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RESEARCH**

APPLICATION NUMBER:

202811Orig1s000

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

Addendum to Clinical Pharmacology Review

NDA: 202811	Original Submission Date: 08/09/2011
Brand Name	Linzess®
Generic Name	Linaclotide
Reviewer	Sandhya Apparaju, Ph.D.
Team Leader	Sue Chih Lee, Ph.D.
OCP Division	DCP3
OND Division	DGIEP
Sponsor	Ironwood Pharmaceuticals, Inc.
Relevant IND(s)	63,290
Submission Type	Original NDA; Standard Review
Formulation; Strength(s)	Oral capsules; 145 µg and 290 µg strengths
Indication	Chronic Idiopathic Constipation (CIC) and Irritable Bowel Syndrome with Constipation (IBS-C)

This is an addendum to the Clinical Pharmacology review signed off in DARRTS on 04/06/2012. Addendum addresses the post-marketing requirement (PMR) for a milk-only lactation study for linaclotide.

Linaclotide (NDA 202811) is a 14-amino acid GC-C agonist that is intended for the treatment of Chronic Idiopathic Constipation (CIC) and IBS-constipation in adults. Systemic exposure was not noted for linaclotide or its active metabolite in phase 1 studies over a range of doses. Similarly sparse sampling following clinically relevant doses of drug in phase 3 clinical trials did not suggest potential for systemic exposure, with the exception of couple of patients who had detectable, albeit very low systemic concentrations at couple of time points post-dose. A Clinical Pharmacology review of the NDA was entered into DARRTS and signed off on 04/06/2012 and concluded acceptability of the NDA from a Clinical Pharmacology perspective.

Subsequently the non-clinical review team identified that an age- and dose-related mortality was noted in juvenile mice dosed with linaclotide. Review team recommended that a post-marketing study (PMR) was to be required to further evaluate the mechanisms of the observed lethality prior to initiating any dosing in pediatric trials. In the meanwhile, strong labeling language was recommended to caution against any use in pediatric patients across the age spectrum, until additional information regarding animal lethality can be obtained and pediatric trials can be initiated.

A related aspect of the labeling that was of relevance to the neonatal/infant population was the issue of lactation or use of linaclotide in nursing mothers. Second to

direct dosing, exposure to a drug can occur in a premature/term neonate and infants via breast-milk. In this regard, the PMHS reviewer Dr. Jeanine Best noted in her review (signed 06/14/2012) that it was not necessary to discourage lactation during treatment with Linzess as drug levels would be anticipated to be very low and likely not detectable in human milk due to the low systemic availability of Linzess in adults. However, PMHS also noted in their review as additional recommendation that the sponsor should be encouraged to conduct a milk-only lactation study, using a validated assay in order to appropriately inform the nursing mothers' subsection of the labeling. The labeling for this section meanwhile was to be worded as follows "it is not known whether linaclotide is excreted in human milk; however, linaclotide and its active metabolite are not measurable in plasma following administration of the recommended clinical doses. Caution should be exercised when LINZESS is administered to a nursing woman".

Labeling language and the PMHS additional recommendation was discussed with the review team and the DCP3 management. The potential for drug transfer into maternal milk appears to be low as drug lacks typical characteristics of a molecule that is likely to undergo uptake into maternal milk such as high maternal plasma concentrations, lipid solubility, low molecular weight etc. However, considering that the consequence of drug dosing in neonatal mice was mortality and that the mechanisms behind this observation are currently not understood, it is important to conclusively establish maternal milk exposure potential for linaclotide and its active metabolite via the conduct of a milk-only lactation (PK) study. The information gained from such a study can ensure that the nursing mothers' section of the labeling can provide appropriate information to both prescribers and patients.

A milk-only lactation study can be conducted in a small cohort of lactating female volunteers (n = 6 – 8). For this particular study considering the current lack of understanding behind the mechanisms of the non-clinical lethality findings, we recommend enrolling only non-nursing female volunteers. Following oral administration of a clinically relevant dose of the drug to the lactating volunteers, milk should be collected over an extended period (e.g. 24 hours) at regular time points and the drug/metabolite levels should be assessed using validated analytical methodologies. Information thus generated should be submitted to the agency and labeling language should be proposed as appropriate for further review and comment.

The following post-marketing requirement (PMR) was communicated to the sponsor and the sponsor has concurred via email as of August 01, 2012:

Conduct a multiple-dose milk-only lactation study to assess concentrations of linaclotide and its active metabolite in the milk of healthy, lactating but non-nursing female volunteers, using a validated assay in order to appropriately inform the nursing mothers' subsection of the labeling.

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/s/

SANDHYA K APPARAJU
08/03/2012

SUE CHIH H LEE
08/03/2012

EDWARD D BASHAW
08/03/2012

Addendum to Clinical Pharmacology Review

NDA: 202811	PPSR Submission Date: 10/07/2011
Brand Name	Linzess®
Generic Name	Linaclotide
Reviewer	Sandhya Apparaju, Ph.D.
Team Leader	Sue Chih Lee, Ph.D.
OCP Division	DCP3
OND Division	DGIEP
Sponsor	Ironwood Pharmaceuticals, Inc.
Relevant IND(s)	63,290
Submission Type	Original NDA; Standard Review
Formulation; Strength(s)	Oral capsules; 145 µg and 290 µg strengths
Indication	Chronic Idiopathic Constipation (CIC) and Irritable Bowel Syndrome with Constipation (IBS-C)

Background: Linaclotide, a 14-amino acid synthetic peptide, is a potent and selective guanylate cyclase-c (GC-C receptor) agonist structurally related to the endogenous guanylin peptide family. It is intended for adult CIC and IBS-C indications. (b) (4)

The sponsor has also been granted waiver for study conduct in pediatric CIC patients < 6 months and IBS-C patients < 6 years of age. The sponsor has requested deferral of the remaining pediatric trials until adequate safety and efficacy have been established in the adult population and the approval of adult indications. In a recent PeRC meeting, nonclinical lethality findings in juvenile mice (corresponding to pediatric ages of 1 to 23 months) were discussed and a decision was made to grant deferral of all pediatric trials, pending need for further non-clinical/mechanistic information to understand the observed lethality in these very young mice. Recommended labeling carries a contraindication for pediatric use in < 6 years of age and a strong caution against using in all remaining pediatric age groups. A post-marketing requirement (PMR) is in place to gather additional information pertaining to the mechanisms of lethality noted in juvenile mice. This review is based on the preliminary proposal by the sponsor in this regard. The actual studies to be conducted and the appropriate grouping of the age cohorts may change when further information regarding pediatric safety is available (b) (4)

(b) (4)

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SANDHYA K APPARAJU
08/03/2012

SUE CHIH H LEE
08/03/2012

BIOPHARMACEUTICS REVIEW
Office of New Drug Quality Assessment

Application No.:	NDA 202-811	Reviewer: Kareen Riviere, Ph.D.	
Submission Dates:	8/9/2011; 11/18/2011; 3/1/2012; 3/26/2012		
Division:	Gastroenterology Products	Acting Biopharmaceutics Supervisory Lead: Angelica Dorantes, Ph.D.	
Applicant:	Ironwood Pharmaceuticals, Inc.	Secondary Signature: Sandra Suarez-Sharp, Ph.D.	
Trade Name:	Linzess	Date Assigned:	8/31/2011
Generic Name:	Linacotide	Date of Review:	4/10/2012
Indication:	Treatment of constipation-predominant irritable bowel syndrome (IBS-C) and chronic constipation (CC).	Type of Submission: Original New Drug Application	
Formulation/strengths:	IR Capsules, 145 µg and 290 µg		
Route of Administration:	Oral		

SUMMARY:

This is a 505(b)(1) New Drug Application for 145 µg and 290 µg capsules of Linzess (linaclotide) indicated for the treatment of constipation-predominant irritable bowel syndrome (IBS-C) and chronic constipation (CC). This submission includes a drug product development section, a dissolution development report with a proposed dissolution specification and acceptance criterion.

The Biopharmaceutics review for this NDA is focused on the evaluation and acceptability of the proposed dissolution methodology, acceptance criterion, and manufacturing site change.

The proposed dissolution method is:

Apparatus:
 Speed of Rotation:
 Media Volume:
 Dissolution Media:
 Sampling Times:

 Sampling:
 Filter:



The proposed acceptance criterion is Q = (b) (4) in (b) (4) minutes.

A. Dissolution Method

Data provided showed that rotation speed did not affect the rate and extent of dissolution. During the review cycle, the ONDQA Biopharmaceutics Team requested the Applicant to justify the use of (b) (4) rotation speed. In a letter dated March 1, 2012, the Applicant revised the dissolution basket rotation speed to 50 rpm.

B. Dissolution Acceptance Criterion

In an IR letter to the Applicant dated January 20, 2012, the ONDQA Biopharmaceutics Team recommended a dissolution acceptance criterion of Q = (b) (4) at 15 minutes based on the mean in-vitro dissolution profiles for all strengths at release and under 15 months stability studies. On March 1, 2012, the Applicant proposed an acceptance criterion of Q = (b) (4) at (b) (4) minutes with a basket rotation speed of 50 rpm. Their proposal was based on the fact that

60% of batches required Stage 2 testing under the criterion of $Q = (b) (4)$ at 15 minutes. The ONDQA Biopharmaceutics Team held a teleconference with the Applicant on March 15, 2012 to discuss the following issues: 1) The dissolution acceptance criterion should be established based on average in vitro dissolution data for each lot under study, equivalent to USP Stage 2 testing (n=12); 2) that having 60% of batches requiring Stage 2 testing under the criterion of $Q = (b) (4)$ at 15 minutes is not an indication of poor product quality, and 3) that an acceptance criterion of $Q = (b) (4)$ at 15 minutes would make the dissolution method more discriminating for quality control purposes. The Applicant accepted the recommended dissolution acceptance criterion and revised the drug product specifications to include a dissolution acceptance criterion of $Q = (b) (4)$ at 15 minutes (refer to submission dated March 26, 2012).

C. Manufacturing Site Change

The Phase 3 drug product was manufactured at the Applicant's site in the U.S. while the commercial drug product was manufactured at the Applicant's site in Ireland. The manufacturing site change from U.S. to Ireland is analogous to a Level 3 site change according to the SUPAC-IR Guidance. The Applicant provided dissolution data using their proposed dissolution method. Although, f_2 testing could not be calculated due to the drug product's rapid dissolution (more than $(b) (4)$ of the drug was released within 15 minutes for all batches), the percentage dissolved at 15, 30 and 45 min is super-imposable. Therefore, the drug products made from the Applicant's U.S. and Ireland manufacturing sites are considered bioequivalent.

RECOMMENDATION:

1. Linaclotide 145 μ g and 290 μ g strength capsules are recommended for approval from a Biopharmaceutics standpoint.
 - The following dissolution method and acceptance criterion have been agreed upon with the Applicant for both strengths (see submissions dated March 1, 2012 and March 26, 2012):
 - i. Dissolution method: Apparatus I, 50 rpm agitation rate, 500 mL media volume, 37 °C, 50 mM phosphate buffer (pH 4.5).
 - ii. Dissolution acceptance criterion: $Q = (b) (4)$ at 15 minutes.

Kareen Riviere, Ph.D.
Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Sandra Suarez-Sharp, Ph.D.
Senior Biopharmaceutics Reviewer
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cc: Angelica Dorantes, Ph.D.

BIOPHARMACEUTICS INFORMATION ASSESSMENT

1. Background

Drug Substance

Linaclotide, a 14-amino acid synthetic peptide, is a minimally absorbed agonist of the guanylate cyclase type-C (GC-C) receptor, which lines the luminal surface of the intestine and is involved in the regulation of intestinal fluid homeostasis and bowel function. The structure of linaclotide is shown in Figure 1.

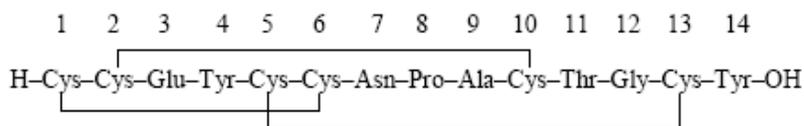


Figure 1. Structure of Linaclotide

Linaclotide is considered by the Applicant to be a BCS Class 3 (high solubility, low permeability) compound. The solubility profile of linaclotide is shown in Table 1.

Table 1. pH Solubility of Linaclotide at 37 °C

Buffer	pH	Solubility (µg/mL)
KCl/HCl	1.0	>100
KCl/HCl	2.0	>100
KCl/HCl	3.0	>100
Sodium Acetate	4.0	>100
Sodium Acetate	5.0	>100
Phosphate buffer saline	6.0	>100
Phosphate buffer saline	7.5	>100

According to the Applicant, the solubility of Linaclotide was determined under the physiological pH range of 1.0 to 7.5. The solubility was found to be greater than 100 µg/mL in this pH range. The highest intended commercial dose of linaclotide of 290 µg is soluble in less than 2.9 mL which is well below the 250 mL limit for high solubility drugs. Therefore, linaclotide can be classified as a highly soluble drug in the entire physiological pH range based on the BCS FDA Guidance for Industry.

Reviewer’s Comments and Evaluation:

Although the solubility profile does not include specific numbers, it sufficiently demonstrates that linaclotide is highly soluble throughout the physiological pH range.

Drug Product

Linaclotide capsules are comprised of linaclotide-coated beads in a hard gelatin capsule. (b) (4) microcrystalline cellulose (b) (4) excipients (calcium chloride, leucine and hypromellose) (b) (4).

Table 2 presents the composition of linaclotide formulation used in phase 3 studies. This is exactly the same as the proposed commercial formulation.

Table 2. Composition of the Formulation Selected for Phase 3 Studies

Component	Theoretical Concentration (w/w)	Theoretical Amount per Unit	
		145 µg capsules	290 µg capsules
Linaclotide	(b) (4)	0.145 mg	0.290 mg
Hypromellose (b) (4)	(b) (4)	(b) (4)	(b) (4)
Calcium Chloride dihydrate	(b) (4)	(b) (4)	(b) (4)
L-leucine	(b) (4)	(b) (4)	(b) (4)
Microcrystalline Cellulose (b) (4)	(b) (4)	(b) (4)	(b) (4)
(b) (4)	(b) (4)	(b) (4)	(b) (4)
Total	100.00	56.25 mg	112.50 mg
White Opaque hard gelatin capsules, Size 2			

Reviewer’s Comments and Evaluation:

The 145 µg and 290 µg strength capsules (b) (4).

