482) increase in tumor incidence of benign granular cell tumor in uterus with cervix in the 1,000 μg/kg/day group when compared to the combined control for the female rats. There were no drug-related neoplastic findings observed in male or female rats, based on Dr. Nagem's statistical analysis (i.e. failure to achieve statistical significance in both pairwise and trend tests).

The Kaplan-Meier survival analysis indicated that there were no statistically significant dose response relationships among treatment groups or differences between the combined vehicle control and any of the treated groups in survival across treatment groups in either male or female rats (see FDA statistical review by Mohamed Nagem, Ph.D. dated 2/07/2012).,

In summary, there were no significant drug-related effects on survival rates, clinical signs, masses noted clinically, body weights, food consumption, hematology, macroscopic observations, or microscopic findings (neoplastic and non-neoplastic).

Adequacy of Carcinogenicity Study

The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The test model used by the Sponsor was appropriate.

Evaluation of Tumor Findings

Benign interstitial cell (Leydig cell) adenomas of the testes (common tumor) were observed in 4/70 males (5.7%) in the 3,500 μg/kg/day group compared to 0/140 control males. Although statistical significance was achieved in the trend test, the study results were considered to be negative based on the following: the incidence in the 3500 μg/kg/day males was not significantly different from control (p=0.0175, α=0.01); the incidence of testicular interstitial adenoma in the 3500 μg/kg/day males (5.7%) was within the historical control range (0-5.7%); the p value in the trend test (0.0049) was

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extremely close to the designated significance level for common tumors (α=0.005); and there is no plausible mechanism for drug-related tumor induction, since no systemic exposure to the drug or its active metabolite would be expected at the doses used in this study. The Exec CAC noted that the study was adequate. The Exec CAC concluded that the study was negative for drug-related neoplasms (see meeting minutes dated January 20, 2012).

Table 24: Date and Site/Type of First Tumor Observed in a 2-year Carcinogenicity Study in Rats

	Male (Day/site of first tumor)	**Female (Day/site of first tumor)**
Cont 1	320/skin, malignant fibrosarcoma	410/mammary gland, malignant adenocarcinoma and pituitary gland, benign pars distalis adenoma (same animal)
Cont 2	360/pituitary gland, benign pars distalis adenoma	380/mammary gland, benign fibroadenoma
300 μg/kg	360/preputial glands, malignant lliposarcoma	303/ pituitary gland, benign pars distalis adenoma
1000 μg/kg	391/heart, benign schwannoma	301/ pituitary gland, benign pars distalis adenoma
3500 μg/kg	420/pituitary gland, benign pars distalis adenoma	238/head, malignant sarcoma

Methods	
Doses:	300, 1,000, and 3,500 μg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	0.5% methylcellulose in deionized water
Basis of dose selection:	Executive Carcinogenicity Assessment Committee recommendations. The Committee concurred with the Sponsor's proposed high dose of 3,500 μg/kg/day. However, the Committee recommended that the intermediate and low doses be changed to 1,000 and 300 μg/kg/day, respectively. The selection of the high dose was based on high multiples of the pharmacologically active dose.
Species/Strain:	Crl:CD® (SD)
Number/Sex/Group:	70
Age:	~8 weeks
Animal housing:	Animals were housed individually in suspended, stainless steel, wire-mesh, type cages
Paradigm for dietary restriction:	Meal Lab Diet® Certified Rodent Diet #5002, PMI Nutrition International, Inc.
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes (see study design below)
Deviation from study protocol:	

Table 25: Study Design (104-week Carcinogenicity Study in Rats)

Group	Dose Level (µg/kg/day)	Dose Concentration (mg/mL/dose)	Number of Animals					
			Initial		Clinical Pathology[a]		Necropsy	
			M	F	M	F	M	F
Main Study								
1	0 (Control 1)	0	70	70	70	70	70	70
2	0 (Control 2)	0	70	70	70	70	70	70
3	300	0.030	70	70	70	70	70	70
4	1000	0.100	70	70	70	70	70	70
5	3500	0.350	70	70	70	70	70	70
Sentinel[b]								
6	NA	NA	30	30	-	-	30	30

M = Male, F = Female

[a]Hematology evaluations will be conducted for all surviving main study animals prior to their scheduled necropsy and from all main study animals euthanized *in extremis*, if possible.

[b]Sentinel animals will be maintained in the study room, but not dosed, for health screen evaluations. Ten animals/sex will be randomly selected from the shipment of animals prior to study assignment. The remaining 20 animals/sex will be utilized throughout the study period.

Observations and Results

Mortality

All animals were observed twice a day for morbidity, mortality, injury, and availability of food and water through week 52. At week 53, a third mortality check was added in the evenings. There were no drug-related effects on survival in males or females. In general, males and females in control group 1 had a decrease in survival rate compared to control group 2. There was a statistically significant ($p<0.05$) decrease in survival rates of females in control group 1 compared to females in control group 2. The test article-treated males had similar or increased survival rates when compared to control groups 1 and 2. However, the test article-treated females had lower survival rates when compared control group 2. The 300 µg/kg/day females had a statistically significant ($p<0.05$) decrease in survival rate compared to control groups 1 and 2. The majority of deaths observed in all animals occurred after week 79 of the study. The survival rate at the end of the treatment period is presented in the Sponsor's table below.

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Table 26: % Survival in a 2-year Carcinogenicity Study in Rats

Dose Level (μg/kg/day)	Males	Females
0 (Control 1)	35.7	28.6
0 (Control 2)	41.4	47.1
Control 1 and 2	38.6	37.9
300	38.6	25.7
1000	42.9	35.7
3500	48.6	28.6
*Does not include animals that died during Week 105 prior to necropsy		

The Sponsor included a summary of probable causes of mortality in control and test article-treated animals. There was no difference in the probable causes of death between the control and test article-treated groups. In males, the Sponsor postulated that pituitary tumors were responsible for 32/83 deaths in control animals, and 12/44, 20/39, and 21/36 deaths in the 300, 1,000, and 3,500 μg/kg/day groups, respectively. In females, a significant number of deaths were caused by either mammary tumors or pituitary tumors. Mammary tumors were the probable cause of death in 20/87 control animals and 19/51, 16/44 and 14/51 deaths in the 300, 1,000, and 3,500 μg/kg/day females, respectively. In addition, pituitary tumors were the probable cause of death in 37/87 control animals and 25/51, 20/44 and 24/51 deaths in the 300, 1,000, and 3,500 μg/kg/day females, respectively.

According to the FDA statistical review by Mohamed Nagem, Ph.D. dated 2/07/2012, the Kaplan-Meier survival analysis indicated that there were no statistically significant dose response relationships among treatment groups or differences between the combined vehicle control and any of the treated groups in survival across treatment groups in both male and female rats.

Clinical Signs

A detailed clinical examination of each main study animal (non-sentinel) was conducted once a week. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, CNS effects (tremors, convulsions, reactivity to handling, bizarre behavior), and the palpation of masses (location, appearance, and size of the masses were documented).

Sparse hair was observed in control and test article-treated groups starting at week 3 in males and females. However, there was an increased incidence (≤ 24) of sparse hair in the 1,000 μg/kg/day males at weeks 9 to 15 and 21 to 39, when compared to control

animals. Sparse hair was observed in the 3,500 μg/kg/day group (12/48, 12/47, and 11/46 females compared to 6/105, 5/103, and 6/102 females in the control groups during weeks 80, 81 and 82, respectively). Black material around the eyes was noted in the 3,500 μg/kg/day females (4/28, 4/26, 4/22, 4/21, and 3/13 females during weeks 98, 101, 103,104, and 105 respectively, compared to none of the control females). An increased incidence of malocclusion was observed in the 1,000 μg/kg/day group from weeks 13 to 59 and weeks 68 to 76. Clinical signs in the 1000 μg/kg/day group included increased incidence of malocclusion (weeks 13 to 59 and weeks 68 to 76) and cut teeth (females only on week 63). Clinical signs in the 300 μg/kg/day males included increased incidence of plantar/palmar ulcers (5-20 mm, weeks 77, 78, and 83).

Swelling was observed in control and test article-treated males starting at approximately week 45 in males and week 57 in females. Swelling was noted in the 1,000 μg/kg/day females (5/66, 5/65, 6/65, 6/63, and 14/54 females during weeks 62, 63, 64, 65, and 81 respectively, compared to 1/127, 1/127, 3/127, 3/126, and 9/103 females in the control groups). In addition, swelling was noted in the 3,500 μg/kg/day females (11/57 and 12/57 females during weeks 72 and 73 respectively, compared to 6/117 and 7/117 females in the control groups). Nodules (>20 mm) were noted in the 1,000 μg/kg/day females (7/66, 7/65, 7/65, and 6/63 females during weeks 62, 63, 64, and 65 respectively, compared to 1/127, 1/127, 1/127, and 1/126 females in the control groups. At the end of the study period (week 105), smaller nodules (1-5 mm) were observed in 6/26 males in the 3,500 μg/kg/day group, compared to 2/38 males in the control groups.

Decreased activity was noted the 3,500 μg/kg/day males during week 72 in (4/65 males compared to 2/124 males in the control groups). In addition, impaired limb function was observed in the 3,500 μg/kg/day males (4/64 males during weeks 71 to 74, compared to 2/125 control males). In week 86, extended penis was noted in the 3,500 μg/kg/day group (4/54 males compared to 0/100 control males). Abrasions were observed in the 3,500 μg/kg/day males (4/62 and 4/61 males on weeks 80 and 81, respectively, compared to 1/112 control males).

In summary, there were modest increases in the incidence of specific clinical signs in test article-treated animals when compared to control animals during certain weeks of the study, as detailed above. However, the total number of animals exhibiting specific clinical signs was similar between the control and test-article treated groups, based on the cumulative incidence over the entire study. Therefore, there were no significant drug-related clinical observations.

There was no treatment-related effect on the incidence of masses. In general, the greatest increased incidence of masses was noted in the 300 and 1,000 μg/kg/day groups. The observed masses are described in the table below.

Table 27: Masses in a 2-year Carcinogenicity Study in Rats

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	Dose (μg/kg/day) (# of times observed/total number of animals affected)			
Mass (males)	**0***	**300**	**1,000**	**3,500**
Dorsal surface, med 2-3.9 cm	0/0	2/1	18/3	0/0
Dorsal surface, small 1-1.9 cm	1/1	3/1	20/2	0/0
Mass (females)				
Anogenital region, large ≥ 4 cm	42/7	25/3	116/9	1/1
Inguinal region/left, large ≥ 4 cm	90/10	12/1	99/7	33/3
Inguinal region/right, med 2-3.9 cm	34/7	74/12	64/8	36/6

* Combined control groups

Body Weights

Body weights for all main study animals were measured and recorded prior to randomization, 2 days before dosing, once weekly for the first 13 weeks, and once every 4 weeks for the remainder of the study. However, the Sponsor did not provide body weight data after week 101. The mean body weight in control group 2 females was consistently higher than control group 1. The increased mean body weights reached statistically significant levels between weeks 2 and 61. Overall, there were minor decreases in mean body weight gains in the 300, 1,000, and 3,500 μg/kg/day females compared to the combined control groups, however the decreased mean body weight gain in females was not considered to be drug-related because of the relatively small magnitude of change (≤10%). Thus, there were no test article-related effects on male or female body weights. The following table shows the absolute body weights (g) and body weight gains (g) for males and females.

Table 28: Body Weight Changes in a 2-year Carcinogenicity Study in Male and Female Rats

Males	**0 μg/kg/day (Control 1)**	**0 μg/kg/day (Control 2)**	**300 μg/kg/day**	**1,000 μg/kg/day**	**3,500 μg/kg/day**
Group	1	2	3	4	5
Wk 0 (g)	255.7	254.0	254.8	253.2	252.9
Wk 21 (g)	635.6	637.0	631.5	632.3	630.9
% of Control 1, Wk 21	100.0	100.2	99.4	99.5	99.3
ΔWk21-Wk0 (g)	379.9	383.0	376.7	379.1	378.0
BW Gain, % of Initial BW	148.6	150.8	147.8	149.7	149.5
BW Gain, % of Control 1	100.0	101.5	99.5	100.8	100.6

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Wk 53 (g)	795	786.3	776.4	782.6	781.8
% of Control 1, Wk 53	100.0	98.9	97.7	98.4	98.3
∆Wk53-Wk0 (g)	539.3	532.3	521.6	529.4	528.9
BW Gain, % of Initial BW	210.9	209.6	204.7	209.1	209.1
BW Gain, % of Control 1	100.0	99.4	97.1	99.1	99.2
Wk 77 (g)	826.6	814.3	833.1	835.9	830.9
% of Control 1, Wk 77	100.0	98.5	100.8	101.1	100.5
∆Wk77-Wk0 (g)	570.9	560.3	578.3	582.7	578
BW Gain, % of Initial BW	223.3	220.6	227.0	230.1	228.5
BW Gain, % of Control 1	100.0	98.8	101.7	103.1	102.4
Wk 101 (g)	793.2	778.2	790.2	828.6	808.3
% of Control 1, Wk 101	100.0	98.1	99.6	104.5	101.9
∆Wk101-Wk0 (g)	537.5	524.2	535.4	575.4	555.4
BW Gain, % of Initial BW	210.2	206.4	210.1	227.3	219.6
BW Gain, % of Control 1	100.0	98.2	100.0	108.1	104.5

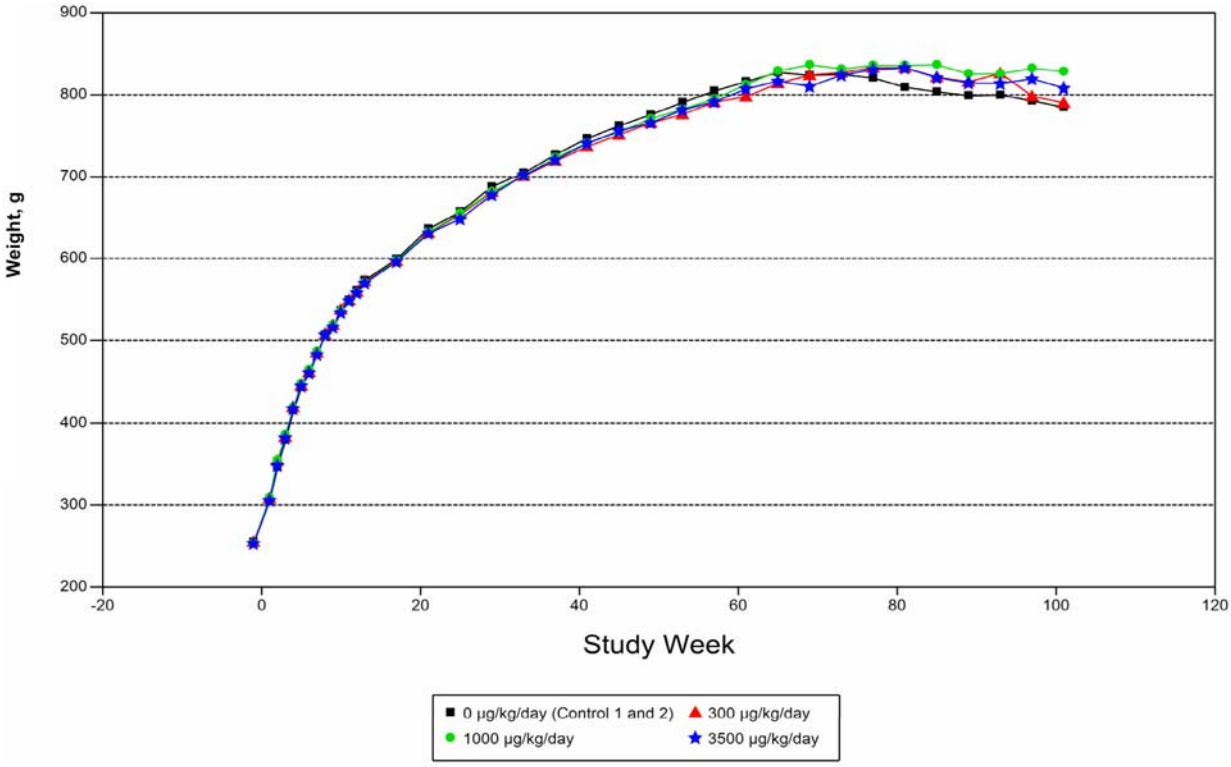
Females	**0 µg/kg/day (Control 1)**	**0 µg/kg/day (Control 2)**	**300 µg/kg/day**	**1,000 µg/kg/day**	**3,500 µg/kg/day**
Group	1	2	3	4	5
Wk 0 (g)	191.7	191.1	191.9	190.7	191.3
Wk 21 (g)	321.4	331.9*	327.5	319.2	325.5
% of Control 1, Wk 21	100.0	103.3	101.9	99.3	101.3
∆Wk21-Wk0 (g)	129.7	140.8	135.6	128.5	134.2
BW Gain, % of Initial BW	67.7	73.7	70.7	67.4	70.2
BW Gain, % of Control 1	100.0	108.9	104.4	99.6	103.7
Wk 53 (g)	416.6	493.3*	421.0	414.3	417.7

% of Control 1, Wk 53	100.0	118.4	101.1	99.4	100.3
∆Wk53-Wk0 (g)	224.9	302.2	229.1	223.6	226.4
BW Gain, % of Initial BW	117.3	158.1	119.4	117.3	118.3
BW Gain, % of Control 1	100.0	134.8	101.8	99.9	100.9
Wk 77 (g)	478.7	496.6	457.5	467	463.9
% of Control 1, Wk 77	100.0	103.7	95.6	97.6	96.9
∆Wk77-Wk0 (g)	287	305.5	265.6	276.3	272.6
BW Gain, % of Initial BW	149.7	159.9	138.4	144.9	142.5
BW Gain, % of Control 1	100.0	106.8	92.4	96.8	95.2
Wk 101 (g)	507.8	523.4	491.2	516.5	468.1
% of Control 1, Wk 101	100.0	103.1	96.7	101.7	92.2
∆Wk101-Wk0 (g)	316.1	332.3	299.3	325.8	276.8
BW Gain, % of Initial BW	164.9	173.9	156.0	170.8	144.7
BW Gain, % of Control 1	100.0	105.5	94.6	103.6	87.8

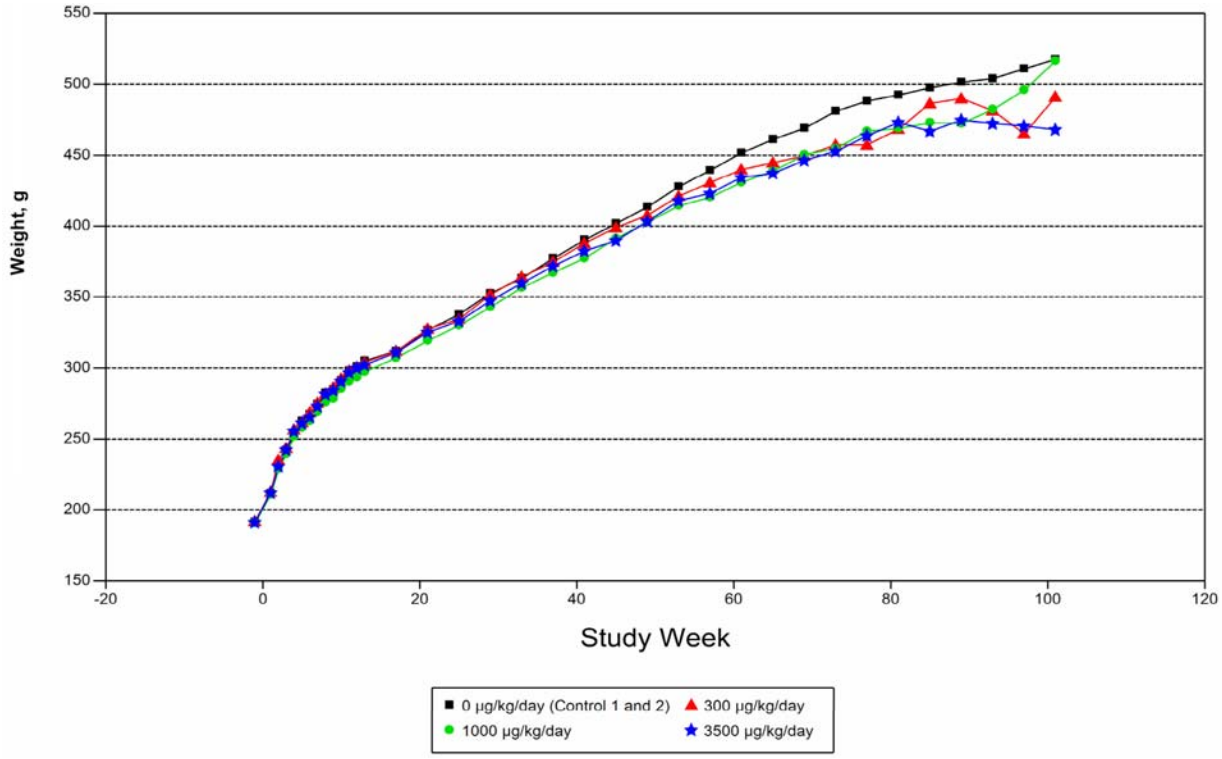
The figures below show mean body weights in males and females over the course of the study period (control groups 1 and 2 are combined in figures below).

Figure 1: Mean Body Weight Values in a 2-year Carcinogenicity Study in Male (A) and Female (B) Rats

A.


Weight, g
Study Week
0 µg/kg/day (Control 1 and 2)
300 µg/kg/day
1000 µg/kg/day
3500 µg/kg/day

B.

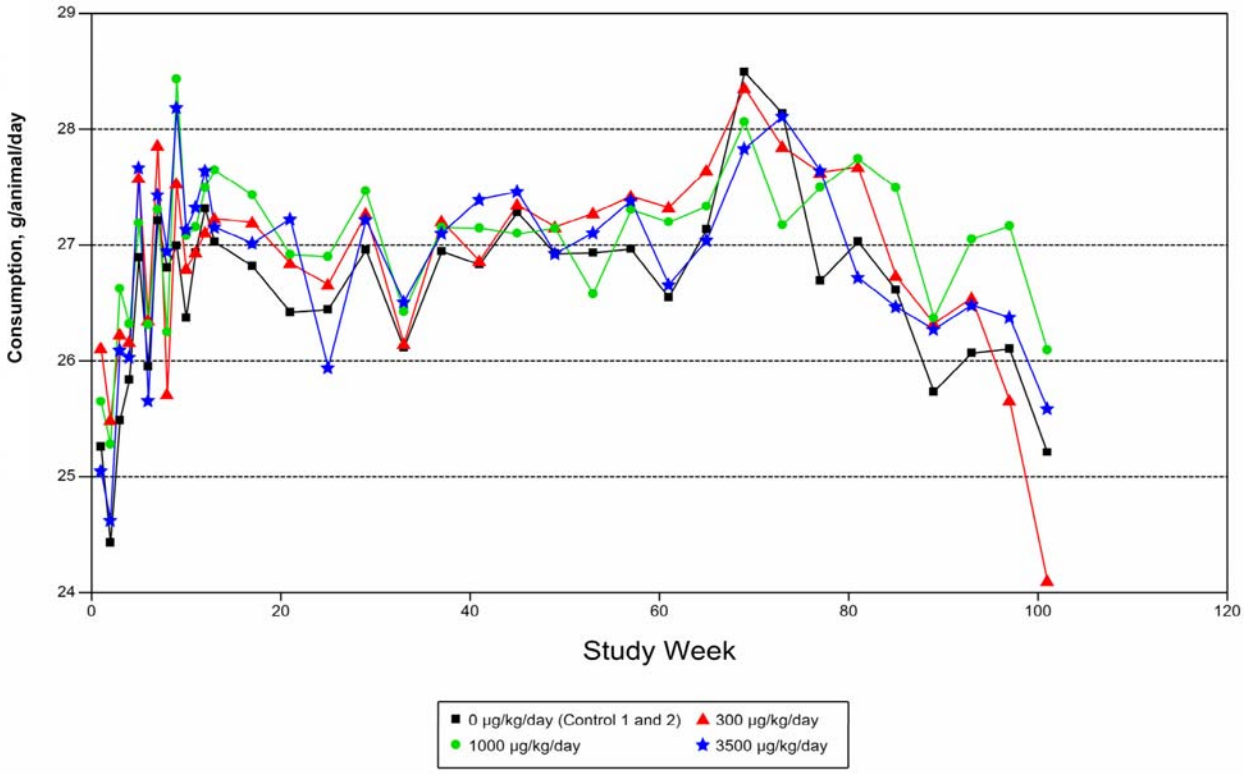

Weight, g
Study Week
0 µg/kg/day (Control 1 and 2)
300 µg/kg/day
1000 µg/kg/day
3500 µg/kg/day

Feed Consumption

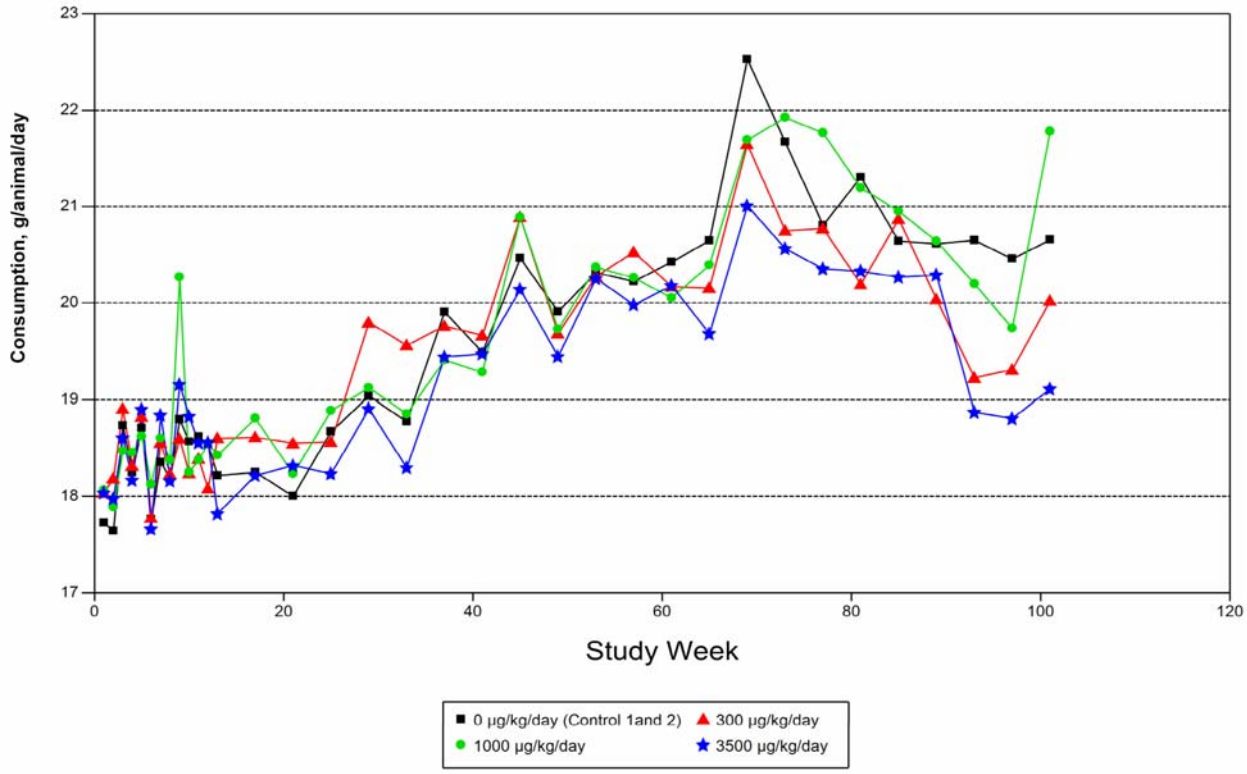
Food consumption was recorded for all main study animals once weekly for the first 13 weeks and then once every 4 weeks for the remainder of the study. The mean food consumption in weeks 1 and 101 in control males (combined groups 1 and 2) was 25.3 and 25.2 g/animal/day, respectively. The mean food consumption in weeks 1 and 101 in control females (combined groups 1 and 2) was 17.7 and 20.6 g/animal/day, respectively. The mean food consumption in control group 2 females was consistently higher than control group 1. There were sporadic increases in food consumption in the 300, 1,000, and 3,500 μg/kg/day males. There were no test article-related effects on food consumption. The figures below show mean food consumption in males and females over the course of the study period (control groups 1 and 2 are combined in figures below).

Figure 2: Mean Food Consumption Values in a 2-year Carcinogenicity Study in Male (A) and Female (B) Rats

A.



B.



Hematology

Blood samples were collected via cardiac puncture from animals euthanized *in extremis* if possible, and from all surviving animals at the scheduled terminal necropsy. The following parameters were analyzed: leukocytes, erythrocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and other cells. The 1,000 µg/kg/day males had a 19% increase in neutrophils and a 20% decrease in eosinophils, compared to the control groups. The 1,000 µg/kg/day females had a 15%, 17%, 23%, 35%, and 46% increase in leukocytes, neutrophils, monocytes, basophils, and other cells respectively, compared to the control groups. These effects are not considered to be drug-related, given the absence of dose-dependency.

Gross Pathology

Necropsy was performed on all animals, including those euthanized in moribund condition or found dead before the end of the dosing period. The animals were examined for palpable masses, and abnormalities were identified and correlated with postmortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities. The Sponsor provided a table with a full list of organs and tissues examined (see table below). There were minor macroscopic changes noted in some test article-treated animals. However, the changes were not dose-dependent, thus there were no drug-related macroscopic changes.

Adrenal glands: Moderate/severe tan discoloration was noted in 3/41 prematurely sacrificed[6] males in the 1,000 μg/kg/day group compared to 0/88 prematurely sacrificed control males. Moderate enlargement was observed in 4/43 prematurely sacrificed males in the 300 μg/kg/day group compared to 2/88 prematurely sacrificed control males.

Thoracic cavity: Mild adhesion was noted in 3/41 prematurely sacrificed males in the 1,000 μg/kg/day group compared to 0/88 prematurely sacrificed control males. Red fluid was observed in 3/41 prematurely sacrificed males in both the 1,000 and 3,500 μg/kg/day groups, compared to 1/88 prematurely sacrificed control males.

Kidney: Pelvic dilation was noted in 5/41 prematurely sacrificed males in the 1,000 μg/kg/day group compared to 2/88 prematurely sacrificed control males.

Lung: Red discoloration was noted in 4/41 prematurely sacrificed males in the 1,000 μg/kg/day group compared to 1/88 prematurely sacrificed control males.

Lymph node (mandibular): Mild/moderate enlarged lymph nodes were noted in 5/43 prematurely sacrificed males in the 300 μg/kg/day group compared to 1/88 prematurely sacrificed control males (severe enlargement observed in control animal).

Skin: A nodule was noted in 4/27 and 8/33 terminal sacrifice males in the 300 and 3,5000 μg/kg/day groups, respectively, compared to 1/52 terminal sacrifice control males.

Stomach: Swollen/thickened (nonglandular) surface was noted in 3/51 prematurely sacrificed females in the 3,500 μg/kg/day group compared to 0/88 prematurely sacrificed control females (severe enlargement observed in control animal).

Histopathology

The tissues listed in the Sponsor's table below were used for histopathological examinations from all main study animals. Minimal cardiomyopathy in the heart, minimal axonal/myelin degeneration of sciatic nerve, and moderate/severe lymphoid depletion in the thymus were observed in a majority of control and test article-treated males and females. A majority of males in all study groups exhibited harderian metaplasia in the lacrimal glands, and minimal/mild chronic progressive nephropathy. A majority of females in all groups exhibited mineralization of the kidneys, mild/moderate lobular hyperplasia of the mammary gland, minimal epithelial cell hyperplasia in the thymus gland, and acute inflammation of the liver. In addition, benign primary fibroadenoma was observed in many of the control and test article-treated females.

[6] "prematurely sacrificed" includes animals that were euthanized in moribund condition and those found dead before the end of the dosing period.

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Table 29: Organs Collected at Necropsy for Macroscopic and Microscopic Examination in a 2-year Carcinogenicity Study in Rats

- Adrenal (2)
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]
- Clitoral gland (2)
- Coagulating gland (2)
- Epididymis (2)
- Eye including optic nerve (2)
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2) and oviduct/fallopian tube (2)
  - testis (2)
- Gross lesions
- Harderian gland (2)
- Heart
- Joint, tibiofemoral
- Kidney (2)
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [3 sections collected; 2 examined]
- Lung [collected whole; 2 sections examined]
- Lymph nodes: mandibular, mesenteric, and regional where applicable
- Mammary gland
- Nose [levels a, b, c, and d]
- Pancreas
- Peyer's patch
- Pharynx
- Pituitary
- Preputial gland (2)
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen
- Thymus
- Thyroid/parathyroid (2)
- Tissue masses
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina
- Zymbal's gland (2)

[a]Bone marrow smears were collected at the scheduled necropsy and held.

(2) Paired organ

Peer Review

Yes

Neoplastic

The Sponsor stated that for the male tumor statistical analysis, the control groups were pooled for comparison to the test article-treated groups, and for female tumor analysis the control groups were compared individually to the test article-treated groups. The Sponsor stated that tumor incidence data was analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number

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of sites examined for each tumor type. The Fisher's exact test was used to compare each treatment group with the control group. The survival adjusted test will be conducted according to the prevalence/mortality methods described by Peto. Evaluation criteria (p-values of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%).