During the development of linaclotide, three different clinical formulations were used. However, no relative bioavailability (BA) or bioequivalence (BE) studies were performed because concentrations of linaclotide and its metabolite are generally undetectable following oral administration and, therefore, standard pharmacokinetic (PK) parameters cannot be calculated. Although a BE study was not necessary because the proposed commercial formulation was used in the Phase 3 trials, comparability of the oral capsule formulations was established based on in vitro dissolution studies. In the CC Phase 3 efficacy trials, the linaclotide doses were 145 µg and 290 µg. In the IBS-C Phase 3 efficacy trials, the linaclotide dose was 290 µg.

2. Dissolution Method

The proposed dissolution method is:

Apparatus:
Speed of Rotation:
Media Volume:
Dissolution Media:
Sampling Times:

Sampling:
Filter:



Reviewer's Comment and Evaluation:

The Applicant did not provide an adequate dissolution method report. There were no dissolution profiles included and no justification/rationale for the selection of dissolution conditions. The following Biopharmaceutics comment was sent to the Applicant in the 74 day letter dated October 21, 2011.

FDA Comment 3

Submit the dissolution method report including the complete dissolution profile data (individual, mean, SD, profiles) collected during the development and validation of the proposed dissolution method.

Applicant's Response

The requested dissolution profile data is provided in reports PRD-RPT-ANL-00315 (Tables 4-1 through 4-6) that is included in *Section 3.2.P.5.6 Justification of Specifications (Linaclotide Capsules, 145 ug)* and PRD-RPT-ANL-00322, submitted under the response to Question #2.

The following information was gathered from the submitted reports:



Figure 2. Multimedia Dissolution Profile for 145 µg Linacotide Capsules

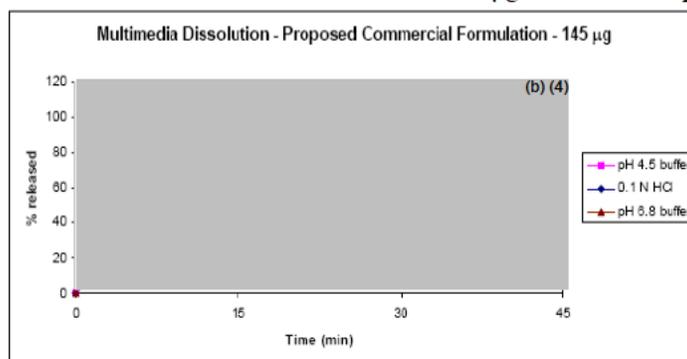
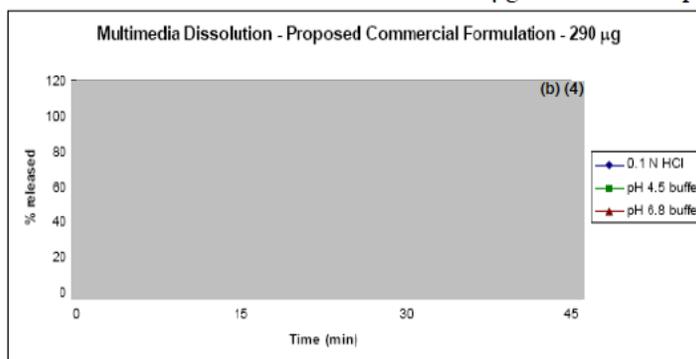


Figure 3. Multimedia Dissolution Profile for 290 µg Linacotide Capsules



The immediate release capsules exhibited rapidly dissolving behavior (b) (4) of drug released in 30 minutes). Actually, the release was greater than (b) (4) in 15 minutes in all media.

Reviewer's Comments and Evaluation:

Since there was no information on the discriminating ability of the dissolution method, the following Biopharmaceutics comment was sent to the Applicant in the 74 day letter dated October 21, 2011.

FDA Comment 2

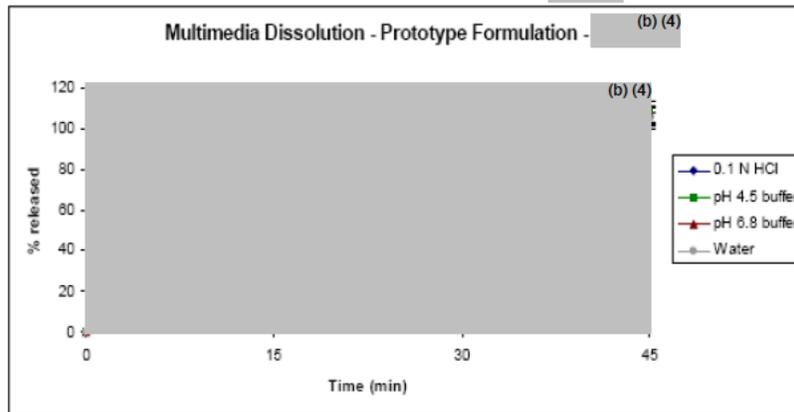
Conduct testing and provide data to demonstrate the discriminating capability of the selected dissolution method.

Applicant's Response

The formulation exhibits dissolution of (b) (4) in less than 30 minutes across the entire physiological pH range (0.1 N HCl, pH 4.5 buffer and pH 6.8 buffer) and was determined to be *rapidly dissolving* according to the FDA BCS guidance. "Given the simplicity / nature of the dosage form design and the very high aqueous solubility of the drug substance across the entire physiological pH range, the options for relevant and meaningful alterations to the Linacotide Capsule formulation are very limited." Therefore the determination of discriminating potential of the proposed regulatory dissolution procedure was not be explored in the traditional manner suitable for low solubility drugs.

Figure 4 illustrates the dissolution profiles for a prototype with formulation changes.

Figure 4. Multimedia Dissolution Profile for Prototype (b) (4) Linaclotide Capsules



The excipients used to manufacture this formulation were identical to the excipients used in the Phase 3/ commercial product formulation; however, the ratios of excipients (b) (4) were slightly different. Compared to the profiles in Figures 2 and 3, the profiles in Figure 4 have faster dissolution (exceed (b) (4) at 15 minutes).

Reviewer's Comments and Evaluation:

Linaclotide is very soluble in the physiological pH range. Therefore, rapid dissolution (b) (4) of drug released in 30 minutes) will be seen in all media in the physiological pH range. As a result, it is difficult to discern whether the proposed method is discriminating.

The following Biopharmaceutics Comment was sent to the Applicant in an IR letter dated January 20, 2012.

FDA Comment 1

It appears that paddle speed does not affect the dissolution profile of your proposed product; therefore, revise your proposed dissolution method to reflect a paddle speed of 50 rpm.

Applicant's Response

We accept the revised dissolution basket rotation speed of 50 rpm.

In a letter dated March 1, 2012, the Applicant accepted to revise their dissolution method to have a basket rotation speed of 50 rpm.

3. Dissolution Acceptance Criterion

The proposed acceptance criterion is $Q = (b) (4)$ in (b) (4) minutes.

Reviewer's Comments:

Since greater than (b) (4) of the product dissolves in 15 minutes, the acceptance criterion could be (b) (4) $Q = (b) (4)$ at 15 minutes.

The following Biopharmaceutics comment was sent to the Applicant in the 74 day letter dated October 21, 2011.

FDA Comment 4

Provide the complete dissolution profile data (raw data and mean values) from the clinical and primary stability batches supporting the selection of the dissolution acceptance criterion (i.e., specification-sampling time point and specification value).

Applicant’s Response

Complete dissolution profile data (raw data, mean, minimum and maximum values) for the primary registration batches at release and on stability is provided in report *PRD-RPT-ANL-00335, Stability Summary Report: Linaclotide Capsules ICH Registration Capsules*. The report is included in Section 3.2.P.8.1 *Stability Summary and Conclusion (Linaclotide Capsules, 145 ug)* and referenced in Section 2.3.P *Quality Overall Summary*. Complete dissolution profiles at time of release (raw data, mean, minimum and maximum values along with % RSD), for all the active drug product batches used in the Phase 3 trials are provided below (Table 4.1–1 through Table 4.1–19).

The data Table 4.1–1 through Table 4.1–19 provided by the Applicant is summarized in Table 3 (Table 3.2.P.2.2.1.4.3-6 in the original submission).

Table 3. In vitro Dissolution Results for Batches Used in Phase 3 and Long-term Safety Studies

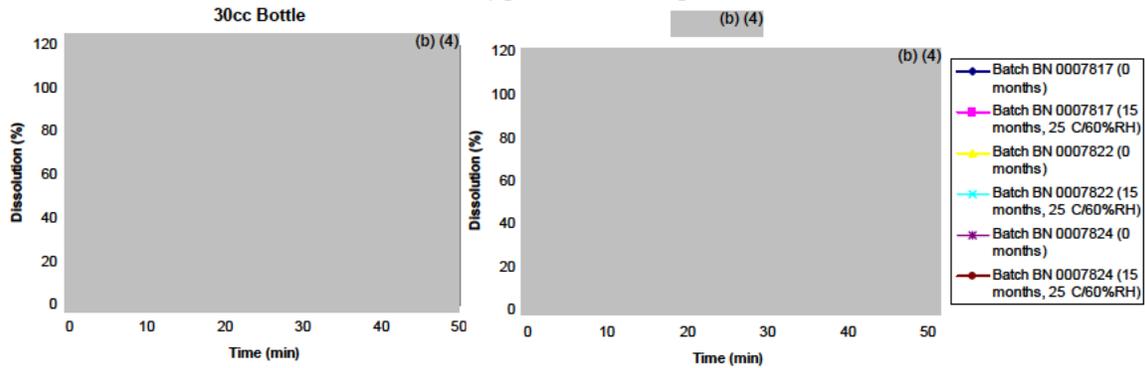
Product ID/ Batch Number	Strength	Number of Units	Collection Times Mean % (Range) RSD %		
			15 Minutes	30 Minutes	45 Minutes
BN0004965	145 ug ^a	12	(b) (4)		
BN0005007	290 ug ^b	12			
BN0005033	290 ug ^b	12			
BN0005034	290 ug ^b	12			
BN0006430	290 ug ^c	12			
BN0005035	145 ug ^a	12			
BN0005032	290 ug ^b	12			
BN0006822	290 ug ^c	12			
BN0008082	145 ug ^d	12			
BN0008230	145 ug ^d	12			
BN0008180	145 ug ^d	12			
BN0007259	290 ug ^c	12			
BN0006986	290 ug ^c	12			

Product ID/ Batch Number	Strength	Number of Units	Collection Times Mean % (Range) RSD %		
			15 Minutes	30 Minutes	45 Minutes
BN0005641	290 ug ^b	12	(b) (4)		
BN0005642	290 ug ^b	12			
BN0007049	290 ug ^c	12			
BN0007738	290 ug ^c	12			
BN0008691 *	290 ug ^c	12			
BN0008794 *	290 ug ^c	12			

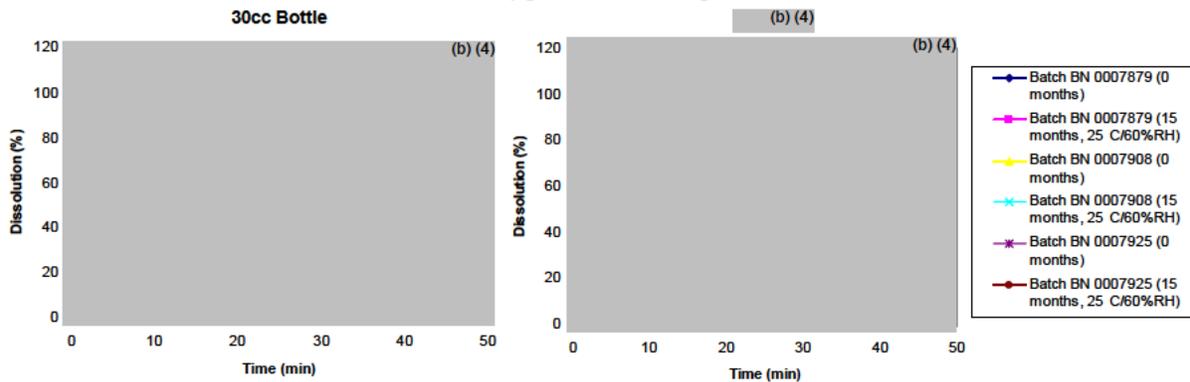
Note: The batches used as to supply Phase 3 were tested using dissolution method PRD-TM-ANL-00771

The dissolution results above demonstrate that batches used in Phase 3 and long-term safety studies would pass an acceptance criterion of $Q = (b) (4)$ at 15 minutes. The dissolution data for primary stability batches taken at 15 months also support a dissolution acceptance criteria of $Q = (b) (4)$ at 15 min is appropriate (refer to Reviewer Figures 1 and 2).

Reviewer's Figure 1. Mean Dissolution Data for Primary ICH Registration Stability Batches of 145 µg Linaclotide Capsules



Reviewer's Figure 2. Mean Dissolution Data for Primary ICH Registration Stability Batches of 290 µg Linaclotide Capsules



The following Biopharmaceutics comment was sent to the Applicant in an IR letter dated January 20, 2012.

FDA Comment 2

The following dissolution acceptance criterion is recommended: $Q = (b) (4)$ at 15 minutes. This recommendation is based on the mean in-vitro dissolution profiles for all strengths at release and under 15 months stability studies. Revise the dissolution acceptance criterion accordingly and submit an updated sheet of specifications for the drug product.

Applicant's Response

As committed to in the preliminary responses, dissolution data was generated at 50 rpm for the Supportive ICH Registration Stability Batches at the 15-month stability time-point. Dissolution data was collected at 15, 30, and 45 minutes, with an "infinity" pull (an additional 15 minutes at 250 rpm). The data are presented in the tables attached.