Benign pituitary adenomas of the pars distalis in the pituitary gland were observed in 50/70 males (71%) in the 3,500 μg/kg/day group compared to 74/140 (53%) in the control males. Benign pituitary adenomas of the pars distalis are considered common tumors. However, according to the Fisher exact test ($p<0.01$ for common tumors), the incidence of adenomas in the 3,500 μg/kg/day males was statistically significant ($p=0.0082$) when compared to the control groups. The Sponsor stated that the percent incidence of benign pituitary adenomas of the pars distalis ranges from 33 to 75% in historical control neoplastic data from 2-year carcinogenicity studies using male Sprague Dawley rats. Only the 1,000 μg/kg/day females had a higher percent incidence (80.0%) of benign pituitary adenomas of the pars distalis than historical control data.

Benign interstitial cell (Leydig cell) adenomas of the testes were observed in 4/70 males (5.7%) in the 3,500 μg/kg/day group compared to 0/140 control males. Interstitial cell adenomas of the testes are considered common tumors. According to the FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012, there was a statistically significant (p=0.0049) dose response relationship in the incidence of the benign Leydig cell adenomas in male rats. However, the tumor incidence in the current study was within the range of historical control neoplastic data from 2-year carcinogenicity studies using Sprague Dawley rats (0.0-5.7%).

In females, primary benign granular cell tumors in the vagina were noted in 5/70 females in the 3,500 μg/kg/day group compared to 2/139 control females. Additionally, primary benign thecoma in the ovaries was noted in 2/70 (2.9%) females in the 3,500 μg/kg/day group compared to 0/140 females in the control groups. Benign thecomas are considered rare tumors, therefore, according to the Peto test ($p<0.025$), the thecomas were statistically significant ($p=0.0189$) when compared to control group 2. However, the p-value for the Peto exact test ($p<0.025$) was not significant. Benign parathyroid adenomas (rare tumor) was observed in 4/52 (7.7%) parathyroid glands retrieved from the 300 μg/kg/day females compared to 3/118 (2.5%) parathyroid glands from the control females. Although there was no dose-related effect, according to the Fisher's exact test ($p<0.05$), the adenomas were statistically significant ($p=0.0436$) when compared to control group 2, but not when compared to control group 1. There was a slight increase in malignant histiocytic sarcomas (multicentric neoplasm) and primary benign granular cell tumor in the uterus (with cervix) of 1,000 μg/kg/day females (see table below). According to FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012, pair-wise comparisons of treated groups and the combined controls indicated there was a statistically significant (p=0.0482) increase in tumor incidence of benign granular cell tumor in uterus with cervix in the 1,000 μg/kg/day group when compared to the combined control for the female rats.

Although a few of the tumors noted reached statistical significance, the percentage of animals with tumors was low and/or within the range of historical controls. There was a statistically significant (p=0.0049) dose response relationship in the incidence of the benign Leydig cell adenomas in male rats (FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012). The neoplastic tumor findings are summarized in the data table below (pathology reported June 17, 2010).

Table 30: Neoplastic Tumor Findings in a 2-year Carcinogenicity Study in Rats

	Cont 1	Cont 2	300 μg/kg	1,000 μg/kg	3,500 μg/kg
Adrenal Gland					
Male: Primary benign subcapsular cell	3/70	6/70	7/70	3/70	3/70
Brain					
Male: Primary malignant astrocytoma	1/70	0/70	2/70	0/70	1/70
Female: Primary malignant astrocytoma	0/70	0/70	1/70	2/70	2/70
Mammary gland					
Female: Primary malignant adenocarcinoma	23/70	16/70	25/70	18/70	17/70
Primary benign fibroadenoma	34/70	30/70	24/70	34/70	22/70
Mutlicentric neoplasm					
Female: Malignant histiocytic sarcoma	0/70	2/70	0/70	4/70	2/70
Ovaries					
Female: Primary benign thecoma	0/70	0/70	0/70	0/70	2/70
Pancreas					
Male: Primary benign islet cell adenoma	8/70	6/70	9/69	4/70	6/70
Primary malignant islet cell adenoma	5/70	5/70	2/70	1/70	6/70
Parathyroid glands					
Female: Primary benign adenoma	3/58	0/60	4/52	0/57	1/53
Pituitary					
Male: Primary benign pars distalis adenoma	31/70	43/70	39/70	40/70	50/70
Female: Primary benign pars distalis adenoma	52/70	44/70	48/70	56/70	52/70
Skin					
Male: Primary benign keratoacanthoma	1/70	3/70	2/70	4/70	6/70
Female: Primary malignant fibrosarcoma	0/5	0/2	2/6	0/3	0/5
Testes					
Male: Primary benign interstitial cell adenoma	0/70	0/70	1/70	0/70	4/70
Thyroid gland					
Female: Primary benign c-cell adenoma	6/70	9/70	3/70	8/70	7/70
Uterus with cervix					
Female: Primary benign granular cell	0/70	1/70	1/70	4/70	2/70

tumor					
Vagina *Female:* Primary benign granular cell tumor	1/69	1/70	2/70	2/70	5/70

Non Neoplastic

Adrenal glands: Minimal/mild focal cortical hyperplasia was noted in 9/33 terminal sacrifice males in the 3,500 μg/kg/day group compared to 6/52 terminal sacrifice control males.

Nose (level b): Minimal/mild nasolacrimal duct inflammation was noted in 8/29 terminal sacrifice males in the 1,000 μg/kg/day group, compared to 5/52 terminal sacrifice control males.

Ovaries: Minimal/mild number of cysts were noted in 18/52, 17/45, and 14/51 prematurely sacrificed females in the 300, 1,000, and 3,500 μg/kg/day group compared to 18/88 prematurely sacrificed control females.

Parathyroid glands: Minimal focal hyperplasia was noted in 4/34 prematurely sacrificed males in the 300 μg/kg/day group compared to 1/74 prematurely sacrificed control males.

Stomach: Acute inflammation was noted in 4/51 prematurely sacrificed females in the 3,500 μg/kg/day group compared to 4/88 prematurely sacrificed control females.

Urinary bladder: Minimal lymphocytic infiltration was noted in 4/25 terminal sacrifice females in the 1,000 μg/kg/day group compared to 3/52 terminal sacrifice control females.

Uterus with cervix: Moderate number of cysts were observed in 3/19 terminal sacrifice females in the 1,000 μg/kg/day group compared to 0/52 terminal sacrifice control females.

Zymbal gland: Subacute/chronic inflammation was noted in 2/33 terminal sacrifice males in the 3,500 μg/kg/day group compared to 0/52 terminal sacrifice control males.

Toxicokinetics

Not conducted.

Dosing Solution Analysis

Samples were analyzed during weeks 1, 4, 12, 26, 52, 78, and 104. The acceptance criteria for concentration verification were that the mean value of each concentration

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was within 10% of the theoretical value (accuracy) and individual results were within 5% from the mean of the replicates (precision). The acceptance criteria for homogeneity analysis was that the mean value of all samples collected were within 7% of the overall mean.

All samples from weeks 4, 26, 52, 78, and 104 met the acceptance criteria for precision and accuracy. One of the group 2 control samples tested positive for the presence of test article. Therefore, the backup samples for group 2 were analyzed and test article was detected.

The week 1 samples for the 300 and 3,500 μg/kg/day groups met the acceptance criteria for precision, accuracy, and homogeneity. The samples for the 1,000 μg/kg/day group met the acceptance criteria for accuracy, but failed for precision and homogeneity, with the top samples having percent recovery values of 121% and 106%. The backup samples for the 1,000 μg/kg/day group were analyzed and met the acceptance criteria for precision and homogeneity. Therefore, with the backup samples, the 1,000 μg/kg/day group met the acceptance criteria for precision, accuracy, and homogeneity.

The week 12 samples for the 1,000 and 3,500 μ/kg/day groups met the acceptance criteria for precision and accuracy. The samples for the 350 μg/kg/day group failed for precision with percent recovery values of 80% and 94%. Therefore, the backup samples for the 350 μg/kg/day group were analyzed and the results met the acceptance criteria for precision and accuracy.

The week 56 samples[7] met the acceptance criteria for precision and homogeneity, but failed for accuracy with mean percent recovery values of 86%, 89%, and 89% for the 350, 1,000, and 3,500 μg/kg/day groups, respectively. However, these samples were analyzed 214 days after preparation.

Study title: 104-Week Oral Carcinogenicity Study of Linaclotide Acetate in Mice	
Study no.:	MNP-103-030
Study report location:	Ironwood Pharmaceuticals, Inc.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 16, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MD1100 Acetate, PPL-MD11000602B, 95% relative to total peptide content MD1100 Acetate, PPL-MD11000801, 94% relative to total peptide content

[7] Additional samples were collected on Week 56 because the protocol required a new homogeneity analysis to be conducted for a batch size greater than 30%.

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CAC concurrence:	Yes (meeting minutes dated January 20, 2012)

Key Study Findings

Dose levels of 0 (two vehicle control groups), 600, 2,000 and 6,000 μg/kg/day were tested in males and females. The survival percentages at the end of the study were consistent among the linaclotide-treated groups and similar to each of the control groups. There were no significant drug-related clinical observations in animals that died before the end of the dosing period or in animals that were terminally sacrificed at the end of the dosing period. The observed clinical signs in the treatment groups were not interpreted as drug-related due to the low incidence and the similarity of incidence in the control and treatment groups.

Small, but statistically significant mean body weight changes (increase or decrease) occurred in the 2,000 and 6,000 μg/kg/day males and females, and 600 μg/kg/day females, compared to combined control values. However, these changes were sporadic and transient. At the end of the dosing period, body weights for both control and drug-treated males and females were similar. Mean food consumption changes (significant increase or decrease) occurred sporadically in males and females at 600, 2,000, and 6,000 μg/kg/day; however these changes were not considered as drug-related due to the transient occurrence and inconsistent magnitude of change.

Hematology analysis showed that leukocyte counts were decreased in a dose-dependent, but not statistically significant manner. The Sponsor stated that the total leukocyte counts compatible with leukemia were observed in relatively equal numbers in all groups. In general, there were modest changes in most of the hematology parameters measured (e.g., basophils, leukocytes, monocytes, eosinophils, lymphocytes, and neutrophils), however most of the changes were not statistically significant or dose-dependent, and some changes were due to a large amount of intra-group variability in control or drug-treated groups.

The macroscopic changes observed in the study were not dose-dependent and were only noted in a small number of animals. Therefore the macroscopic findings were not considered as drug-related. The microscopic examination showed minor non-neoplastic changes in drug-treated animals. Mild/moderate extramedullary hematopoiesis in the liver was observed in 22% of the prematurely sacrificed males in the 6,000 μg/kg/day group compared to 7% of the prematurely sacrificed control males. Minimal/mild lymphocytic infiltration in the lungs was observed in 15% of the terminal sacrifice males in the 6,000 μg/kg/day group compared to 3% of the terminal sacrifice control males. Mild and severe generalized lymphoid necrosis was noted in 12% of the prematurely sacrificed males in the 6,000 μg/kg/day group compared to 2% of the prematurely sacrificed control males. Minimal/mild subacute/chronic inflammation was noted in the clitoral glands in 27% of the terminal sacrifice females in the 6,000 μg/kg/day group

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compared to 4% of the terminal sacrifice control females. The other minor non-neoplastic changes observed were unrelated to dose, and occurred in only a few animals.

The neoplastic changes included a dose-dependent increase in the incidence of benign bronchiolar alveolar adenomas in female mice. Specifically, the incidence of lung adenomas was 9% (pooled control groups 1 and 2), 14%, 16%, and 21% in the 0, 600, 2,000, and 6,000 μg/kg/day females, respectively. According to the FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012, pair-wise comparison of treated groups and the combined controls indicated the incidence of bronchiolar alveolar adenomas in the 6,000 μg/kg/day females was statistically significant (p=0.0064) when compared to the control groups. The Sponsor also noted that there was a statistically significant positive trend in the occurrence of female lung adenomas, and that the 21% incidence of lung adenomas in 6,000 μg/kg/day females is comparable to the upper range of 20% from the historical control incidences of lung adenomas in female mice. In addition, the Sponsor stated that there were no increases in bronchiolar alveolar hyperplasia (which is considered a pre-neoplastic finding) or malignant lung bronchiolar alveolar carcinoma in females that received linaclotide on this study, which would have been expected if there was any involvement of linaclotide in the induction of lung neoplasia in females. According to the Sponsor's statistical analysis, there were no other pair-wise or trend test p-values that were statistically significant according to the evaluation criteria for common and rare tumors. Therefore, there were no significant drug-related neoplastic findings observed in males or females.

According to the FDA statistical review by Mohamed Nagem, Ph.D. dated 2/07/2012, the Kaplan-Meier survival analysis indicated that there were no statistically significant dose response relationships among treatment groups or differences between the combined vehicle control and any of the treated groups in survival across treatment groups in both male and female rats.

There were no significant drug-related effects on survival rates, clinical signs, masses noted during the treatment period, body weights, food consumption, hematology, macroscopic observations, or microscopic findings (neoplastic and non-neoplastic).

Adequacy of Carcinogenicity Study

The carcinogenicity study was conducted appropriately.

Appropriateness of Test Models

The test model used by the Sponsor was appropriate.

Evaluation of Tumor Findings

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The Exec CAC noted that the study was adequate. The Exec CAC concluded that the study was negative for drug-related neoplasms (see meeting minutes dated January 20, 2012).

Table 31: Date and Site/Type of First Tumor Observed in a 2-year Carcinogenicity Study in Mice

	Male (Day/site of first tumor)	**Female (Day/site of first tumor)**
Cont 1	460/multicentric neoplasm, malignant hemangiosarcoma	411/Lung, benign bronchiolar alveolar adenoma
Cont 2	496/multicentric neoplasm, malignant lymphoma	214/multicentric neoplasm, malignant hemangiosarcoma
600 μg/kg	381/multicentric neoplasm, malignant lymphoma	427/multicentric neoplasm, malignant lymphoma
2000 μg/kg	391/multicentric neoplasm, malignant histiocytic sarcoma	367/Lung, malignant bronchiolar alveolar carcinoma
6000 μg/kg	282/multicentric neoplasm, malignant lymphoma	371/multicentric neoplasm, malignant histiocytic sarcoma

Methods	
Doses:	600, 2,000, and 6,000 μg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	0.5% methylcellulose in deionized water
Basis of dose selection:	Executive Carcinogenicity Assessment Committee recommendations. The Committee concurred with the Sponsor's proposed high dose of 6,000 µg/kg/day. However, the Committee recommended that the intermediate and low doses be changed to 2,000 and 600 µg/kg/day, respectively. The selection of the high dose was based on high multiples of the pharmacologically active dose.
Species/Strain:	Crl:CD1®(Icr)
Number/Sex/Group:	70
Age:	~8 weeks
Animal housing:	Animals were housed (~3-4 per cage) in suspended, stainless steel, wire-mesh, type cages
Paradigm for dietary restriction:	Meal Lab Diet® Certified Rodent Diet #5002, PMI Nutrition International, Inc.
Dual control employed:	Yes
Interim sacrifice:	No
Satellite groups:	Yes (see study design below)
Deviation from study protocol:	None

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Table 32: Study Design (104-week Carcinogenicity Study in Mice)

Group	Dose Level (µg/kg/day)[a]	Dose Concentration (mg/mL/dose)	Number of Animals					
			Initial		Clinical Pathology[a]		Necropsy	
			M	F	M	F	M	F
Main Study								
1	0 (Control 1)	0	70	70	70	70	70	70
2	0 (Control 2)	0	70	70	70	70	70	70
3	600	0.060	70	70	70	70	70	70
4	2000	0.200	70	70	70	70	70	70
5	6000	0.600	70	70	70	70	70	70
Sentinel[b]								
6	NA	NA	30	30	-	-	30	30

M = Male, F = Female

[a]Hematology evaluations will be conducted for all surviving main study animals prior to their scheduled necropsy and from all main study animals euthanized *in extremis*, if possible.

[b]Sentinel animals will be maintained in the study room, but not dosed, for health screen evaluations. Ten animals/sex will be randomly selected from the shipment of animals prior to study assignment. The remaining 20 animals/sex will be utilized throughout the study period.

Observations and Results

Mortality

All animals were observed twice a day for morbidity, mortality, injury, and availability of food and water through week 52. At week 53, a third mortality check was added in the evenings. There were no drug-related effects on survival in males or females. In general, the control and drug-treated males had less mortality compared to females. However, there was no dose-dependent effect on mortality in the test article-treated animals. In almost all of the groups, the majority of deaths occurred after week 79 with the exception of the 2,000 µg/kg/day females, in which a majority of deaths occurred after week 66. The survival rate at the end of the treatment period is presented in the Sponsor's table below.

Table 33: Mortality Results in a 2-year Carcinogenicity Study in Mice

Cumulative Percent Survival at the Beginning of Week 105		
Dose Level (μg/kg/day)	Males	Females
0 (Control 1)	51.0	32.9
0 (Control 2)	48.6	40.0
Control 1 and 2	49.8	36.4
600	60.0	42.9
2000	44.3	48.6
6000	48.6	32.9
*Does not include animals that died during Week 105 prior to necropsy		

The Sponsor included a summary of probable causes of mortality in control and test article-treated animals. In general, there was no specific tumor type that was associated with a high number of deaths in the control or test article-treated groups, and there were no substantial differences in the probable causes of death between the control and test article-treated groups. The Sponsor postulated that lung tumors were responsible for 2/69 deaths in control males, and in 3/30, 3/39, and 6/35 deaths in the 600, 2,000, and 6,000 μg/kg/day males, respectively. Urogenital inflammation/obstruction/calculi were the probable causes of death in 17/69 control males and in 6/30, 11/35 and 5/35 deaths in the 600, 2,000, and 6,000 μg/kg/day males, respectively. According to the Sponsor's summary of mortality data, most of the causes of death in the control and test article-treated animals were undetermined.

According to the FDA statistical review by Mohamed Nagem, Ph.D. dated 2/07/2012, the Kaplan-Meier survival analysis indicated that there were no statistically significant dose response relationships among treatment groups or differences between the combined vehicle control and any of the treated groups in survival across treatment groups in both male and female rats.

Clinical Signs

A detailed clinical examination of each main study animal was conducted once a week. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling, and bizarre behavior. Palpation of masses was also performed, with recording of location, appearance, and size of the masses.

Yellow discolored hair and sparse hair was noted throughout the study period in both control and test-article treated males, and a scabbed area and distended abdomen were noted sporadically in both control and test article-treated females. In addition, sparse hair was noted in both control and test article-treated females throughout the study period (most incidences after week 51). After week 65, small skin nodules (1-5 mm) and scabbed areas on the skin were observed in a similar number of males in both the control and test article-treated groups. A distended abdomen was observed in a similar number of males in both the control and test-article treated groups after 78 weeks of treatment. Additionally, in both the control and test-article treated males after week 91, a similar number of animals were observed with incidences of lacrimation. In females, after week 39, both control and test article-treated groups had mice with portions of ear missing.

A portion of the ear was missing on 4-7 males in the 2,000 μg/kg/day group starting on week 11. However, after week 31, control and test article-treated males had similar numbers of animals with portions of ear missing. Skin abrasions were observed in 3-6 males in the 2,000 μg/kg/day group during weeks 9-28, and in 3-4 males in the 2,000 μg/kg/day group during weeks 84-90. In addition, skin abrasions were observed in at least 3 males in the 6,000 μg/kg/day group during weeks 20 to 26, and in 4-6 males in the 6,000 μg/kg/day group during weeks 30 to 37.

During weeks 69-72 and 79-91, the 2,000 and 6,000 μg/kg/day males had an increase (4-8) in the incidence of bent tails compared to control males (0-3). During weeks 81 and 82, 7/56 males in the 2,000 μg/kg/day males had a swollen appearance compared to 4/99 control males. Extended penis was noted in a small number of males (3/38 to 4/34) in the 3,500 μg/kg/day during weeks 100-102 compared to 1/75 to 1/72 control males during the same period. Unkempt appearance was also noted in a small number of males in the 3,500 μg/kg/day group (3/32 to 4/36 males) during weeks 100-102, compared to 0/70 to 1/74 control males during the same period..

There were modest increases in the incidence of specific clinical signs in test article-treated animals when compared to control animals (as detailed above)., However, the total numbers of animals exhibiting clinical signs were similar between the control and test-article treated groups, and there was no dose-dependent relationship in the test article-treated animals. Overall, the adverse events noted occurred in both the control and test article-treated animals, therefore there were no significant test article-related clinical observations. Additionally, there were no test article-related effects on the incidence of masses.

Body Weights

Body weights for all main study animals were measured and recorded prior to randomization, 2 days before dosing, once weekly for the first 13 weeks, and once every 4 weeks for the remainder of the study. However, the Sponsor did not provide body weight data after week 101. During week 53, body weight gains were decreased

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by 12% and 13% in the 2,000 and 6,000 μg/kg/day males respectively, compared to control group 1 males. In addition, the 2,000 μg/kg/day females had a 19% decrease in body weight gain during week 53 when compared to control group 1 females. Overall, there were no test article-related effects on male or female body weights. The following table shows the absolute body weights (g) and body weight gains (g) for males and females.

Table 34: Body Weight Changes in a 2-year Carcinogenicity Study in Mice

Males	**0 μg/kg/day (Control 1)**	**0 μg/kg/day (Control 2)**	**600 μg/kg/day**	**2,000 μg/kg/day**	**6,000 μg/kg/day**
Group	1	2	3	4	5
Wk 0 (g)	27.97	28.1	28.06	28.06	28.12
Wk 21 (g)	40.28	39.8	39.74	40.16	39.13
% of Control 1, Wk 21	100.0	98.9	98.7	99.7	97.1
ΔWk21-Wk0 (g)	12.31	11.8	11.68	12.1	11.0
BW Gain, % of Initial BW	44.0	41.9	41.6	43.1	39.2
BW Gain, % of Control 1	100.0	95.3	94.6	98.0	89.0
Wk 53 (g)	44.08	43.5	44.04	42.21*	42.23*
% of Control 1, Wk 53	100.0	98.7	99.9	95.8	95.8
ΔWk53-Wk0 (g)	16.11	15.44	15.98	14.15	14.11
BW Gain, % of Initial BW	57.6	55.0	56.9	50.4	50.2
BW Gain, % of Control 1	100.0	95.5	98.9	87.6	87.1
Wk 77 (g)	43.29	43.07	43.49	44.09	44.16
% of Control 1, Wk 77	100.0	99.5	100.5	101.8	102.0
ΔWk77-Wk0 (g)	15.32	15.01	15.43	16.03	16.04
BW Gain, % of Initial BW	54.8	53.5	55.0	57.1	57.0
BW Gain, % of Control 1	100.0	97.7	100.4	104.3	104.1
Wk 101 (g)	41.03	42.00	42.08	41.87	40.96
% of Control 1, Wk 101	100.0	102.4	102.6	102.0	99.8

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ΔWk101-Wk0 (g)	13.06	13.94	14.02	13.81	12.84
BW Gain, % of Initial BW	46.7	49.7	50.0	49.2	45.7
BW Gain, % of Control 1	100.0	106.4	107.0	105.4	97.8

Females	**0 μg/kg/day (Control 1)**	**0 μg/kg/day (Control 2)**	**600 μg/kg/day**	**2,000 μg/kg/day**	**6,000 μg/kg/day**
Group	1	2	3	4	5
Wk 0 (g)	23.49	23.5	23.52	23.52	23.55
Wk 21 (g)	33.19	33.1	32.57	32.64	32.63
% of Control 1, Wk 21	100.0	99.6	98.1	98.3	98.3
ΔWk21-Wk0 (g)	9.7	9.6	9.05	9.12	9.1
BW Gain, % of Initial BW	41.3	40.7	38.5	38.8	38.6
BW Gain, % of Control 1	100.0	98.5	93.2	93.9	93.4
Wk 53 (g)	36.83	36.81	36.34	34.37**	36.99
% of Control 1, Wk 53	100.0	99.9	98.7	93.3	100.4
ΔWk53-Wk0 (g)	13.34	13.31	12.82	10.85	13.44
BW Gain, % of Initial BW	56.8	56.6	54.5	46.1	57.1
BW Gain, % of Control 1	100.0	99.7	96.0	81.2	100.5
Wk 77 (g)	37.98	38.1	37.73	37.12	37.15
% of Control 1, Wk 77	100.0	100.3	99.3	97.7	97.8
ΔWk77-Wk0 (g)	14.49	14.6	14.21	13.6	13.6
BW Gain, % of Initial BW	61.7	62.1	60.4	57.8	57.7
BW Gain, % of Control 1	100.0	100.7	97.9	93.7	93.6
Wk 101 (g)	36.87	37.71	37.36	37.29	36.58
% of Control 1, Wk 101	100.0	102.3	101.3	101.1	99.2
ΔWk101-Wk0 (g)	13.38	14.21	13.84	13.77	13.03

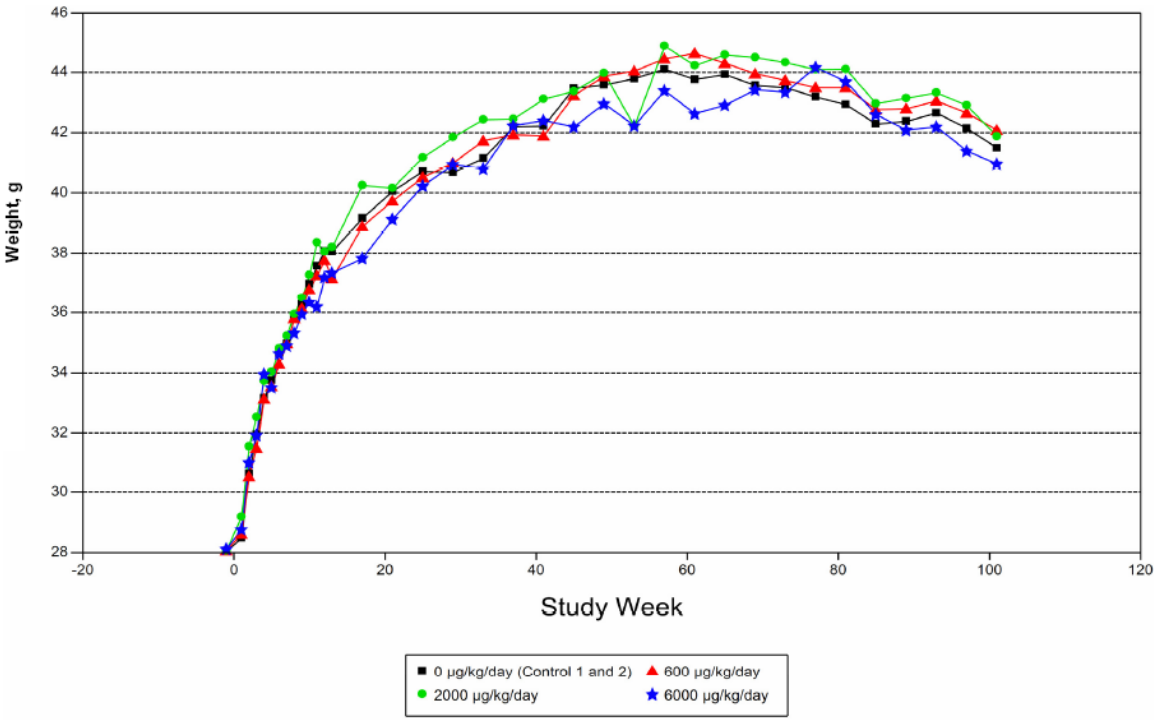
BW Gain, % of Initial BW	57.0	60.5	58.8	58.5	55.3
BW Gain, % of Control 1	100.0	106.2	103.3	102.8	97.1

* Significantly different from 0 μg/kg/day (Control 1); $p<0.05$
** Significantly different from 0 μg/kg/day (Control 1 and 2); $p<0.01$

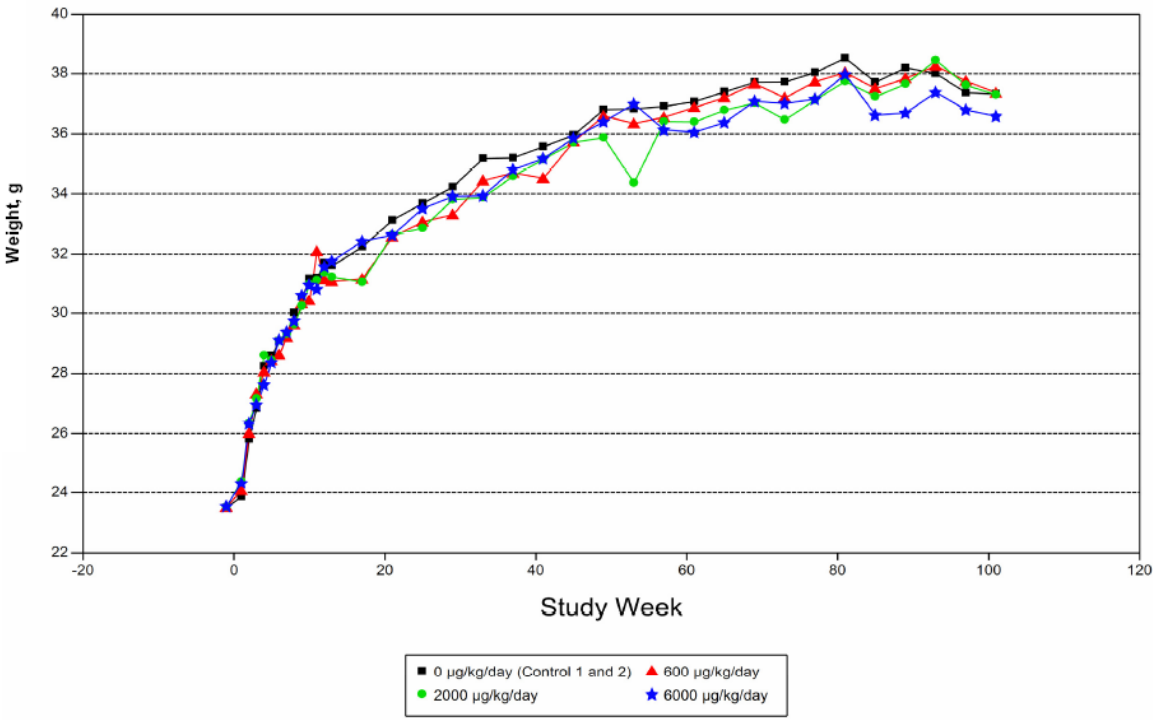
The figures below show the mean body weights in males and females over the course of the study period (control groups 1 and 2 are combined in the figures below).

Figure 3: Mean Body Weight Values in a 2-year Carcinogenicity Study in Male (A) and Female (B) Mice

A.



B.