Based on a criteria of $Q = (b) (4)$ at 15 minutes at 50rpm, a majority of batches (13 out of 20; 65%) required Stage 2 testing. The %RSD values are up to $(b) (4)$ and individual capsule dissolution ranges of a high as $(b) (4)$ for the 145 ug capsules indicating that the evaluation of the drug product against a limit ($Q = (b) (4)$ at 15 minutes) is sub-optimal for the product and therefore not indicative of product quality.

Based on a criteria of $Q = (b) (4)$ at $(b) (4)$ minutes at 50 rpm, a considerable number of batches (6 out of 20, 30%) required Stage 2 testing, with %RSD values of up to $(b) (4)$ and individual capsule dissolution ranges of up to $(b) (4)$ for the 145 ug capsules. These results illustrate that a specification $Q = (b) (4)$ at $(b) (4)$ minutes is appropriately discriminating, and is reflective of product quality.

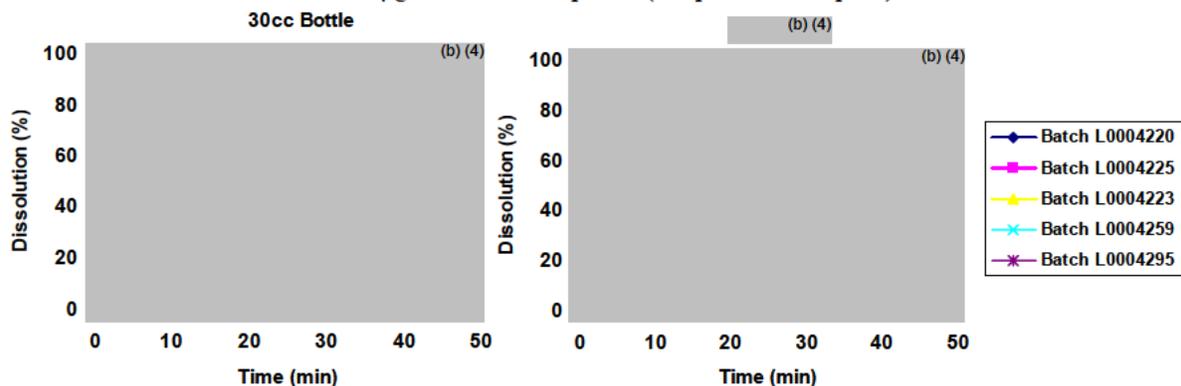
Based on these results, a regulatory acceptance criterion of $Q = (b) (4)$ at $(b) (4)$ minutes with a basket rotation speed of 50 rpm is proposed for the commercial product.

Reviewer's Comments and Evaluation:

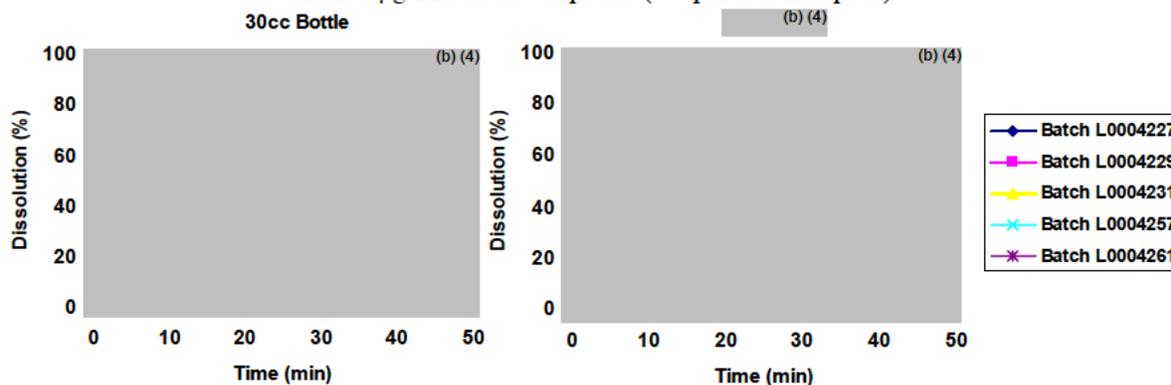
This reviewer confirmed that indeed 12/20 = 60% of batches needed to perform Stage 2 testing based on the recommended specification of $Q = (b) (4)$ at 15 minutes using 50 rpm rotation speed. Additionally, this reviewer confirmed that indeed 6/20 = 30% of batches needed to perform Stage 2 testing based on a specification of $Q = (b) (4)$ at $(b) (4)$ minutes using 50 rpm rotation speed.

Reviewer's Figures 3 and 4 illustrate the dissolution data for 15-month stability batches tested with a 50 rpm basket rotation speed. The shape of the dissolution profiles for these batches (in Reviewer's Figures 3, 4) is similar to those for 15-month stability batches tested with 100 rpm rotation speed (in Reviewer's Figures 1 and 2). Also the dissolution profiles of the batches achieve complete dissolution with both 50 rpm and 100 rpm basket rotation speeds.

Reviewer's Figure 3. Mean Dissolution Data for Primary ICH Registration 15 month Stability Batches of 145 µg Linaclotide Capsules (50 rpm rotation speed)



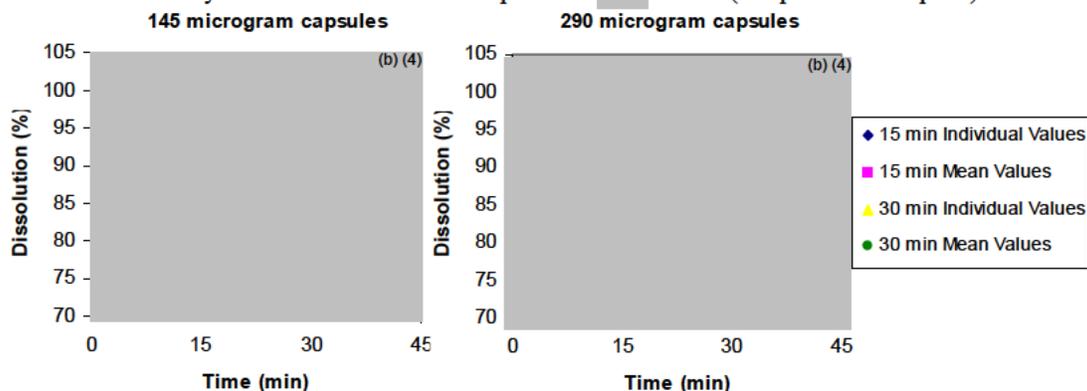
Reviewer's Figure 4. Mean Dissolution Data for Primary ICH Registration 15 month Stability Batches of 290 µg Linaclotide Capsules (50 rpm rotation speed)



The average value of the mean percent dissolved at 15 minutes for all batches is (b) (4)%. The minimum value of the percent dissolved at 15 minutes for all batches is (b) (4) (for two 290µg capsules in the 30cc bottle). Thus, all batches would pass the USP Stage 2 testing criterion: Average of 12 units is equal or greater than Q and no unit is less than Q - (b) (4)

A comparison of the individual versus mean dissolution data for the 15 month stability batches of 145 µg and 290 µg linaclotide capsules in the (b) (4) (Reviewer's Figure 5) demonstrates that the majority of capsules achieve greater than (b) (4) dissolution at both 15 minute and 30 minute time points.

Reviewer's Figure 5. Individual vs. Mean Dissolution Data for Primary ICH Registration 15 month Stability Batches of Linaclotide Capsules in (b) (4) Bottle (50 rpm rotation speed)



The ONDQA Biopharmaceutics Team held a teleconference with the Applicant on March 15, 2012 to discuss 1) Dissolution specifications should be established based on average in vitro dissolution data for each lot under study, equivalent to USP Stage 2 testing (n=12); 2) that having 60% of batches requiring Stage 2 testing under the criterion of $Q = (b) (4)$ at 15 minutes is not an indication of poor product quality, and 3) that an acceptance criterion of $Q = (b) (4)$ at 15 minutes would make the dissolution method more discriminating for quality control purpose, specially since the discriminating ability of the dissolution method was questionable due to lack of appropriate information. In a letter dated March 26, 2012, the Applicant accepted the recommended dissolution acceptance criterion and revised the drug product specifications to include a dissolution acceptance criterion of $Q = (b) (4)$ at 15 minutes

4. Manufacturing Site Change

Dr. Jane Chang, the ONDQA CMC reviewer, confirms that the Phase 3 and commercial drug products have the same formulation and manufacturing process. However, the Phase 3 drug product was manufactured at the

Applicant's site in the U.S. while commercial drug product was manufactured at the Applicant's site in Ireland. The Applicant provided dissolution data (see Tables 4-6), for one drug product batch manufactured at the US site and three drug product batches manufactured at the Ireland site, for each strength.

Table 4. Summary of the Drug Product Batches Evaluated for the Comparability Evaluation

Drug Substance Supplier (Lot Number)	Linacotide Beads			Linacotide Capsules		
	Batch Number	Strength	Manufacturing Site and Scale	Batch Number	Strength	Encapsulation Site and Scale
PPL Sweden (109062-01)	LDN043	(b) (4)	Forest Ireland (b) (4)	BN0007824	145 ug ^b	Forest Commack (b) (4)
				BN0007925	290 ug ^c	
PPL Sweden (109113-01)	LDN047			BN0007817	145 ug ^b	
				BN0007879	290 ug ^c	
PPL Sweden (109133-01)	LDN048			BN0007822	145 ug ^b	
				BN0007908	290 ug ^c	
PPL Sweden (109113-01)	LDN047			L0004295	145 ug ^b	Forest Ireland (b) (4)
				L0004261	290 ug ^c	

Table 5. Batch Analysis Data at Time of Release for 145 µg Batches Evaluated for Comparability Assessment

Test	Acceptance Criteria	Results			
		BN0007824	BN0007817	BN0007822	L0004295
Description *	Locked, size 3, white to off-white opaque capsules with gray radial bar imprint on the cap. Upon opening the capsules, contents inside should confirm the presence of white to off-white beads	Complies	Complies	Complies	Complies
Identification	The retention time of the major peak in the sample conforms to reference standard	Complies	Complies	Complies	Complies
Content Uniformity	Complies with USP <905> Uniformity of Dosage Units	Complies: Stage 1 requirements (b) (4)			
		AV: (b) (4)	AV: (b) (4)	AV: (b) (4)	AV: (b) (4)
		Mean (b) (4)	Mean (b) (4)	Mean (b) (4)	Mean (b) (4)
		%RSE (b) (4)	%RSE (b) (4)	%RSE (b) (4)	%RSD (b) (4)
		Range (b) (4)	Range (b) (4)	Range (b) (4)	Range (b) (4)
Assay	(b) (4) Label Claim	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Degradation Products	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Unspecified (each)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Total (Specified and Unspecified)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Total	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Microbiology	Total aerobic microbial count	NMT (b) (4)	(b) (4)	(b) (4)	(b) (4)
	Total combined molds and yeasts count	NMT (b) (4)	(b) (4)	(b) (4)	(b) (4)
	Escherichia coli	Absence in 1 g	Absent in 1 g	Absent in 1 g	Absent in 1 g
Dissolution	NLT (b) (4) in (b) (4) minutes	15 minutes: (b) (4)			
		30 minutes: (b) (4)			
		45 minutes: (b) (4)			

Table 6. Batch Analysis Data at Time of Release for 290 µg Batches Evaluated for Comparability Assessment

Test	Acceptance Criteria	Results			
		BN0007925	BN0007879	BN0007908	L0004261
Description *	Locked, size 2, white to off-white opaque capsules with gray radial bar imprint on the cap. Upon opening the capsules, contents inside should confirm the presence of white to off-white beads.	Complies	Complies	Complies	Complies
Identification	The retention time of the major peak in the sample conforms to reference standard	Complies	Complies	Complies	Complies
Content Uniformity	Complies with USP <905> Uniformity of Dosage Units	Complies: Stage 1 requirements			
		AV: (b) (4)	AV: (b) (4)	AV: (b) (4)	AV: (b) (4)
		Mean (b) (4)	Mean (b) (4)	Mean (b) (4)	Mean (b) (4)
		%RSD	%RSD	%RSD	%RSD
		Range	Range	Range	Range (b) (4)
Assay	(b) (4) Label Claim				(b) (4)
Degradation Products					(b) (4)
	(b) (4)				(b) (4)
	(b) (4)				(b) (4)
	(b) (4)				(b) (4)
	(b) (4)				(b) (4)
	(b) (4)				(b) (4)
	(b) (4)				(b) (4)
Unspecified (each)					(b) (4)
Total (Specified and Unspecified)					(b) (4)
Total	(b) (4)				(b) (4)
Microbiology	Total aerobic microbial count	NMT (b) (4)			(b) (4)
	Total combined molds and yeasts count	NMT (b) (4)			(b) (4)
	Escherichia coli	Absence in 1 g			(b) (4)
Dissolution	NLT (b) (4) in (b) (4) minutes	15 minutes= (b) (4)			
		30 minutes=	30 minutes=	30 minutes=	30 minutes=
		45 minutes=	45 minutes=	45 minutes=	45 minutes=

Reviewer’s Comments and Evaluation:

The manufacturing site change from U.S. to Ireland is analogous to a Level 3 site change according to the SUPAC-IR Guidance. To show that the drug products from both manufacturing sites are bioequivalent, multi-point dissolution data and f2 analysis are required. The Applicant provided dissolution data using their proposed dissolution method. Although, f2 testing could not be calculated due to the drug product’s rapid dissolution (more than (b) (4) of the drug was released within 15 minutes for all batches), the percentage dissolved at 15, 30 and 45 min is super-imposable. Therefore, the drug products made from the Applicant’s U.S. and Ireland manufacturing sites are considered bioequivalent.