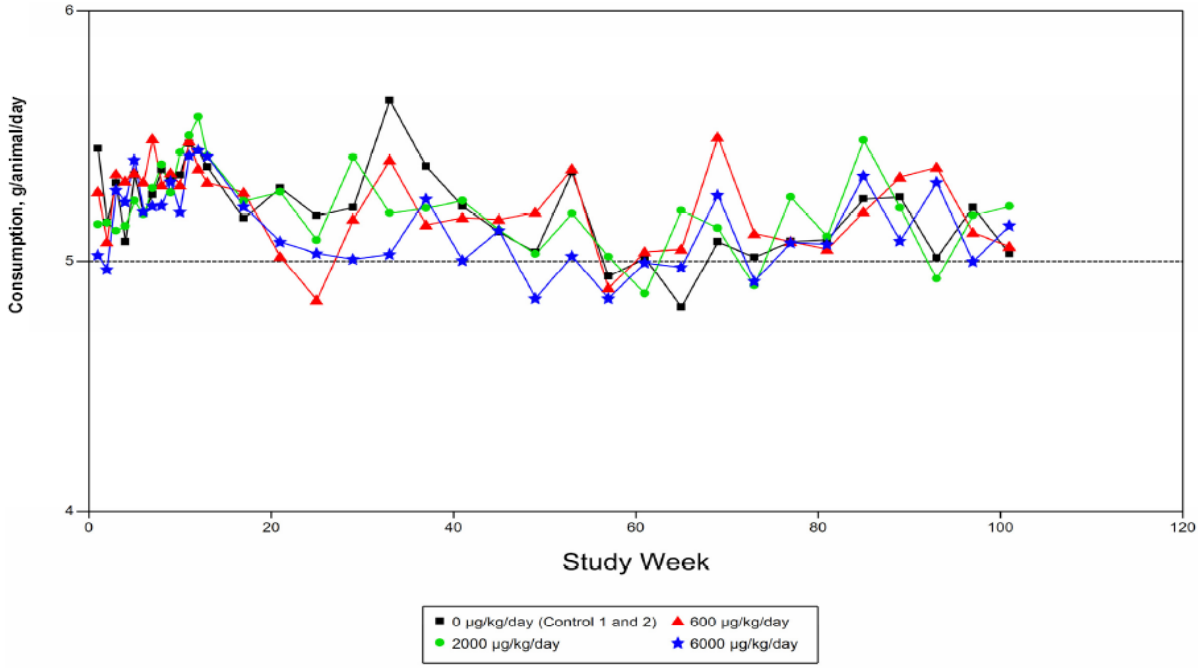


Feed Consumption

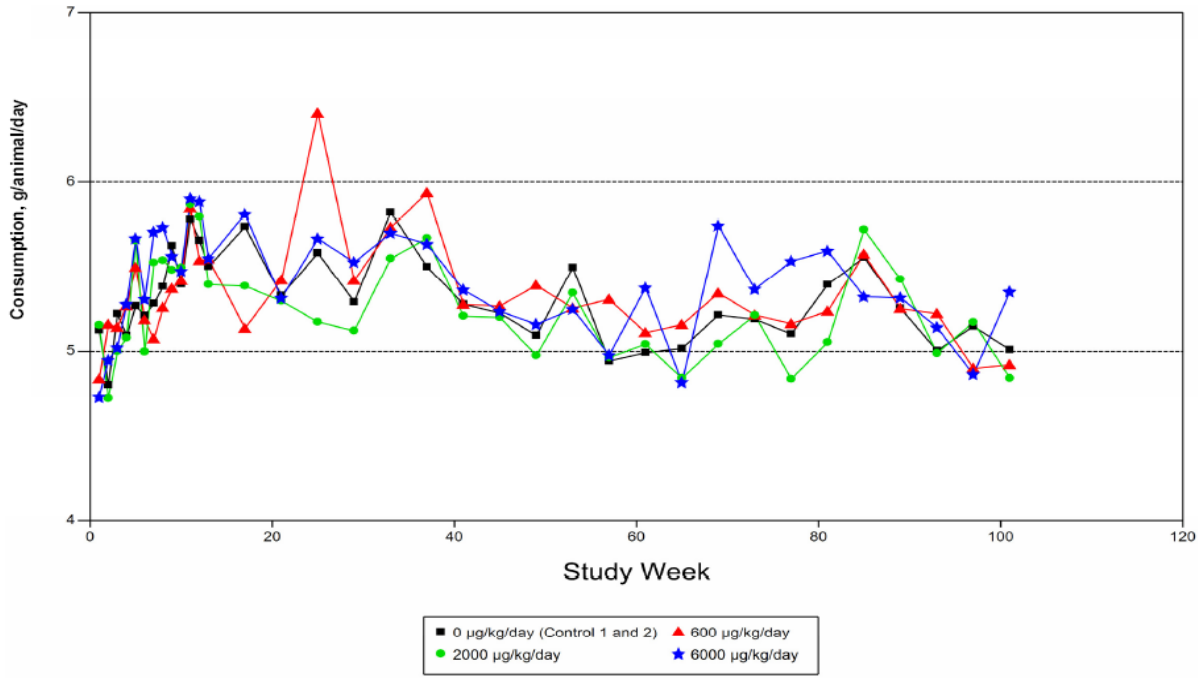
Food consumption was recorded for all main study animals once weekly for the first 13 weeks and then once every 4 weeks for the remainder of the study. The mean food consumption in weeks 1 and 101 in control males was 5.5 and 5.0 g/animal/day, respectively. The mean food consumption in weeks 1 and 101 in control females was 5.1 and 5.0 g/animal/day, respectively. Small, sporadic reductions (11-15%) in food consumption were observed in the treatment groups, but these changes are not considered as drug-related. The figures below show mean food consumption in males and females over the course of the study period (control groups 1 and 2 are combined in figures below).

Figure 4: Mean Food Consumption Values in a 2-year Carcinogenicity Study in Male (A) and Female (B) Mice

A.



B.



Hematology

Blood samples were collected via cardiac puncture from animals euthanized *in extremis* if possible, and from all surviving animals at the scheduled terminal necropsy. The following parameters were analyzed: leukocytes, erythrocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, and other cells.

Basophils increased by 20% in the 600 μg/kg/day males, and other cells increased by 20% and 46% in the 2,000 and 6,000 μg/kg/day males, respectively, compared to the control males. Neutrophils were increased by 45% and 37% in the 600 and 6,000 μg/kg/day males, respectively. Lymphocytes were decreased by 13% and 16% in the 600 and 2,000 μg/kg/day males respectively. In addition, monocytes were decreased by 30%, 42%, and 40% in the 600, 2,000 and 6,000 μg/kg/day males, respectively, compared to the control males. However, neutrophil counts in the test article-treated males, and lymphocyte and monocyte counts in the control group had a large amount of intra-group variability.

In females, leukocyte counts were decreased in a dose-dependent, but not statistically significant manner. Specifically, leukocytes were decreased by 15%, 17%, and 21% in the 600, 2,000, and 6,000 μg/kg/day females, respectively, compared to the control females. There was a statistically significant ($p<0.01$) decrease of 54% in eosinophils in the 600 μg/kg/day females, and a 31% decrease (not significant) in the 6,000 μg/kg/day females. Neutrophils were decreased by 15% and 26% in the 2,000 and 6,000 μg/kg/day females respectively. Lymphocytes were decreased by 19%, 19%, and 17%, and monocytes were decreased by 34%, 15%, and 38% in the 600, 2,000, and 6,000 μg/kg/day females, respectively. Basophils were decreased by 43% in the 600 μg/kg/day females and increased by 35% in the 2,000 μg/kg/day females. Other cells were increased by 17% and 25% in the 2,000 and 6,000 μg/kg/day females respectively.

Although there were modest changes in most of the hematology parameters measured, most of the changes were not statistically significant or dose-dependent.

Gross Pathology

All animals, including those euthanized in moribund condition or found dead before the end of the dosing period, were submitted to full necropsy. The animals were examined for palpable masses, and abnormalities were identified and correlated with postmortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities. The Sponsor provided a table with a full list of organs and tissues examined (see table below). There were minor macroscopic changes noted in both control and test article-treated animals, however these changes were not dose-dependent. Therefore, there were no test article-related macroscopic changes. The observed macroscopic changes are described below.

Lung: A nodule was noted in 7/28, 5/33, 4/22 terminal sacrifice females in the 600, 2,000, and 6,000 μg/kg/day group, respectively, compared to 3/51 terminal sacrifice control females.

Lymph node (mediastinal): Mild/moderate enlarged lymph nodes were noted in 4/22 terminal sacrifice females in the 6,000 μg/kg/day group, compared to 1/51 terminal sacrifice control females.

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Thymus gland: Mild/moderate enlargement was noted in 3/33 and 3/22 terminal sacrifice females in the 2,000 and 6,000 μg/kg/day group respectively, compared to 0/51 terminal sacrifice control females. Mild/severe decrease in size was observed in 6/22 prematurely sacrificed[8] females in the 6,000 μg/kg/day group, compared to 5/51 prematurely sacrificed control females.

Histopathology

The tissues listed in the Sponsor's table below were used for histopathological examinations from all main study animals. Minimal to moderate subcapsular cell hyperplasia in the adrenal glands, minimal/mild axonal/myelin degeneration in the sciatic nerve, and minimal to severe generalized lymphoid depletion in the thymus gland, was observed in a majority of control and test article-treated males and females. In addition, cystic endometrial hyperplasia in the uterus was noted in a majority of females treated with the control or test articles.

Minimal to severe granulocytic hyperplasia in bone marrow (femur/sternum), minimal focal mineralization in the brain, minimal/mild lymphocytic infiltration in the harderian, salivary, and lacrimal glands, minimal/mild mononuclear cell infiltration in the liver, and minimal to severe extramedullary hematopoiesis in the spleen were observed in many control and test article-treated males and females.

In males, mild/severe bilateral oligospermia/germ cell debris in the epididymides, and subacute/chronic inflammation and minimal to severe bilateral degeneration/atrophy of the seminiferous tubules in the testes were noted in both the control and test article-treated groups. Minimal/mild lymphocytic infiltration in the kidneys was noted in prematurely sacrificed males. In addition, minimal/mild cysts and chronic progressive nephropathy in the kidneys were observed in both the control and test article-treated males. Minimal/mild cardiomyopathy in the heart was noted in both the control and test-article treated males.

The following changes were noted in many control and test-article treated females: minimal to moderate fibro-osseous proliferation in the bone (femur and sternum) and nose (level a, b, c, and d), minimal to severe chronic progressive nephropathy in the kidneys, minimal to moderate alveolar histiocytosis in the lungs, minimal to severe generalized lymphoid depletion and sinus erythrocytosis/erythrophagocytosis in the mesenteric lymph node, mild to severe generalized lymphoid depletion in the spleen, and minimal to severe medulla lymphoid hyperplasia in the thymus. Minimal to severe cysts and hemorrhages in the ovaries were noted in both the control and test article-treated females. Additionally, minimal/mild lymphocytic infiltration in the mandibular salivary gland, urinary bladder and uterus was noted in the control and test article-treated females.

[8] "prematurely sacrificed" includes animals that were euthanized in moribund condition and those found dead before the end of the dosing period.

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Table 35: Organs Collected at Necropsy for Macroscopic and Microscopic Examination in a 2-year Carcinogenicity Study in Mice

- Adrenal (2)
- Aorta
- Bone with marrow [femur]
- Bone with marrow [sternum]
- Bone marrow smear [2 collected][a]
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]
- Clitoral gland (2)
- Coagulating gland (2)
- Eye including optic nerve (2)
- Epididymis (2)
- Gallbladder
- Gastrointestinal tract:
  - esophagus
  - stomach [glandular and nonglandular]
  - duodenum
  - jejunum
  - ileum
  - cecum
  - colon
  - rectum
- Gonads:
  - ovary (2) with oviducts/fallopian tube (2)
  - testes (2)
- Gross lesions
- Harderian gland (2)
- Heart
- Joint, tibofemoral
- Kidney (2)
- Lacrimal gland, exorbital (2)
- Larynx
- Liver [collected whole; 2 examined]
- Lung [collected whole; all lobes examined]
- Lymph nodes: mandibular, mesenteric, and regional where applicable
- Mammary gland
- Nasal tissue [Levels A, B, C, and D]
- Pancreas
- Peyer's patch
- Pharynx
- Pituitary
- Preputial gland (2)
- Prostate and seminal vesicle (2)
- Salivary gland, mandibular/sublingual [2 collected; 1 examined]
- Salivary gland, parotid [2 collected; 1 examined]
- Sciatic nerve
- Skeletal muscle, biceps femoris
- Skin
- Spinal cord [cervical, thoracic, and lumbar]
- Spleen
- Thymus
- Thyroid/parathyroid (2)
- Tissue masses
- Tongue
- Trachea
- Ureter (2)
- Urinary bladder
- Uterus [both horns]/Cervix
- Vagina
- Zymbal's gland (2)

Peer Review

Yes

Neoplastic

The Sponsor stated that for the tumor statistical analysis the control groups were pooled for comparison to the test article-treated groups. The Sponsor stated that tumor incidence data was analyzed using both survival adjusted and unadjusted tests. The unadjusted tests will be based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto. Evaluation criteria (p-values of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%).

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There was a dose-dependent increase in the incidence of primary benign bronchiolar alveolar adenoma in the lungs of test article-treated females. The incidence of lung adenomas was 9% (pooled control groups 1 and 2), 14%, 16%, and 21% in the 0, 600, 2,000, and 6,000 μg/kg/day females, respectively. Benign bronchiolar alveolar adenomas are considered common tumors in CD-1 mice. According to the FDA statistical review by Dr. Mohamed Nagem dated 2/07/2012, pair-wise comparison of treated groups and the combined controls indicated the incidence of bronchiolar alveolar adenomas in the 6,000 μg/kg/day females was statistically significant (p=0.0064) when compared to the control groups. Furthermore, the Sponsor noted that statistically significant positive trends in the occurrence of female lung adenomas were also present (Cochran-Armitage trend test p=0.0049, Peto trend test p=0.0031)[9]. The 21% incidence of lung adenomas in the 6,000 μg/kg/day females is comparable to the upper range of 20% from the historical control incidences of lung adenomas in female mice from 15 other studies (with a total of 21 control groups)[10]. Additionally, the Sponsor stated that the incidences of benign bronchiolar alveolar adenomas in the two female control groups in this study were 10.0% and 7.1%, which is lower than the average historical percentage of lung adenomas in female control mice from other studies conducted at this laboratory. In males, there was no similar increased incidence of lung adenomas; instead, the incidence was variable and the highest incidence occurred in control males (23%, 19%, 13%, and 9% in males at 0, 600, 2,000, and 6,000 μg/kg/day, respectively).

The Sponsor stated that there were no other pair-wise or trend test p-values that were statistically significant according to the evaluation criteria for common and rare tumors. The pair-wise p-value of three common tumors was greater than 0.01, thus not statistically significant; malignant multicentric hemangiosarcoma in the 600 μg/kg/day females (p=0.0305), malignant uterine stromal sarcoma in the 2,000 μg/kg/day females (p=0.0346), and malignant lung bronchiolar alveolar carcinoma in the 6,000 μg/kg/day males (p=0.0402).

There were no significant test article-related effects on neoplastic lesions, based on Dr. Nagem's statistical analysis (i.e. failure to achieve statistical significance in both pairwise and trend tests). The neoplastic tumor findings are summarized in the data table below.

Table 36: Neoplastic Tumor Findings in a 2-year Carcinogenicity Study in Mice

	Cont 1	Cont 2	600 μg/kg	2,000 μg/kg	6,000 μg/kg
Adrenal Gland *Male:* Primary benign subcapsular cell adenoma	2/68	3/67	6/70	3/70	1/67
Bone (sternum) *Male:* Multicentric malignant lymphoma	0/69	1/70	0/70	0/70	2/70

[9] For common tumors, p<0.005 for trend tests and p<0.01 for pair-wise comparisons are statistically significant.

[10] (b) (4) historical control data

Bone marrow, femur *Female:* Multicentric malignant lymphoma	1/70	2/70	5/70	3/70	5/70
Bone marrow, sternum *Female:* Multicentric malignant lymphoma	0/69	3/70	6/70	4/70	5/70
Harderian glands *Male:* Primary benign adenoma	9/69	6/70	12/70	9/70	2/69
Multicentric malignant lymphoma	0/69	1/70	2/70	1/70	0/69
Female: Primary benign adenoma	5/70	1/70	3/70	0/69	6/70
Heart *Male:* Multicentric malignant lymphoma	1/69	1/70	2/70	2/70	3/70
Large intestine, colon *Female:* Multicentric malignant lymphoma	0/70	1/70	1/70	0/70	2/70
Liver *Male:* Primary benign hepatocellular adenoma	4/69	5/70	7/70	10/70	9/70
Multicentric malignant hemangiosarcoma	4/69	3/70	5/70	5/70	2/70
Multicentric malignant lymphoma	1/69	4/70	2/70	3/70	5/70
Multicentric malignant histiocytic sarcoma	0/69	0/70	2/70	0/70	2/70
Female: Multicentric malignant hemangiosarcoma	1/70	2/70	3/70	1/70	6/70
Multicentric malignant lymphoma	3/70	6/70	8/70	6/70	7/70
Lung *Male:* Primary benign bronchiolar alveolar adenoma	15/69	17/70	13/70	9/70	6/70
Primary malignant bronchiolar alveolar carcinoma	7/69	4/70	8/70	6/70	12/70
Multicentric malignant lymphoma	2/69	3/70	2/70	3/70	4/70
Female: Primary benign bronchiolar alveolar adenoma	7/70	5/70	10/70	11/70	15/70
Multicentric malignant lymphoma	7/70	8/70	8/70	5/70	9/70
Lymph node (hepatic) *Female:* Multicentric malignant lymphoma	0/2	0/1	0/0	1/3	2/4
Lymph node (mandibular) *Female:* Multicentric malignant lymphoma	8/68	7/69	9/69	3/69	6/69
Lymph node (mediastinal) *Female:* Multicentric malignant lymphoma	2/4	0/3	3/3	0/2	4/8

Lymph node (mesenteric) *Male:* Multicentric malignant histiocytic sarcoma	0/69	0/70	2/70	0/70	1/70
Multicentric malignant lymphoma	1/68	3/70	4/70	2/70	3/70
Female: Multicentric malignant lymphoma	5/70	6/70	10/70	5/70	8/67
Multicentric neoplasm *Female:* Multicentric malignant hemangiosarcoma	4/19	5/16	11/25	8/18	8/19
Multicentric malignant lymphoma	11/19	11/17	14/25	11/19	14/19
Pancreas *Female:* Multicentric malignant lymphoma	3/70	5/70	9/70	5/70	5/70
Pituitary *Female:* Primary benign pars distalis adenoma	3/70	3/70	3/70	2/69	4/69
Multicentric malignant lymphoma	0/70	0/70	3/70	0/69	0/69
Salivary gland, sublingual *Female:* Multicentric malignant lymphoma	2/69	3/70	2/70	1/69	4/69
Spleen *Male:* Multicentric malignant lymphoma	1/69	3/70	4/70	2/70	5/70
Multicentric malignant histiocytic sarcoma	0/69	0/70	2/70	0/70	1/70
Female: Multicentric malignant lymphoma	8/70	7/70	12/70	5/70	9/70
Multicentric malignant hemangiosarcoma	2/70	0/70	1/70	1/70	3/70
Testes *Male:* Primary benign interstitial cell adenoma	0/69	3/70	4/70	5/70	0/70
Ureters *Female:* Multicentric malignant lymphoma	7/70	6/70	10/70	4/69	8/70
Urinary bladder *Female:* Multicentric malignant lymphoma	5/70	5/70	7/70	4/70	7/70
Uterus with cervix *Female:* Multicentric malignant hemangiosarcoma	1/70	2/70	5/70	4/70	1/70
Multicentric malignant lymphoma	4/70	6/70	5/70	6/70	8/70
Primary malignant stromal sarcoma	2/70	2/70	4/70	7/70	1/70
Zymbal's gland *Male:* Multicentric malignant lymphoma	1/65	1/68	2/60	1/64	0/66

Non Neoplastic

Epididymides: Minimal to severe unilateral oligospermia/germ cell debris was noted in 9/40 terminal sacrifice males in the 600 μg/kg/day group compared to 6/67 terminal sacrifice control males.

Eye: Minimal/moderate cataract was noted in 6/40 terminal sacrifice males in the 600 μg/kg/day group compared to 2/67 terminal sacrifice control males.

Clitoral glands: Minimal/mild subacute/chronic inflammation was noted in 6/22 terminal sacrifice females in the 6,000 μg/kg/day group compared to 2/50 terminal sacrifice control females.

Gallbladder: Mild to severe mucosal hyperplasia was noted in 4/40 terminal sacrifice males in the 600 μg/kg/day group compared to 0/67 terminal sacrifice control males.

Liver: Mild/moderate biliary cysts were noted in 6/40 and 4/33 terminal sacrifice males in the 600 and 6,000 μg/kg/day groups respectively, compared to 2/67 terminal sacrifice control males. Mild/moderate extramedullary hematopoiesis was observed in 8/37 prematurely sacrificed males in the 6,000 μg/kg/day group, compared to 5/72 prematurely sacrificed control males.

Lung: Mild to severe alveolar histiocytosis was noted in 7/40 and 6/33 terminal sacrifice males in the 600 and 6,000 μg/kg/day group respectively, compared to 4/67 terminal sacrifice control males. Minimal/mild lymphocytic infiltration was observed in 5/33 terminal sacrifice males in the 6,000 μg/kg/day group compared to 2/67 terminal sacrifice control males.

Lymph node (mesenteric): Mild/moderate lymphoid depletion was noted in 6/38 prematurely sacrificed males in the 2,000 μg/kg/day group compared to 3/72 prematurely sacrificed control males. Minimal to moderate sinus erythocytosis/erythrophagocytosis was noted in 11/37 prematurely sacrificed males in the 6,000 μg/kg/day group compared to 8/71 prematurely sacrificed control males.

Mammary gland: Minimal to moderate lobular hyperplasia was noted in 6/47 prematurely sacrificed females in the 2,000 μg/kg/day group compared to 3/88 prematurely sacrificed control females

Nose (level A): Minimal to moderate odontodysplasia was noted in 10/70 males in the 6,000 μg/kg/day group compared to 11/139 control males.

Nose (level B): Minimal to moderate odontodysplasia was noted in 7/70 males in the 6,000 μg/kg/day group compared to 4/139 control males.

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Pancreas: Minimal/mild lymphocytic infiltration was noted in 9/33 and 4/22 prematurely sacrificed males in the 2,000 and 6,000 μg/kg/day groups, respectively, compared to 2/51 prematurely sacrificed control females.

Preputial glands: Minimal to moderate subacute/chronic inflammation was noted in 4/40 and 3/31 terminal sacrifice males in the 600 and 2,000 μg/kg/day groups respectively, compared to 0/67 terminal sacrifice control males.

Stomach: Minimal to moderate erosion/ulcer was noted in 7/70 females in the 6,000 μg/kg/day group compared to 6/140 control females.

Testes: Minimal to severe unilateral, degeneration/atrophy in the seminiferous tubules was noted in 11/40 terminal sacrifice males in the 2,000 μg/kg/day group compared to 6/67 terminal sacrifice control males.

Thymus gland: Mild and severe generalized lymphoid necrosis was noted in 4/34 prematurely sacrificed males in the 6,000 μg/kg/day group compared to 1/59 prematurely sacrificed control males.

Vagina: Minimal/mild lymphocytic infiltration was noted in 5/33 terminal sacrifice females in the 2,000 μg/kg/day group compared to 0/51 terminal sacrifice control females.

Toxicokinetics

Not conducted.

Dosing Solution Analysis

Samples were analyzed during weeks 1, 4, 12, 26, 52, 78, and 104. The acceptance criteria concentration verification were that the mean value of each concentration was within 10% of the theoretical value (accuracy) and individual results were within 5% from the mean of the replicates (precision). The acceptance criteria for homogeneity analysis was that the mean value of all samples collected were within 7% of the overall mean.

During weeks 4, 26, 52, and 104, all dose formulations met the acceptance criteria for precision, accuracy and homogeneity.

The week 1 samples for the 2,000 and 6,000 μg/kg/day formulations met the acceptance criteria for precision, accuracy, and homogeneity. The samples for the 600 μg/kg/day group met the acceptance criteria for accuracy, but failed for precision and homogeneity, with the bottom samples having percent recovery values of 103% and 78%. The backup samples for the 600 μg/kg/day formulation were analyzed and met

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the acceptance criteria for precision and homogeneity. Therefore, with the backup samples, the 600 μg/kg/day formulation met the acceptance criteria for precision, accuracy, and homogeneity.

The week 12 samples for the 600 and 6,000 μg/kg/day formulations met the acceptance criteria for precision and accuracy. The samples for the 2,000 μg/kg/day formulation failed for precision, with percent recovery values of 87% and 98%. This was not noted until the preparation of the analytical report. Therefore, the backup samples for the 2,000 μg/kg/day formulation were not requested nor analyzed. The mean percent recovery for the 2,000 μg/kg/day formulation was 93% and was considered to meet the acceptance criteria by the Study Director and Principal Investigator.

The week 78 samples for the 600 and 2,000 μg/kg/day formulations met the acceptance criteria for precision and accuracy. The samples from the 6,000 μg/kg/day group failed for precision, with percent recovery values of 104% and 57%. Therefore the backup samples for the 6,000 μg/kg/day group were analyzed and these results met the acceptance criteria for precision and accuracy.

THE FOLLOWING IS THE EXECUTIVE CAC MEETING MINUTES FOR THE SPA:

Executive CAC
Date of Meeting: April 17, 2007
Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Lynnda Reid, Ph.D., DRUP, Alternate Member
Sushanta Chakder, Ph.D., DGP Acting Supervisory Pharmacologist
David Joseph, Ph.D., DGP Presenting Reviewer

Author of Draft: David Joseph, Ph.D.
The following information reflects a brief summary of the Committee discussion and its recommendations.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 63,290
Drug Name: Linaclotide acetate
Sponsor: Microbia, Inc.
Background: Linaclotide acetate is a gastrointestinal prokinetic drug that acts through stimulation of guanylate cyclase C in the intestinal mucosa, leading to increased secretion of intestinal fluid. This drug is a 14-amino acid peptide, and is under

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development for treatment of constipation-predominant irritable bowel syndrome and chronic constipation.

Mouse Carcinogenicity Study Protocol and Dose Selection:
The Sponsor proposes a 104-week carcinogenicity study in Crl:CD1®(Icr) mice using dose levels of 0 (vehicle), 0 (vehicle), 240, 1200, and 6000 µg/kg/day linaclotide acetate, administered by oral gavage. The Sponsor cited the ICH option of a 25-fold rodent to human exposure ratio for selection of the high dose (ICH S1C(R)). The proposed high dose is 49-fold greater than the predicted human dose (10 µg/kg/day) on a µg/m2 basis. However, the ICH 25-fold the human exposure option requires actual AUC data, and linaclotide has not been detected in human plasma at any dose level. The Sponsor selected 240 µg/kg/day as the low dose, because this dose is approximately 2-fold greater than the human dose on a µg/m2 basis. The proposed intermediate dose, 1200 µg/kg/day, is the geometric mean of the low- and high-dose levels.

Rat Carcinogenicity Study Protocol and Dose Selection:
The Sponsor proposes a 104-week carcinogenicity study in Crl:CD®(SD) rats using dose levels of 0 (vehicle), 0 (vehicle), 140, 700, and 3500 µg/kg/day linaclotide acetate, administered by oral gavage. The basis of dose selection was the same as described above for the mouse carcinogenicity study. As noted above, linaclotide has not been detected in human plasma.

Executive CAC Recommendations and Conclusions:
Mouse:
1. The Committee concurred with the proposed high dose of 6000 µg/kg/day. However, the Committee recommended that the intermediate and low doses be changed to 2000 and 600 µg/kg/day, respectively. The selection of the high dose was based on high multiples of the pharmacologically active dose.
2. The Committee agrees that histopathologic examination should be performed on fallopian tubes, in addition to all tissues listed in the draft protocol.

Rat:
1. The Committee concurred with the proposed high dose of 3500 µg/kg/day. However, the Committee recommended that the intermediate and low doses be changed to 1000 and 300 µg/kg/day, respectively. The selection of the high dose was based on high multiples of the pharmacologically active dose.

2. The Committee agrees that histopathologic examination should be performed on fallopian tubes, in addition to all tissues listed in the draft protocol.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC
cc:\
/IND 63,290/Division File, DGP
/Jasti Choudary/Supervisory Pharmacologist, DGP

Reference ID: 3114166

/Sushanta Chakder/Acting Supervisory Pharmacologist, DGP
/David Joseph/Reviewer, DGP
/Cristi Stark/CSO/PM, DGP
/ASeifried, OND IO

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/s/

David Jacobson-Kram
4/23/2007 08:02:56 AM

THE FOLLOWING IS THE EXECUTIVE CAC MEETING MINUTES FROM THE FINAL REPORT:

Executive CAC
Date of Meeting: January 17, 2012
Committee: David Jacobson-Kram, Ph.D., OND IO, Chair
Abby Jacobs, Ph.D., OND IO, Member
Paul Brown, Ph.D., OND IO, Member
David B. Joseph, Ph.D., DGIEP, Alternate Member/Team Leader
Niraj R. Mehta, Ph.D., DGIEP Presenting Reviewer
Author of Draft: Niraj R. Mehta

The following information reflects a brief summary of the Committee discussion and its recommendations.
NDA # 202811
Drug Name: LINZESS (linaclotide acetate) tablets
Sponsor: Ironwood Pharmaceuticals, Inc.

Background: Linaclotide is a first-in-class, orally active, synthetic 14-amino acid peptide structurally related to the guanylin peptide family. Linaclotide is a potent activator of GC-C (guanylate cyclase C), which increases the intracellular concentrations of the second messenger cyclic guanosine monophosphate (cGMP). The known pharmacologic effects of linaclotide include increased intestinal fluid secretion, acceleration of intestinal transit, and antinociception in rodent models of visceral pain secondary to colorectal injury. The proposed indications for linaclotide are the treatment of chronic constipation and irritable bowel syndrome with constipation. Linaclotide was negative in the Ames assay and in the *in vitro* chromosome aberration assay. The *in vivo* micronucleus test was not conducted.

Mouse Carcinogenicity Study:
Linaclotide was administered to Crl:CD-1 mice (70/sex/group) at doses of 0 (0.5% methylcellulose in deionized water), 600, 2000, and 6000 µg/kg/day, given by oral

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gavage for 104 weeks. There were no significant drug-related effects on survival rates, clinical signs, masses noted during the treatment period, body weights, food consumption, hematology, macroscopic observations, or microscopic findings (neoplastic and non-neoplastic.)

Rat Carcinogenicity Study: Linaclotide was administered to Crl:CD(SD) rats (70/sex/group) at doses of 0 (0.5% methylcellulose in deionized water), 300, 1000, and 3500 µg/kg/day, given by oral gavage for 104 weeks. Survival in control group 1 females (28.6%) was significantly lower than control group 2 females (47.1%). The test article-treated females had lower Reference ID: 3074568 survival rates when compared control group 2. The 300 µg/kg/day females had a statistically significant decrease in survival rate (25.7%) compared to the combined control groups. In general, there were no significant drug-related effects on survival rates, clinical signs, masses noted during the treatment period, body weights, food consumption, hematology, macroscopic observations, or microscopic findings (neoplastic and non-neoplastic). Neoplastic findings showed that benign interstitial cell (Leydig cell) adenomas of the testes (common tumor) were observed in 4/70 males (5.7%) in the 3,500 µg/kg/day group compared to 0/140 control males. Although statistical significance was achieved in the trend test, the study results were considered to be negative based on the following: the incidence in the 3500 µg/kg/day males was not significantly different from control ($p=0.0175$, $\alpha=0.01$); the incidence of testicular interstitial adenoma in the 3500 µg/kg/day males (5.7%) was within the historical control range (0-5.7%); the p value in the trend test (0.0049) was extremely close to the designated significance level for common tumors ($\alpha=0.005$); and there is no plausible mechanism for drug-related tumor induction, since no systemic exposure to the drug or its active metabolite would be expected at the doses used in this study.

Executive CAC Conclusions and Recommendations:
Rat:
• The Committee agreed that the study was adequate, noting prior approval of the study protocol.
• The Committee concluded that the study was negative for drug-related neoplasms.

Mice:
• The Committee agreed that the study was adequate, noting prior approval of the study protocol.
• The Committee concluded that the study was negative for drug-related neoplasms.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC
cc:\
/Division File, DGIEP
/D. Joseph, DGIEP
/N. Mehta, DGIEP
/B. Strongin, DGIEP
/ASeifried, OND-IO

Reference ID: 3074568

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/s/

ADELE S SEIFRIED
01/20/2012
DAVID JACOBSON KRAM
01/20/2012
Reference ID: 3074568

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: An Oral Gavage Fertility Study of Linaclotide Acetate in the Rat	
Study no.:	MNP-103-026-RTR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 5, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide acetate (MD-1100 acetate), lot #PPL-MD11000602A, 94.9% relative to total peptide content.

Key Study Findings

Male and female rats were dosed with 10, 50, or 100 mg/kg/day test article. There were no effects on reproduction and fertility of rats and no effects on early embryonic development. The NOAEL was ≥ 100 mg/kg/day.

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Methods	
Doses:	10, 50, and 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg/day
Route of administration:	Oral
Formulation/Vehicle:	0.5% methylcellulose 400 cps in deionized water
Species/Strain:	Crl:CD(SD) rats
Number/Sex/Group:	22
Satellite groups:	None
Study design:	Males were dosed for 29 days prior to mating, during mating, and until necropsy. Females were dosed for 14 days prior to mating, during mating, and up to gestation day 7 (see table below for design).
Deviation from study protocol:	none

Table 37: Study Design (Fertility Study of Linaclotide in Rats)

Group Number Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Animal Numbers	
			Males	Females
1/ Vehicle Control	0	10	1001 to 1022	1501 to 1522
2/ Linaclotide	10	10	2001 to 2022	2501 to 2522
3/ Linaclotide	50	10	3001 to 3022	3501 to 3522
4/ Linaclotide	100	10	4001 to 4022	4501 to 4522

Observations and Results

Mortality

All animals were examined twice daily for mortality and signs of ill health or reaction to treatment. There were no deaths.

Clinical Signs

A detailed examination was performed weekly. Stained fur was observed in both control and test article-treated animals. Thin cover of fur was noted on the right and left forelimb of 1/22, 3/22, 5/22, and 7/22 females in the control, 10, 50, and 100 mg/kg/day groups, respectively.

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Body Weight

Individual body weights were measured twice weekly starting at randomization and extending through the treatment period. Mated females were weighed on gestation days 0, 3, 7, 10, and 13. During the premating period (between study days 0 and 28), body weight gains in the 10 and 100 mg/kg/day males were significantly ($p \leq 0.05$) lower (20% and 21%, respectively) than the control males from days 3 to 7, and body weight gains in the 100 mg/kg/day males were significantly lower (25%) than the control males from days 10 to 14. During the mating period, the overall gains were 96.7 g, 99.5 g, 97.4 g and 87.5 g for the control, 10, 50 and 100 mg/kg/day males, respectively. However, there was no significant difference in body weight in the test article-treated groups at the end of the 28-day mating period.

During the mating and post-mating periods (between study days 28 and 63), body weight gains were generally lower (10-36%) in the 100 mg/kg/day males when compared to control males with differences reaching statistical significance (e.g. ↓36% on days 38 to 42. However, the overall gains in males during the mating and post-mating periods were similar (70.7 g, 69.6 g, 71.3 g and 72.9 g in the control, 10, 50 and 100 mg/kg/day groups, respectively.