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/s/

KAREEN RIVIERE
04/10/2012

SANDRA SUAREZ
04/10/2012

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA: 202811	Submission Date(s): 08/09/2011
Brand Name	Linzess®
Generic Name	Linaclotide
Reviewer	Sandhya Apparaju, Ph.D.
Team Leader	Sue Chih Lee, Ph.D.
OCP Division	DCP3
OND Division	DGIEP
Sponsor	Ironwood Pharmaceuticals, Inc.
Relevant IND(s)	63,290
Submission Type	Original NDA; Standard Review
Formulation; Strength(s)	Oral capsules; 145 µg and 290 µg strengths
Indication	Chronic Constipation (CC) and Irritable Bowel Syndrome with Constipation (IBS-C)

An optional inter-divisional level Office of Clinical Pharmacology briefing was held for NDA 202811 on April 02, 2012 from 2 to 3 PM, in White Oak Bldg 51, Conf Room 3300. Attendees included, Drs'. E. Dennis Bashaw, Hae Young Ahn, Sue Chih Lee, Erica Wynn, Chinmay Shukla, Insook Kim, Jie Wang, Sandhya Apparaju and others.

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1 Executive Summary

1.1 Recommendation

NDA 202811, linaclotide for CC and IBS-C is acceptable from a Clinical Pharmacology perspective.

1.2 Phase IV Commitments

None

1.3 Summary of Important Clinical Pharmacology and Biopharmaceutics Findings

Linaclotide, a chemically synthesized 14-amino acid peptide is an agonist at the Guanylate Cyclase C (GC-C) receptor at the luminal surface of the intestinal epithelium. It is proposed for the treatment of Chronic Constipation (CC) and Irritable Bowel Syndrome related constipation (IBS-C). Drug is available as capsules in 145 and 290 µg strengths.

Systemic bioavailability of linaclotide and its active metabolite MM-419447 (formed via removal of the carboxy terminal tyrosine) was found to be negligible following administration of clinically relevant doses of the drug. Systemic exposures were assessed during phase 1 single dose and multiple dose PK/PD studies, and via sparse sampling in Phase 3 trials following a change in the formulation. Validated LC-MS/MS methods were used for the detection of drug and metabolite in plasma.

In a food-effect PK/PD study, concomitant food intake did not result in detectable concentrations of drug or metabolite in plasma. Dosing with food resulted in the formation of looser and more frequent stools suggestive of increased pharmacodynamic effects (based on PD endpoints such as stool consistency and frequency). In Phase 2 and Phase 3 clinical trials of CC and IBS-C, linaclotide was dosed at least 30 minutes prior to breakfast. The proposed labeling recommends dosing on an empty stomach. We recommend revisions to note that drug should be dosed on an empty stomach ‘at least 30 minutes prior to the first meal of the day’.

There was no metabolism of linaclotide in the human intestinal microsomes suggesting absence of gut CYP450 involvement. Intestinal stability results suggest that the peptide is broken down into smaller amino acid fragments by the action of local proteases. In vitro findings from Caco-2 studies were inconclusive with regard to permeability characteristics of linaclotide due to conflicting results from two separate studies in this regard.

Systemic drug-drug interactions with oral linaclotide are unlikely to be a concern due to negligible systemic exposure of parent or metabolite after oral administration of clinically relevant doses. Nevertheless, sponsor has conducted several in vitro studies to evaluate drug-drug interaction potential of linaclotide via effects on CYP450 enzymes and transporters. The concentrations in these in vitro trials (0.05 – 50 ng/mL) were chosen to encompass the maximum concentrations noted in two individuals who’d received a supratherapeutic (10-fold higher; 2897 µg) dose of linaclotide in a food-effect PD study. In vitro inhibition studies evaluating all major drug metabolizing enzymes

suggest that linaclotide or its metabolite cannot be expected to cause clinically meaningful inhibition of the metabolism of co-administered drugs.

In addition, sponsor has conducted in vitro assessments that include concentrations of linaclotide encompassing local (I_{gut}) concentrations. No clinically relevant inhibition of CYP3A4 (direct or mechanism-based) was noted when linaclotide concentrations encompassing local (I_{gut}) concentrations were investigated in a separate study.

Neither linaclotide nor its primary metabolite induced CYP1A2, 2B6 or 3A4/5 in the in vitro investigations which included concentrations of up to 50 ng/mL for CYP1A2 and 2B6 and up to 5000 ng/mL for CYP3A4.

Linaclotide doesn't appear to be a substrate of P-gp nor did it inhibit P-gp mediated efflux of digoxin. Linaclotide doesn't cause inhibition of BCRP, MRP2 and MRP3 transporters. Some inhibition was noted on MRP4 at all concentrations of linaclotide tested (18 – 24 %). The active metabolite did not inhibit MRP 2/3/4 transporters but had weak inhibitory effect (18 %) on BCRP transporter at the highest concentration. BCRP and MRP2 are the efflux transporters expressed at the intestine. As IC_{50} values for inhibition of BCRP by the metabolite are $> 10 \mu\text{M}$ and likely gut (local) concentrations of linaclotide (parent) are $< 1 \mu\text{M}$, this finding is unlikely to be of clinical relevance for drug interactions.

Linaclotide does not interact with OATP1B1, OATP1B3, PEPT1 and OCTN1 uptake transporters at the highest concentration tested ($10 \mu\text{M}$). A maximum inhibition of about 55 % was observed for linaclotide at $10 \mu\text{M}$ on OATP2B1 uptake transporter. The active metabolite MM-419447 did not interact with OATP1B1, OATP1B3, OATP2B1, or OCTN1 uptake transporters. However, this metabolite weakly inhibited PEPT1 (~ 18 – 21 %). As systemic exposure potential of linaclotide and its major metabolite are negligible following clinically relevant doses, the observed inhibition of OATP2B1 (uptake transporter expressed at hepatocytes) at the highest concentration evaluated may not be clinically relevant. The modest effect of metabolite on PEPT1 may not be relevant as no IC_{50} could be computed at concentrations tested (exceeding ten times the local linaclotide concentrations following maximum dose).

Results suggest that linaclotide and its metabolite are unlikely to inhibit, induce major CYP450 enzymes and transporters and therefore are unlikely to interact with co-administered drugs. PK and safety studies in specific subpopulations have not been conducted due to the absence of systemic exposure following clinical doses.

Dose-finding of linaclotide was conducted during Phase 2b trials in CC and IBS-C patients. Linaclotide doses of 75, 150, 300 and 600 μg were evaluated against placebo. Endpoints evaluated in the Phase 2b trials were similar to the clinical efficacy endpoints and included frequency of spontaneous bowel movements (SBM), complete spontaneous bowel movements (CSBM), % responders, stool consistency, stool frequency, straining, abdominal pain etc.

For CC, dose-related trends for various efficacy endpoints were noted. Safety information also suggested dose-related trends in AEs, in particular diarrhea. Based on this information, the two middle doses (150 μg and 300 μg) were selected for Phase 3 trials in CC. Dose-related trends for efficacy were not noted in the Phase 2b trial for IBS-C. In this population, the 300 μg dose provided greatest benefit compared to the two lower doses and even the highest dose evaluated, for most endpoints. Based on the information from Phase 2b, only the 300 μg dose was progressed into phase 3 trials for

IBS-C. The 600 µg dose was not continued into Phase 3 for either indication due to a higher incidence of diarrhea and other GI adverse events as well as discontinuations due to diarrhea. Note that due to revised potency expression for linaclotide, the 150 µg and 300 µg dose strengths are renamed as 145 µg and 290 µg in the Phase 3 trials as well as in the proposed labeling.

In Phase 3 trials linaclotide doses demonstrated markedly higher and statistically significant improvements from baseline relative to placebo. The effects on bowel movement endpoints were typically noted within the first week and were sustained throughout the dosing duration. A trend for higher efficacy at 290 µg dose was noted during the Phase 2b trial in CC and in one of the two Phase 3 trials. The other Phase 3 trial showed no further benefit of the 290 µg dose relative to 145 µg dose. (b) (4). Although the potential utility of the higher dose cannot be ruled out in all CC patients, tolerability may become an issue, particularly as it relates to incidence of severe diarrhea. (b) (4)

One possibility could be to start with the lower 145 µg dose, with an option to increase dose to 290 µg if the lower dose is well tolerated and a need for greater efficacy has been identified. However, this needs to be discussed further within the review team.

The proposed labeling recommends a dose of 290 µg in IBS-C. An option for a lower starting dose or dose reduction in case of adverse events has not been suggested by the sponsor for IBS-C, nor has it been evaluated in the double-blind primary efficacy phases of the pivotal IBS-C trials. However, the Clinical Reviewer has noted information from the long-term phases of the IBS-C trials where dose reductions were allowed. (b) (4)

Because of linaclotide's limited systemic bioavailability, a thorough QT study was not performed. Based on recommendations received from FDA during development, triplicate ECGs were obtained from a subset of patients in the Phase 3 CC and IBS-C trials. The Clinical reviewer Dr. Lara Dimick has noted absence of QT prolongation effects by linaclotide in these data. Please refer to the Medical Officers' review in this regard for an interpretation of the ECG information in Phase 3 trials of CC and IBS-C.

Formal testing for assessing immunogenicity potential was not conducted for linaclotide. Sponsor considers immunogenicity not to be a concern for the drug as it is a small peptide for oral administration, and has no measurable systemic exposure following clinically relevant doses. A clinical consult with the Office of Biotechnology Products (OBP) is pending to determine whether the sponsor will need to investigate immunogenicity issue in a more formal setting.

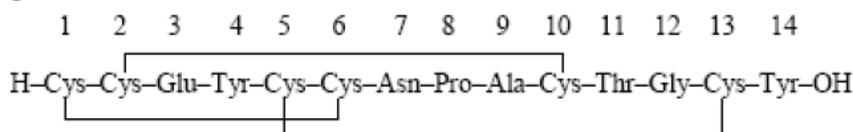
2 Summary of CPB Findings

2.1 General Attributes of the Drug

2.1.1. What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Linaclotide is a chemically synthesized 14-amino acid peptide with 3 disulfide bonds. The molecular formula is $C_{59}H_{79}N_{15}O_{21}S_6$ and the molecular weight is 1526.8 Da.

Figure 1. Structural Formula of Linaclotide



The solubility in aqueous solutions over a pH range of 1.0 to 7.5 is $> 100 \mu\text{g/mL}$.

The proposed commercial product comprises hard gelatin capsules (145 μg and 290 μg strengths) containing linaclotide drug substance coated onto microcrystalline cellulose beads along with (b) (4) (hypromellose) and (b) (4) (calcium chloride dihydrate and L-leucine).

Table 1: Composition (b) (4)

Component	Function	Quality Standard	Theoretical Weight (mg/capsule)		Theoretical Weight (% w/w)
			Linaclotide Capsules, 145 μg	Linaclotide Capsules, 290 μg	
Linaclotide	Active Ingredient	In House Standard ^a	0.145 ^b	0.29 ^b	0.26
Calcium chloride dihydrate	(b) (4)	USP	(b) (4)		
L-leucine	(b) (4)	USP			
Hypromellose	(b) (4)	USP			
Microcrystalline cellulose	(b) (4)	NF			
(b) (4)	(b) (4)	USP			
(b) (4)	(b) (4)	NF			
Total	(b) (4)		56.36	112.70	100.00

The two strengths of the capsule product (145 μg and 290 μg) (b) (4)

The proposed (b) (4) composition for the commercial

product is identical to the formulation used in the Phase 3 clinical trials (efficacy and safety studies).

Dose-strength expression of linaclotide in the NDA submission:

Sponsor notes that during the course of product development, a major improvement was made to the approach used to determine the potency of the linaclotide primary reference standard. (b) (4)



This led to the depiction of the same dose strengths in many different ways throughout the NDA studies. Sponsor notes that these modifications represent changes only in the dose-strength expression, and do not reflect changes in the actual dose strength administered to patients. The potency expression that was used in a particular study has been left intact during the write-up of this review. The table below will enable further understanding of how linaclotide doses compare using the different potency methods:

Table 2: Changes in the dose strength expression of linaclotide during development

<i>Clinical Study Number</i>	<i>Linaclotide Dose (ug)</i>			
	<i>Original Dose</i>	<i>Revised Dose 1</i>	<i>Revised Dose 2</i>	<i>Nominal Dose</i>
	(b) (4)			
MCP-103-001 ^b	30, 100, 300, 1000, 3000	24, 81, 244, 812, 2436	27, 88, 265, 884, 2653	29, 97, 290, 966, 2897
MCP-103-002 ^b	30, 100, 300, 1000	24, 81, 244, 812	27, 88, 265, 884	29, 97, 290, 966
MCP-103-004 ^b	100, 300, 1000	93, 281, 927	101, 306, 1010	97, 290, 966
MCP-103-005 ^b	100, 1000	81, 812	88, 884	97, 966
MCP-103-103	300, 3000	266, 2660	290, 2897	
MCP-103-201	75, 150, 300, 600	67, 133, 266, 532	72, 145, 290, 579	
MCP-103-202	75, 150, 300, 600	67, 133, 266, 532	72, 145, 290, 579	
MCP-103-303	150 and 300	133 and 266	145 and 290	
LIN-MD-01	150 and 300	133 and 266	145 and 290	
MCP-103-302	300	266	290	
LIN-MD-31	300	266	290	
MCP-103-305	150 and 300	133 and 266	145 and 290	
LIN-MD-02	150 and 300	133 and 266	145 and 290	

- a. Based on a nominal linaclotide content to facilitate comparison of doses across linaclotide studies.
- b. Drug substance lots were re-assayed as part of the first revision for the dose reassignment.

2.1.2. What are the proposed mechanism(s) of action and therapeutic indication(s)?

Linaclotide is a first-in-class guanylate cyclase C (GC-C) receptor agonist, structurally related to the endogenous guanylin peptide family. The guanylin peptide family is involved in the regulation of intestinal fluid homeostasis and bowel function. This family includes the hormones guanylin and uroguanylin. Activation of the GC-C receptor increases GI secretion and transit.