There were no significant adverse effects on body weights or body weight gains in females at any dose level. There was a small decrease (11-21%) in body weight gain in the test article-treated premating females, however there was no difference in overall body weights between test article-treated females and control females.

The Sponsor's figures below summarize overall weight gain throughout the study period.

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Figure 5: Group Mean Body Weights of Male Rats in the Fertility Study

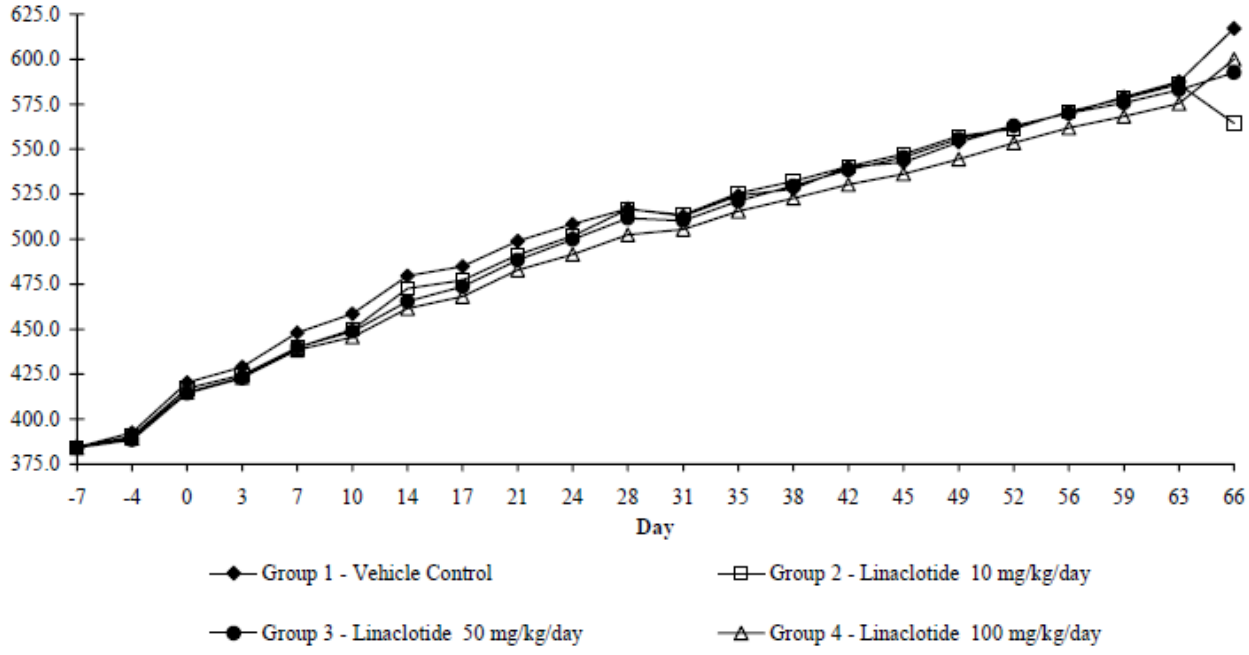


Figure 6: Group Mean Body Weights (Premating) of Female Rats in the Fertility Study

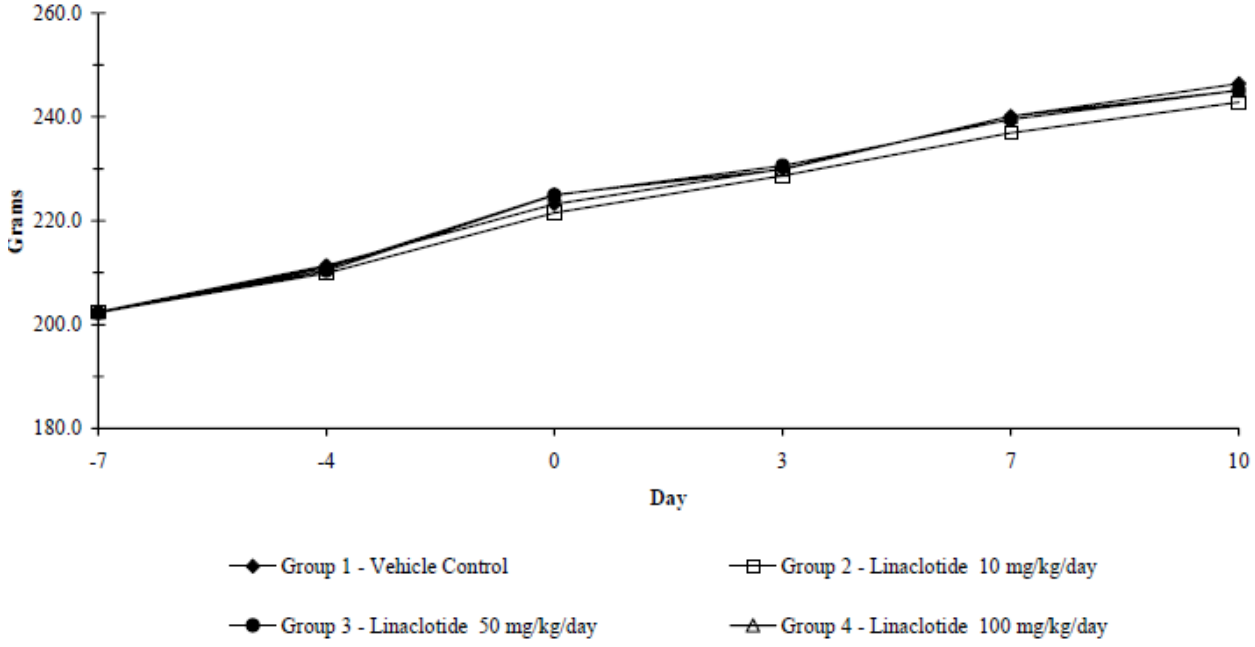
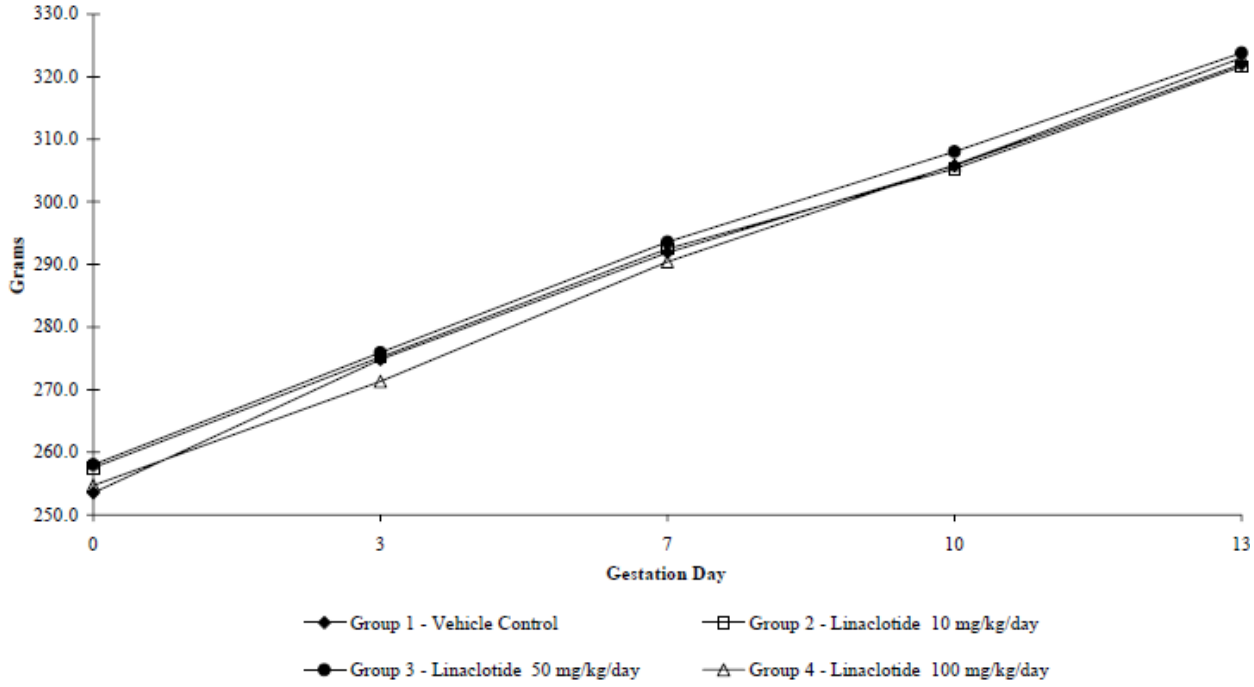


Figure 7: Group Mean Body Weights of Pregnant Females in the Fertility Study



Feed Consumption

Individual food consumption was measured twice weekly (except during the cohabitation period) commencing the day of randomization and extending through the treatment period. In mated females, food consumption was measured on days 0 to 3, 3 to 7, 7 to 10 and 10 to 13 of gestation. There were no test article-related effects on food consumption

Estrous Cycles

The estrous cycles of all females were determined for the 14 days before cohabitation for mating and during mating until the day of positive identification of mating. There were no test article-related effects on the estrous cycles.

Toxicokinetics

Not conducted.

Dosing Solution Analysis

No linaclotide was detected in the control solutions. The individual results for the homogeneity analysis were between 99.2% and 108.0% of the target, and the results of

concentration verification analyses were between 87.4% and 128.8% of the target. All samples met the acceptance criteria for precision and homogeneity. All samples, with the exception of the 50 mg/kg/day sample on week 10, met the acceptance criteria for precision and accuracy. The week 10 samples in the 50 mg/kg/day group failed the acceptance criteria for accuracy with a mean percent target value of 128.6%. However, according to the Sponsor, this had no impact on the outcome of the study since there was no significant toxicological effect.

Necropsy

Macroscopic examinations were conducted shortly after euthanization of animals. For each animal, the following organs were weighed; epididymides, ovaries, prostate, seminal vesicles, and testes (organ weight ratios relative to body weights were calculated). The following organs were preserved; cervix, epididymis (right), mammary glands (cervical and inguinal), ovaries, prostate, seminal vesicles, testes, uterine horns, uterus, and vagina.

There were no significant test article-treated effects on gross pathology or organ weights. Dark material was noted in the vagina of 3/22 females in the 100 mg/kg/day group compared with 0/22 control females.

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

Parental performance

Following the pre-mating treatment period, one female was placed with one male in the same dose group for up to 18 days. The females were examined for mating by examination of the vaginal lavage for spermatozoa. There were no test article-related effects on reproductive performance. The mean number of days needed to mate was 2.6, 4.1, 3.3 and 4.4 for the control, 10, 50, and 100 mg/kg/day groups, respectively. The mating index was 100% for all groups. The fertility index and conception rate were 100%, 90.9%, 100% and 90.9% for the control, 10, 50 and 100 mg/kg/day groups, respectively.

There were no test article-related effects on the numbers of corpora lutea, implantation sites, live embryos, dead embryos, or resorptions. Additionally, there were no test article-related effects on pre- and post-implantation losses.

The left cauda epididymis was used to assess sperm motility, sperm count, and sperm morphology. There was a 15% decrease in sperm count in the 100 mg/kg/day males compared to control males.

9.2 Embryonic Fetal Development

Study title: A Study of the Effects of Linaclotide Acetate on Embryo-Fetal

Reference ID: 3114166

Development in Mice	
Study no.:	MNP-103-033-RTR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.2.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 27, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide acetate (MD-1100 acetate), lot #PPL-MD11000603C, 92.6% relative to total peptide content.

Key Study Findings

A dose range-finding study (MNP-103-032-RTR-01) evaluating the effects of the test article on embryo-fetal development in mice showed that doses of 5, 20, and 80 mg/kg/day produced no significant clinical signs, and no body weight or food consumption changes. Additionally, intrauterine growth and survival were unaffected by test article administration with the exception of tarsal flexure and open eyelid noted in one fetus in the 5 and 80 mg/kg/day groups. The Sponsor indicated that, based on the lack of maternal or embryo-fetal developmental effects, oral doses of 5, 40, and 100 mg/kg/day (expressed as peptide content) were selected for the embryo-fetal developmental toxicity study in pregnant Crl:CD1 mice.

In the present study, during gestation days 7 to 10, 5 females (administered 1 to 4 doses of test article total) in the 100 mg/kg/day group were found dead or sacrificed *in extremis*. Thus, the 100 mg/kg/day dose level was lowered to 40 mg/kg/day (100/40 mg/kg/day group). In the 40 mg/kg/day group, 5 females (administered 1 to 10 doses of test article total) were found dead or sacrificed *in extremis*. Due to the mortality in the 40 mg/kg/day group, 7 pregnant mice (gestation day 6) from a stock colony were added to this group. Treatment-related maternal toxicity (i.e. mortality and moribund condition) was evident in the 40 and 100/40 mg/kg/day groups, with clinical signs such as hunched posture, hypoactivity, flattened body, tremors, cold to touch, and/or half-closed eyes. Additionally, treatment-related lower mean body weights and body weight gains were noted in the 40 and 100/40 mg/kg/day groups.

Developmental toxicity was noted in the 40 and 100/40 mg/kg/day groups. Statistically significant lower mean fetal weights were noted in the 40 and 100/40 mg/kg/day groups compared to the vehicle control groups, resulting in lower mean gravid uterine weights. In addition, test article-related increases in proportions of open eyelid (by mean litter) were noted at 40 mg/kg/day, and vertebral anomaly with or without associated rib anomaly were noted in the 40 and 100/40 mg/kg/day groups. The mean litter proportions of these findings exceeded the maximum mean values in the historical control data.

Reference ID: 3114166

Based on clinical signs, mortality, moribund condition, decrease in body weight gains, intrauterine growth and fetal morphology at doses of ≥40 mg/kg/day, the no-observed-adverse-effect level (NOAEL) for maternal and embryo-fetal developmental toxicity in mice was 5 mg/kg/day.

Methods	
Doses:	5, 40, and 100/40 (the 100 mg/kg/day dose level was reduced to 40 mg/kg/day beginning on gestation day 6, 7, 8, 9, 10 or 11.
Frequency of dosing:	Daily (days 6 to 15 of gestation)
Dose volume:	10 mL/kg
Route of administration:	Oral
Formulation/Vehicle:	0.5% methylcellulose 400 cps in deionized water
Species/Strain:	Crl:CD1 mice
Number/Sex/Group:	25 mated females/group (7 additional mice were added to the 40 mg/kg/day group during the study)
Satellite groups:	TK group (48 mated females/group)
Study design:	See below
Deviation from study protocol:	No

Table 38: Study Design of an Embryo-fetal Developmental Study in Mice

Embryo/fetal Development Phase

Group Number	Test Article	Dosage Level[a] (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control 1	0	10	25
2	Vehicle Control 2	0	10	25
3	Linaclotide acetate	5	10	25
4	Linaclotide acetate	40	10	32[c]
5	Linaclotide acetate	100/40[b]	10	25

Toxicokinetic Phase

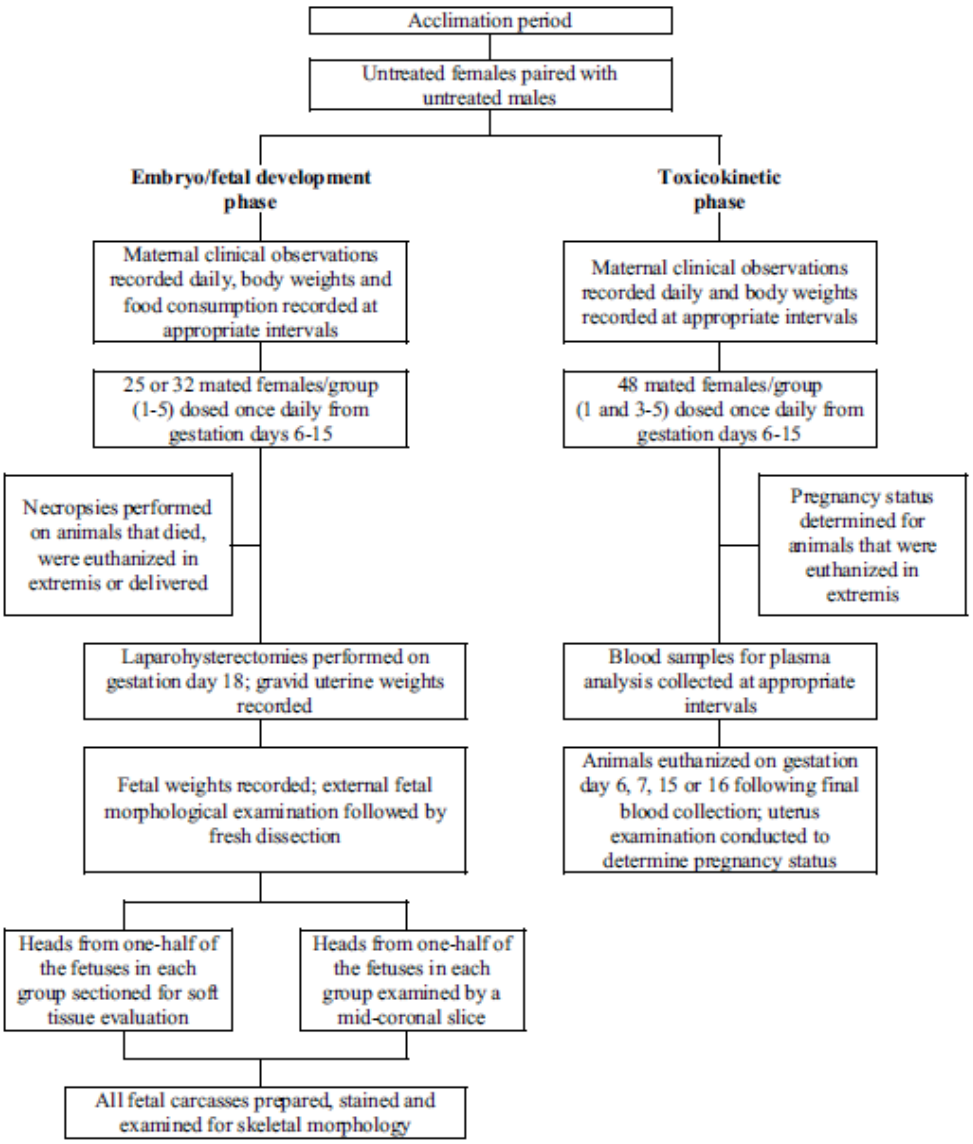
Group Number	Test Article	Dosage Level[a] (mg/kg/day)	Dosage Volume (mL/kg)	Number of Females
1	Vehicle Control 1	0	10	48
3	Linaclotide acetate	5	10	48
4	Linaclotide acetate	40	10	48
5	Linaclotide acetate	100/40[b]	10	48

[a] = Dosage levels and concentrations are expressed as peptide content. A correction factor of 1.08 was used to account for the peptide content.

[b] = The 100 mg/kg/day dosage level was lowered to 40 mg/kg/day beginning on 21 January 2008 (Gestation Day 6, 7, 8, 9, 10 or 11) and continuing throughout the remainder of the study.

[c] = Seven additional mice (from the stock colony) were added to Group 4 (embryo/fetal development phase) during the study.

Figure 8: Study Design of an Embryo-fetal Developmental Study in Mice



Observations and Results

Mortality

All mice were observed for mortality twice daily, once in the morning and once in the afternoon. During gestation days 7 to 10, 5 females (administered 1 to 4 doses of test article total) in the 100 mg/kg/day group were found dead or sacrificed *in extremis*. Thus, the 100 mg/kg/day dose level was lowered to 40 mg/kg/day, starting on gestation days 6-11 (pregnant mice were at varying days of gestation when dose reduction was implemented). An additional female in the 100/40 mg/kg/day group was sacrificed on gestation day 13. All the females that were found dead or sacrificed were pregnant. In

the 40 mg/kg/day group (group 4 in the table above), 5 females (administered 1 to 10 doses of test article total) were found dead or sacrificed *in extremis*. Due to the mortality in the 40 mg/kg/day (group 4) group, 7 pregnant mice (gestation day 6) from a stock colony were added to this group. Of the 5 females that were found dead or sacrificed, 4 were pregnant.

In the toxicokinetic phase, one female was sacrificed *in extremis* on gestation day 7, after a single dose of 100 mg/kg/day linaclotide. After the dose reduction from 100 to 40 mg/kg/day, 3 females in the 40 mg/kg/day group and one female in the 100/40 mg/kg/day were sacrificed *in extremis* between gestation days 13 and 15.

A majority of the females in the 40 and 100/40 mg/kg/day groups that were found dead or sacrificed had drug-related clinical signs including hunched posture, hypoactivity, tremors, cool to the touch, and half closed eyes noted on the day prior and/or on the day of death/sacrifice.

Clinical Signs

Individual detailed clinical observations were recorded 0.5 hours following dose administration from gestation days 0 through 18. Including those animals that were found dead or sacrificed, hunched posture was noted in 3/25 females in the 100/40 mg/kg/day group. Hypoactivity was observed in 4/32 and 5/25 females in the 40 and 100/40 mg/kg/day groups, respectively. A flattened body was noted in 5/32 and 6/25 females in the 40 and 100/40 mg/kg/day groups, respectively. Tremors were observed in 4/32 and 5/25 females in the 40 and 100/40 mg/kg/day groups, respectively. Cold body was noted in 5/32 and 6/25 females in the 40 and 100/40 mg/kg/day groups, respectively. Half-closed eyes were noted in 6/32 and 7/25 females in the 40 and 100/40 mg/kg/day group, respectively. Labored breathing was noted in 3/25 females in the 100/40 mg/kg/day group. None of the listed clinical signs were noted in any of the control females.

Body Weight

Maternal body weights were recorded on gestation days 0 and days 6 to 18 (daily). Initial body weights of the two control groups were 28.6 and 28.5 g, and the terminal body weights were 55.3 and 55.1 g. There was a 14-18% decrease in body weight gain between gestation days 12 and 16 in both the 40[11] and 100/40 mg/kg/day females when compared to the combined control groups. During the entire treatment period (gestation days 6 to 16), there was a ~12% decrease in body weight gain in the 40 and 100/40 mg/kg/day females when compared to the combined control groups. There was no significant difference in body weights between the test article-treated group and combined control groups on gestation day 16.

[11] Statistically significant ($p<0.05$) when compared to control group 2.

Additionally, gravid uterine weights were ~14% lower in the 40 and 100/40 mg/kg/day females compared to control groups 1 and 2.

Feed Consumption

Individual food consumption was recorded on gestation days 0, and days 6 to 18 (daily). Food intake was reported as g/animal/day and g/kg/day for the corresponding body weight change intervals. There were no test article-related effects on food consumption.

Toxicokinetics

Blood samples were collected via the vena cava on gestation days 6 and 15 prior to dose administration and at 1, 2, 4, 8, and 24 hours after dose administration.

The plasma concentrations of linaclotide and MM-419447 (active metabolite) were below the lower limits of quantitation (0.5 ng/mL for linaclotide, 5 ng/mL for MM-419447) in the control and treatment groups prior to gestation day 6 (i.e., predose). Following administration of linaclotide acetate to pregnant female mice, plasma concentrations of linaclotide and MM-419447 increased (between 1 and 2 hours) and then decreased rapidly to below the lower limits of quantitation (within 4 hours). Detection of linaclotide in plasma at time-points beyond 4 hours was sporadic, and was limited to the 40 and 100/40 mg/kg/day groups. The same groups also had quantifiable concentrations of MM-419447 beyond 4 hours. The half-lives of linaclotide and MM-419447 could not be estimated accurately for most dosing groups due to the abundance of points below the lower limit of quantitation, but were calculated to range from 0.5 to 1 hour for linaclotide.

C_{max} and AUC_{24hr} increased with dose, but dose proportionality could not be assessed due to the abundance of time-points below the limit of quantitation and the reduction of the 100 mg/kg/day dose to 40 mg/kg/day. The highest average C_{max} and AUC_{24hr} for linaclotide was 11.8 ng/mL and 25.2 ng·h/mL, respectively, observed in the high-dose group (100/40 mg/kg/day). Similarly, the highest average C_{max} and AUC_{24hr} for MM-419447 was 7.7 ng/mL and 12.3 ng·h/mL, respectively, in the 100/40 mg/kg/day dose group. No accumulation of linaclotide or MM-419447 was observed after repeated administration. The results are presented in the Sponsor's table below.

Reference ID: 3114166

Table 39: Mean Toxicokinetic Parameters of Linaclotide and MM-419447 Analyzed in the Embryo-fetal Developmental Study in Mice

Analyte	Daily Dose (mg/kg)	Gestation Day	$t_{1/2}$ (h)	t_{max} (h)	C_{max} (ng/mL)	C_{max}/D	AUC_2 (ng h/mL)	AUC_t (ng h/mL)	AUC_{24} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)	AUC Ratio
Linaclotide	5	6	NC	1.00	1.49	0.30	1.74	1.74	2.25	NC	NA
		15	NC	1.00	1.95	0.39	2.85	2.85	4.64	NA	NA
	40	6	1.13	2.00	8.12	0.20	6.55	17.3	18.6	17.5	NA
		15	NC	2.00	8.13	0.20	10.2	10.2	18.4	NA	NA
	100/40	6	NC	1.00	10.7	0.11	15.5	15.5	25.2	NC	NA
		15	0.46	1.00	11.8	0.12	14.9	21.4	21.7	NA	NA
MM-419447	5	6	NC	NC	0.00	NC	NC	0.00	0.00	NC	0.00
		15	NC	NC	0.00	NC	NC	0.00	0.00	NA	0.00
	40	6	NC	NC	0.00	NC	NC	0.00	0.00	NC	0.00
		15	NC	1.00	2.85	0.07	3.70	3.70	5.40	NA	0.29
	100/40	6	NC	1.00	5.42	0.05	7.72	7.72	12.3	NC	0.49
		15	NC	1.00	7.70	0.08	9.02	9.02	11.6	NA	0.54

Notes: NC = not calculated (not enough concentrations above LOQ in terminal phase), NA = not applicable, AUC
The dose normalized Cmax was calculated based on the doses of 5, 40 and 100 mg/kg.
Ratio = $AUC_{24,metabolite}/AUC_{24,parent}$

Dosing Solution Analysis

The individual results for the pre-initiation homogeneity analysis were between 97.3% and 100.7% for the 10 mg/mL formulation, and between 95.9% and 98.2% for the 0.5 mg/mL formulation. For the dosing formulations prepared at 0.5, 4.0 and 10 mg/mL linaclotide, the individual results were between 92.2% and 105.1% (week 0, week 1, and week 2). All samples met acceptance criteria for homogeneity, precision, and accuracy.

Necropsy

A gross necropsy was performed on females that died, were sacrificed *in extremis*, delivered early during the course of the study, or survived to the scheduled necropsy. There were no test article-related macroscopic findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Laparohysterectomies were performed with blinding to treatment group on gestation day 18. The number of corpora lutea on each ovary was recorded. The uterus was weighed and opened, and the number and location of all fetuses, early and late resorptions and the total number of implantation sites were recorded. The placentas were also examined. All implantation sites, including resorptions, were numbered in consecutive order beginning with the left distal to the left proximal uterine horn, noting the position of the cervix, and continuing from the right proximal to the right distal

uterine horn. Mean fetal weights were significantly (p<0.05 or p<0.01) decreased (7-9% per litter) in the 40 and 100/40 mg/kg/day groups when compared to the either control group. There was a slight increase (~5-7% per litter) in mean early resorptions and total resorptions, and post-implantation loss in the 40 and 100/40 mg/kg/day groups. These changes were primarily attributable to one female in each group. The data is summarized in the Sponsor's tables below.

Table 40: Summary of Cesarean Section Data in the Embryo-fetal Development Study in Mice

GROUP		SEX M	SEX F	VIABLE FETUSES	DEAD FETUSES	RESORPTIONS EARLY	RESORPTIONS LATE	POST IMPLANTATION LOSS	IMPLANTATION SITES	CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
1	TOTAL	140	127	267	3	13	6	22	289	311	22	NA	22
	MEAN	6.4	5.8	12.1	0.1	0.6	0.3	1.0	13.1	14.1	1.0	1.35	
	S.D.	2.06	1.93	2.25	0.64	0.91	0.63	1.45	2.21	2.12	1.02	0.062	
	S.E.	0.44	0.41	0.48	0.14	0.19	0.13	0.31	0.47	0.45	0.22	0.013	
2	TOTAL	139	130	269	1	11	2	14	283	304	21	NA	22
	MEAN	6.3	5.9	12.2	0.0	0.5	0.1	0.6	12.9	13.8	1.0	1.34	
	S.D.	2.08	2.47	1.60	0.21	0.74	0.29	0.79	1.46	1.65	1.00	0.092	
	S.E.	0.44	0.53	0.34	0.05	0.16	0.06	0.17	0.31	0.35	0.21	0.020	
3	TOTAL	151	134	285	0	15	5	20	305	335	30	NA	23
	MEAN	6.6	5.8	12.4	0.0	0.7	0.2	0.9	13.3	14.6	1.3	1.29	
	S.D.	2.78	2.71	3.30	0.00	1.30	0.60	1.32	2.42	2.97	1.29	0.080	
	S.E.	0.58	0.56	0.69	0.00	0.27	0.13	0.28	0.50	0.62	0.27	0.017	
4-A	TOTAL	144	159	303	1	26	3	30	333	368	35	NA	26
	MEAN	5.5	6.1	11.7	0.0	1.0	0.1	1.2	12.8	14.2	1.3	1.24df	
	S.D.	2.28	2.34	3.64	0.20	1.62	0.33	1.69	2.61	2.56	1.47	0.152	
	S.E.	0.45	0.46	0.71	0.04	0.32	0.06	0.33	0.51	0.50	0.29	0.030	
5-B	TOTAL	101	89	190	0	25	2	27	217	234	17	NA	17
	MEAN	5.9	5.2	11.2	0.0	1.5	0.1	1.6	12.8	13.8	1.0	1.23ce	
	S.D.	1.98	2.39	3.41	0.00	3.08	0.33	3.14	2.31	1.86	1.12	0.150	
	S.E.	0.48	0.58	0.83	0.00	0.75	0.08	0.76	0.56	0.45	0.27	0.036	

MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
A - SEVEN ADDITIONAL MICE WERE ADDED TO THIS GROUP ON 21 JANUARY 2008 (GESTATION DAYS 6)
B - THE 100 MG/KG/DAY DOSE LEVEL WAS LOWERED TO 40 MG/KG/DAY ON 21 JANUARY 2008 (GESTATION DAYS 6, 7, 8, 9, 10 OR 11)
c - Significantly different from control group 1 at 0.05
d - Significantly different from control group 1 at 0.01
e - Significantly different from control group 2 at 0.05
f - Significantly different from control group 2 at 0.01
NA - NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY

Table 41: Summary of Cesarean Section Data in the Embryo-fetal Development Study in Mice (% per litter)

GROUP:	VEHICLE CONTROL1	VEHICLE CONTROL2	5 MG/KG/DAY	40 MG/KG/DAY-A	100/40 MG/KG/DAY-B
CORPORA LUTEA					
MEAN	14.1	13.8	14.6	14.2	13.8
S.D.	2.12	1.65	2.97	2.56	1.86
S.E.	0.45	0.35	0.62	0.50	0.45
N	22	22	23	26	17
IMPLANTATION SITES					
MEAN	13.1	12.9	13.3	12.8	12.8
S.D.	2.21	1.46	2.42	2.61	2.31
S.E.	0.47	0.31	0.50	0.51	0.56
N	22	22	23	26	17
VIABLE FETUSES (%)					
MEAN	92.7	95.1	91.2	88.7	88.2
S.D.	10.20	6.32	20.54	20.97	21.29
S.E.	2.17	1.35	4.28	4.11	5.16
N	22	22	23	26	17
DEAD FETUSES (%)					
MEAN	1.1	0.4	0.0	0.2	0.0
S.D.	4.92	1.77	0.00	1.24	0.00
S.E.	1.05	0.38	0.00	0.24	0.00
N	22	22	23	26	17
EARLY RESORPTIONS (%)					
MEAN	4.5	3.9	7.4	10.2	10.9
S.D.	6.69	5.88	20.71	20.85	20.69
S.E.	1.43	1.25	4.32	4.09	5.02
N	22	22	23	26	17

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
CORPORA LUTEA AND IMPLANTATION SITES COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group
A = SEVEN ADDITIONAL MICE WERE ADDED TO THIS GROUP ON 21 JANUARY 2008 (GESTATION DAY 6)
B = THE 100 MG/KG/DAY DOSE LEVEL WAS LOWERED TO 40 MG/KG/DAY ON 21 JANUARY 2008 (GESTATION DAYS 6, 7, 8, 9, 10 OR 11)

GROUP:	VEHICLE CONTROL1	VEHICLE CONTROL2	5 MG/KG/DAY	40 MG/KG/DAY-A	100/40 MG/KG/DAY-B
LATE RESORPTIONS (%)					
MEAN	1.8	0.7	1.5	0.8	0.9
S.D.	4.12	2.12	3.91	2.34	2.66
S.E.	0.88	0.45	0.82	0.46	0.64
N	22	22	23	26	17
TOTAL RESORPTIONS (%)					
MEAN	6.3	4.6	8.8	11.0	11.8
S.D.	9.67	5.81	20.54	20.99	21.29
S.E.	2.06	1.24	4.28	4.12	5.16
N	22	22	23	26	17
PRE-IMPLANTATION LOSS (%)					
MEAN	7.1	6.6	8.4	9.3	7.6
S.D.	7.29	6.69	7.37	9.90	9.26
S.E.	1.55	1.43	1.54	1.94	2.25
N	22	22	23	26	17
POST-IMPLANTATION LOSS (%)					
MEAN	7.3	4.9	8.8	11.3	11.8
S.D.	10.20	6.32	20.54	20.97	21.29
S.E.	2.17	1.35	4.28	4.11	5.16
N	22	22	23	26	17
MALES (%)					
MEAN	52.2	52.3	53.2	47.1	53.7
S.D.	14.95	17.99	17.57	11.61	12.58
S.E.	3.19	3.83	3.75	2.32	3.05
N	22	22	22	25	17

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group
A = SEVEN ADDITIONAL MICE WERE ADDED TO THIS GROUP ON 21 JANUARY 2008 (GESTATION DAY 6)
B = THE 100 MG/KG/DAY DOSE LEVEL WAS LOWERED TO 40 MG/KG/DAY ON 21 JANUARY 2008 (GESTATION DAYS 6, 7, 8, 9, 10 OR 11)

GROUP:	VEHICLE CONTROL1	VEHICLE CONTROL2	5 MG/KG/DAY	40 MG/KG/DAY-A	100/40 MG/KG/DAY-B
FEMALES (%)					
MEAN	47.8	47.7	46.8	52.9	46.3
S.D.	14.95	17.99	17.57	11.61	12.58
S.E.	3.19	3.83	3.75	2.32	3.05
N	22	22	22	25	17
MALE FETAL WEIGHTS (g)					
MEAN	1.37	1.37	1.31	1.26de	1.24df
% DIFFERENCE-G		0.0	-4.4	-8.0	-9.5
% DIFFERENCE-H	0.0		-4.4	-8.0	-9.5
S.D.	0.070	0.101	0.098	0.155	0.148
S.E.	0.015	0.022	0.021	0.031	0.036
N	22	22	22	25	17
FEMALE FETAL WEIGHTS (g)					
MEAN	1.32	1.32	1.27	1.21de	1.22ce
% DIFFERENCE-G		0.0	-3.8	-8.3	-7.6
% DIFFERENCE-H	0.0		-3.8	-8.3	-7.6
S.D.	0.056	0.102	0.081	0.155	0.152
S.E.	0.012	0.022	0.017	0.031	0.037
N	22	22	22	25	17
COMBINED FETAL WEIGHTS (g)					
MEAN	1.35	1.34	1.29	1.24de	1.23ce
% DIFFERENCE-G		-0.7	-4.4	-8.1	-8.9
% DIFFERENCE-H	0.7		-3.7	-7.5	-8.2
S.D.	0.062	0.092	0.080	0.152	0.150
S.E.	0.013	0.020	0.017	0.030	0.036
N	22	22	22	25	17

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
FETAL WEIGHTS COMPARED USING DUNNETT'S TEST
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
A - SEVEN ADDITIONAL MICE WERE ADDED TO THIS GROUP ON 21 JANUARY 2008 (GESTATION DAY 6)
B - THE 100 MG/KG/DAY DOSE LEVEL WAS LOWERED TO 40 MG/KG/DAY ON 21 JANUARY 2008 (GESTATION DAYS 6, 7, 8, 9, 10 OR 11)
c - Significantly different from control group 1 at 0.05
d - Significantly different from control group 1 at 0.01
e - Significantly different from control group 2 at 0.05
f - Significantly different from control group 2 at 0.01
G - PERCENT DIFFERENCE FROM VEHICLE CONTROL GROUP 1
H - PERCENT DIFFERENCE FROM VEHICLE CONTROL GROUP 2

Offspring (Malformations, Variations, etc.)