In the gastrointestinal (GI) tract, linaclotide is metabolized to a single primary active metabolite, MM-419447, which is a 13-amino acid peptide lacking the C-terminal tyrosine present in linaclotide. Both linaclotide and its metabolite bind to and activate the GC-C receptor locally, on the luminal surface of the intestinal epithelium. Activation of GC-C results in an increase in concentrations of cyclic guanosine monophosphate (cGMP), both extracellularly and intracellularly. Extracellular cGMP decreases pain-fiber activity, resulting in reduced visceral pain. Intracellular cGMP causes secretion of chloride and bicarbonate into the intestinal lumen, through activation of the cystic fibrosis transmembrane conductance regulator (CFTR), which results in increased intestinal luminal fluid and accelerated transit. Linaclotide has been shown to reduce visceral pain and increase GI transit in animal models.

Proposed Indications:

- Treatment of Chronic Constipation (CC) in adults
- Treatment of Irritable Bowel Syndrome with Constipation (IBS-C) in adults

2.1.3. What are the proposed dosages and route of administration?

Linaclotide capsules are intended for once-daily oral administration, on an empty stomach. The sponsor's proposed doses for Chronic Constipation are 145 µg (b) (4). The proposed dose for IBS-C is 290 µg.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

NDA submission includes results from three Clinical Pharmacology (PK and food-effect) studies in healthy volunteers, four Phase 3 randomized, placebo-controlled, double-blind safety and efficacy trials (two in patients with CC and two in IBS-C patients), as well as from two Phase 2b dose-ranging studies (one in each population) to provide evidence of linaclotide's safety and efficacy in the treatment of IBS-C and CC. Reports of 8 *in vitro* investigations of linaclotide's intestinal stability, metabolism, permeability and drug-drug interaction potential via enzymes and transporters are also included in the NDA. Analytical method validation reports are included for the parent and active metabolite.

The study designs and doses for the Clinical Pharmacology studies are shown:

- Study MCP-103-001, a single oral, ascending dose, placebo-controlled study in healthy volunteers for evaluation of safety, tolerability, PK and gastrointestinal PD. Study evaluated 30, 100, 300, 1000, 3000 µg doses of linaclotide.
- Study MCP-103-002, a multiple-ascending dose, placebo controlled study in healthy volunteers to evaluate safety, tolerability, PK and gastrointestinal PD. Study evaluated 30, 100, 300 and 1000 µg once-daily doses of linaclotide.
- Study MCP-103-103, a randomized, two-period, two-sequence, crossover trial of oral linaclotide acetate administered under fed and fasting conditions in healthy volunteers, to evaluate safety and gastrointestinal PD under fed and fasted conditions. Study evaluated 300 µg once-daily for seven days. A 10-fold higher dose (single dose of 2897 µg) was included at the end of the multiple dose phase, to evaluate systemic exposures if any, following suprathreshold dose of Linaclotide.

The design and dosing aspects of the key dose-ranging (Phase 2b) studies are shown:

- Study MCP-103-201, a 4-week, randomized, double-blind, placebo-controlled, dose-finding trial in patients with Chronic Constipation. Study evaluated 75, 150, 300 and 600 µg doses of linaclotide or placebo. CC patients were enrolled if patient met criteria for CC: reported < 3 SBMs per week and met 1 or more of the following symptoms for 12 weeks in the 12 months preceding the Screening Visit: Straining during > 25% of BMs, Lumpy or hard stools (BSFS score of 1 or 2) during > 25% of BMs, Sensation of incomplete evacuation during > 25% of BMs. Study included four distinct periods: a screening period (Day -42 to -15; includes drug washout), pre-treatment period (Day -14 to Day -1; includes baseline bowel habit, daily patient symptoms severity, and weekly patient global assessments), treatment period (Day 1 to Day 28; daily dosing of treatments; includes baseline bowel habit, daily patient symptoms severity, and weekly patient global assessments and rescue medication details) and post-treatment period (Day 29- Day 43; Daily Bowel Habits, Daily Patient Symptom Severity Assessments, Weekly Patient Global Assessments, and per-protocol rescue medication use).
- Study MCP-103-202, a 12-week, randomized, double-blind, parallel-group, placebo-controlled, dose finding study in patients with IBS-C. Study evaluated 75, 150, 300 and 600 µg doses of linaclotide. During the pre-treatment, treatment and post-treatment periods, Daily Patient Bowel Habits, Daily Patient Symptom Severity Assessments, Weekly Patient Global Assessments, and per-protocol rescue medication use were to be collected through the IVRS.

Phase 3 safety and efficacy trials: Two trials were conducted for each of the two indications: MCP-103-303 and LIN-MD-01 in CC and MCP-103-302 and LIN-MD-31 in IBS-C. All trials were randomized, double-blind, placebo-controlled, parallel group studies in the target populations. Each trial included a screening period for confirming enrollment eligibility, a pre-treatment period for obtaining baseline information, a 12 week or 26 week (IBS trial 302) double-blind treatment period for primary efficacy evaluation and a randomized withdrawal period in at least one trial within each indication. In the two CC Phase 3 efficacy trials, the linaclotide doses were 145 µg and 290 µg. In the two IBS-C Phase 3 efficacy trials, the linaclotide dose was 290 µg. Sparse

sampling was included in these trials in an attempt to identify detectable drug concentrations at clinically relevant doses.

Within each indication, the enrollment criteria and rules for the use of concomitant medications were identical in both Phase 3 efficacy trials, and similar to the Phase 2b trial. To enroll in the Phase 2b and 3 studies, patients were required to meet modified Rome II criteria specific for either CC or IBS-C, which were similar to the criteria used in other CC and IBS-C drug development programs. In both the Phase 2b and Phase 3 trials, linaclotide was administered as an oral capsule once-daily at least 30 minutes prior to breakfast. This was based on the finding of increased PD effects (and a potential for higher incidence of diarrhea) when linaclotide was dosed with food in the phase 1 food-effect study.

2.2.2 What is the basis for selecting the response endpoints (i.e., clinical or surrogate endpoints) or biomarkers (collectively called pharmacodynamics (PD)) and how are they measured in clinical pharmacology and clinical studies?

Phase 1: The gastrointestinal PD endpoints evaluated in these Clinical Pharmacology studies were stool consistency using Bristol Stool Form Scale (BSFS), stool frequency, straining (ease of passage) and stool weight.

Phase 2b: For the 4-week dose-ranging study in CC, the primary efficacy parameter was the mean change (overall) from baseline in the weekly spontaneous bowel movement (SBM) frequency rate during the 4 weeks of the Treatment Period. The secondary efficacy responder parameters were SBM overall 75% responder and complete SBM (CSBM) overall 75% responder rates. Responder is defined as patient whose SBM or CSBM for the week was ≥ 3 and the patient's SBM or CSBM rate for the week was increased ≥ 1 from the baseline weekly rate. A patient was a 75 % responder if the patient was a SBM or CSBM responder for ≥ 3 of the 4 treatment period weeks. The secondary efficacy change-from-baseline parameters were overall weekly CSBM Frequency Rate, Stool Consistency (BSFS score), and overall Severity of Straining score.

For the 12-week dose-ranging Phase 2b trial in IBS-C, the primary efficacy parameter was the change from baseline in the weekly CSBM Frequency Rate during Weeks 1 through 12 of the Treatment Period. The secondary efficacy parameters were CSBM Overall 75% Responder, and overall change from baseline in the following: weekly SBM Rate, BSFS score, Severity of Straining score, Degree of Relief of IBS Symptoms, and Abdominal Pain.

Phase 3: The primary and secondary efficacy parameters for the CC and IBS-C Phase 3 trials are shown. The IBS-C indication include four co-primary endpoints with two of them being composite endpoints for assessing both CSBM and abdominal pain.

CC	IBS-C
<p><i>Primary Efficacy Parameters:</i></p> <p>1. 12-week CSBM Overall Responder (9/12 Week CSBM 3+1 Responder)^a</p>	<p><i>Primary Efficacy Parameter:</i></p> <p>1. 9/12 Week Abdominal Pain and CSBM (APC) 3+1 Responder</p> <p>2. 9/12 Week CSBM 3+1 Responder</p> <p>3. 9/12 Week Abdominal Pain Responder</p> <p>4. 6/12 Week APC +1 Responder (primary parameter based on FDA draft guidance)</p>
<p><i>Secondary Efficacy Parameters:</i></p> <p>1. Change from Baseline in 12-week CSBM Frequency Rate</p> <p>2. Change from Baseline in 12-week SBM Frequency Rate</p> <p>3. Change from Baseline in 12-week Stool Consistency</p> <p>4. Change from Baseline in 12-week Severity of Straining</p> <p>5. Change from Baseline in 12-week Abdominal Discomfort</p> <p>6. Change from Baseline in 12-week Bloating</p> <p>7. Change from Baseline in 12-week Constipation Severity</p>	<p><i>Secondary Efficacy Parameters:</i></p> <p>1. Change from Baseline in 12-week CSBM Frequency Rate</p> <p>2. Change from Baseline in 12-week SBM Frequency Rate</p> <p>3. Change from Baseline in 12-week Stool Consistency</p> <p>4. Change from Baseline in 12-week Severity of Straining</p> <p>5. Change from Baseline in 12-week Abdominal Discomfort</p> <p>6. Change from Baseline in 12-week Bloating</p> <p>7. Change from Baseline in 12-week Abdominal Pain</p> <p>8. 6/12 Week CSBM +1 Responder</p> <p>9. 6/12 Week Abdominal Pain Responder</p> <p>10. Change from Baseline in 12-week Percent of Abdominal Pain-free Days</p>

- A weekly CSBM 3+1 Responder was defined as a patient who had at least 3 CSBMs per week and a minimum level of improvement of at least 1 CSBM per week over baseline (CC and IBS-C)
- A weekly CSBM +1 Responder was defined as a patient who had a minimum level of improvement of at least 1 CSBM per week over baseline (i.e., there was no requirement for a minimum absolute number of CSBMs during the week) (IBS-C)
- A weekly Abdominal Pain Responder was a patient who had at least a 30% improvement over his or her average weekly baseline score for “worst abdominal pain in the past 24 hours” in a particular week.
- Based on a recommendation from the FDA, to be a responder for each of the 3 weekly IBS-C responder definitions, IBS-C patients also had to complete at least 4 daily calls into the IVRS system for that week.

Sponsor notes that the efficacy parameters for the CC and IBS-C Phase 3 efficacy trials were selected based upon qualitative and quantitative research in patients, consultation with gastroenterologists, Phase 2b study results, input from FDA, and the FDA’s patient-reported outcomes (PRO) and IBS guidance documents.

2.2.3 Are the active moieties in the plasma appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships? (If yes, refer to 2.6, Analytical Section; if no, describe the reasons.)

An LC/MS/MS method with an LLOQ of 0.2 ng/mL for linaclotide and an LLOQ of 2.0 ng/mL for the active metabolite MM-419447 was developed and used in the analysis of PK samples from the Phase 1 food-effect study and the Phase 3 trials. Systemic exposures of parent linaclotide or its active metabolite were negligible following clinically relevant doses.

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy? If relevant, indicate the time to the onset and offset of the desirable pharmacological response or clinical endpoint.

There were no concentration-response assessments due to the lack of systemic exposure for linaclotide. Results of the dose-response [Phase 2b (CC and IBS-C) and Phase 3 (CC only)] studies are summarized below:

Dose-response information in Chronic Constipation (CC):

MCP-103-201 (Phase 2b in CC): This study was a multicenter, randomized, double-blind, parallel-group, placebo-controlled, multiple oral dose study of 75, 150, 300, and 600 µg capsules of linaclotide or placebo administered to patients with CC. Doses were administered once daily in the morning on an empty stomach and fasting continued for at least 30 minutes after dosing. The primary efficacy endpoint was the change in the weekly normalized spontaneous bowel movement (SBM) rate during weeks 1 through 4 of the Treatment Period from the weekly normalized SBM frequency during the Pretreatment Period.

Results: Results for the primary and key secondary endpoints are shown here.

At pre-treatment, all groups had comparable weekly normalized SBM rates. Following placebo or active treatments for four weeks, the overall weekly normalized SBM rates were higher for all groups compared to baseline, with a trend towards dose-response for absolute values. The change from baseline (pre-treatment) was also dose-related, with placebo providing the smallest increase in weekly SBM rates over baseline (1.55) and the 600 µg linaclotide providing the maximum change (4.54) in the evaluable population. Least square mean differences from placebo were significant for all active treatment groups except 75 µg in the evaluable population. In the ITT population, the change from baseline relative to placebo was significant for all doses evaluated. The 75 µg dose often provided statistically significant changes from baseline, albeit numerically smaller improvements for the key primary and secondary endpoints evaluated.

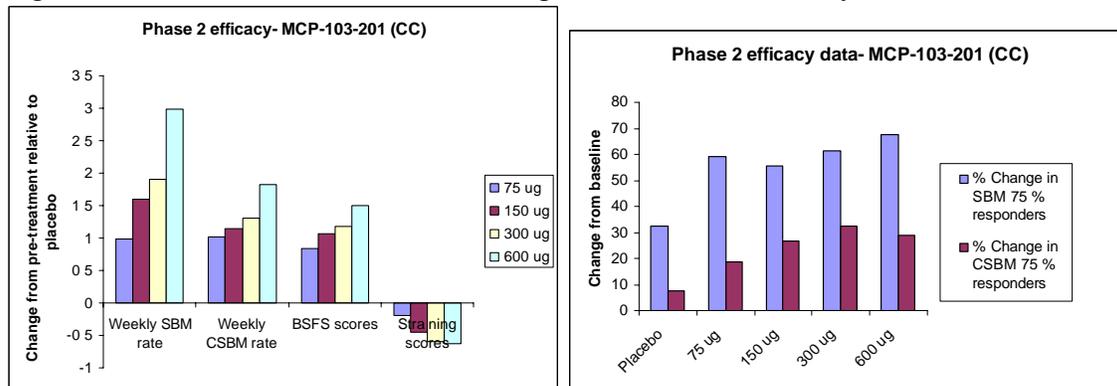
The following summary table provides findings for the primary and various secondary as well as exploratory endpoints for the ITT population:

Table 3: Efficacy results from the Phase 2b trial in CC population

Endpoint ¹	Placebo (n=68)	Linaclotide			
		75 ug (n=59)	150 ug (n=56)	300 ug (n=62)	600 ug (n=62)
SBM Frequency ² (SBM/week)	1.45	2.59*	3.25*	3.57***	4.29***
SBM 75%Responder ³	32.4%	59.3%*	55.4%*	61.3%*	67.7%***
CSBM Frequency ² (CSBM/week)	0.45	1.47*	1.59*	1.75**	2.26***
CSBM 75%Responder ³	7.4%	18.6%	26.8%*	32.3%**	29.0%**
Stool Consistency (BSFS score)	0.50	1.35**	1.57***	1.68***	2.00***
Straining (5-point ordinal scale)	-0.52	-0.71	-0.97**	-1.11***	-1.14***
Abdominal Pain ⁴ (5-point ordinal scale)	-0.08	-0.28*	-0.27*	-0.19	-0.24
Abdominal Discomfort ⁴ (5-point ordinal scale)	-0.04	-0.32*	-0.30*	-0.24*	-0.28*
Bloating ⁴ (5-point ordinal scale)	-0.02	-0.40**	-0.42**	-0.27*	-0.26*
Constipation Severity ⁴ (5-point ordinal scale)	-0.17	-0.78**	-0.89**	-0.88**	-0.95**
Degree of Relief CC Symptoms ⁵ (7-point balanced scale)	-0.50	-0.99*	-1.12**	-1.13**	-1.26***

p-values: * ≤0.05, ** ≤0.001 *** ≤0.0001

Figure 2 and 3: Phase 2b in CC: Dose-response trends for efficacy



Onset and duration of therapeutic effects: The effects on key primary and secondary endpoints were typically seen by the first week of dosing, and these effects were sustained throughout the 4-week duration with linaclotide treatment. At cessation of dosing, values returned toward pre-treatment, with no evidence for worsening of symptoms (rebound) during the evaluated post-treatment period.