Fetal examinations were performed with blinding to treatment group. Each viable fetus was examined externally, individually sexed, and weighed. The detailed external examination of each fetus included, but was not limited to, an examination of the eyes, palate and external orifices. Nonviable fetuses were examined, weighed, sexed, and the crown-rump length was measured. Visceral and skeletal findings were recorded. The number of fetuses available for morphological evaluation in the 0, 0, 5, 40, and 100/40 mg/kg/day groups were 267 (22 litters), 269 (22 litters), 285 (22 litters), 303 (25 litters) and 190 (17 litters), respectively. Malformations were observed in 2 (2 litters), 4 (4 litters), 5 (3 litters), 24 (8 litters) and 14 (5 litters) fetuses in the 0, 0, 5, 40, and 100/40 mg/kg/day groups, respectively. The results are presented in the Sponsor's table below.

Table 42: Summary of Fetal Examinations and Litters with Malformations in the Embryo-fetal Developmental Study in Mice

	FETUSES					LITTERS				
DOSE GROUP:	1	2	3	4	5	1	2	3	4	5
NUMBER EXAMINED EXTERNALLY	267	269	285	303	190	22	22	22	25	17
SHORT TAIL	0	0	0	0	1	0	0	0	0	1
AMELIA	0	0	0	0	1	0	0	0	0	1
CLEFT PALATE	1	0	4	9	0	1	0	2	1	0
PAW HYPERFLEXION	0	0	1	0	0	0	0	1	0	0
OPEN EYELID	0	0	0	5	0	0	0	0	3	0
POLYDACTYLY	0	1	0	0	0	0	1	0	0	0
BENT TAIL	0	1	0	0	0	0	1	0	0	0
NUMBER EXAMINED VISCERALLY	267	269	285	303	190	22	22	22	25	17
RIGHT-SIDED AORTIC ARCH	0	1	0	0	0	0	1	0	0	0
NUMBER EXAMINED SKELETALLY	267	269	285	303	190	22	22	22	25	17
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	1	0	0	8	10	1	0	0	4	3
STERNEBRA(E) MALALIGNED (SEVERE)	0	1	0	1	1	0	1	0	1	1
RIB ANOMALY	0	0	0	2	1	0	0	0	1	1
COSTAL CARTILAGE ANOMALY	0	0	0	0	2	0	0	0	0	2
VERTEBRAL CENTRA ANOMALY	0	0	0	1	0	0	0	0	1	0
TOTAL NUMBER WITH MALFORMATIONS										
EXTERNAL :	1	2	5	14	1	1	2	3	4	1
SOFT TISSUE:	0	1	0	0	0	0	1	0	0	0
SKELETAL :	1	1	0	12	14	1	1	0	5	5
COMBINED :	2	4	5	24	14	2	4	3	8	5

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY

The results from the examination of external malformations and variations of the fetuses showed that open eyelid was noted in 5 females in the 40 mg/kg/day group compared to 0 from the control group (see table above). The difference from the vehicle control group values was not statistically significant; however, the mean proportion (1.4% of fetuses) of this finding exceeded the maximum mean value in the historical control data (0.3%). In addition, cleft palate was observed in 1.4% and 2.6% pups in the 5 and 40 mg/kg/day groups respectively, compared to 0.3% and 0% in control groups 1 and 2, respectively. These values exceeded the maximum mean value in the historical control data (0.8% of fetuses), however the 9 fetuses in the 40 mg/kg/day group with cleft palate came from a single litter, which had a lower mean fetal body weight (0.92 g) than other litters in the 40 mg/kg/day group (1.25 g) and the control groups (1.3-1.4 g). Also, cleft palate was not noted in the 100/40 mg/kg/day group, thus the observed cleft palate was not attributed to the test article. In the 100/40 mg/kg/day group, one fetus was noted with a short tail (only 6 caudal vertebrae present) and amelia (humerus, radius, ulna and all digits absent and bent scapula; unilateral). These findings were noted in a single fetus with a low mean fetal weight (0.79 g) and therefore, not attributed to the test article.

The visceral examination for malformations and variations showed a developmental variation (hemorrhagic ring around the left iris) in one fetus in the 40 mg/kg/day group. The difference in the mean litter proportion of this finding (0.3%) from the vehicle control groups (0% for both groups) was not significant, and due to its occurrence in a single fetus, was not attributed to maternal test article administration.

The skeletal examination for malformations and variations showed a vertebral anomaly with or without associated rib anomaly (generally consisting of fused sternebrae, ribs or arches, absent ribs, centra or arches, smaller than normal centra or arches, malaligned sternebrae, malpositioned centra) in ~3% (8 pups from 4 litters) and 5% (10 pups from 3

litters) in the 40 and 100/40 mg/kg/day groups respectively, compared to 0.4% and 0% in control groups 1 and 2, respectively.

Overall, the 40 and 100/40 mg/kg/day groups had higher percentages of fetuses with malformations (~8% and 7% in the 40 and 100/40 mg/kg/day groups, respectively) when compared to control groups 1 (0.7%) and 2 (1.4%). There was no difference in fetal variations (external, visceral, or skeletal) between control and treatment groups. The fetal examination data is presented in the Sponsor's table below.

Table 43: External, Visceral, and Skeletal Examination for Malformations of Fetuses in the Embryo-fetal Developmental Study in Mice (% per litter)

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED EXTERNALLY		22	22	22	25	17
SHORT TAIL	MEAN	0.0	0.0	0.0	0.0	2.9
	S.D.	0.00	0.00	0.00	0.00	12.13
	S.E.	0.00	0.00	0.00	0.00	2.94
AMELIA	MEAN	0.0	0.0	0.0	0.0	2.9
	S.D.	0.00	0.00	0.00	0.00	12.13
	S.E.	0.00	0.00	0.00	0.00	2.94
CLEFT PALATE	MEAN	0.3	0.0	1.4	2.6	0.0
	S.D.	1.52	0.00	4.88	12.86	0.00
	S.E.	0.32	0.00	1.04	2.57	0.00
PAW HYPERFLEXION	MEAN	0.0	0.0	0.3	0.0	0.0
	S.D.	0.00	0.00	1.42	0.00	0.00
	S.E.	0.00	0.00	0.30	0.00	0.00
OPEN EYELID	MEAN	0.0	0.0	0.0	1.4	0.0
	S.D.	0.00	0.00	0.00	4.42	0.00
	S.E.	0.00	0.00	0.00	0.88	0.00
POLYDACTYLY	MEAN	0.0	0.4	0.0	0.0	0.0
	S.D.	0.00	1.94	0.00	0.00	0.00
	S.E.	0.00	0.41	0.00	0.00	0.00
BENT TAIL	MEAN	0.0	0.3	0.0	0.0	0.0
	S.D.	0.00	1.52	0.00	0.00	0.00
	S.E.	0.00	0.32	0.00	0.00	0.00

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED VISCERALLY		22	22	22	25	17
RIGHT-SIDED AORTIC ARCH	MEAN	0.0	0.4	0.0	0.0	0.0
	S.D.	0.00	1.94	0.00	0.00	0.00
	S.E.	0.00	0.41	0.00	0.00	0.00

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED SKELETALLY		22	22	22	25	17
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	MEAN	0.4	0.0	0.0	2.9	9.3
	S.D.	1.78	0.00	0.00	8.29	26.33
	S.E.	0.38	0.00	0.00	1.66	6.39
STERNEBRA(E) MALALIGNED (SEVERE)	MEAN	0.0	0.3	0.0	0.4	0.5
	S.D.	0.00	1.33	0.00	2.22	1.87
	S.E.	0.00	0.28	0.00	0.44	0.45
RIB ANOMALY	MEAN	0.0	0.0	0.0	0.6	0.5
	S.D.	0.00	0.00	0.00	2.86	1.87
	S.E.	0.00	0.00	0.00	0.57	0.45
COSTAL CARTILAGE ANOMALY	MEAN	0.0	0.0	0.0	0.0	1.0
	S.D.	0.00	0.00	0.00	0.00	2.78
	S.E.	0.00	0.00	0.00	0.00	0.67
VERTEBRAL CENTRA ANOMALY	MEAN	0.0	0.0	0.0	0.3	0.0
	S.D.	0.00	0.00	0.00	1.43	0.00
	S.E.	0.00	0.00	0.00	0.29	0.00

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED		22	22	22	25	17
TOTAL MALFORMATIONS						
PERCENT PER LITTER WITH EXTERNAL MALFORMATIONS	MEAN	0.3	0.7	1.7	4.0	2.9
	S.D.	1.52	2.41	4.99	13.31	12.13
	S.E.	0.32	0.51	1.06	2.66	2.94
PERCENT PER LITTER WITH SOFT TISSUE MALFORMATIONS	MEAN	0.0	0.4	0.0	0.0	0.0
	S.D.	0.00	1.94	0.00	0.00	0.00
	S.E.	0.00	0.41	0.00	0.00	0.00
PERCENT PER LITTER WITH SKELETAL MALFORMATIONS	MEAN	0.4	0.3	0.0	4.2	11.1
	S.D.	1.78	1.33	0.00	9.35	26.30
	S.E.	0.38	0.28	0.00	1.87	6.38
TOTAL PERCENT PER LITTER WITH MALFORMATIONS	MEAN	0.7	1.4	1.7	7.7	11.1
	S.D.	2.28	3.16	4.99	15.18	26.30
	S.E.	0.49	0.67	1.06	3.04	6.38

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

Table 44: External, Visceral, and Skeletal Examination for Variations of Fetuses in the Embryo-fetal Developmental Study in Mice (% per litter)

DOSE GROUP:	1	2	3	4	5
NUMBER OF LITTERS EXAMINED EXTERNALLY	22	22	22	25	17
NUMBER OF LITTERS WITH FINDINGS	0	0	0	0	0

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED VISCERALLY		22	22	22	25	17
HEMORRHAGIC RING AROUND THE IRIS	MEAN	0.0	0.0	0.0	0.3	0.0
	S.D.	0.00	0.00	0.00	1.33	0.00
	S.E.	0.00	0.00	0.00	0.27	0.00

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED SKELETALLY		22	22	22	25	17
14TH FULL RIB(S)	MEAN	17.0	21.7	16.3	23.0	20.4
	S.D.	17.98	18.17	19.09	28.14	23.78
	S.E.	3.83	3.87	4.07	5.63	5.77
7TH CERVICAL RIB(S)	MEAN	21.2	29.1	25.5	22.7	16.0
	S.D.	20.78	21.54	22.36	23.41	18.00
	S.E.	4.43	4.59	4.77	4.68	4.37
14TH RUDIMENTARY RIB(S)	MEAN	19.8	20.1	13.3	23.4	18.5
	S.D.	14.87	14.57	11.23	19.43	17.27
	S.E.	3.17	3.11	2.40	3.89	4.19
ACCESSORY SKULL BONE(S)	MEAN	16.5	12.5	17.1	11.8	18.4
	S.D.	16.18	14.45	12.92	10.95	12.78
	S.E.	3.45	3.08	2.75	2.19	3.10
25 PRESACRAL VERTEBRAE	MEAN	1.8	1.1	1.4	1.0	1.5
	S.D.	6.36	3.90	3.21	3.57	4.76
	S.E.	1.36	0.83	0.69	0.71	1.16
7TH STERNEBRA	MEAN	7.1	3.5	5.0	7.5	4.4
	S.D.	12.76	8.06	15.27	14.31	8.22
	S.E.	2.72	1.72	3.26	2.86	1.99
STERNEBRA(E) MALALIGNED(SLIGHT OR MODERATE)	MEAN	4.7	3.8	2.2	3.4	5.0
	S.D.	7.27	9.29	3.32	6.96	9.80
	S.E.	1.55	1.98	0.71	1.39	2.38
EXTRA SITE OF OSSIFICATION ANTERIOR TO CERVICAL ARCH #2	MEAN	0.0	0.3	0.9	0.0	0.5
	S.D.	0.00	1.52	2.39	0.00	1.87
	S.E.	0.00	0.32	0.51	0.00	0.45

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED SKELETALLY		22	22	22	25	17
27 PRESACRAL VERTEBRAE	MEAN	0.8	0.0	0.0	0.3	0.5
	S.D.	3.55	0.00	0.00	1.43	1.87
	S.E.	0.76	0.00	0.00	0.29	0.45
REDUCED OSSIFICATION OF THE 13TH RIB(S)	MEAN	0.0	0.0	0.0	0.0	0.4
	S.D.	0.00	0.00	0.00	0.00	1.73
	S.E.	0.00	0.00	0.00	0.00	0.42
EXTRA SITE OF OSSIFICATION VENTRAL TO CERVICAL CENTRUM #2	MEAN	0.0	0.3	1.8	0.3	1.0
	S.D.	0.00	1.52	4.89	1.43	4.04
	S.E.	0.00	0.32	1.04	0.29	0.98
VERTEBRAL CENTRA UNOSSIFIED	MEAN	0.0	0.3	0.0	1.5	0.0
	S.D.	0.00	1.64	0.00	3.61	0.00
	S.E.	0.00	0.35	0.00	0.72	0.00
EXTRA SITE OF OSSIFICATION DORSAL TO CERVICAL CENTRUM #2	MEAN	0.4	0.0	0.0	0.0	0.0
	S.D.	1.78	0.00	0.00	0.00	0.00
	S.E.	0.38	0.00	0.00	0.00	0.00
STERNEBRA(E) #1,#2,#3 AND/OR #4 UNOSSIFIED	MEAN	0.0	0.0	0.0	0.3	2.9
	S.D.	0.00	0.00	0.00	1.67	12.13
	S.E.	0.00	0.00	0.00	0.33	2.94
BENT RIB(S)	MEAN	0.0	0.0	0.0	0.7	0.0
	S.D.	0.00	0.00	0.00	3.33	0.00
	S.E.	0.00	0.00	0.00	0.67	0.00

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED SKELETALLY		22	22	22	25	17
STERNEBRA(E) #5 AND/OR #6 UNOSSIFIED	MEAN	0.0	0.0	0.0	0.9	0.0
	S.D.	0.00	0.00	0.00	3.25	0.00
	S.E.	0.00	0.00	0.00	0.65	0.00

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

DOSE GROUP:		1	2	3	4	5
NUMBER OF LITTERS EXAMINED		22	22	22	25	17
TOTAL VARIATIONS						
PERCENT PER LITTER WITH EXTERNAL VARIATIONS	MEAN	0.0	0.0	0.0	0.0	0.0
	S.D.	0.00	0.00	0.00	0.00	0.00
	S.E.	0.00	0.00	0.00	0.00	0.00
PERCENT PER LITTER WITH SOFT TISSUE VARIATIONS	MEAN	0.0	0.0	0.0	0.3	0.0
	S.D.	0.00	0.00	0.00	1.33	0.00
	S.E.	0.00	0.00	0.00	0.27	0.00
PERCENT PER LITTER WITH SKELETAL VARIATIONS	MEAN	62.6	67.6	60.5	67.8	63.8
	S.D.	24.74	21.95	18.15	20.99	21.70
	S.E.	5.27	4.68	3.87	4.20	5.26
TOTAL PERCENT PER LITTER WITH VARIATIONS	MEAN	62.6	67.6	60.5	67.8	63.8
	S.D.	24.74	21.95	18.15	20.99	21.70
	S.E.	5.27	4.68	3.87	4.20	5.26

1-VEHICLE CONTROL1 2-VEHICLE CONTROL2 3- 5 MG/KG/DAY 4- 40 MG/KG/DAY 5-100/40 MG/KG/DAY
MODIFIED STATISTICS USED.
For statistical analyses, control group 1 was compared to groups 3, 4 and 5; control group 2 was compared to groups 3, 4 and 5.
None significantly different from control group

Study title: MD-1100 Acetate: An Oral (Gavage) Embryo-Fetal Development Study in the Rat	
Study no.:	MNP-103-014-RTR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.2.1
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	June 26, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide acetate (MD-1100 acetate), lot #PPL-MD11000601A, 95.5% relative to total peptide content.

Key Study Findings

A dose range-finding study (MNP-103-052-RTR-01) evaluated the effects of daily administration of the test article (via oral gavage) on maternal and *in utero* toxicity in pregnant rats during the period of organogenesis. There were no test article-related effects on mortality, clinical signs, body weight gain, food consumption, or macroscopic observations in the mated females at doses of 1, 5, 10, and 20 mg/kg/day. At doses ≤ 20 mg/kg/day, the test article produced no maternal toxic effects, and there was no indication of effects on embryo/fetal viability or growth and development. The no observed effect level (NOEL) in pregnant rats was ≥ 20 mg/kg/day.

In the present embryo-fetal development study, rats were dosed with 10, 50, or 100 mg/kg/day test article. There were no test article-related effects on mortality, clinical signs, body weight gain, food consumption, or macroscopic observations. At doses up to 100 mg/kg/day, the test article produced no maternal toxic effects, and there were no differences in fetal malformations or variations between control and treatment groups.

No evidence of embryo-fetal toxicity was observed. Therefore, the NOAEL for maternal and embryo-fetal developmental toxicity in rats was 100 mg/kg/day.

Methods	
Doses:	10, 50, and 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral (days 6 to 17 of gestation)
Formulation/Vehicle:	0.5% methylcellulose 400 cps in deionized water
Species/Strain:	Crl:CD® (SD) rats
Number/Sex/Group:	22 mated females/group
Satellite groups:	TK group (2 mated females/group in vehicle and 8 females/group in test article-treated groups)
Study design:	See table below
Deviation from study protocol:	No

Table 45: Study Design of the Embryo-fetal Developmental Study in Rats

Group Number Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Number of Animals	
			Main Study	Toxicokinetic
			Females	Females
1/ Vehicle Control	0	10	22	2
2/ MD-1100 Acetate	10	10	22	8
3/ MD-1100 Acetate	50	10	22	8
4/ MD-1100 Acetate	100	10	22	8

Observations and Results

Mortality

All rats were observed twice daily for mortality. There was no mortality in the study.

Clinical Signs

Individual detailed clinical observations were recorded on all main study animals on the days of body weight assessment (see below) during gestation. Soft feces was noted in 2/22 females in the 100 mg/kg/day group compared to none in the control females. There were no test article-related clinical signs.

Body Weight

Maternal body weights were recorded on gestation days 0, 3, 6, 9, 12, 15, 18, and 21.

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The mean initial body weight (gestation day 0) of the control group was 219 g and the mean terminal body weight was 406 g. There was a 17% decrease in body weight gain in the 100 mg/kg/day group compared to the control group on day 17. However, over the course of the study period, there was no difference in body weights between the test article-treated groups and the control group. Thus, there were no test article-related effects on body weight.

Feed Consumption

Individual food consumption was recorded for all main study animals on days 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, and 18 to 21. Food intake was reported as g/animal/day. There were no test article-related effects on food consumption.

Toxicokinetics

Blood samples were collected via the jugular vein on gestation days 6 and 17 at 0.5, 1, 2, and 4 hours after dose administration, and plasma drug levels were measured. The lower limits of quantitation were 0.5 ng/mL for linaclotide and 5 ng/mL for MM-419447 (active metabolite). There was limited exposure to linaclotide in the treated groups. Linaclotide was readily absorbed with t_{max} values ranging from 0.5 to 4 h on gestation days 6 and 17. There were no consistent differences in C_{max} and AUC_{0-t}. The results indicate that there was no accumulation of linaclotide after multiple administrations. Neither linaclotide nor MM-419447 were detected in the control group. The results are presented in the table below.

Table 46: Mean Toxicokinetic Parameters of Linaclotide Analyzed in the Embryo-fetal Developmental Study in Rats

Reference ID: 3114166

Dose Group	Gender	Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0.5-2}$ (ng•hr/mL)
			Gestation Day 6			
2	F	10	2.53	1.00	NC	NC
3	F	50	33.2	4.00	62.3	17.3
4	F	100	26.9	0.500	37.2	30.5
			Gestation Day 17			
2[a]	F	10	NC	NC	NC	NC
3	F	50	21.4	0.500	NC[b]	19.2
4	F	100	38.7	0.500	NC[b]	39.8

NC Not calculated.
a All samples were below the limit of quantitation.
b Calculation of AUC_{0-t} was not possible because no pre-dose samples were collected.

Exposure to MM-419447 increased with the increasing doses of linaclotide (50 to 100 mg/kg/day). MM-419447 was either below the lower limit of quantitation or not calculated in the 10 mg/kg/day group. MM-419447 was readily absorbed with t_{max} values of 0.5 hr on gestation days 6 and 17. C_{max} and AUC_{0-t} increased in an almost dose proportional manner between the 50 and 100 mg/kg/day groups. The results indicate that there was no accumulation of MM-419447 after multiple administrations. The results are presented in the table below.

Table 47: Mean Toxicokinetic Parameters of MM-419447 Analyzed in the Embryo-fetal Developmental Study in Rats

Dose Group	Gender	MD-1100 Acetate Dose Level (mg/kg/day)	C_{max} (ng/mL)	T_{max} (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0.5-2}$ (ng•hr/mL)	$AUC_{0.5-2}$ Metabolite/Parent Ratio
			Gestation Day 6				
2	F	10	NC	NC	NC	NC	NC
3	F	50	11.2	0.500	12.9	10.1	0.583
4	F	100	22.0	0.500	29.2	23.7	0.778
			Gestation Day 17				
2[a]	F	10	NC	NC	NC	NC	NC
3	F	50	15.0	0.500	NC[b]	17.3	0.899
4	F	100	33.5	0.500	NC[b]	28.1	0.705

NC Not calculated.
a All samples were below the limit of quantitation.
b Calculation of AUC_{0-t} was not possible because no pre-dose samples were collected.

Dosing Solution Analysis

No linaclotide was detected in the control solutions. All dosing formulations were found to be homogenous. Results of the homogeneity and concentration verification analyses indicated all MD-1100 acetate dosing solutions were within 22.6% of the targeted concentrations.

Necropsy

Main study animals were sacrificed on day 21. There were no test article-related macroscopic findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

On gestation day 21, the reproductive tract, including the ovaries were removed. Corpora lutea were counted, gravid uterus was weighed, uterine contents were examined, and the number and position of live fetuses, dead fetuses, and early, middle and late resorptions were recorded. There were no test article-related findings. The number of corpora lutea, implantation sites, live and dead fetuses, early, middle or late resorptions, sex ratio, and the pre- and post implantation losses in each treatment group were comparable to that of the control group. The data is summarized in the Sponsor's tables below.

Table 48: Summary of Maternal Performance in the Embryo-fetal Development Study in Rats

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 10 mg/kg/day
Group 3 - MD-1100 Acetate 50 mg/kg/day
Group 4 - MD-1100 Acetate 100 mg/kg/day

	Group			
	1	2	3	4
Number of Rats Mated	22	22	22	22
Number of Pregnant Rats	21	21	21	22
Number of Rats Identified as Pregnant by Ammonium Sulfide	0	0	0	0
Number of Pregnancy Status not Determinable	0	0	0	0
Number of Rats Dying/Euthanized on Study	0	0	0	0
Number of Rats with Total Resorptions	0	0	0	0
Number of Rats Littering Prior to Scheduled Cesarean	0	0	0	0
Pregnancy Rate (%)	95.5	95.5	95.5	100.0

* Excluding toxicokinetic animals

Table 49: Summary of Group Mean Uterine Findings in the Embryo-fetal Development Study in Rats

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 10 mg/kg/day
Group 3 - MD-1100 Acetate 50 mg/kg/day
Group 4 - MD-1100 Acetate 100 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)
1	Mean	16.2	13.9	6.5	6.8	49.29
	SD	2.3	1.4	2.2	2.3	16.16
	N	21	21	21	21	21
2	Mean	16.0	14.1	6.6	6.7	51.12
	SD	2.0	1.6	2.1	2.2	15.91
	N	21	21	21	21	21
3	Mean	16.6	14.0	6.0	7.0	46.31
	SD	3.1	1.9	2.1	2.4	12.49
	N	21	21	21	21	21
4	Mean	15.7	14.0	6.1	7.4	45.01
	SD	2.4	2.0	1.8	1.8	10.52
	N	22	22	22	22	22

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Middle Resorptions	Late Resorptions
1	Mean	13.3	0.0	0.5	0.05	0.00
	SD	1.6	0.0	0.9	0.22	0.00
	N	21	21	21	21	21
2	Mean	13.2	0.0	0.9	0.00	0.05
	SD	2.8	0.0	2.0	0.00	0.22
	N	21	21	21	21	21
3	Mean	13.0	0.0	1.0	0.00	0.00
	SD	3.0	0.0	2.1	0.00	0.00
	N	21	21	21	21	21
4	Mean	13.5	0.0	0.5	0.00	0.00
	SD	2.3	0.0	0.7	0.00	0.00
	N	22	22	22	22	22

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

Group	Summary Information	Sum of Resorptions	Pre-implantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	0.6	13.39	4.19	99.7
	SD	0.9	12.39	6.73	10.1
	N	21	21	21	21
2	Mean	0.9	10.80	6.90	99.0
	SD	1.9	9.31	16.22	19.4
	N	21	21	21	21
3	Mean	1.0	14.13	7.46	97.5
	SD	2.1	11.22	16.45	19.2
	N	21	21	21	21
4	Mean	0.5	10.63	3.49	100.0
	SD	0.7	9.32	5.60	15.7
	N	22	22	22	22

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)

Offspring (Malformations, Variations, etc.)

Each fetus was weighed, given a detailed external examination, and the sex was recorded. Abnormalities were classified as major malformations, minor external, visceral or skeletal anomalies, or common skeletal variants. Fetal weights (males, females, and total) in each treatment group were comparable to the control group (see Sponsor's table below).

Table 50: Group Mean Fetal Weights in the Embryo-fetal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 10 mg/kg/day
Group 3 - MD-1100 Acetate 50 mg/kg/day
Group 4 - MD-1100 Acetate 100 mg/kg/day

Group	Summary Information	Males	Females	Total
1	Mean	5.766	5.480	5.621
	SD	0.295	0.300	0.273
	N	21	21	21
2	Mean	5.697	5.419	5.569
	SD	0.365	0.410	0.391
	N	21	20	21
3	Mean	5.826	5.509	5.662
	SD	0.415	0.408	0.414
	N	21	21	21
4	Mean	5.700	5.432	5.548
	SD	0.312	0.323	0.311
	N	22	22	22

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

The numbers of fetuses available for morphological evaluation were 279 (21), 278 (21 litters), 273 (21 litters), and 297 (22 litters) in the control, 10, 50, and 100 mg/kg/day groups, respectively. Major malformations were observed in one fetus in both the 10 and 100 mg/kg/day groups. Specifically, the 10 mg/kg/day fetus had reduced lower and upper jaws, stenosis of the pulmonary trunk, irregular formation of the palate, a shortened tongue, and an incompletely formed nasal pharyngeal canal. The 100 mg/kg/day fetus was missing an aortic arch and portion of the descending aorta, and had a shortened tail. The results are presented in the Sponsor's table below.

Examination of fetuses showed minor external and visceral abnormalities in the 10, 50, and 100 mg/kg/day groups (3, 1, and 1 fetus, respectively). In the 10 mg/kg/day group, the malformations and variations included: innominate artery of the heart absent, variations in minor vessels of the heart, reduction of the renal papilla(e), and dilated ureter or mega-ureter. In the 50 mg/kg/day group, variations included reduction of the renal papilla(e) and dilated ureters. In the 100 mg/kg/day group, malformations and variations included innominate artery of the heart absent and dilated ureters. There were significantly fewer fetuses with minor skeletal abnormalities in the 100 mg/kg/day group compared to the control group. Evaluation of common skeletal variants showed a dose-dependent decrease in the incidence of reduced ossification of sternebra 5 and xiphisternum at each dose level, compared with the controls. However, these percentages remained within the historical control ranges (i.e., 5.1 - 30.8%).

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Overall, there was no difference in fetal malformations, minor anomalies, or variations between control and treatment groups. The fetal examination data is presented in the Sponsor's table below.