MCP-103-303 [Phase 3 in CC]: This was a Phase 3, Randomized, Double-blind, Placebo-controlled, Parallel-group, Trial of Linaclotide Administered Orally for 12 Weeks Followed by a 4-Week Randomized Withdrawal Period in Patients with Chronic Constipation. 209 in the placebo group, 217 in the 150 µg linaclotide group and 217 in the 300 µg group were randomized; oral capsules once daily taken at least 30 min before

breakfast. The primary efficacy parameter was the 12-week complete spontaneous BM (CSBM) Overall Responder (a patient who was a CSBM Weekly Responder [CSBM rate ≥ 3 with an increase from baseline of ≥ 1] for ≥ 9 of the 12 wks of the Treatment Period).

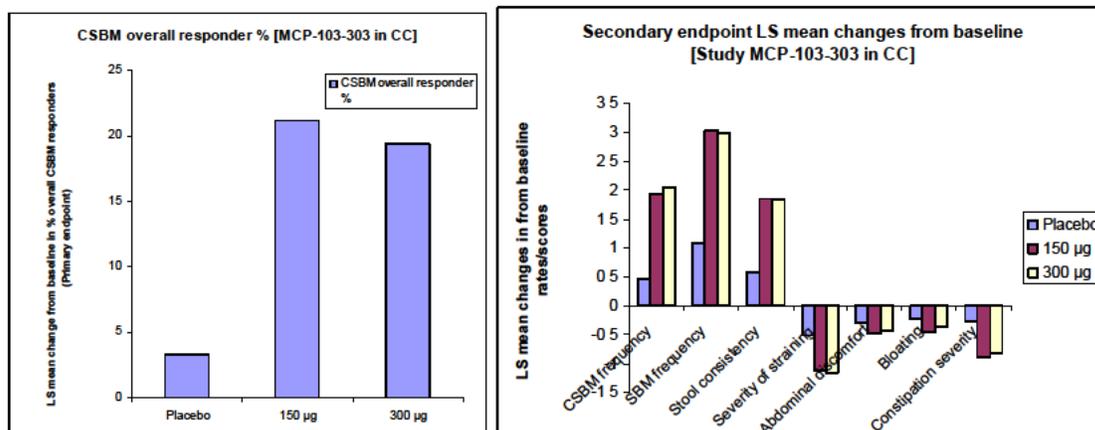
Results of the primary and various secondary efficacy parameters in the ITT population are summarized in the table below. Albeit slightly different in final values, in general FDA statisticians' analyses of phase 3 data were deemed at the time of writing of this review to be consistent in magnitude and trends to the sponsor's data that is presented below. The number and percentage of patients who were 12-week CSBM Overall Responders (primary efficacy) was numerically greater for both the 150 (46 patients, 21.2%) and 300 μg (42 patients, 19.4%) [or 145 and 290 μg per revised potency notation] dose groups when compared to placebo (7 patients, 3.3%), and the comparison of each active treatment groups to placebo was statistically significant ($p < 0.0001$). The data indicate that the odds of being a 12-week CSBM Overall Responder are approximately 7 times higher in patients on linaclotide than in patients on placebo.

There did not appear to be marked benefit of using the higher dose of linaclotide (300 μg) for the primary efficacy endpoint. Additional analyses to support primary endpoint show that the proportion of patients who were CSBM Weekly Responders (patients who had ≥ 3 CSBMs and a change from baseline of ≥ 1 during the particular week) was greater with each dose of linaclotide than with placebo. Secondary endpoints demonstrated similar results [superiority of both linaclotide doses over placebo] without a trend for dose-response.

Table 4: Efficacy outcomes during the Phase 3 trial in CC

Efficacy parameter	Placebo	150 μg Linaclotide	300 μg Linaclotide
Primary endpoint			
CSBM overall responder	3.3 %	21.2 %	19.4 %
Secondary endpoints (changes from baseline)			
CSBM frequency	0.453	1.935	2.042
SBM frequency	1.075	3.034	2.982
Stool consistency	0.576	1.851	1.838
Severity of straining	-0.512	-1.119	-1.150
Abdominal discomfort	-0.303	-0.478	-0.435
Bloating	-0.223	-0.464	-0.373
Constipation severity	-0.271	-0.897	-0.810
For all endpoints, both linaclotide doses produced statistically significant differences relative to placebo; $p < 0.0001$			

Figure 4 and 5: Dose-response trends for efficacy during Phase 3 trial MD103-303 in CC



LIN-MD-01 [Phase 3 in CC]: This was a Phase III, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Trial of Linaclotide Administered Orally for 12 Weeks in Patients With Chronic Constipation. A total of 633 patients were randomized, all of whom were included in the Safety Population; 630 of these patients were included in the Intent-to-Treat Population. Patients were randomized 1:1:1 to placebo: 145µg: 290 µg linaclotide. Study drug was administered once daily at least 30 minutes prior to breakfast. The primary efficacy parameter was 12-week CSBM overall responders. Numerous secondary and exploratory endpoints were also assessed.

Primary and secondary efficacy outcomes are summarized in the table below. In this Phase 3 trial, both linaclotide doses demonstrated statistically significant improvement relative to placebo for all primary and key secondary endpoints. In addition, a trend for dose-response was noted for the two doses of linaclotide evaluated in this trial, although the trial was not powered to demonstrate statistical significance of differences between linaclotide dose groups.

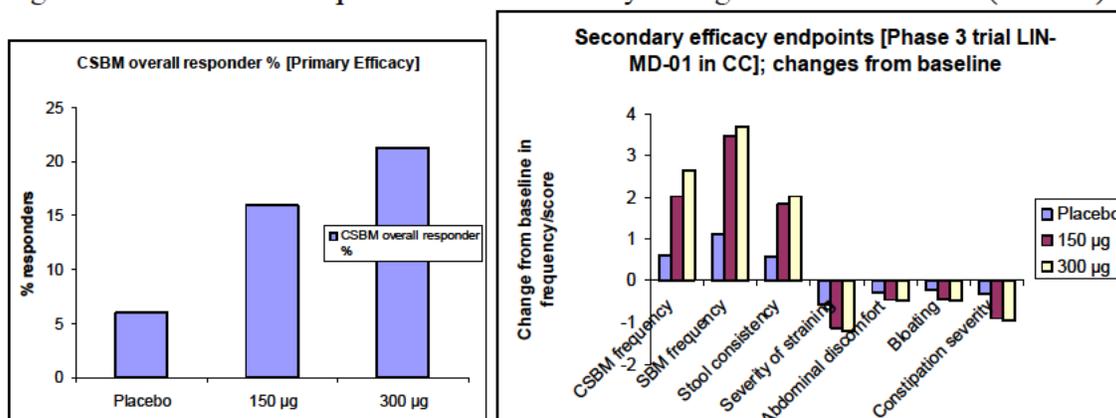
For the primary efficacy endpoint, patients treated with placebo had a responder rate of 6.0% compared with 16.0% for linaclotide 150 µg/day patients, and 21.3% for linaclotide 300 µg/day patients. The placebo responder rate was greater compared to the earlier Phase 3 trial in CC. These data indicate that the odds of linaclotide patients being 12-week CSBM overall responders are approximately 3 to 4 times those of patients receiving placebo. The increases relative to placebo were significant for both linaclotide treatment groups. Weekly data for CSBM responders as well as the data for various secondary endpoints demonstrated similar findings i.e. statistically superior benefit of linaclotide doses over placebo and a trend for greater efficacy with the higher dose of linaclotide evaluated.

Table 5: Efficacy outcomes during the Phase 3 trial LIN-MD-01 in CC

Efficacy parameter	Placebo N = 215	150 µg Linaclotide N = 213	300 µg Linaclotide N = 202
Primary endpoint			
CSBM overall responder	6.0 %	16 %	21.3 %
Secondary endpoints (Changes from baseline)			
CSBM frequency	0.614	2.011	2.653
SBM frequency	1.113	3.446	3.675

Stool consistency	0.572	1.823	2.009
Severity of straining	-0.554	-1.141	-1.208
Abdominal discomfort	-0.271	-0.455	-0.485
Bloating	-0.224	-0.432	-0.485
Constipation severity	-0.306	-0.908	-0.954
Linaclotide doses are significantly different relative to placebo; p < 0.0001 (all endpoints)			

Figures 5 and 6: Dose response trends in efficacy during LIN-MD-01 in CC (Phase 3)



Efficacy and dose-response conclusions from the Phase 2b and Phase 3 trials in CC:

- Linaclotide demonstrated therapeutic efficacy in relieving constipation as demonstrated by improvements over baseline in the various primary and secondary efficacy endpoints such as CSBM responder rates, changes from baseline in CSBM/SBM frequencies, BSFS stool consistency scores, straining scores, abdominal pain/discomfort, bloating scores, constipation severity, relief scores and PAC-QOL scores.
- Statistically significant changes from baseline relative to placebo were noted for most primary and secondary endpoints at all doses evaluated in these studies. Statistical significance was noted in the weekly response outcomes relative to placebo and the effect was generally achieved within the first week of dosing and sustained throughout the double-blind treatment period. Withdrawal of linaclotide did not appear to result in worsening (rebound) of baseline symptoms during the 2 to 4 week randomized withdrawal periods investigated.
- Dose-related trends for efficacy were noted for several endpoints over the dose-range investigated in the Phase 2b study (75 – 600 µg) and during the Phase 3 trial LIN-MD-01 that evaluated two doses of linaclotide (150 and 300 µg). No trend for dose-response was noted for these two doses in the phase 3 trial MCP-103-303 in which the higher dose of linaclotide provided no additional benefit over the lower dose.

Dose-response information in the IBS-C population:

MCP-103-202 (Phase 2b in IBS-C): This was a multicenter, randomized, double-blind, parallel-group, placebo-controlled, dose-range-finding oral dose study of 75, 150, 300,

and 600 µg linaclotide administered to patients with IBS-C. Approximately 80 patients were randomized per group. Patients were to self-administer once daily doses of study drug for 12 weeks. Study drug was to be administered at approximately the same time each day, with water, ≥ 30 minutes before the first meal of the day. The primary efficacy endpoint in this study was the change from Pretreatment Period (baseline) in weekly normalized Complete Spontaneous Bowel Movement (CSBM) Rate during the 12-week Treatment Period.

Results: The table below summarizes the change from baseline in bowel habits and IBS-C symptoms in the ITT population. Please refer to the individual study review in the appendix for complete details:

Table 6: Efficacy outcomes during the Phase 2b trial in IBS-C patients

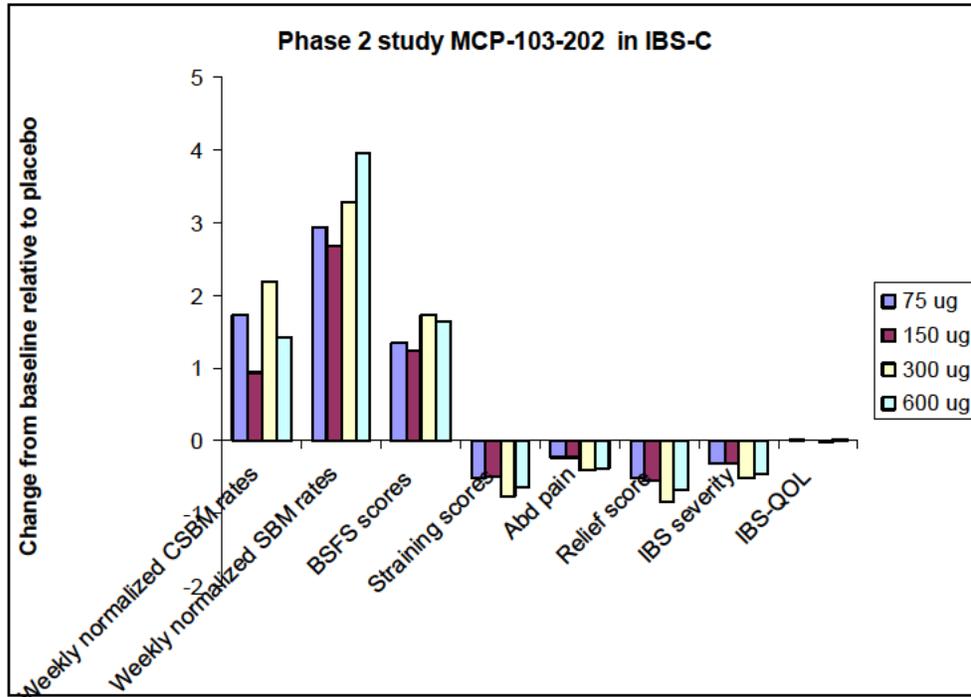
Endpoint ^a	Placebo (n=85)	Linaclotide			
		75 µg (n=79)	150 µg (n=82)	300 µg (n=84)	600 µg (n=89)
CSBM Frequency ^b (CSBM/week)	1.01	2.90**	2.49*	3.61***	2.68**
CSBM 75% Responder ^c	11.8%	25.3%*	19.5%	32.1%**	23.6%*
SBM Frequency ^b (SBM/week)	1.68	4.62***	4.36***	4.97***	5.64***
SBM 75% Responder ^c	29.4%	54.4%*	39.0%	65.5%***	52.8%*
Stool Consistency (7-point ordinal BSFS ^d)	0.56	1.91***	1.80***	2.28***	2.20***
Straining ^e (5-point ordinal scale)	-0.71	-1.23***	-1.20***	-1.48***	-1.35***
Abdominal Pain ^f (5-point ordinal scale)	-0.49	-0.71*	-0.71*	-0.90***	-0.86**
Abdominal Discomfort ^f (5-point ordinal scale)	-0.45	-0.65	-0.68*	-0.90***	-0.81**
Bloating ^f (5-point ordinal scale)	-0.38	-0.64*	-0.59	-0.88***	-0.75**
Degree of Relief of IBS Symptoms ^g (7-point balanced scale)	-0.81	-1.33**	-1.37**	-1.66***	-1.49***
Adequate Relief IBS Symptoms 50% Responder (≥50% of 12 wks)	29.4%	50.6%*	51.2%*	67.9%***	62.9%***
Adequate Relief IBS Symptoms 75% Responder (≥75% of 12 wks)	22.4%	38.0%*	32.9%	51.2%**	42.7%*
Average IBS Severity ^h (5-point ordinal scale)	-0.56	-0.87*	-0.88*	-1.08**	-1.02**
Constipation Severity ^h (5-point ordinal scale)	-0.65	-1.09**	-1.04*	-1.42***	-1.23***

n-values: * ≤ 0.05. ** < 0.001. *** < 0.0001

Most changes in efficacy endpoints were statistically significant relative to placebo, especially for the two highest doses evaluated. In this study, the 300 µg dose of linaclotide consistently provided greater response for the IBS-C endpoints, compared to the lower doses (75, 150 µg) or even the highest dose (600 µg) evaluated in this study. For the primary endpoint (CSBM rates) and two key secondary endpoints (CSBM/SBM 75 % responders), the 75 µg dose provided better outcomes compared to the 150 µg and 600 µg doses of linaclotide. For most other secondary endpoints, the efficacy findings were comparable for the 75 and 150 µg doses but still numerically smaller compared to

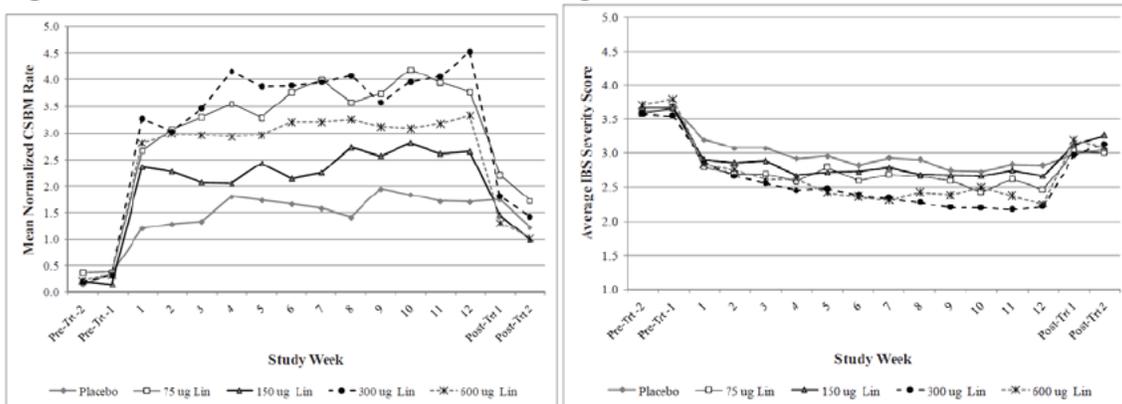
either the 300 or 600 µg. Thus a clear dose-response for efficacy endpoints was not noted in this Phase 2b trial for linaclotide in IBS-C.

Figure 7: Dose-response trends in efficacy during phase 2b trial in IBS-C



Onset and duration of effects: Improvement over baseline in the various primary and secondary endpoints was seen as early as week 1 and effects were sustained throughout the 12 week treatment period. Following cessation of therapy, values returned toward baseline without worsening (rebound) of symptoms during the investigated period.

Figure 8: Onset and duration of effects during Phase 2b trial in IBS-C



Efficacy and dose-response conclusions (Phase 2b in IBS-C):

- Linaclotide demonstrated therapeutic efficacy in relieving constipation in IBS-C as demonstrated by improvements over baseline in the primary efficacy endpoint

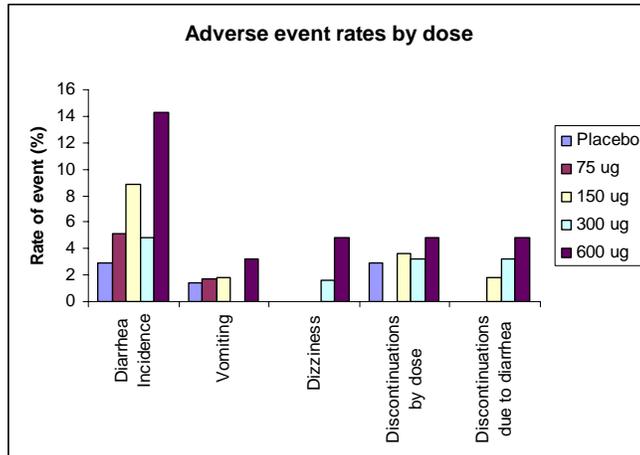
- (weekly normalized complete spontaneous bowel movement rates) and various other secondary and exploratory endpoints such as SBM rates, SBM/CSBM 75 % responder rates, changes in BSFS stool consistency scores, straining scores, abdominal pain/discomfort, bloating scores, constipation severity, and degree of relief of IBS-C scores.
- Statistically significant changes from baseline relative to placebo were noted for most primary and secondary endpoints at all doses evaluated in this study.
 - Dose-related trends for efficacy were not noted in this study (75 µg, 150 µg, 300 µg and 600 µg). The 300 µg dose provided greatest benefit compared to the two lower doses and even the highest dose evaluated, with the exception of the weekly SBM rates.
 - Only one dose (290 µg) was evaluated relative to placebo in the Phase 3 trial for IBS-C. Hence, no dose-response information is available from Phase 3 studies. Please refer to the Medical Officer's review for additional information in this regard.

2.2.4.2 What are the characteristics of the dose-response relationships for safety? If relevant, indicate the time to the onset and offset of the undesirable pharmacological response or clinical endpoint.

MCP-103-201 [Phase 2b in CC]: Safety Information

- Treatment-emergent adverse events (TEAEs) were reported by 31.9 %, 35.6 %, 32.1 %, 29 %, and 38.1 % of patients in the placebo, 75, 150, 300 and 600 µg groups, respectively.
- TEAEs leading to discontinuation were noted in 2.9 %, 0 %, 3.6 %, 3.2 %, and 4.8 % of patients receiving the placebo, 75, 150, 300 and 600 µg doses.
- Incidence of diarrhea varied with linaclotide dose with the lowest incidence (4.8%) occurring in the 300 µg dose group and the highest incidence (14.3%) occurring in the 600 µg dose group. Dizziness was not reported in any patient who experienced diarrhea as a TEAE.
- There was a dose-related increase in the number of patients who discontinued study drug due to diarrhea [0 %, 1.8 %, 3.2 % and 4.8 % at 75, 150, 300 and 600 µg doses, respectively].

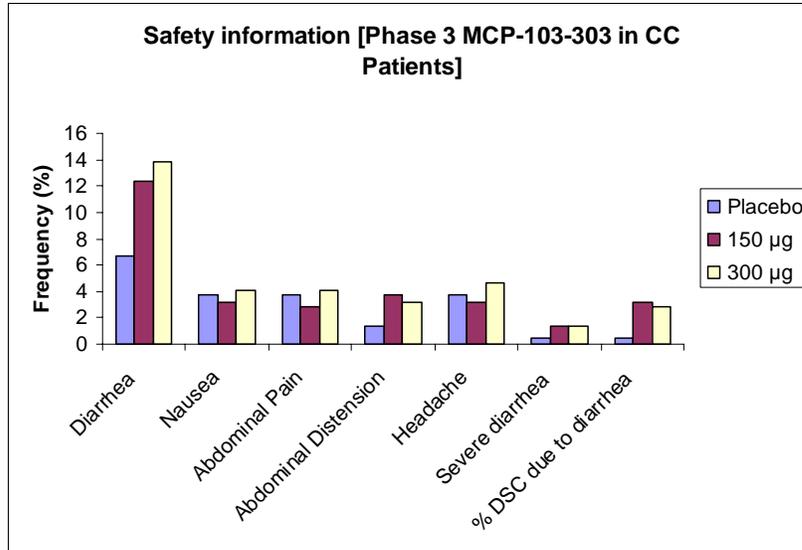
Figure 9: Dose-response trends for safety during phase 2b trial in CC



MCP-103-303 [Phase 3 in CC]: Safety Information

- Patients experiencing at least 1 TEAE were somewhat higher in the linaclotide groups, with no dose-related trends [50.2 %, 56.2 % and 54.8 % for placebo, linaclotide 145 µg and 290 µg respectively].
- % of patients experiencing serious AEs (SAEs) was 2.4 %, 1.4 % and 1.8 % in the above groups.
- Discontinuations due to TEAE were higher in the two linaclotide groups compared to placebo [3.8 %, 5.1 % and 5.1 % respectively for placebo, 145 µg and 290 µg]. But there was no dose-related trend in discontinuation rates.
- Diarrhea occurred at a greater (twice) frequency in the linaclotide groups compared to placebo. The frequency of diarrhea was slightly higher in the 290 µg dose group [6.7 %, 12.4 % and 13.8 %].
- Three patients (1.4%) in each linaclotide dose group had severe diarrhea, and 1 patient (0.5%) in the placebo group had severe diarrhea.
- Discontinuation rates for diarrhea were 0.5 %, 3.2 % and 2.8 % for placebo, 145 µg and 290 µg doses respectively.
- In general, incidence of diarrhea, nausea, abdominal pain and headache were highest in the 290 µg dose group, although the difference between the two groups was not marked for these AEs.

Figure 10: Dose-response trends for safety AEs in Phase 3 trial MCP-103-303 in CC



Timing of onset of diarrhea: The majority of linaclotide patients with diarrhea reported that they experienced the onset of diarrhea during the first 2 weeks of dosing. Four of 27 (14.8%) and 5 of 30 (16.7%) 145 µg and 290 µg linaclotide-treated patients, respectively reported diarrhea onset on Day 1, compared to 0 placebo patients. After Week 5, the incidence of first onset of diarrhea was comparable in patients treated with linaclotide 145 µg (5/217, 2.3%), linaclotide 290 µg (5/217, 2.3%) and placebo (4/209, 1.9%).

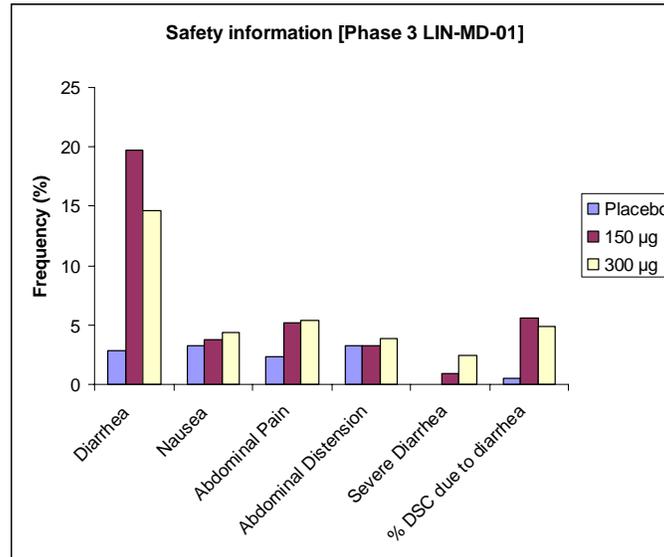
LIN-MD-01 [Phase 3 in CC]: Safety Information

- While overall AEs were higher in the linaclotide groups compared to placebo (60.8 % vs. 54 %), there was no trend for dose-response for overall TEAE frequency with increasing dose [54 %, 64.8 % and 56.6 % for placebo, low dose and high dose linaclotide respectively].
- Serious AEs (SAEs) were 1.9 %, 1.4 % and 3.4 % respectively, for these cohorts.
- The % discontinuation due to AEs was 4.7 %, 9.9 % and 9.8 % in the placebo, low dose and high dose groups, respectively.
- The incidence of diarrhea was markedly higher in the linaclotide group (17.2 %) compared to placebo (2.8 %). Again, there was no dose-response trend for diarrhea [2.8 %, 19.7 % and 14.6 % for placebo, low dose and high dose linaclotide].
- % of patients whose diarrhea was reported as ‘severe’ were more frequent in the higher linaclotide dose group compared to placebo or lower dose [0 %, 0.9 % and 2.4 % with placebo, 145 µg and 290 µg doses]. The highest dose also had two instances of defecation urgency and flatulence that were coded as severe, compared to none in the placebo or lower linaclotide dose.
- Of the total patients who discontinued due to AEs, the % of patients who discontinued due to diarrhea did not demonstrate dose-related trend [0.5 %, 5.6 % and 4.9 % in the placebo, low dose and high dose respectively].
- The time (mean ± SD) from the first dose of double-blind treatment to the first TEAE of diarrhea was 14.6 ± 19.7 days for the linaclotide patients compared with 29.8 ± 24.0 days for the placebo patients. Of the 72 linaclotide patients who had

TEAEs of diarrhea, 42 (58.3%) experienced their first episode in the first week of treatment.