Table 51: External, Visceral, and Skeletal Examination for Malformations of Fetuses in the Embryo-fetal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 10 mg/kg/day
Group 3 - MD-1100 Acetate 50 mg/kg/day
Group 4 - MD-1100 Acetate 100 mg/kg/day

	Group 1		Group 2		Group 3		Group 4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	21	279	21	278	21	273	22	297
Visceral (VIS)	21	141	21	139	21	135	22	149
Skeletal (SKE)	21	138	21	140	21	138	22	149
Technique of Wilson (WT)	21	141	21	139	21	135	22	149
Major Malformations (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Heart								
Stenosis of the pulmonary trunk (VIS)	0	0	1	1	0	0	0	0
Aortic arch and portion of descending aorta absent (VIS)	0	0	0	0	0	0	1	1
Tail								
Tail shortened (microcaudia) (EXT, SKE)	0	0	0	0	0	0	1	1

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		Group 2		Group 3		Group 4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	21	279	21	278	21	273	22	297
Visceral (VIS)	21	141	21	139	21	135	22	149
Skeletal (SKE)	21	138	21	140	21	138	22	149
Technique of Wilson (WT)	21	141	21	139	21	135	22	149
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Minor External and Visceral Anomalies (Total)	0	0	3	3	1	1	1	1
Heart								
Innominate artery absent (VIS)	0	0	1	1	0	0	1	1
Minor vessels variations (VIS)	0	0	1	1	0	0	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	21	279	21	278	21	273	22	297
Visceral (VIS)	21	141	21	139	21	135	22	149
Skeletal (SKE)	21	138	21	140	21	138	22	149
Technique of Wilson (WT)	21	141	21	139	21	135	22	149
Minor External and Visceral Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Kidney(s)								
Reduction of the renal papilla(e) (VIS)	0	0	1	1	1	1	0	0
Ureter(s)								
Ureter(s): Dilated (VIS)	0	0	1	1	1	1	1	1
Megaureter (VIS)	0	0	1	1	0	0	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	21	279	21	278	21	273	22	297
Visceral (VIS)	21	141	21	139	21	135	22	149
Skeletal (SKE)	21	138	21	140	21	138	22	149
Technique of Wilson (WT)	21	141	21	139	21	135	22	149
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Minor Skeletal Anomalies (Total)	16	43	13	31	16	39	17	26 **
Skull								
Parietal bone(s): Incomplete ossification	5	5	4	5	3	6	3	4
Interparietal bone: Incomplete ossification	11	22	9	14	7	17	10	10
Supraoccipital bone: Incomplete ossification	1	1	1	1	3	3	1	1
Hyoid bone: Incomplete ossification	13	26	6	10 **	10	15	3 **	3 ***

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	21	279	21	278	21	273	22	297
Visceral (VIS)	21	141	21	139	21	135	22	149
Skeletal (SKE)	21	138	21	140	21	138	22	149
Technique of Wilson (WT)	21	141	21	139	21	135	22	149
Minor Skeletal Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Vertebral Column								
Thoracic centrum misaligned	0	0	0	0	0	0	1	1
Thoracic vertebral arches fused	0	0	1	1	0	0	0	0
Thoracic vertebral arch(es) absent	0	0	1	1	0	0	0	0
Ossification center on 1st lumbar or14th thoracic vertebra	4	6	5	10	7	12	5	9
Lumbar centrum bipartite	0	0	1	1	0	0	0	0
Lumbar vertebral arch(es): Fused	0	0	1	1	0	0	0	0
Sacral vertebral centrum: Incomplete ossification	0	0	0	0	0	0	1	1
Sacral vertebral arch(es): Absent	0	0	0	0	0	0	1	1
Sacral vertebral arch(es): Incomplete ossification	0	0	0	0	0	0	1	1

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	21	279	21	278	21	273	22	297
Visceral (VIS)	21	141	21	139	21	135	22	149
Skeletal (SKE)	21	138	21	140	21	138	22	149
Technique of Wilson (WT)	21	141	21	139	21	135	22	149
Minor Skeletal Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Sternebrae								
Sternebrae: Misaligned	0	0	1	1	0	0	0	0
Ribs								
Fused rib(s)	0	0	1	1	0	0	0	0
Rudimentary 14th rib(s)	1	1	0	0	2	2	1	1
Rib(s): Incomplete ossification	1	2	0	0	0	0	0	0
Ossification center(s) on 7th cervical vertebra	2	2	1	1	0	0	0	0
Pelvic Girdle								
Pubic bone(s): Incomplete ossification	0	0	0	0	0	0	1	1

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Table 52: Group Incidence of External, Visceral and Skeletal Findings (Major Malformations, Minor Anomalies and Common Variants) in the Embryo-fetal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 10 mg/kg/day
Group 3 - MD-1100 Acetate 50 mg/kg/day
Group 4 - MD-1100 Acetate 100 mg/kg/day

	Affected Fetuses/Litters Mean % (SD) Group			
	1	2	3	4
Common Skeletal Variants				
Thoracic centrum variants (unossified/incomplete/ semi-bipartite/bipartite)	20.4 (27.50)	22.8 (26.75)	26.3 (27.22)	23.6 (21.85)
Sternebrae 1 to 4 (unossified/incomplete/ semi-bipartite/bipartite)	0.0 (0.00)	1.1 (3.56)	1.7 (5.54)	0.6 (2.67)
Sternebra 5 and Xiphisternum (unossified/incomplete/ semi-bipartite/bipartite)	29.2 (26.85)	26.9 (29.32)	19.0 (22.38)	14.5 (16.98)

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Study title: MD-1100 Acetate: An Oral (Gavage) Embryo-Fetal Development Study in the Rabbit	
Study no.:	MNP-103-015-RTR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.2.1
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	June 21, 2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide acetate (MD-1100 acetate), lot # PPL-MD11000601A, 95.5% relative to total peptide content.

Key Study Findings

A dose range-finding study (MNP-103-053-RTR-01) evaluated the effects of daily administration of MD-1100 acetate (via oral gavage) on maternal and developmental toxicity in pregnant rabbits during the period of organogenesis. There was a dose-dependent increase in soft feces in the 1, 5, and 20 mg/kg/day groups. However, there was no significant drug-related effects on clinical signs, body weight gain, food consumption, or macroscopic observations in the mated females. There was no indication of effects on embryo-fetal growth or viability and development. The NOAEL in pregnant rabbits was ≥ 20 mg/kg/day.

The Sponsor conducted an additional 13-day oral dose range finding toxicology study (MNP-103-029-RTR-01) in female rabbits. The test article was administered at doses of 5 and 40 mg/kg/day to provide information on dosage selection for a subsequent embryo-fetal development study in rabbits. Soft feces and/or brown fur staining were observed for all treated animals at 5 and 40 mg/kg/day. Additionally, there was a decrease (non dose-dependent) in food intake in the 5 and 40 mg/kg/day groups. There were no other test article-related effects on mortality, clinical signs, body weight gain or macroscopic observations. Based on the results of this study, the Sponsor concluded that 40 mg/kg/day was suitable as the high dose level for the subsequent embryo-fetal development study in the rabbit.

In the present embryo-fetal developmental study, rabbits were dosed with 5, 10, or 40 mg/kg/day MD-1100 acetate. There were no drug-related effects on mortality, body weight gain, food consumption, or macroscopic observations. At doses up to 40 mg/kg/day, the test article produced no maternal toxic effects, and there were no differences in fetal malformations or variations (external, visceral, or skeletal) between the control and treatment groups. No evidence of embryo-fetal toxicity was observed. Therefore, the NOAEL for maternal and embryo-fetal developmental toxicity in rabbits was 40 mg/kg/day.

Methods	
Doses:	5, 10, and 40 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg
Route of administration:	Oral (days 7 to 19 of gestation)
Formulation/Vehicle:	0.5% methylcellulose 400 cps in deionized water
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	22 mated females/group
Satellite groups:	None
Study design:	See table below
Deviation from study protocol:	None

Table 53: Study Design of the Embryo-fetal Developmental Study in Rabbits

Group Number Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Animal Numbers Females
1/ Vehicle Control	0	10	1501-1522
2/ MD-1100 Acetate	5	10	2501-2522
3/ MD-1100 Acetate	10	10	3501-3522
4/ MD-1100 Acetate	40	10	4501-4522

Observations and Results

Mortality

All rabbits were observed twice daily for mortality. There was no mortality in the study.

Clinical Signs

Individual detailed clinical observations were recorded on all main study animals on the days of body weight assessment (see below) during gestation. Soft feces was noted in 11, 7, and 16 females in the 5, 10, and 40 mg/kg/day groups respectively, compared to 3 females in the control group. Brown fur staining was noted with higher incidence and frequency in the 5 and 40 mg/kg/day groups when compared to the control group. There were no other significant test article-related clinical signs.

Body Weight

Maternal body weights were recorded on gestation days 0, 5, 7, 10, 13, 16, 20, 23, 26, and 29. The initial body weight (gestation day 5) of the control group was 3.1 kg and the terminal body weight was 3.6 kg. On days 20 to 23, there was a 25%, 42% (statistically significant; $p \leq 0.05$), and 33% decrease in body weight gain in the 5, 10, and 40 mg/kg/day groups compared to the control group. However, over the course of the study period, there were no differences in body weights between the test article-treated groups and control group. Thus, there were no test article-related effects on body weight gain.

Feed Consumption

Individual food consumption was measured daily from gestation day 5 onwards. Food intake was reported as g/animal/day. There was a 15% and 12% decrease in food consumption on days 16 to 17 in the 10 and 40 mg/kg/day groups compared to the control group. Additionally, a statistically significant decrease of 14% was noted on days 17 to 18 in the 10 mg/kg/day group. Since the observed differences in food consumption were sporadic, these changes are not considered to be drug-related.

Toxicokinetics

Blood samples were collected via the jugular vein on gestation days 7 and 19 at 0.5, 1, 2, 4, and 8 hours after dose administration. Linaclotide was readily absorbed with t_{max} values ranging from 0.5 to 1.0 hours on gestation day 7 and 0.5 hours on gestation day 19. Exposure to linaclotide increased with dose level from 5 to 40 mg/kg/day. However, the increases in C_{max} and $AUC_{0.5\text{-}8}$ were not consistently dose proportional. Since exposure to linaclotide was similar after multiple doses, there was no evidence of drug accumulation in plasma. TK analysis of MM-419447 (active metabolite) was limited to the 40 mg/kg/day group, given that plasma levels were generally below the limit of quantitation in the 5 and 10 mg/kg/day groups. The plasma concentrations of linaclotide and MM-419447 were below the lower limits of quantitation (not specified in

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the study report) in the control group. The TK analysis results for linaclotide and MM-419447are presented in the Sponsor's tables below.

Table 54: Mean Toxicokinetic Parameters of Linaclotide in the Embryo-fetal Developmental Study in Rabbits

Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0.5-4}$ (ng•hr/mL)	$AUC_{0.5-8}$ (ng•hr/mL)	$t_{1/2}$ (hr)
			Gestation Day 7				
2	5	34.3	0.500	36.0	35.1	35.1	NC
3	10	50.2	1.00	110	99.5	124	NC
4	40	199	0.500	634	474	584	1.38
			Gestation Day 19				
2	5	25.2	0.500	NC	46.0	55.0	1.36
3	10	72.8	0.500	NC	101	118	1.63
4	40	364	0.500	NC	503	636	1.83

NC: not calculated due to absence of predose plasma drug concentration

Table 55: Mean Toxicokinetic Parameters of MM-419447 in the Embryo-fetal Developmental Study in Rabbits

Dose Group	Dose Level (mg/kg/day)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-t} (ng•hr/mL)	$AUC_{0.5-4}$ (ng•hr/mL)	$AUC_{0.5-8}$ (ng•hr/mL)	$AUC_{0.5-4}$ Metabolite to Parent Ratio
			Gestation Day 7				
4	40	5.56	2.00	16.5	15.4	24.6	0.0326
			Gestation Day 19				
4	40	6.59	2.00	NC	20.1	30.3	0.0400

Dosing Solution Analysis

No MD-1100 acetate was detected in the control solutions. All dosing formulations were found to be homogenous. Results of the homogeneity and concentration verification analyses indicated that all linaclotide dosing solutions were within 19.8% above the targeted concentrations. The Sponsor stated that these deviations were consistent between concentrations and were observed on both occasions. The deviations from the

targeted concentrations were considered to have no impact on the interpretation of the study data.

Necropsy

Main study animals were sacrificed on day 29. There were no test article-related macroscopic findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

On gestation day 29, the reproductive tract, including the ovaries were removed. Corpora lutea were counted, gravid uterus was weighed, uterine contents were examined, and the number and position of live fetuses, dead fetuses, and early, middle and late resorptions were recorded. There were no test article-related findings. The number of corpora lutea, implantation sites, live and dead fetuses, early, middle or late resorptions, sex ratio, and the pre- and post implantation losses in each treatment group were comparable to that of the control group. The data is summarized in the Sponsor's tables below.

Table 56: Summary of Maternal Performance in the Embryo-fetal Development Study in Rabbits

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 5 mg/kg/day
Group 3 - MD-1100 Acetate 10 mg/kg/day
Group 4 - MD-1100 Acetate 40 mg/kg/day

	Group 1	2	3	4
Number of Rabbits Inseminated/Mated	22	22	22	22
Number of Pregnant Rabbits	20	22	21	20
Number of Rabbits Identified as Pregnant by Ammonium Sulfide	0	0	0	0
Number of Pregnancy Status not Determinable	0	0	0	0
Number of Rabbits Dying/Euthanized on Study	0	0	0	0
Number of Rabbits with Total Resorptions	0	0	0	0
Number of Rabbits Littering Prior to Scheduled Cesarean	0	0	0	0
Number of Rabbits Aborting Prior to Scheduled Cesarean	0	0	0	0
Pregnancy Rate (%)	90.9	100.0	95.5	90.9

Reference ID: 3114166

Table 57: Summary of Group Mean Uterine Findings in the Embryo-fetal Development Study in Rabbits

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 5 mg/kg/day
Group 3 - MD-1100 Acetate 10 mg/kg/day
Group 4 - MD-1100 Acetate 40 mg/kg/day

Group	Summary Information	Total Number of Corpora Lutea	Total Implantation Sites	Male Fetuses	Female Fetuses	Sex Ratio (% Males)
1	Mean	9.0	7.8	3.1	4.4	41.98
	SD	1.7	1.8	1.4	1.8	18.05
	N	20	20	20	20	20
2	Mean	9.4	7.9	3.9	3.7	50.05
	SD	1.5	1.9	2.0	1.6	21.93
	N	22	22	22	22	22
3	Mean	9.7	8.6	3.9	4.6	48.35
	SD	2.7	2.8	1.8	2.4	22.77
	N	21	21	21	21	21
4	Mean	8.6	7.6	3.9	3.6	53.09
	SD	1.7	1.9	1.3	1.7	18.90
	N	20	20	20	20	20

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Group	Summary Information	Live Fetuses	Dead Fetuses	Early Resorptions	Middle Resorptions	Late Resorptions
1	Mean	7.5	0.0	0.15	0.1	0.05
	SD	1.7	0.0	0.49	0.2	0.22
	N	20	20	20	20	20
2	Mean	7.6	0.0	0.23	0.0	0.05
	SD	1.9	0.0	0.43	0.0	0.21
	N	22	22	22	22	22
3	Mean	8.5	0.0	0.05	0.0	0.05
	SD	2.7	0.0	0.22	0.0	0.22
	N	21	21	21	21	21
4	Mean	7.5	0.0	0.05	0.1	0.00
	SD	1.9	0.0	0.22	0.3	0.00
	N	20	20	20	20	20

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Group	Summary Information	Sum of Resorptions	Number of Empty Implantation Sites	Pre-implantation Loss (%)	Post Implantation Loss (%)	Gravid Uterus Weight (g)
1	Mean	0.3	0.0	13.53	2.85	450.1
	SD	0.6	0.0	13.62	6.21	97.2
	N	20	20	20	20	20
2	Mean	0.3	0.0	16.01	3.40	464.4
	SD	0.5	0.0	14.76	5.74	96.2
	N	22	22	22	22	22
3	Mean	0.1	0.0	11.80	0.87	493.8
	SD	0.3	0.0	12.71	2.81	119.6
	N	21	21	21	21	21
4	Mean	0.2	0.0	10.82	1.81	461.0
	SD	0.4	0.0	16.21	4.42	96.5
	N	20	20	20	20	20

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

Offspring (Malformations, Variations, etc.)

Each fetus was weighed, given a detailed external examination, and the sex was recorded. Abnormalities were classified as major malformations, minor external, visceral or skeletal anomalies, or common skeletal variants. Fetal weights (males, females, and total) in each treatment group were comparable to control group (see table below).

Table 58: Group Mean Fetal Weights in the Embryo-fetal Developmental Study in Rabbits

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 5 mg/kg/day
Group 3 - MD-1100 Acetate 10 mg/kg/day
Group 4 - MD-1100 Acetate 40 mg/kg/day

Group	Summary Information	Males	Females	Total
1	Mean	44.87	44.18	44.48
	SD	4.80	5.00	4.76
	N	20	20	20
2	Mean	44.77	44.80	44.67
	SD	4.92	5.48	4.76
	N	22	22	22
3	Mean	44.52	42.45	43.56
	SD	6.05	6.27	6.01
	N	21	20	21
4	Mean	45.64	44.20	44.93
	SD	3.93	3.65	3.44
	N	20	19	20

Significantly different from control group (group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P \leq 0.05$ E - $P \leq 0.01$ F - $P \leq 0.001$ (Dunn)

The numbers of fetuses available for morphological evaluation were 150 (20 litters), 167 (22 litters), 178 (21 litters), and 149 (20 litters) in the control, 5, 10, and 40 mg/kg/day groups, respectively. A major malformation (diaphragmatic hernia) was observed in one fetus in both the control and 5 mg/kg/day groups. The results are presented in the Sponsor's table below.

Examination of external malformations and variations noted that 3 fetuses in the 10 mg/kg/day group had retrovacal ureter(s) compared to 1 fetus in the control group. Evaluation of minor skeletal variants noted rib(s) on the 7th cervical vertebra in 3 fetuses in the 40 mg/kg/day group compared to 2 fetuses in the control group. Additionally, 2 fetuses in the 10 mg/kg/day group had incomplete ossification of the pubic bone(s) compared to 2 fetuses in the control group.

Overall, there was no difference in fetal malformations, minor anomalies, or variations between control and treatment groups. The fetal examination data is presented in the Sponsor's table below.

Reference ID: 3114166

Table 59: External, Visceral, and Skeletal Examination for Malformations of Fetuses in the Embryo-fetal Developmental Study in Rabbits

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 5 mg/kg/day
Group 3 - MD-1100 Acetate 10 mg/kg/day
Group 4 - MD-1100 Acetate 40 mg/kg/day

	Group 1		Group 2		Group 3		Group 4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	20	150	22	167	21	178	20	149
Visceral (VIS)	20	150	22	167	21	178	20	149
Skeletal (SKE)	20	150	22	167	21	178	20	149
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Major Malformations (Total)	2	2	1	1	0	0	0	0
Uterus Ovaries								
Ovary absent (VIS)	1	1	0	0	0	0	0	0
Diaphragm								
Diaphragmatic hernia (VIS)	1	1	1	1	0	0	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected

	Group 1		Group 2		Group 3		Group 4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	20	150	22	167	21	178	20	149
Visceral (VIS)	20	150	22	167	21	178	20	149
Skeletal (SKE)	20	150	22	167	21	178	20	149
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Minor External and Visceral Anomalies (Total)	4	5	2	2	5	5	0	0
Ureter(s)								
Ureter(s) retrocaval (VIS)	1	1	1	1	3	3	0	0
Lungs and Thymus								
Accessory lung lobe absent (VIS)	2	3	1	1	0	0	0	0
Liver								
Supernumerary liver lobe (VIS)	1	1	0	0	0	0	0	0
Vestigial liver lobe (VIS)	0	0	0	0	1	1	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - $P \leq 0.05$ ** - $P \leq 0.01$ *** - $P \leq 0.001$ (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	20	150	22	167	21	178	20	149
Visceral (VIS)	20	150	22	167	21	178	20	149
Skeletal (SKE)	20	150	22	167	21	178	20	149
Minor External and Visceral Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Spleen								
Spleen reduced (VIS)	0	0	0	0	1	1	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	20	150	22	167	21	178	20	149
Visceral (VIS)	20	150	22	167	21	178	20	149
Skeletal (SKE)	20	150	22	167	21	178	20	149
	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Minor Skeletal Anomalies (Total)	16	50	19	50	18	46	19	49
Skull								
Nasal bone(s) incomplete ossification	1	1	1	1	1	1	0	0
Parietal bone(s) incomplete ossification	1	1	0	0	0	0	0	0
Frontal bone(s) incomplete ossification	7	13	11	18	10	14	9	11
Interparietal bone incomplete ossification	0	0	0	0	0	0	1	1
Interparietal bone bipartite	0	0	0	0	1	1	0	0
Hyoid bone incomplete ossification	5	15	9	20	8	21	9	19
Hyoid bone irregular ossification	1	4	2	4	4	4	6	9

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	20	150	22	167	21	178	20	149
Visceral (VIS)	20	150	22	167	21	178	20	149
Skeletal (SKE)	20	150	22	167	21	178	20	149
Minor Skeletal Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Sternebrae								
Sternebrae fused	4	4	3	5	2	2	2	2
Extra sternebra(e)	3	3	1	1	1	1	0	0
Ribs								
Fused rib(s)	1	1	0	0	0	0	0	0
Ossification center(s) on 7th cervical vertebra	1	1	0	0	1	1	1	1
Rib(s) on 7th cervical vertebra	2	2	1	1	1	1	3	4
Bifurcated rib(s)	1	1	0	0	0	0	0	0
Detached rib(s)	1	1	0	0	0	0	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

	Group 1		2		3		4	
	L/E	F/E	L/E	F/E	L/E	F/E	L/E	F/E
External (EXT)	20	150	22	167	21	178	20	149
Visceral (VIS)	20	150	22	167	21	178	20	149
Skeletal (SKE)	20	150	22	167	21	178	20	149
Minor Skeletal Anomalies (Cont'd)	L/A	F/A	L/A	F/A	L/A	F/A	L/A	F/A
Pelvic								
Pubic bone(s) incomplete ossification	2	2	3	3	2	5	0	0
Pubic bone(s) unossified	0	0	0	0	1	1	0	0

L/E = Litters examined L/A = Litters affected
F/E = Fetuses examined F/A = Fetuses affected
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Table 60: Group Incidence of Fetal External, Visceral and Skeletal Findings (Major Malformations, Minor Anomalies and Common Variants) in the Embryo-fetal Developmental Study in Rabbits

Group 1 - Vehicle Control
Group 2 - MD-1100 Acetate 5 mg/kg/day
Group 3 - MD-1100 Acetate 10 mg/kg/day
Group 4 - MD-1100 Acetate 40 mg/kg/day

	Affected Fetuses/Litters Mean % (SD) Group			
	1	2	3	4
Common Skeletal Variants				
Unilateral 13th rib (extra/rudimentary)	12.89 11.35	14.80 10.44	15.65 16.14	21.36 19.05
Bilateral 13th rib (extra/rudimentary/ ossification center)	56.80 25.43	40.08 27.20	32.32 b 25.35	40.00 a 30.74
Ribs - total 13th (unilateral and bilateral) extra/rudimentary/ossification center	69.67 24.88	54.89 29.17	47.98 27.02	61.36 29.30
Sternebrae (unossified/incomplete/semi-bipartite/ bipartite)	35.54 28.86	43.27 27.33	25.08 18.84	50.07 30.06

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

9.3 Prenatal and Postnatal Development

Study title: Linaclotide Acetate: An Oral Pre and Postnatal Study in the Rat	
Study no.:	MNP-103-041-RTR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.3.1
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	July 7, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide acetate, lot # PPL MD11000604A, PPL-MD11000601D, PPL-MD11000601A , 89.9%, 90.1%, 95.5%, respectively relative to total peptide content

Key Study Findings

Linaclotide acetate was administered daily to time-mated rats at doses of 10, 50 and 100 mg/kg/day from gestation day 6 to *post partum* day 21. There were no drug-related mortalities, clinical signs, or body weight changes in the F_0 dams, F_1 pups and adults, or

the F_2 pups. There was no maternal toxicity observed in the F_0 or F_1 pups. There were no drug-related effects on the survival, viability, physical development, behavior, or reproductive performance of F_1 pups and adults. Furthermore, there were no drug-related effects on the F_2 pups, based on external and internal examination. The NOAEL for the F_0, F_1, and F_2 generations is ≥ 100 mg/kg/day.

Methods	
Doses:	10, 50, and 100 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	10 mL/kg/day
Route of administration:	Oral (gestation day 6 to *post partum* day 21, 22, or 23)
Formulation/Vehicle:	0.5% methylcellulose 400 cps in deionized water
Species/Strain:	Crl:CD® (SD) rats
Number/Sex/Group:	24 mated females/group
Satellite groups:	None
Study design:	See table below
Deviation from study protocol:	None

Table 61: Study Design of the Pre- and Post-natal Developmental Study in Rats

Group Number Identification	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Animal Number Females
1/ Vehicle Control	0	10	1501 to 1524
2/ Linaclotide	10	10	2501 to 2524
3/ Linaclotide	50	10	3501 to 3524
4/ Linaclotide	100	10	4501 to 4524

Observations and Results

F_0 Dams	
Survival:	All animals were observed twice daily for mortality. There was no test article-related mortality.
Clinical signs:	All F_0 animals were observed on the days of body weight assessment (see below). During gestation, broken toe nails (2nd digit right forepaw) were observed in 2/24 animals in the 100 mg/kg/day group compared to 0 females in the control group. There were 8 incidences of thin fur cover on the right forepaw in the 50 mg/kg/day group compared to 1 incidence in the control group. Overall, there were increased incidences of thin fur cover during the gestation and lactation period in the 50 mg/kg/day group compared to the control group.
Body weight:	Individual body weights were recorded on gestation days 1, 3, 6, 9, 12, 15, 18, and 20 and on *post partum* days 0, 4, 7, 10, 14, 17 and 21. There was a dose-dependent decrease in body weight gain of 16%, 18%, and 19% in the 10, 50, and 100 mg/kg/day groups compared to the control on *post partum* days 0 to 4. However, over the course of the study period, there was no difference in body weights between the treatment groups and the control group. Thus, there were no drug-related effects on body weight.
Feed consumption:	Individual food consumption was measured on gestation days 3 to 6, 6 to 9, 9 to 12, 12 to 15, and 15 to 18, and on *post partum* days 0 to 4, 4 to 7, 7 to 10, and 10 to 14. There were no drug-related effects on food consumption.
Uterine content:	There was a significantly greater incidence of dead pups in 10 mg/kg/day litters when compared to the control group (see table below). The length of gestation and duration of parturition at each dose level were comparable to controls. The sex ratio, number of live, dead and malformed pups were unaffected by treatment with the test article. The results are summarized in the Sponsor's table below.
Necropsy observation:	Mated females which failed to deliver pups were sacrificed on day 26 *post coitum*. All other females were sacrificed on *post partum* day 22. A dark area of the lung was observed in 4/24 and 3/24 females in the 10 and 100 mg/kg/day groups respectively, compared to 1/24 females in the control group. There were no drug-related effects on macroscopic findings.
Toxicokinetics:	Not conducted
Dosing Solution Analysis	The week 1 homogeneity and concentration

	verification results for the 10 and 100 mg/kg/day groups met acceptance criteria for precision, accuracy, and homogeneity. The 50 mg/kg/day group samples did not meet the acceptance criteria for precision (% recovery values = 107% to 118%). However, the retention samples were analyzed and met acceptance criteria for precision, accuracy, and homogeneity. The week 2 concentration verification samples met acceptance criteria for precision and accuracy. The week 5 concentration verification samples prepared using lot PPL-MD11000601D met acceptance criteria for precision, but failed for accuracy with mean % recovery values of 112%, 112%, and 115% for the 10, 50 and 100 mg/kg/day samples, respectively. The Sponsor stated that this "minor deviation" from the acceptance criteria is not considered to have an impact on the interpretation of the data or the study outcome. The week 6 homogeneity and concentration verification results for samples prepared using lot PPL-MD11000601D met acceptance criteria for precision and homogeneity, but failed for accuracy with mean % recovery values of 111%, 112%, and 112% for the 10, 50, and 100 mg/kg/day samples, respectively. Analysis of the retention samples confirmed the initial results. As stated above, the Sponsor stated that this "minor deviation" from the acceptance criteria is not considered to have an impact on the interpretation of the data or the study outcome. The week 6 homogeneity and concentration verification results for samples prepared using lot PPL-MD11000601A met acceptance criteria for precision, accuracy, and homogeneity.
Other:	None

Reference ID: 3114166

Table 62: Summary of Maternal Performance in the Pre- and Post-natal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	No. of Mated Females	No. of Pregnant Females	Pregnancy Rate (%)	Gestation Index (%)	Dead Pups		Malformed Pups	
					Litters Affected	Pups Affected	Litters Affected	Pups Affected
1	24	24	100.0	100.0	0	0	0	0
2	24	24	100.0	95.8	4	5 *	0	0
3	24	24	100.0	100.0	1	1	0	0
4	24	24	100.0	100.0	2	2	0	0

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)
Note: Litter 2524 Excluded from Dead Pups and Malformed Pups (Litter Partially Cannibalized)

Group	Summary Information	Length of Gestation (Days)	Duration of Parturition (h)	Sex Ratio (% Males)	Number of Pups at Birth/Litters			No. of Implant Scars	Live Birth Index (%)
					Live	Dead	Malformed		
1	Mean	21.4	2.22	49.08	12.5	0.0	0.0	13.5	92.52
	SD	0.5	1.18	14.74	1.9	0.0	0.0	1.6	11.63
	N	24	5	24	24	24	24	24	24
2	Mean	21.7	1.82	49.60	11.5	0.2	0.0	13.0	88.65
	SD	0.5	0.28	16.05	2.0	0.5	0.0	1.8	12.15
	N	24	2	23	23	23	23	23	23
3	Mean	21.6	2.56	47.50	12.8	0.0	0.0	13.6	94.41
	SD	0.5	1.03	13.33	1.6	0.2	0.0	1.3	5.89
	N	24	6	24	24	24	24	24	24
4	Mean	21.7	1.42	45.73	12.3	0.1	0.0	12.7	95.69 b
	SD	0.5	0.53	12.70	2.5	0.3	0.0	2.1	8.72
	N	24	3	24	24	24	24	24	24

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon)
Note: Litter 2524 Excluded from Sex Ratio, Live, Dead, Malformed, No. of Implant Scars and Live Birth Index (Litter Partially Cannibalized)

F_1 Generation (On *post partum* day 4, each litter was randomly culled to 8 pups (4 animals/sex))	
Survival:	On *post partum* day 0, the pups were examined for malformations, sexed, and the numbers of live and dead pups were recorded. Mortality and clinical signs were examined twice daily for F_1 adults. In the F_1 pups, there were no test article-related effects on pup viability, survival or lactation indices. In the F_1 adults, there was no test article-related mortality.
Clinical signs:	The general condition of the F_1 pups was evaluated each day during the lactation period (*post partum* days 0-21). Thereafter, a complete detailed examination was performed weekly. In the F_1 pups, thin fur on the cranium was observed in 7/264 and 8/308 pups in the 10 and 50 mg/kg/day groups respectively, compared to 0/299 pups in the control group. An anal skin scab and a urogenital skin scab were noted in 5/294 and 6/294 pups respectively, in the 100 mg/kg/day group compared to 0/299 pups in the control group. In the F_1 adults, there was an increased number of males with thin fur cover in the 100 mg/kg/day group compared to the control group. There were no test article-related effects on clinical signs in F_1 adult females during the premating, gestation or lactation periods. In general, there were no drug-related effects on clinical signs in the F_1 pups and adults.
Body weight:	Body weights were recorded at birth and on *post partum* days 4, 7, 10, 14 and 21. Thereafter, body weights were recorded weekly during the post-weaning period. Mated females were weighed on gestation days 0, 3, 6, 9, 12, 15, 18, and 20, and on *post partum* days 0 and 4. There were no drug-related effects on body weight. Overall, there were no treatment-related effects on body weight in the F_1 pups. In F_1 adults, there were significant decreases in body weight gains in the 50 mg/kg/day group (13% on days 49 to 56, 16% on days 77 to 84). Decreased weight gain of 10-13% was noted in the 50 mg/kg/day males on days 49 to 84. In addition, body weight gain was reduced by 18% in the 100 mg/kg/day males on days 77 to 84. In F_1 adult

	females, there was a 17% and 15% decrease in body weight gain in the 50 and 100 mg/kg/day groups respectively, during gestation days 9 to 12. The effects on weight gain in F_1 adults were sporadic, and are not considered as drug-related.

Feed consumption:	Not conducted
Physical development:	The following parameters were assessed: pinna unfolding on *post partum* days 1 to 4 only, tooth eruption from *post partum* day 7, and eye opening from *post partum* day 12. The pupillary closure and visual placing responses of the F_1 generation were tested on *post partum* day 21. Vaginal opening was evaluated starting on *post partum* day 26 until development for females, and preputial separation was evaluated starting on *post partum* day 35 for males. There was a small, but statistically significant decrease of 13% and 17% in the mean day of development of pinna unfolding in the 50 and 100 mg/kg/day groups respectively, compared to the control group. There were no delays in the development of tooth eruption or eye opening in the treatment groups, and there were no drug-related effects on pupillary closure and visual placing. Furthermore, there were no drug-related effects on vaginal opening or preputial separation. Additionally, there were no delays in the development of the righting reflex, negative geotaxis and auricular startle in the treatment groups.
Neurological assessment:	Motor activity was assessed for 1 hour on *post partum* days 35 ± 2 and 60 ± 2 in the F_1 rats. There were no significant drug-related effects on motor activity in the F_1 adult rats. On *post partum* day 55 (±2), startle habituation responses were measured in F_1 rats. There were no significant drug-related effects on the auditory startle habituation response in the F_1 adult rats. On *post* partum days 60 and 70, swimming abnormalities were tested in a water maze. The time to exit the maze and the number of errors (incorrect turns) were recorded in 5 tests. There were no significant drug-related effects in the water maze test in the F_1 adult rats.
Reproduction:	At approximately 85 days of age, 1 female was placed with 1 male (sibling matings were avoided) in the same dosage group for 14 days. The females were examined for mating by examination of a vaginal lavage for spermatozoa. Females were observed at least 3 times each day from gestation day 20 for signs of parturition. Where possible, parturition was observed, and the time of onset and completion of parturition were recorded. Behavior of the females immediately *post partum* was noted.

Reference ID: 3114166

	The mating and fertility indices, the conception rate, and the mean day to mating were unaffected by treatment. The pregnancy rate varied between 75.0% and 95.5% and the gestation rate was 100% in each group (lowest pregnancy rate was in the control group). The gestation index was 100% in each group. The length of gestation was comparable in the control and test article-treated groups. The number of implant scars and live birth index were unaffected by treatment. There were no malformed pups at any dose level. There were no drug-related effects on parturition or parental/maternal performance. The results are summarized in the Sponsor's table below.
Other:	There were no drug-related effects on macroscopic findings. Mottled kidney was noted in 6/24 males in the 50 mg/kg/day group compared to 3/24 males in the control group. Dark area in the lung was noted in 3/23 males in the 10 mg/kg/day group compared to 0/24 males in the control group. Dark foci in the thymus was noted in 4/23 and 4/24 males in the 10 and 50 mg/kg/day groups compared to 1/24 control group males. A parasite in the rectum was noted in 5/21 females in the 50 mg/kg/day group compared to 1/15 control group females.

Table 63: Development of Pinna Unfolding in F_1 Pups in the Pre- and Postnatal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	Summary Information	Males	Females	Total
1	Mean	2.49	2.44	2.46
	SD	0.6	0.6	0.6
	N	24	24	24
2	Mean	2.23	2.23	2.23
	SD	0.5	0.5	0.5
	N	23	23	23
3	Mean	2.17 a	2.17 a	2.17 a
	SD	0.4	0.4	0.4
	N	24	24	24
4	Mean	2.07 b	2.07 b	2.07 b
	SD	0.3	0.3	0.3
	N	24	24	24

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)
Values are the mean *post partum* day of development.

Table 64: Development of Tooth Eruption in F_1 Pups in the Pre- and Postnatal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	Summary Information	Males	Females	Total
1	Mean	11.55	11.51	11.53
	SD	0.8	0.8	0.7
	N	24	24	24
2	Mean	11.51	11.62	11.55
	SD	0.6	0.8	0.7
	N	21	21	21
3	Mean	11.20	11.31	11.26
	SD	0.7	0.8	0.7
	N	24	24	24
4	Mean	11.04	11.26	11.17
	SD	0.8	0.8	0.8
	N	24	24	24

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)
Values are the mean *post partum* day of development.

Table 65: Development of Eye Opening in F_1 Pups in the Pre- and Postnatal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	Summary Information	Males	Females	Total
1	Mean	13.94	13.74	13.84
	SD	0.7	0.6	0.6
	N	24	24	24
2	Mean	13.90	13.65	13.77
	SD	0.6	0.5	0.5
	N	23	23	23
3	Mean	13.75	13.63	13.69
	SD	0.4	0.5	0.4
	N	24	24	24
4	Mean	13.60	13.45	13.52
	SD	0.6	0.6	0.5
	N	24	24	24

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)
Values are the mean *post partum* day of development.

Table 66: Vaginal Opening in F_1 Generation in the Pre- and Postnatal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	Summary Information	Day of Development
1	Mean	32.6
	SD	2.1
	N	24
2	Mean	32.0
	SD	1.3
	N	23
3	Mean	31.8
	SD	0.7
	N	23
4	Mean	32.2
	SD	1.6
	N	24

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)
Values are the mean *post partum* day of development.

Table 67: Preputial Separation in F_1 Generation in the Pre- and Postnatal Developmental Study in Rats

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	Summary Information	Day of Development
1	Mean	42.7
	SD	1.7
	N	24
2	Mean	43.1
	SD	1.9
	N	23
3	Mean	42.1
	SD	1.4
	N	24
4	Mean	42.3
	SD	1.4
	N	24

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)
Values are the mean *post partum* day of development.

Table 68: Summary of Parental/Maternal Performance in F_1 Rats in the Pre- and Post-natal Developmental Study

F1 Generation - Adults

Group 1 - Vehicle Control
Group 2 - Linaclotide 10 mg/kg/day
Group 3 - Linaclotide 50 mg/kg/day
Group 4 - Linaclotide 100 mg/kg/day

Group	Number Placed For Mating		Number Mating	Mean (SD) Day to Mating	Number Females Pregnant	Mating Index (%)	Fertility Index (%)	Conception Rate (%)
	Males	Females						
1	24	24	20	3.6 2.6 (N = 20)	15	83.3	62.5	75.0
2	23	23	18	1.9 a 1.3 (N = 18)	17	78.3	73.9	94.4
3	24	24	22	3.2 2.1 (N = 22)	21	91.7	87.5	95.5
4	24	24	21@*	4.1 2.4 (N = 20)	20	87.5	83.3	95.2

Significantly different from control group (group 1) value: a - P ≤ 0.05 b - P ≤ 0.01 c - P ≤ 0.001 (Wilcoxon) (Day to mating only)
Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)
@* Including female No. 471 with mating undetected

Group	No. of Mated Females	No. of Pregnant Females	Gestation Index (%)	Dead Pups		Malformed Pups	
				Litters Affected	Pups Affected	Litters Affected	Pups Affected
1	20	15	100.0	3	5	0	0
2	18	17	100.0	3	4	0	0
3	22	21	100.0	3	5	0	0
4	21	20	100.0	5	8	0	0

Significantly different from control group (group 1) value: * - P ≤ 0.05 ** - P ≤ 0.01 *** - P ≤ 0.001 (Fisher's)

Group	Summary Information	Length of Gestation (Days)	Duration of Parturition (h)	Sex Ratio (% Males)	Number of Pups at Birth/Litters			No. of Implant Scars	Live Birth Index (%)
					Live	Dead	Malformed		
1	Mean	21.8	2.76	60.59	14.4	0.3	0.0	15.9	91.45
	SD	0.4	1.13	13.27	2.2	0.8	0.0	2.8	8.02
	N	15	3	15	15	15	15	15	15
2	Mean	21.8	3.54	49.19 a	15.6	0.2	0.0	16.7	93.75
	SD	0.4	0.60	13.12	2.1	0.6	0.0	1.9	7.29
	N	17	3	17	17	17	17	17	17
3	Mean	21.8	2.56	49.83 a	14.5	0.2	0.0	16.1	90.25
	SD	0.4	0.49	9.17	2.7	0.6	0.0	2.9	9.16
	N	21	3	21	21	21	21	21	21
4	Mean	21.9	2.44	50.21 a	14.2	0.4	0.0	15.4	91.92
	SD	0.3	0.72	12.92	3.9	0.8	0.0	3.7	9.73
	N	19	6	20	20	20	20	20	20

Significantly different from control group (group 1) value: a - $P \leq 0.05$ b - $P \leq 0.01$ c - $P \leq 0.001$ (Wilcoxon)

F_2 Generation	
Survival:	The general condition of the F_2 pups was evaluated each day during the lactation period. There was no difference in viability index on *post partum* day 4 in the control and test article-treated groups. The mean litter sizes were comparable in control and test article-treated groups.
Body weight:	Body weights were recorded at birth and *post partum* day 4. There were no drug-related effects on body weight gain.
External/Internal evaluation:	The F_2 pups were sacrificed on *post partum* day 4, 5, or 6. There were no drug-related effects on external or internal findings
Male/Female ratio:	The sex ratio (%males) in each treatment group was significantly lower than the controls. This effect appears to be due to an unusually high control value (61%). The results are summarized in the table above.
Other:	There were no test article-related clinical signs.

Study title: Linaclotide: An Oral Range-finding Study in the Juvenile Mouse	
Study no.:	MNP-103-046
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.4
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	6/29/2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide, PPL-MD11000803, 85.4% relative to total peptide content

Key Study Findings

Linaclotide was administered (via oral gavage) at doses of 0.1, 10, 50, 100 or 600 µg/kg/day to juvenile mice for up to 5 days starting on *post partum* day 7, 14 or 21 (followed by a 2-day observation period) to evaluate the effects of daily administration in juvenile mice. Treatment resulted in adverse clinical effects and mortality within 24 hours after the first dose when dosing was initiated on *post partum* day 7 at ≥ 50 µg/kg/day, on *post partum* day 14 at 100 µg/kg/day, and on *post partum* day 21 at 600 µg/kg/day. The test article was well tolerated in juvenile mice when treatment was initiated on *post partum* day 7 at 10 µg/kg/day, on *post partum* day 14 at 50 µg/kg/day and on *post partum* day 21 at 100 µg/kg/day. Based on these results, the maximum tolerated doses of the test article were 10, 50, and 100 µg/kg/day when treatment was initiated on *post partum* days 7, 14 and 21, respectively.

Methods	
Doses:	0.1, 10, 100, 50, 600 µg/kg/day (no control group was included)
Frequency of dosing:	Once daily for 5 days
Route of administration:	Oral (gavage)
Dose volume:	2 ml/kg/day
Formulation/Vehicle:	Deionized water
Species/Strain:	Crl:CD1®(ICR) mice
Number/Sex/Group:	8/sex/group
Age:	Post partum days 7, 14, and 21
Weight:	*pp* 7: 3.8-6.6g; *pp* 14: 6.5-10.1g; *pp* 21:7.8-14.2g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minor deviations did not affect the quality or integrity of the study.

Observations and Results

Reference ID: 3114166

Mortality

All pups were examined twice daily for mortality and signs of ill health. Mortality attributed to linaclotide was observed within 24 hours of the first dose when treatment was initiated on *pp* day 7 at ≥ 50 µg/kg/day, on *pp* day 14 at 100 µg/kg/day, and on *pp* day 21 at 600 µg/kg/day. In these groups, most or all of the male and female pups were either found dead or euthanized in poor condition. The few remaining animals in these groups, although showing no clinical signs, were euthanized within 2 days after treatment. No evidence of linaclotide-related changes was observed at necropsy in pups from any of these groups. One female pup, which started dosing on *pp* day 21 at 0.1 µg/kg/day, was found dead on *pp* day 25. There were no gross changes observed at necropsy. As no linaclotide-related mortality occurred in the animals of the same age treated at 10 µg/kg/day, the death was considered incidental. One female, which started dosing on *pp* day 21 at 10 µg/kg/day, was found dead on *pp* day 24. The cause of death was attributed to perforation of the esophagus observed at gross pathological examination. The following table (taken from Sponsor's report) summarizes the mortality rate.

Text Table 6 Summary of Mortality/Early Termination

Age at Dosing					
7 *pp*		14 *pp*		21 *pp*	
Group Number / dose level - Number of pups preterminally euthanized, found dead					
Males					
1 / 0.1 µg/kg	0	2 / 0.1 µg/kg	0	3 / 0.1 µg/kg	0
4 / 10 µg/kg	0	5 / 10 µg/kg	0	6 / 10 µg/kg	0
7 / 100µg/kg	**8/8**	8 / 100 µg/kg	**6/8**	9 / 100 µg/kg	0
10 / 50 µg/kg	**8/8**	11 / 50 µg/kg	0	12 / 600 µg/kg	**8/8**
Females					
1 / 0.1 µg/kg	0	2 / 0.1 µg/kg	0	3 / 0.1 µg/kg	1**
4 / 10 µg/kg	0	5 / 10 µg/kg	0	6 / 10 µg/kg	1*
7 / 100µg/kg	**8/8**	8 / 100 µg/kg	**5/8**	9 / 100 µg/kg	0
10 / 50 µg/kg	**8/8**	11 / 50 µg/kg	0	12 / 600 µg/kg	**8/8**

* gavage accident; not test article-related ** incidental, not test article-related

Clinical Signs

A detailed clinical examination was performed once daily before initiation of treatment, hourly for the first 4 hours following each treatment, and daily during the observation period.

When treatment was initiated on *pp* day 7 at ≥ 50 µg/kg/day, on *pp* day 14 at 100 µg/kg/day, and on *pp* day 21 at 600 µg/kg/day, the following clinical signs were observed: decreased activity, cold to touch, and lying on side. There were no clinical observations attributed to linaclotide in groups that started dosing on *pp* day 7 at ≤ 10 µg/kg/day, on *pp* day 14 at ≤ 50 µg/kg/day and on *pp* day 21 at ≤ 100 µg/kg/day.

Weaning

Reference ID: 3114166

On *pp* day 21, the pups from *pp* day 21 treatment groups were separated from their dams and were housed per sex per litter until study termination.

Body Weights

Body weights were measured on *pp* days 7, 10, 14, 17, 21, 24, and 28 as applicable in all groups. A transient decrease in body weight gains in males and females was observed following initiation of treatment on *pp* day 14 at 50 µg/kg/day. Thereafter (*i.e. pp* days 17 to 21), body weight gains were comparable to those from untreated animals (*i.e.*, the groups designated to begin dosing on *pp* day 21). A transient decrease in body weight gains in the 10 µg/kg/day males and females that started treatment on *pp* day 21 was not considered to be drug-related due to low incidence and the absence of such an effect at 100 µg/kg/day. There were no linaclotide-related effects on body weights in groups that started dosing on *pp* day 7 or 14 at ≤ 10 µg/kg/day. No bodyweight measurements were made for pups that died preterminally.

Gross Pathology

Necropsy was performed on treated animals euthanized following the post-treatment 2-day observation period, on all pups found dead during the study, the surviving pups euthanized preterminally, and those euthanized for humane reasons. There were no linaclotide-related macroscopic findings.

Study title: Linaclotide: An Oral Range-finding Study in the Juvenile Rabbit	
Study no.:	MNP-103-TXR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.4
Conducting laboratory and location:	(b) (4)
Date of study initiation:	8/30/2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Linaclotide, PPL-MD11000803, 85.4% relative to total protein content

Key Study Findings

Linaclotide was administered orally at 2, 10, and 40 mg/kg/day on post partum (*pp*) day 14 (acute phase) and at 0.6, 2, 10, and 40 mg/kg/day on *pp* days 14 to 18 (subacute phase) in rabbits. The treatments were followed by a 2-day observation period. There

Reference ID: 3114166

were no deaths. Linaclotide produced a higher incidence of yellow fur staining at doses ≥ 2 mg/kg/day in the acute and subacute phases. Macroscopic findings in the intestinal tract included watery digestive cecal content and dark discoloration/area of cecum in males and/or females in the acute phase at ≥ 10 mg/kg/day, and cecal dilatation and watery digestive contents of the cecum/colon in males and/or females in the subacute phase at ≥ 10 mg/kg/day. Based on these findings, linaclotide was well tolerated in juvenile rabbits at doses up to 40 mg/kg/day, administered as a single dose or for 5 days. A maximum tolerated dose was not identified. Macroscopic findings at doses ≥ 10 mg/kg/day appear to be consistent with the anticipated exaggerated pharmacology of linaclotide.

Methods	
Doses:	Acute phase: 2, 10, 40 mg/kg/day Subacute phase: 0.6, 2, 10, 40 mg/kg/day (no control groups)
Frequency of dosing:	Acute phase: a single dose Subacute phase: once daily for 5 days (dosing was followed by a 2-day observation period)
Route of administration:	Oral (gavage)
Dose volume:	2 ml/kg/day
Formulation/Vehicle:	Deionized water and 0.5% methylcellulose 400 cps in deionized water
Species/Strain:	New Zealand White rabbits
Number/Sex/Group:	Acute phase: 1/sex/group; subacute phase: 4/sex/group
Age:	*Post partum (pp)* day 14
Weight:	195 to 349 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Minor deviations did not affect the quality or integrity of the study.

Observations and Results

Mortality

All pups were examined twice daily for mortality and signs of ill health. There were no deaths.

Clinical Signs

Reference ID: 3114166

A detailed clinical examination was performed once daily before initiation of treatment, hourly for the first 4 hours following each treatment, and daily during the observation period.

In the acute and subacute phases, a higher incidence of yellow staining of the fur was noted in the rabbits administered linaclotide at 2, 10 and 40 mg/kg/day, as compared to rabbits administered linaclotide at 0.6 mg/kg/day. These changes were also observed during the 2-day observation period and were considered as likely drug-related; however, they are not considered adverse.

Body Weights

There were occasional decreases in body weight gains at 2, 10, and 40 mg/kg/day in males. Occasional decreases in body weights occurred at 10 and 40 mg/kg/day in males, and at 2, 10, and 40 mg/kg/day in females. Similar decreases were noted for the body weight gains in females at ≥ 2 mg/kg/day. These differences were not clearly attributable to treatment with linaclotide.

Gross Pathology

In the acute phase, watery digestive cecal content and dark discoloration/area of cecum were found in one 40 mg/kg/day female and in one 10 mg/kg/day male. In the subacute phase, cecal dilatation (10 mg/kg/day: 2/4 M, 1/4 F; 40 mg/kg/day: 2/4 M, 2/4 F) and watery digestive contents in cecum and colon (10 mg/kg/day: 3/4 M, 4/4 F ; 40 mg/kg/day: 3/4 M, 4/4 F) were present in groups treated with 10 and 40 mg/kg/day.

Study title: Linaclotide: A 9-week Oral Gavage Toxicity Study in the Juvenile Mouse Followed by a 4-Week Recovery Period	
Study no.:	MNP-103-054-TXR-01
Study report location:	(Original amendment) 8/9/11: 4.2.3.5.4.1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 10, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MD-1100 Acetate, lot # PPL-MD11000803, 85.4% relative to total peptide content.

Key Study Findings

MD-1100 acetate was administered at doses of 1[12], 3, or 10 μg/kg/day from *post partum* day 7 to *post partum* day 70. After 1 or 2 doses of test article, increased mortality was observed in the 10 μg/kg/day group, however after *post partum* day 9 the 10 μg/kg/day dose was well tolerated for the remainder of the treatment period. The hematology analysis showed a robust increase in white blood cell counts, neutrophils, eosinophils, lymphocytes, monocytes and basophils in the 10 μg/kg/day females, but the increased levels were due in part to a high amount of intragroup variability. After the recovery period, the hematology values in the 10 μg/kg/day females were similar to those in control females. The clinical chemistry analysis indicated an increase in the alanine aminotransferase levels in 10 μg/kg/day females, but the Sponsor indicated that the increased level was within the historical range. In general, there were no drug-related changes in clinical observations, body weight gain, hematology, clinical chemistry, bone measurements, gross pathology, or histopathology. In addition, there were no drug-related effects on the physical development (vaginal opening or preputial separation) or neurobehavioral assessments (functional observation battery). Based on mortality, the NOAEL in male and female juvenile mice was 3 μg/kg/day.

Methods	
Doses:	1, 3, 10, and 30 μg/kg/day
Frequency of dosing:	Daily
Route of administration:	Oral (*post partum* day 7 to day 70)
Dose volume:	Varied (see table below)
Formulation/Vehicle:	0.5% methylcellulose in deionized water
Species/Strain:	Crl:CD1®(ICR) female mice with litters of ≤ 5 pups/sex
Number/Sex/Group:	20 (5 litters)
Age:	*Post partum* day 7 (dosing begins)
Weight:	2.8 to 6.0 g
Satellite groups:	Yes (see tables below)
Unique study design:	Following body weight assessment on *post partum* day 4, each litter was culled to a sex ratio of 4:4, where possible. Pups in poor health, with obvious abnormalities (malformations), not developing normally, or at a lower extreme of the body weight range were culled.
Deviation from study protocol:	Minor deviations did not affect the quality or integrity of the study.

[12] Due to pup mortality in the 30 μg/kg/day TK groups, the 30 μg/kg/day was discontinued and the untreated litters were dosed with 1 μg/kg/day instead.

Reference ID: 3114166

Table 69: Study Design (9-week Oral Toxicity Study in Juvenile Mice)

Group Number/ Identification	Dose Level (ug/kg/day)	Concentration (ug/mL)[b]	Minimum Number of Litters (Toxicology + TK)	Number of Animals (Toxicology + TK)	
				Males	Females
1/ control	0	0 / 0	5 + 30	20 + 120	20 + 120
2/ linaclotide	3	1.5 / 3	5 + 30	20 + 120	20 + 120
3/ linaclotide	10	5 / 10	5 + 30	20 + 120	20 + 120
4/ linaclotide[a]	30	15 / -	0 + 12	0 + 96	0 + 96
5/ linaclotide[a]	1	- / 1	5 + 30	20 + 120	20 + 120

a As a result of pup mortality observed at 30 ug/kg/day in the TK groups, treatment at 30 ug/kg/day was discontinued. The remaining untreated litters assigned to Group 4 (30 ug/kg/day) were transferred to Group 5 (1 ug/kg/day).

b Concentration based on corresponding dose volume, i.e., 2 mL/kg/day from 10 to 13 May 2010/1 mL/kg/day from 17 May 2010 onwards.

In groups 1, 2, 3 and 5, pups from assigned litters were assigned to subsets using a randomization procedure as described below before initiation of treatment (see below).

Subset Identification	Duration of Dosing	Recovery Period	Number of Animals per Group	
			Males	Females
A Toxicology - Main Study	9 weeks	-	10	10
B Toxicology - Recovery	9 weeks	4 weeks	10	10
C Toxicokinetic - Day 70 *pp*	9 weeks	-	24	24
D Toxicokinetic - Day 7 *pp*	1 day	-	96	96

Observations and Results

Mortality

The study groups were observed twice daily. During the lactation period, the dams were also observed. A number of TK litters were replaced due to insufficient male/female pups, an insufficient amount of blood, and/or incorrect dose volume due to technical error. One dam associated with the control group was sacrificed for "humane reasons" on *post partum* day 15, and one dam associated with the 10 μg/kg/day group was found dead on *post partum* day 13 (Sponsor did not provide any additional information on cause of deaths). F_1 pups in the 10 μg/kg/day group, from a single dam, were sacrificed due to cannibalism by the dam on *post partum* day 17. One female pup in the 3 μg/kg/day group, two female pups and one male pup in the 1 μg/kg/day group were found dead or sacrificed due to deteriorating condition (attributed to gavage accident between *post partum* days 8 and 11). The animals that died due to gavage death or dam's death were replaced. Four female and two male pups in the 10 μg/kg/day group were found dead between *post partum* days 8 and 9. One of the 10 μg/kg/day males died from an apparent gavage error, however the cause of death of the other pups could not be determined based on clinical signs, or macroscopic and microscopic examinations. Therefore, the deaths in the 10 μg/kg/day were considered to be treatment-related.

During the post-weaning period, one female in the 1 μg/kg/day group was sacrificed in moribund condition. Clinical signs in this animal included weakness, labored breathing, decreased activity, cold to touch, slight tremors, reduced feces output, and moderate dehydration. No abnormal macroscopic observations were noted. However, microscopic findings indicative of a gavage accident were seen, which included acute inflammation with presence of bacteria in the aorta and esophagus, acute inflammation of thymus, and inflammation of the lungs with signs of necrosis and hemorrhage.

Clinical Signs

A detailed clinical examination was performed daily. Yellow staining of the urogenital fur was noted in 5/20, 6/20, and 5/18 males in the 1, 3, and 10 μg/kg/day groups respectively, compared to 2/20 males in the control group. For a majority of these animals, this observation occurred on the last day of dosing or during the recovery period. No significant drug-related clinical signs were observed.

Body Weights

The body weights were measured and recorded on *post partum* days 4, 7, 10, 14, 17, 21, 24, 28, 31, and 35, and twice weekly thereafter until the scheduled termination (*post partum* day 70). Body weight changes are summarized in the table below.

Table 70: Body Weight Changes at Study Termination in the 9-week Oral Toxicity Study in Juvenile Mice

Dose (μg/kg)	Initial Body Weight (g)	Final Body Weight (g)	Body Weight Gain (g)	% Control Group Weight Gain
	Males			
0	2.65 ± 0.53	37.9 ± 2.82	35.25	100%
1	2.59 ± 0.46	35.7 ± 3.62	33.11	94%
3	2.69 ± 0.55	35.7 ± 3.15	33.01	94%
10	2.62 ± 0.65	37.5 ± 2.52	34.88	99%
	Females			
0	2.69 ± 0.45	28.0 ± 3.05	25.31	100%
1	2.63 ± 0.53	27.6 ± 2.34	24.97	99%
3	2.58 ± 0.55	27.1 ± 2.03	24.52	97%
10	2.69 ± 0.56	27.8 ± 2.65	25.11	99%

During the pre-weaning period (*post partum* days 7 to 21), the 3 μg/kg/day males and females had a significant decrease of 20% and 26% respectively, in body weight gain when compared to the control males. These changes were not observed during the

post-weaning and recovery periods. Furthermore, the overall body weights and body weight gains of the test article-treated animals were very similar to the control groups at the end of the dosing period (see table above). Therefore, no significant test-article related changes on body weight gains were observed.

Feed Consumption

Food consumption in the toxicology groups were measured on *post partum d*ays 21 to 24, 24 to 28 (per cage basis), 28 to 31, 31 to 35, 35 to 38, 38 to 42 *post partum*, then twice weekly thereafter until the day before scheduled sacrifice. No significant drug-related changes in food consumption were observed.

Physical Development

Vaginal opening and preputial separation were assessed in the toxicology groups, beginning on *post partum* day 21 until development. The average time of development for vaginal opening (~ 29 to 31 days) or preputial separation (~27 to 29 days) was comparable in the control and treatment groups.

Functional Observation Battery (FOB)

On *post partum* day 28 (±1) and again at the end of the treatment (day 71) and recovery (day 98) periods, an observational battery was performed for toxicology groups. The qualitative observational assessment included observations in home cage (e.g., body position, convulsions, tremors, bizarre behavior, restlessness, writhing, and grooming), removal from home cage (e.g., ease of removal), observations in arena (e.g., rearing, gait, convulsions, tremors, bizarre behavior, straub tail, arousal, papebral closure, olfactory response, locomotor activity level, grooming, respiration rate, defecation/urination, and escape response), handling observations (e.g., lacrimation, pupil size, salivation, urinary staining, diarrhea, body tone, corneal reflex, pinna reflex, tail pinch, cutaneous blood flow, passivity, paralysis, catalepsy, righting reflex, aggressiveness, and vocalization), and observations on surface (e.g., auricular startle). The quantitative observational assessment included a measurement of grip strength and body temperature.

A moderately decreased escape response was noted in 4/10 and 3/10 males[13] in the 1 and 10 μg/kg/day groups respectively, compared to 0/10 control males. Upon handling, 2/10 males in both the 3 and 10 μg/kg/day groups showed a slight increase in aggressiveness compared to 0/10 control males. From the arena observations, 2/8 females in the 10 μg/kg/day group had an increase in respiration rate compared to 0/10 control females. No drug-related changes of toxicologic significance in the FOB tests were observed.

[13] Statistically significant change in the 1 and 10 μg/kg/day groups ($p \leq 0.01$)

Hematology

Blood samples (from the abdominal aorta) were collected from the toxicology groups at scheduled termination. The following tables list the parameters that were measured and a summary of the data.

Table 71: Hematology Parameters in the 9-week Oral Toxicity Study in Juvenile Mice

Reticulocytes	Red blood cells	Hemoglobin
Hematocrit	Mean corpuscular hemoglobin	Mean corpuscular volume
Mean corpuscular hemoglobin concentration	Mean platelet volume	Platelet count
White blood cells	Neutrophils	Eosinophils
Lymphocytes	Monocytes	Basophils
Large unstained cells	Red cell volume distribution width	

Table 72: Effects on Hematology Parameters in the 9-week Oral Toxicity Study in Juvenile Mice

Parameter	Dose (μg/kg/day)	Change at termination compared to control
Reticulocytes	1, 3, 10 (female)	↑ 26%, ↑ 24%, ↑ 22%
White blood cells	3, 10 (male) 1, 3, 10 (females)	↑ 39%, ↓ 37% ↑ 39%, ↑ 29%, ↑ 152%
Neutrophils	3, 10 (male) 1, 10 (females)	↑ 25%, ↓ 60% ↑ 24%, ↑ 96%
Eosinophils	1, 3, 10 (male) 1, 3, 10 (female)	↓ 44%, ↑ 27%, ↓ 39% ↑ 22%, ↑ 51%, ↑ 126%
Lymphocytes	3, 10 (male) 1, 3, 10 (female)	↑ 41%, ↓ 32% ↑ 41%, ↑ 33%, ↑ 156%*
Monocytes	1, 3, 10 (male) 1, 3, 10 (female)	↓ 27%, ↑ 58%, ↓ 52% ↑ 175%, ↑ 113%, ↑ 338%
Basophils	10 (male) 10 (female)	↓ 62% ↑ 550%*
Large unstained cells	1, 10 (male) 1, 10 (female)	↓ 23%, ↓ 53% ↑ 71%, ↑ 386%

* Statistically significant: ($p \leq 0.05$)

The changes noted in males were not statistically significant and inconsistent across the dose groups. There was a high amount of intragroup variability in the control and 3 μg/kg/day groups, and the changes were not noted after the 4-week recovery period. In the 10 μg/kg/day females, there were robust increases in white blood cell counts

(152%), neutrophils (96%), eosinophils (126%), lymphocytes (156%; statistically significant), monocytes (338%), and basophils (550%; statistically significant). The increased cell counts in the 10 μg/kg/day females were due in part to a high amount of intragroup variability (especially in animal #3502). After the recovery period, hematology values in the 10 μg/kg/day females were similar to those in control females. Most of the changes noted above were not dose-dependent or statistically significant, thus the hematology changes are not considered to be drug-related.

Clinical Chemistry

Blood samples (from the abdominal aorta) were collected from the toxicology groups at scheduled termination. The following tables list the parameters that were measured and a summary of the data.

Table 73: Clinical Chemistry Parameters in the 9-week Oral Toxicity Study in Juvenile Mice

Alkaline phosphatase	Total bilirubin	Total cholesterol
Aspartate aminotransferase	Creatinine	Calcium
Alanine aminotransferase	Total protein	Phosphorus
Creatine kinase	Albumin	Globulin
Albumin/globulin ratio	Glucose	Electrolytes (sodium, potassium, chloride)
Urea nitrogen	Triglycerides	

Table 74: Effects on Clinical Chemistry Parameters in the 9-week Oral Toxicity Study in Juvenile Mice

Parameter	**Dose (μg/kg/day)**	**Change at termination compared to control**
Alanine aminotransferase	3 (male) 3, 10 (female)	↓ 18% ↑ 18%, ↑ 37%*
Alkaline phosphatase	3 (male) 1, 3, 10 (female)	↑ 18% ↓ 29%, ↓ 16%, ↓ 21%
Aspartate aminotransferase	1, 10 (female)	↑ 14%, ↑ 20%
Creatine kinase	10 (male)	↓ 20%
Glucose	1, 10 (male) 3 (female)	↓ 24%, ↓ 14% ↑ 24%
Total bilirubin	10 (male) 10 (female)	↓ 20% ↓ 14%
Triglycerides	3, 10 (male) 1, 3, 10 (female)	↑ 20%, ↑ 24% ↑ 33%, ↑ 64% ↑ 90%
Urea nitrogen	10 (male) 1, 3, 10 (female)	↑ 17% ↑ 27%, ↑ 19%, ↑ 21%
Phosphorous	1, 3, 10 (male)	↑ 14%, ↑13%, ↑ 41%
Cholesterol	1, 3, 10 (female)	↓ 17%, ↓ 21%, ↓ 27%

* Statistically significant: ($p \leq 0.05$)

The minor changes in males were not dose-dependent or statistically significant. In females, there was a significant increase in alanine aminotransferase (ALT) levels in the 10 μg/kg/day females, however this change was not observed after the 4-week recovery period. The Sponsor also stated that the ALT level in 10 μg/kg/day females was within the historical range (20 to 53 U/L). There were dose-dependent increases in triglyceride levels and dose-dependent decreases in cholesterol levels in the females. Triglyceride levels were 122% higher in the 10 μg/kg/day recovery females when compared to control recovery females, however there was a high amount of intragroup variability due to a small sample size in the 10 μg/kg/day females. No drug-related changes of toxicologic significance were observed.

Gross Pathology

All animals in the toxicology groups were subjected to a complete gross necropsy examination. The Sponsor's table below lists the tissues preserved for microscopic examination.

Table 75: Organs Collected at Necropsy for Macroscopic and Microscopic Examination in the 9-week Oral Toxicity Study in Juvenile Mice

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adrenals
aorta (thoracic)
[g]bone (right femur)
[b]bone and marrow (sternum)
[g]bone and joint (left femoral-tibial)
brain (forebrain, midbrain, cerebellum and medulla oblongata)
cecum
colon
duodenum
[c]epididymides
esophagus
[d]eyes
gallbladder
harderian glands
heart (including section of aorta)
ileum
jejunum
kidneys
lacrimal glands
liver (sample of 2 lobes)
[e]lungs (sample of 2 lobes)
lymph nodes (mandibular, unilateral; mesenteric)
[f]mammary gland (inguinal)
[d, f]optic nerves
ovaries
pancreas
pituitary
prostate
[a]rectum
salivary gland (mandibular, unilateral)
sciatic nerve
seminal vesicles
skeletal muscle
skin (inguinal)
spinal cord (cervical)
spleen

stomach
[c]testes
thymus
[f]thyroid lobes (and parathyroids)
tongue
trachea
urinary bladder
uterus (horns, body and cervix)
vagina

a Retained but not processed.
b Bone decalcified prior to sectioning.
c Fixed in modified Davidson's fluid (euthanized animals only).
d Fixed in Davidson's fluid (euthanized animals only).
e Infused with neutral buffered 10% formalin (all animals).
f Examined histopathologically only if present in routine sections of eyes (optic nerves), thyroid lobes (parathyroid glands), or skin (mammary gland).
g The left femoral tibial joint was decalcified for histology evaluation. The right bone femur was retained whole in saline soaked gauze, plastic film and stored frozen in a freezer set to maintain -20°C

No treatment-related macroscopic changes were observed.

Organ Weights

Organs were collected from the main study and recovery groups. The organs listed in the Sponsor's table below were weighed and appropriate organ weight ratios were calculated (relative to body weight). Paired organs were weighed together. The organ weight changes are summarized below. '*' and '**' denotes values that are statistically different from the control group; $p \leq 0.05$ and $p \leq 0.01$, respectively.

Table 76: Organs Weighed after Necropsy in the 9-week Oral Toxicity Study in Juvenile Mice

adrenal glands
brain
epididymides
heart
kidneys
liver
lungs
ovaries/testes
pituitary
prostate
salivary gland, mandibular
spleen
thymus
thyroid lobes and parathyroid glands
uterus

Epididymides: Absolute weight and organ/body weight ratio were decreased by 14%, and 8%** respectively, in the 10 μg/kg/day males.

Heart: Organ/body weight ratio was increased by 11%** in the 10 μg/kg/day males.

Ovaries: Absolute weight was decreased by 23% and 12% in the 1 and 10 μg/kg/day females, respectively. Organ/body weight ratio was decreased by 18% in the 1 μg/kg/day females.

Pituitary gland: Absolute weight was increased by 11% and 12% in the 1 and 3 μg/kg/day males, respectively. Organ/body weight ratio was increased by 15% in the 1 μg/kg/day males.

Absolute weight and organ/body weight ratio were decreased by 19% and 14% respectively, in the 1 μg/kg/day females.

Spleen: Organ/body weight ratio was increased by 21% in the 10 μg/kg/day females.

Testes: Absolute weight was decreased by 13%, 11%, and 10% in the 1, 3, and 10 μg/kg/day males, respectively. Organ/body weight ratio was decreased by 10% and 15% in the 1 and 3 μg/kg/day males, respectively.

Thymus: Absolute weight was decreased by 14% in the 1 μg/kg/day males. Organ/body weight ratio was decreased by 12% both the 1 and 3 μg/kg/day males.

Absolute weight and organ/body weight ratio were decreased by 18% and 13% respectively, in the 1 μg/kg/day females.

Thyroid/parathyroid: Absolute weight and organ/body weight ratio were decreased by 35% and 34% respectively, in the 1 μg/kg/day males.

Absolute weight and organ/body weight ratio were increased by 20% and 21% respectively, in the 3 μg/kg/day females.

Uterus: Absolute weight and organ/body weight ratio were increased by 13% and 18% respectively, in the 1 μg/kg/day females.

Histopathology

The tissues collected for microscopic examination are listed in the table under "Gross Pathology".

Adequate Battery

Yes.

Peer Review

Yes.

Histological Findings

No significant drug-related microscopic findings were observed.

Kidney: Minimal tubular dilatation was noted in 2/10 males in both the 1 and 10 μg/kg/day groups, compared to 0/10 control males.

Special Evaluation

Bone Measurements

The right femur was retained at scheduled necropsy for bone measurements (length and width recorded). There were no drug-related effects on bone length or width.

Toxicokinetics

Blood samples were collected via cardiac puncture after a single dose on *post partum* day 7 from 4 pups/sex/time-point (4 litters/group) at 0.5, 1, 2, 4, 8 and 24 hours after test article administration. In addition, at the termination of dosing (*post partum* day 70), blood samples were collected via the abdominal aorta from 4 animals/sex/time-point in the 1, 3, and 10 μg/kg/day groups at 0.5, 1, 2, 4, 8 and 24 hours after test article

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administration. Plasma samples were analyzed for concentration of MD-1100 and its metabolite (MM-419447). There were no plasma samples with quantifiable levels of test article in any of the dose groups, including the 30 μg/kg/day group on *post partum* day 7. One pooled plasma sample from the 30 μg/kg/day males had quantifiable levels (13.9 ng/ml) of MM-419447 at 2 hours after a single dose (*post partum day 7*). All remaining plasma samples in all dose groups were below the limits of quantitation. Therefore, toxicokinetic analysis could not be performed. Plasma concentrations of MD-1100 and MM-419447 were below the limits of quantitation (2 ng/ml and 5 ng/ml, respectively) in the control group and prior to dosing in the test article treated groups.

10 Special Toxicology Studies

None

11 Integrated Summary and Safety Evaluation

Linaclotide is a synthetic 14-amino acid peptide that is structurally related to the guanylin peptide family, which consists of three endogenous peptides, including guanylin, uroguanylin, and lymphoguanylin, and one exogenous peptide toxin, the bacterial enterotoxin ST_a. Linaclotide contains 3 disulfide bonds, instead of the 2 disulfide bonds found in the endogenous guanylin peptides, which increase the stability of linaclotide. Receptor binding and receptor function studies showed that guanylate cyclase C (GC-C), a cell surface receptor, is the molecular target for linaclotide in the GI tract. Linaclotide binds with selectivity and high affinity (K_i in low nanomolar range) to GC-C and stimulates the production of cGMP (cyclic guanosine 3', 5'-monophosphate) in T84 cells, similar to the actions of guanylin peptides. Linaclotide (10 μM) exhibited no binding activity when tested in a panel of 50 receptors, ion channels, and transporters. Although linaclotide was not tested in binding or functional assays with the other known guanylate cyclase subtypes, the receptor domain for GC-C is markedly different from that of the other receptor guanylate cyclases. Therefore, there is no reason to expect that linaclotide binds with sufficient affinity to the other receptor guanylate cyclases. Linaclotide binds with high-affinity to mouse intestinal mucosal brush-border membranes derived from WT mice, but not to membranes derived from GC-C KO mice, confirming that the GC-C receptor is the molecular target of linaclotide. Stimulation of cGMP production in the intestine results in increased secretion of chloride, bicarbonate, and fluid. Oral administration of linaclotide in mice and rats stimulates gastrointestinal transit and stimulates intestinal secretion. When tested in a colorectal distension model in rats, orally administered linaclotide produced relief of inflammation- and stress-induced colorectal hyperalgesia, as indicated by a reduction in the number of abdominal contractions. MM-419447 is an active metabolite of linaclotide. It is a 13-amino acid peptide created by hydrolytic cleavage of linaclotide in the intestine by

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carboxypeptidase A. The pharmacological activity of MM-419447 in the *in vitro and in vivo* assays is equivalent to that of linaclotide.

Oral bioavailability of linaclotide in rats and mice is extremely limited, ranging from 0.08% to 0.20%. Following oral administration of linaclotide, MM-419447 was present in the plasma of mice, rats, rabbits, and monkeys, and in the feces of humans. The oral bioavailability of this metabolite also has been found to be extremely low in rats. Linaclotide and MM-419447 have no detectable accumulation in brain, liver, or adipose tissues after oral administration in mice. The degradation pathway of linaclotide in the intestine involves formation of the active metabolite MM-419447 by enzymatic removal of the C-terminal tyrosine of linaclotide. Under reducing conditions in the intestinal fluids, the disulfide bonds of linaclotide and MM-419447 are reduced, resulting in the loss of tertiary structure and pharmacologic activity. Linaclotide was rapidly metabolized and degraded when injected into the ligated intestinal loops of anesthetized rats and when incubated *in vitro* in intestinal fluid from mice, rats, and humans. Both linaclotide and MM-419447 are mainly cleared in the kidneys following intravenous injection. However, even in nephrectomized rats, the concentration of linaclotide and MM-419447 decreased with time, suggesting the presence of non-renal clearance pathways. A very small proportion of orally administered linaclotide is excreted as active peptide, predominately in the form of the metabolite, in the feces of rats (< 1%) and humans (≤ 6%). Neither linaclotide nor MM-419447 is an inducer or inhibitor of the hepatic cytochrome P450 enzymes. Furthermore, linaclotide is not an inhibitor of the intestinal cytochrome P450 enzymes. Oral bioavailability of two linaclotide (b) (4) degradants, (b) (4) ((b) (4) and (b) (4) (b) (4) is also very low. These degradants were rapidly metabolized in the intestinal fluid. Overall, these findings suggest that linaclotide and MM-419447 have the potential to interact with GC-C receptors lining the intestinal tract and elicit their pharmacological effects through local actions in the intestinal mucosa.

In the safety pharmacology studies, linaclotide had no significant effects on hERG channel current at concentrations up to 100 µM, and it had no significant effects on cardiovascular (including ECG) or respiratory parameters at IV doses up to 4.5 mg/kg, corresponding with an AUC of about 1000 µg•min/ml. No specific neurobehavioral safety pharmacology studies were conducted; however, the Sponsor reported that no neurobehavioral effects were observed in any of the repeated-dose oral toxicology studies in mice, rats, or monkeys.

The toxicity of linaclotide (MD-1100 acetate) was evaluated in single-dose studies. Oral and intravenous administration of linaclotide at 5 mg/kg in rats did not produce any evidence of toxicity. A single oral dose of up to 5 mg/kg in monkeys produced marked changes in stool consistency (i.e. non-formed or liquid feces), which was likely due to an exaggerated pharmacological effect of linaclotide. Similarly, a single intravenous dose of up to 15 mg/kg in monkeys was associated with reversible stool consistency changes.

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Linaclotide was evaluated in repeated-dose oral toxicity studies in rats, mice, and monkeys. In all three species, linaclotide was generally well tolerated. In the 2-week oral toxicity study in rats, animals were treated with 0, 1, 5, or 20 mg/kg/day linaclotide. Only the 20 mg/kg/day group had detectable plasma levels of the drug. No toxic effects were observed in this study. The NOAEL was 20 mg/kg/day. In the 2-week oral toxicity study in Cynomolgus monkeys, animals were treated with 0, 0.5, 2.5, or 5 mg/kg/day linaclotide. Liquid and/or non-formed stool occurred in all drug-treated groups. Only trace levels of the drug were detected in plasma. The NOAEL was considered to be 0.5 mg/kg/day based on the increased plasma ALT levels at 2.5 and 5 mg/kg/day linaclotide.

In the 13-week oral toxicity study in rats, animals were treated with 0, 10, 50, or 100 mg/kg/day linaclotide. One male in the 100 mg/kg/day group was found dead on day 85. The cause of death could not be determined from necropsy or microscopic examination, although focal necrosis (minimal) in liver was observed. This death is considered as possibly drug related, given that it occurred at the highest dose. Target organs of toxicity were not identified in animals sacrificed at study termination. The NOAEL (no observed adverse effects level) in males was considered to be 50 mg/kg/day, based on the single death at 100 mg/kg/day. The NOAEL in females was considered to be 100 mg/kg/day.

In the 13-week oral toxicity study in Cynomolgus monkeys, animals were treated with 0, 5, 10, or 50 mg/kg/day linaclotide. A single death in the 10 mg/kg/day group appeared to be unrelated to treatment. Hematuria and hemoglobinuria was observed at 10 and 50 mg/kg/day. These urine abnormalities were associated with kidney lesions in the 50 mg/kg/day group (10 mg/kg/day group was not examined). Therefore, kidney was considered to be a target organ of toxicity in the 50 mg/kg/day group. However, it is noted that kidney lesions were not apparent in the subsequent 39-week oral toxicity study in Cynomolgus monkeys at doses up to 50 mg/kg/day (see review below). Reversible changes in stool consistency were observed and were considered to be an exaggerated pharmacological effect of linaclotide. A NOAEL cannot be determined due to the deficiencies in the study methods, although the drug was tolerated at all doses.

In order to support dose selection in a carcinogenicity study, a 13-week oral toxicity study in mice was conducted. Animals were treated with linaclotide at 0, 20, 100, or 200 mg/kg/day. Drug-related deaths occurred in the 100 and 200 mg/kg/day groups. Deaths also occurred in the 0 and 20 mg/kg/day groups (4 and 6 mice, respectively). All dead animals in the 20 mg/kg/day group were toxicokinetic animals. Histopathologic evaluation was not performed on any of the dead animals in the 20 mg/kg/day group. Thus, the relationship of mortality at 20 mg/kg/day to treatment with linaclotide is uncertain. Clinical signs in the 100 and 200 mg/kg/day groups included decreased activity, tremors, convulsions, absence of or reduced feces, partial or complete closure of eyelids, and cold skin. Target organs of toxicity in dead animals included kidneys, spleen, and glandular stomach (100 and 200 mg/kg/day). Cecum and heart were also target organs of toxicity in dead animals in the 200 mg/kg/day group. Among the surviving animals, lesions were observed in eyes (200 mg/kg/day), heart (100 and 200

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mg/kg/day), kidneys (100 and 200 mg/kg/day), liver (200 mg/kg/day), and several other organs. An MTD (maximum tolerated dose) cannot be estimated from this study due to mortality and target organ toxicity in the 100 and 200 mg/kg/day groups, and the absence of histopathologic evaluation of the 20 mg/kg/day group.

In the 26-week oral toxicity study in mice, animals were treated with 0, 5, 20, or 100/80 mg/kg/day linaclotide. The 100 mg/kg/day dose was reduced to 80 mg/kg/day due to the mortality observed in both sexes during the first week of dosing. A total of 8 animals in the 100/80 mg/kg/day main study group were found dead or euthanized after 2 weeks of dosing. Most of the deaths were attributed to dosing errors, although a relationship to treatment with linaclotide could not be ruled out because the cause of death was not determined for all animals. There were no significant drug-related effects on clinical observations, body weight, food consumption, ophthalmoscopic evaluation, clinical pathology parameters, or macroscopic or microscopic evaluations. There were significant dose-dependent decreases in absolute and relative thyroid/parathyroid gland weights; however, there were no correlated changes noted in the microscopic findings. Organ weight changes in the 100/80 mg/kg/day group were reversible after the 4-week recovery period. Based on mortality, the NOAEL was considered to be 20 mg/kg/day.

In the 39-week oral toxicity study in Cynomolgus monkeys, animals were treated with 0, 5, 10, or 50 mg/kg/day linaclotide. There were two drug-related deaths; a 10 mg/kg/day male and a 50 mg/kg/day female. Clinical signs in both animals included severe watery feces, hunched appearance, low/no food consumption, lethargy, and decreased activity. In addition, the 10 mg/kg/day male and 50 mg/kg/day female had a 24% and 14% decrease in body weight at sacrifice, respectively, compared to their starting body weights. Treatment-related microscopic findings in the dead animals included: necrosis/degeneration in the cecal, colonic, and rectal mucosa; lymphoid depletion in the mandibular lymph node and spleen; thymic cortical atrophy; and pancreatic zymogen depletion. Mortality in these monkeys appears to be related to an exaggerated pharmacological effect of linaclotide. The main clinical sign noted in the other drug-treated animals was watery feces. Bodyweight gain was decreased at all doses with the exception of the 5 mg/kg/day females. After 39 weeks of dosing, the 10 and 50 mg/kg/day females had a 37% and 48% decrease in bodyweight gain, respectively. $AUC_{0\text{-}24\ hr}$ values for linaclotide and its metabolite, MM-419447, increased with dose. Based on the mortality and decrease in bodyweight gain, the NOAEL was considered to be 5 mg/kg/day. This dose is approximately 1000-fold higher than the proposed maximum recommended human dose of 290 µg/day, or 4.8 µg/kg/day based on a 60-kg bodyweight.

Oral toxicity of linaclotide was also evaluated in neonatal/juvenile mice and rabbits. In the dose range-finding study, linaclotide was administered (via oral gavage) at doses of 0.1, 10, 50, 100 or 600 µg/kg/day to neonatal/juvenile mice for up to 5 days starting on *post partum* day 7, 14 or 21 (followed by a 2-day observation period). Linaclotide produced adverse clinical effects and mortality within 24 hours after the first dose when treatment was initiated on *post partum* day 7 at ≥ 50 µg/kg/day, on *post partum* day 14 at 100 µg/kg/day, and on *post partum* day 21 at 600 µg/kg/day. Clinical signs included

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decreased activity, cold to touch, and lying on side. No drug-related changes were observed at necropsy in pups from any of these groups. By contrast, linaclotide was well tolerated in mice when treatment was initiated on *post partum* day 7 at 10 µg/kg/day, on *post partum* day 14 at 50 µg/kg/day, and on *post partum* day 21 at 100 µg/kg/day. Based on these results, the maximum tolerated doses of linaclotide were 10, 50, and 100 µg/kg/day when treatment was initiated on *post partum* days 7, 14 and 21, respectively.

In the repeated-dose oral toxicity study in neonatal/juvenile mice, animals were treated with 1, 3, or 10 µg/kg/day from *post partum* day 7 to *post partum* day 70. Increased mortality was observed in the 10 µg/kg/day group after 1 or 2 doses of linaclotide. However, after *post partum* day 9 the 10 µg/kg/day dose was well tolerated for the remainder of the treatment period. Gavage-related injury was determined to be the cause of death in some pups in linaclotide-treated groups. However, the cause of death in pups without signs of gavage-related injury could not be determined due to the absence of clinical signs, macroscopic lesions, or microscopic lesions. These deaths were considered to be treatment-related, and the incidence of drug-related deaths at 10 µg/kg/day was determined to be 5/40. There were no toxicologically significant drug-related changes in clinical observations, bodyweight gain, hematology, clinical chemistry, bone measurements, gross pathology, or histopathology. The hematologic changes in the 10 µg/kg/day females were reversible after the recovery period. In addition, there were no drug-related effects on physical development (vaginal opening or preputial separation) or neurobehavioral assessments (functional observation battery). Based on mortality, the NOAEL in mice less than 9 days of age was considered to be 3 µg/kg/day. Because the 10 µg/kg/day dose was well tolerated after *post partum* day 9, the NOAEL in juvenile mice age 9 days or older is considered to be at least 10 µg/kg/day. The minimum lethal dose in neonatal mice less than 9 days of age (10 µg/kg/day) is approximately 2 times the maximum recommended human dose, based on a µg/kg comparison.

The oral toxicity studies in neonatal/juvenile mice reveal that the toxicity of linaclotide in mice is highly age dependent. The Sponsor hypothesized that the increased sensitivity of neonatal/juvenile mice to linaclotide may be related to the increased expression of intestinal GC-C receptors in young animals (Al-Majali et al, Lab Animal Sci., 49: 254-259, 1999; Cohen et al., Pediatr Res., 20: 555-560, 1986), or possibly to other factors such as those related to an immature GI system (Walthall et al., Birth Defects Research (Part B), 74:132-156, 2005; Heller, Arch Dis. Child, 26: 195-204, 1951). It is noteworthy that doses as low as 2.5 µg/kg were shown to be pharmacologically active in a study in suckling mice. However, based on the limited information available in the toxicity studies in neonatal/juvenile mice, it is not possible to determine whether the drug-related deaths were due to exaggerated pharmacological activity (i.e. excessive intestinal fluid secretion and loss of electrolytes). The relevance of the data from neonatal/juvenile mice to the potential for lethality or other adverse events in infants or young pediatric patients is unknown. It is noted that the levels of expression of the receptor for heat-stable enterotoxin (i.e. GC-C) in the small and large intestine of children is age dependent; a greater number of receptors are present in infants and the

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number decreases with increasing age (Cohen et al., Gastroenterology, 94: 367-373, 1988).

In the dose range-finding study in juvenile rabbits, linaclotide was administered orally at 2, 10, and 40 mg/kg/day on day 14 *post partum (pp)* (acute phase) and at 0.6, 2, 10, and 40 mg/kg/day from days 14 to 18 *pp* (subacute phase). There were no deaths. A higher incidence of drug-related clinical signs of yellow fur staining was noted at doses ≥ 2 mg/kg/day for the acute and subacute phases. Macroscopic findings in the intestinal tract included watery digestive cecal content and dark discoloration/area of cecum in males and/or females of the acute phase at ≥ 10 mg/kg/day, and cecal dilatation and watery digestive contents of the cecum/colon for males and/or females from the subacute phase at ≥ 10 mg/kg/day. Thus, administration of a single dose or 5 daily doses of linaclotide were well tolerated at up to 40 mg/kg/day in juvenile rabbits, and a maximum tolerated dose was not identified. Macroscopic findings at doses ≥ 10 mg/kg/day are consistent with the anticipated exaggerated pharmacology of linaclotide.

The pharmacokinetics of linaclotide and its active metabolite, MM-419447, appear similar in rats, mice, and Cynomolgus monkeys, as revealed by the toxicokinetic studies. After oral dosing, plasma linaclotide concentrations increased rapidly, reaching maximum concentration within 0.5 to 3 hours (0.5 to 1 hour in rats and mice, and 1 to 3 hours in monkeys). The plasma levels declined rapidly thereafter, with a mean half-life of 1.8 to 4.6 hr in monkeys. Plasma linaclotide exposure in general increased with dose. Female mice and females monkeys tended to have a higher plasma exposure than males. There appears to be a small accumulation of plasma linaclotide in monkeys. Plasma concentrations of MM-419447 also increased quite rapidly after dosing, reaching maximum levels between 0.5 to 1 hour in rats and mice, and 2 to 8 hours in monkeys. Plasma levels of MM-419447 decreased rapidly thereafter in all three species, with a half-life of 1.6 to 6.6 hours in monkeys. Plasma exposure of MM-419447 also increased with dose in rats and monkeys, and there were no definitive differences in exposure between the males and females. There was no accumulation of plasma MM-419447 in all three species examined. It should be noted that the achievement of quantifiable plasma levels of linaclotide or MM-419447 (LLQ = 0.5-3.0 ng/ml) in animals required oral dose levels that were at least 500 times the proposed maximum recommended human dose (4.8 μg/kg based on a 60-kg bodyweight).

In reproductive toxicity studies, linaclotide was evaluated in a segment 1 fertility and early embryonic developmental study in rats. Male and female rats were dosed orally with 10, 50, or 100 mg/kg/day linaclotide. Males were dosed for 29 days prior to mating, during mating, and until necropsy. Females were dosed for 14 days prior to mating, during mating, and up to gestation day 7. Linaclotide produced no adverse effects in the mated males and females, no effects on mating or fertility, and no effects on early embryonic development. The NOAEL was considered to be ≥ 100 mg/kg/day.

The developmental toxicity of linaclotide was studied in rats, mice, and rabbits. In the segment 2 embryo-fetal developmental study in rats, females were dosed orally with 10, 50, or 100 mg/kg/day linaclotide during gestation days 6 to 17. There were no drug-

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related effects on mortality, clinical signs, bodyweight gain, food consumption, or macroscopic observations. At doses up to 100 mg/kg/day, linaclotide produced no signs of maternal toxicity, and there were no differences in fetal malformations or variations between control and treatment groups. No evidence of embryo-fetal toxicity was observed. The NOAEL for maternal and embryo-fetal developmental toxicity in rats was considered to be 100 mg/kg/day.

In the segment 2 embryo-fetal developmental study in rabbits, females were treated orally with 5, 10, or 40 mg/kg/day linaclotide on gestation days 7 to 19. There were no drug-related effects on mortality, body weight gain, food consumption, or macroscopic observations. At doses up to 40 mg/kg/day, linaclotide produced no signs of maternal toxicity. No treatment-related fetal malformations, variations, or embryo-fetal toxicity was observed. The NOAEL for maternal and embryo-fetal developmental toxicity in rabbits was 40 mg/kg/day.

In the segment 2 embryo-fetal developmental study in mice, females were dosed orally with 5, 40, or 100/40 mg/kg/day linaclotide during gestation days 6 to 15. Treatment-related maternal toxicity, which included mortality, was evident in the 40 and 100/40 mg/kg/day groups, with clinical signs such as hunched posture, hypoactivity, flattened body, tremors, cold to touch, and/or half-closed eyes. The 40 and 100/40 mg/kg/day groups also exhibited lower mean body weights and bodyweight gains. Developmental toxicity was noted in the 40 and 100/40 mg/kg/day groups. Lower mean fetal weights were noted in the 40 and 100/40 mg/kg/day groups compared to the control groups, resulting in lower mean gravid uterine weights. A drug-related increase in mean litter proportion of open eyelid was noted in the 40 mg/kg/day group, and vertebral anomaly with or without associated rib anomaly was noted in the 40 and 100/40 mg/kg/day groups. Based on clinical signs, mortality, moribund condition, decreases in bodyweight gains, intrauterine growth, and fetal morphology at doses of ≥40 mg/kg/day, the NOAEL for maternal and embryo-fetal developmental toxicity in mice was considered to be 5 mg/kg/day. Results from the embryo-fetal developmental studies demonstrate again that mouse is the most sensitive to linaclotide among the species studied.

In the segment 3 pre- and postnatal developmental study in rats, females were dosed orally with 10, 50, or 100 mg/kg/day linaclotide from gestation day 6 to *post partum* day 21, 22, or 23. There were no drug-related mortalities, clinical signs, or body weight changes in the F_0 dams, F_1 pups and adults, or the F_2 pups. Also, there were no drug-related effects on the survival, viability, physical development, behavior, or reproductive performance of F_1 pups and adults. Furthermore, there were no drug-related effects on the F_2 pups, based on external and internal examination. The NOAEL for the F_0, F_1, and F_2 generations was considered to be ≥ 100 mg/kg/day.

It is not known whether linaclotide is excreted in milk. Based on the low oral bioavailability and the low dose levels recommended in humans (145 and 290 μg/day), it appears unlikely that measurable levels of linaclotide or its active metabolite would be present in the milk of lactating women. It should be noted that neither linaclotide nor its

active metabolite were detected in human plasma after administration of the recommended dose levels.
Linaclotide does not appear to be mutagenic or clastogenic; it tested negative in the reverse mutation assay in bacterial cells and in the chromosomal aberration assay in cultured human peripheral blood lymphocytes.

Linaclotide was not tumorigenic in rats or mice. In the 104-week oral carcinogenicity studies, linaclotide at doses up to 3,500 µg/kg/day in rats and 6,000 µg/kg/day in mice had no significant effects on survival rates, clinical signs, masses noted clinically, body weights, food consumption, hematology, macroscopic observations, or microscopic findings (neoplastic and non-neoplastic) in the males or females.

The Sponsor reported the presence of two (b) (4) degradation products, (b) (4) ((b) (4) and (b) (4) at levels (b) (4) the ICH qualification threshold in the drug product. (b) (4) . The toxicity and mutagenic potential of these degradants were evaluated. In the 28-day oral toxicity study in mice, animals were treated with 20 mg/kg/day linaclotide alone or 20 mg/kg/day linaclotide spiked with (b) (4) (b) (4)) and (b) (4) (b) (4) at dose levels of 0.04 mg/kg/day and 0.08 mg/kg/day, respectively. There were higher incidences of oily fur and dehydration in males treated with linaclotide or spiked linaclotide; body weights and bodyweight gains were higher in the females treated with linaclotide or spiked linaclotide. Microscopic lesions occurred at very low frequencies and no target organs of toxicity were identified. Thus, the impurities (b) (4) ((b) (4) and (b) (4) (b) (4) do not appear to have significant toxicity in mice at the doses tested. The Sponsor's proposed limits for (b) (4) are (b) (4) and (b) (4) respectively. At the maximum recommended clinical dose of 290 µg/day, this corresponds to a maximum daily intake of (b) (4) and (b) (4) for (b) (4) and (b) (4) , respectively, based on a 60-kg bodyweight. Thus, the doses of (b) (4) and (b) (4) that were studied in mice were approximately (b) (4) and (b) (4) times, respectively, the maximum potential daily intake of these degradants at the proposed limits. (b) (4) and (b) (4) both tested negative in the bacterial reverse mutation assay (Ames assay). Thus, the degradant impurities, (b) (4) and (b) (4) are considered to be qualified at the proposed limits.

(b) (4) linaclotide (b) (4) are (b) (4) which can also form as degradants of linaclotide in solution, particularly at a (b) (4) . These (b) (4) are pharmacologically inactive impurities which are rapidly degraded in rat intestinal fluid. The proposed acceptance criterion for total (b) (4) content in the (b) (4) is (b) (4) This corresponds to the administration of up to (b) (4) of the (b) (4) , based on a 60-kg bodyweight, at the proposed maximum recommended clinical dose of (b) (4) (b) (4) based on peptide content). (b) (4) were present in the linaclotide lots ((b) (4)

(b) (4)) used in repeat-dose toxicity studies (13-39 weeks). The (b) (4) dose levels at the linaclotide NOAEL were in the range of (b) (4) mg/kg/day, (b) (4) the maximum possible daily dose of (b) (4) by (b) (4) In addition, (b) (4) were present in the linaclotide lot used in the Ames test, where the maximum dose of (b) (4) was (b) (4) In the chromosomal aberration test for linaclotide, the maximum dose of (b) (4) was (b) (4) The results from the nonclinical studies support the Sponsor's proposed acceptance limit of (b) (4) for (b) (4).

In summary, the molecular target of linaclotide is the membrane guanylate cyclase-C (GC-C) receptor, located on the luminal surface of the intestine. Linaclotide stimulates production of cGMP through direct activation of the GC-C receptor, leading to an increase in intestinal secretion and GI transit, and a reduction of visceral pain in animal models. Linaclotide appears to act locally in the intestine, and the oral bioavailability is extremely limited in all animal species studied. The metabolite, MM-419447, has the same pharmacological activity as linaclotide, and contributes to the therapeutic effects. Nonclinical studies in rats, mice, and monkeys show that linaclotide is well tolerated in all three species at dose levels markedly higher (1000-21,000-fold) than the proposed maximum recommended clinical dose on a bodyweight basis (see table below), with the exception of neonatal/juvenile mice. Linaclotide had no effects on mating or fertility in rats. Linaclotide produced severe maternal toxicity in pregnant mice, which was associated with developmental toxicity. However, no developmental toxicity was observed in the segment 2 studies in rats and rabbits, or in the segment 3 study in rats. Linaclotide is neither mutagenic nor tumorigenic.

Toxicity	Species	NOAEL (mg/kg) M/F	Multiples of maximum recommended clinical dose
13-week oral	Rat	M: 50; F: 100	10,400; 20,800
	Mouse	Not determined	-
	Monkey	Not determined	-
26-week oral	Mouse	M & F: 20	4,170
39-week oral	Monkey	M & F: 5	1,040
9-week oral juvenile	Mouse	M & F: 0.003 (< 9 days) M & F: 0.010 (≥ 9 days)	0.6 2
Segment 1 Reproductive	Rat	M & F: 100	20,800
Segment 2 Reproductive	Rat	F: 100	20,800
	Rabbit	F: 40	8,330
	Mouse	F: 5	1,040
Segment 3 Reproductive	Rat	M & F: 100	20,800

Recommendations:

From a nonclinical standpoint, this application should be approved.

Suggested labeling: The labeling should be changed as described in the "EXECUTIVE SUMMARY" section of this review.

REVIEWER SIGNATURE _____
YUK-CHOW NG, PH.D.
PHARMACOLOGIST
Division of Gastroenterology and Inborn Errors Products

SUPERVISOR SIGNATURE_____
DAVID B. JOSEPH, PH.D.
PHARMACOLOGY TEAM LEADER
DIVISION OF GASTROENTEROLOGY AND INBORN ERRORS PRODUCTS

Reference ID: 3114166

cc:
ORIG NDA 202,811
DGIEP
DGIEP/PM
DGIEP/DR. JOSEPH
DGIEP/DR. NG
OSI/DR. MEHTA
DGIEP/DR. FIORENTINO
DGIEP/DR. WYNN
DGIEP/DR. HE
DGIEP/DR. DIMICK
OND IO/DR. JACOBS

R/D INIT.: D. JOSEPH 4/9/12

12 Appendix/Attachments

None

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/s/

YUK-CHOW NG
04/10/2012
The application should be approved.

DAVID B JOSEPH
04/10/2012
I concur. Please see my Team Leader memo.

Comments on NDA 202811

From A. Jacobs, Pharm/Tox AD
Date: 4/10/12

1. I concur with the proposed pregnancy category—C

2. The issue of deaths in neonatal mice: dosing starting on d 7, but not rabbits (dosing starting on d 14): Presumably, human neonates would not be given the dose by gavage and thus not be at risk and the human neonates are considerably larger than mouse neonates, and at less risk for aspiration into the lung. Furthermore the minimal age for future study in pediatrics is 6 months, so perhaps this is not an issue for this NDA.

Some mice died of gavage injury, and the cause of deaths of the other neonatal mice is not clear. Deaths were not seen in the rats in the pre-postnatal study in rats, but were not direct-dosed in the pre/postnatal study. It is difficult to gavage neonatal mice of only a few days of age, and it is possible that some of the drug was deposited into or aspirated into the lung, resulting in suffocation or systemic exposure. Exaggerated pharmacologic activity and increased systemic exposure (e.g., effects on bronchial smooth muscle, increased gut permeability) are also a few of the many possibilities, and deaths could be due to a combination of factors. There appears to be insufficient information to evaluate the relevance to human neonates.

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/s/

ABIGAIL C JACOBS
04/09/2012

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 202,811 **Applicant: Ironwood Pharmaceuticals** **Stamp Date: 08/09/11**

Drug Name: linaclotide **NDA/BLA Type: NDA**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	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		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

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

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

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

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

Niraj R. Mehta, Ph.D. 10/06/11
Reviewing Pharmacologist Date

David B. Joseph, Ph.D. 10/06/11
Team Leader/Supervisor Date

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

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/s/

NIRAJ R MEHTA
10/06/2011

DAVID B JOSEPH
10/06/2011
I concur.