- Abdominal pain [5.3 % vs. 2.3 % in placebo], nausea [4.1 % vs. 3.3 %] also were more frequent in the linaclotide group but no distinct dose response trends were noted.

Figure 11: Dose-response trends for safety in Phase 3 trial LIN-MD-01 in CC



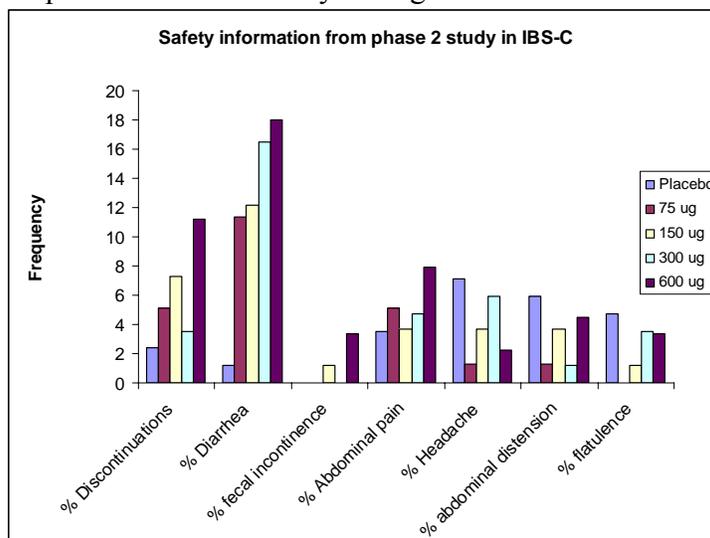
Safety conclusions in CC: While there is no overall increase in AE frequency at 300 µg compared to 150 µg linaclotide, the frequency of diarrhea may be more at this dose. In addition, diarrhea related discontinuations, as well as % severe cases of diarrhea were seen more frequently at the higher doses, though not consistently across CC studies.

Phase 2b study MCP-103-202 in IBS-C: Safety information

- Pre-treatment adverse event rates and severities were similar across the groups. With treatment, the increase in adverse events was obvious for all groups including placebo. The active groups had a higher incidence of TEAEs related to study drug, exhibiting a dose-related increase with linaclotide treatment [15.3 %, 16.5 %, 22 %, 25.9 % and 30.3 % with placebo, 75, 150, 300 and 600 µg doses].
- The TEAE rates leading to discontinuation were highest in the 600 µg group, and lowest in placebo.
- Diarrhea incidence was markedly higher in the four linaclotide treatment groups compared to placebo, with a dose-related increase in the incidence (1.2 % in placebo vs. 11.4, 12.2, 16.5 and 18 % in the 75, 150, 300 and 600 µg doses of linaclotide). The highest dose 600 µg also had the greatest incidence of fecal incontinence (3.4 %) and abdominal pain (7.9 %).
- The discontinuation rates due to diarrhea were roughly dose related, with incidence of 2.5 %, 4.9 %, 1.2 % and 6.7 % in the 75, 150, 300 and 600 µg dose groups. The other reasons for study drug discontinuation that exhibited dose related trends included abdominal pain, fecal incontinence and headache. In

addition, at the 600 µg dose drug was withheld temporarily due to diarrhea in three patients and fecal incontinence in two patients during this study.

Figure 12: Dose-response trends for safety during Phase 2b trial in IBS-C



Safety conclusions (phase 2b in IBS-C): The 600 µg exhibited the highest incidence of total and specific AEs, including discontinuation due to AEs. The % diarrhea, headache and abdominal pain were seen more frequently at the 300 µg dose compared to lower doses.

2.2.4.3 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-response, and are there any unresolved dosing or administration issues?

Chronic Constipation: In the phase 2b study MCP-103-201, a dose-response trend was noted for several of the efficacy endpoints. Numerically larger improvements were noted with the three higher doses of linaclotide compared to the lowest dose evaluated (75 µg) for various endpoints. These included the change in weekly SBM rate, weekly CSBM rate, 75 % responder rate (CSBM), stool consistency scores, and straining scores. A dose-response trend for the incidence of diarrhea and higher study discontinuation rates due to this AE were noted, particularly at the 600 µg dose level. Thus the sponsor has continued the 150 and 300 µg doses of linaclotide into phase 3 program for CC. Phase 2b findings support the doses advanced into phase 3 for CC.

In the two Phase 3 studies for CC, MCP-103-303 and LIN-MD-01, the two linaclotide doses demonstrated statistically significant improvement over placebo for several primary and secondary endpoints. Only one trial demonstrated a trend for increasing efficacy with increasing dose (LIN-MD-01), while in the Phase 3 trial MCP-103-303 results suggested no additional benefit of the higher dose. The studies were not powered to demonstrate difference between the two doses. Safety information from Phase 3 trials in CC suggest that there was no marked trend for overall increase in AEs with increasing

dose of the drug. However, the incidence of diarrhea may be greater with the higher dose of linaclotide, including the incidence of severe grade of diarrhea.

(b) (4)

One option for consideration could be to initiate treatment at a lower starting dose (145 µg) in all CC patients, with an option to increase dose to 290 µg if the lower dose is well tolerated and a need for greater efficacy has been identified. This needs to be further discussed with the clinical review team.

IBS-C: In the 12 week phase 2b study MCP-103-202, dose-response for efficacy was noted for the various endpoints evaluated. The 300 µg dose, followed by the 600 µg dose were found to be most-effective. The 150 µg dose often provided efficacy outcomes that were numerically inferior to that of the other doses including the lowest dose evaluated. As dose-related increase in drug-related TEAEs, particularly diarrhea, fecal incontinence and abdominal pain as well as discontinuation rates due to diarrhea were noted, particularly at the 600 µg dose, this dose was not progressed into the Phase 3 trials of IBS-C. Sponsor has continued only the 300 µg dose (or 290 µg per revised dose strength expression) of linaclotide into phase 3 based on this study. The 75 µg dose demonstrated efficacy (next to the 300 µg dose) for one or more primary and secondary outcomes but was not progressed into the Phase 3 trial for IBS-C.

The proposed labeling recommends a dose of 290 µg in IBS-C. An option for a lower starting dose or dose reduction in case of AEs has not been suggested by the sponsor for IBS-C, nor has it been evaluated in the double-blind primary efficacy phases of the pivotal IBS-C trials. However, the Clinical Reviewer has information from the long-term phases of the IBS-C trials where dose reductions were allowed. (b) (4)

2.2.4.4 Does this drug prolong the QT or QTc interval?

Because of linaclotide's limited systemic bioavailability, a thorough QT study was not performed. Based on recommendations received from FDA during development, triplicate ECGs were obtained on a cohort of patients prior to dosing and at post-treatment time points following dosing at steady-state and when clinically indicated, to increase the robustness of the ECG data collected in Phase 3 CC and IBS-C trials. Medical reviewer Dr. Lara Dimick has noted absence of QT effects based on this data. Please refer to the Medical Officers' review for an interpretation of the ECG information in Phase 3 trials of CC and IBS-C.

2.2.5 Pharmacokinetics

2.2.5.1 What are the single dose and multiple dose PK parameters of the drug and its major metabolite?

Linaclotide and its primary (active) metabolite (formed by the loss of terminal tyrosine) were not detected in plasma of healthy volunteers (Phase 1) or patients (Phase 3 sparse sampling) using validated LC-MS/MS analytical methods. This indicates insignificant absorption of the peptide or its major metabolite following oral route of administration. PK studies are summarized briefly:

MCP-103-001: This was a Single Oral, Ascending Dose, Placebo-Controlled Study in Healthy Males and Postmenopausal Females. The objectives were to evaluate PK, PD, safety and tolerability of linaclotide. Study evaluated under fasting conditions 30, 100, 300, 1000, and 3000 µg (equivalent to doses of 29, 97, 290, 966 and 2897 µg of peptide content per revised method of representing dose) of linaclotide conducted in 30 normal healthy male and postmenopausal female subjects. Blood samples for determination of plasma concentrations of drug and its metabolite, MM-419447, were collected at the following time points: 0 hour (prior to dose) and at 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12, 24, 36, and 48 hours post-dose. Plasma concentrations were analyzed by a validated LC-MS/MS method with a 3 ng/mL lower limit of quantitation.

PK findings: Levels of MD-1100 Acetate and its metabolite were below the limit of detection (3 ng/mL) at all dose levels; thus, no PK analyses could be performed.

MCP-103-002: This was a 7-Day, Oral, Multiple-Ascending Dose, Placebo-Controlled Study of linaclotide in Healthy Subjects for evaluating PK, PD, safety and tolerability following multiple daily dosing of linaclotide under fasted conditions. 48 subjects were enrolled (16 per dose group; 8 on drug and 4 on placebo). Subjects were randomized to receive either linaclotide (30, 100, 300, 1000 µg) or placebo. Regular samples were collected for PK on day 1 and day 7; Plasma samples were assayed using validated LC-MS/MS method with a LOQ of 3 ng/mL. Appropriate calibration curve and QC data during the analytical run batches suggests acceptability of assay method used.

PK findings: Plasma samples assayed for drug and metabolite concentrations after 7 days of daily dosing with various doses of linaclotide did not reveal measurable concentrations of either moiety in any of the individuals.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

In the two Phase 3 trials in CC, sparse sampling for PK was included. Plasma concentrations were assessed for linaclotide and its active metabolite using a revised validated LC-MS/MS method with a LLOQ of 0.2 ng/mL for linaclotide and 2 ng/mL for the active metabolite. Plasma samples in both trials did not reveal measurable concentrations of either moiety.

Of the subset of PK patients in the two similarly conducted IBS-C Phase 3 trials,

165 (18 males and 147 females) received placebo and 162 (11 men and 151 women) received 290 µg of linaclotide. Approximately 2 hours following the initial dose of linaclotide, two (1%) of the 162 linaclotide patients had measurable concentrations of linaclotide, which were just above the LLOQ (0.2 ng/mL). No samples collected after four weeks of once-daily dosing showed linaclotide concentrations greater than the LLOQ, and none of the plasma samples collected during the study had measurable concentrations of MM-419447 (LLOQ = 2 ng/mL) at either time point.

2.2.5.3 What are the characteristics of drug absorption?

Pharmacokinetic studies did not reveal systemic exposure potential for linaclotide or its active metabolite.

In vitro permeability data across Caco-2 cell layers was inconclusive, as the permeability of linaclotide tested at three different concentrations, in two separate in vitro investigations provided different results, although similar test systems were used and comparable performance of the permeability standards was noted.

In the first investigation, in vitro permeability of linaclotide across Caco-2 monolayers was assessed at 0.24, 2.4 and 24 µg/mL concentrations [assumes 0.1, 1, 10 fold concentrations achieved locally following a dose of 600 µg taken with 250 mL water]. Apical to basolateral (A→B) and basolateral to apical (B→A) permeability of linaclotide were assessed. In addition, labetalol was evaluated at 100 µg/mL as a high permeability marker, while ¹⁴C-mannitol was used as a low permeability marker.

In this study, linaclotide permeability across Caco-2 monolayers was lower than that of the accepted low permeability marker drug mannitol. Permeability couldn't be assessed at the two lower concentrations citing that the signal is below limit of detection.

Table 7a: Permeability results for linaclotide across Caco-2 monolayers

Compound	Concentration (µg/mL)	$P_{app} A \rightarrow B$ (n=6) ($\times 10^{-7}$ cm/s)	$P_{app} B \rightarrow A$ (n=6) ($\times 10^{-7}$ cm/s)	Mass Balance (%)
Linaclotide	24	0.32 ± 0.58	1.10 ± 2.07	93.8 (A → B) 95.2 (B → A)
Linaclotide	2.4	ND ¹	ND ¹	NA ¹
Linaclotide	0.24	ND ¹	ND ¹	NA ¹
Labetalol	100	263.2 ± 8.2	294.5 ± 10.9	82.2 (A → B) 96.6 (B → A)
Mannitol	0.36	6.41 ± 0.71	4.46 ± 0.43	99.1 (A → B) 102.5 (B → A)

¹ ND: The signal is below LOD; NA: not applicable

In a separate study evaluating P-gp substrate potential for linaclotide, permeability across AP to BL and BL to AP directions was assessed in Caco-2 cell system at 0.12, 1.2 and 12 µg/mL concentrations [assuming 0.1, 1, 10 fold the concentrations seen after maximum therapeutic dose of 300 µg dissolved in 250 mL water].

In this study, detectable permeability was noted in the apical to basolateral (AP to BL) directions for all three concentrations tested, with the highest values noted for the lowest concentration evaluated (144×10^{-7} at 0.12 $\mu\text{g/mL}$).

Table 7b: Permeability findings for linaclotide across Caco-2 cell layers

Compound	Concentration ($\mu\text{g/mL}$)	P_{app} AP-to-BL (n=6) ($\times 10^{-7}$ cm/s)	P_{app} BL-to-AP (n=6) ($\times 10^{-7}$ cm/s)	Mass Balance (%)
Linaclotide	0.12	144.2 ± 12.0	1.88 ± 4.61	118.1 (AP-to-BL) 105.6 (BL-to-AP)
Linaclotide	1.2	13.4 ± 1.5	ND ¹	98.0 (AP-to-BL) ND ¹ (BL-to-AP)
Linaclotide	12	1.70 ± 1.05	0.23 ± 0.14	87.3 (AP-to-BL) 98.3 (BL-to-AP)
Linaclotide (+ 25 μM CsA)	12	1.79 ± 1.09	4.47 ± 7.74	89.3 (AP-to-BL) 98.2 (BL-to-AP)
Labetalol	100	334.1 ± 8.7	445.5 ± 7.0	87.5 (AP-to-BL) 95.4 (BL-to-AP)
Mannitol	0.36	6.54 ± 0.65	8.75 ± 7.56	94.7 (AP-to-BL) 102.1 (BL-to-AP)

¹ ND: The signal is below LOD

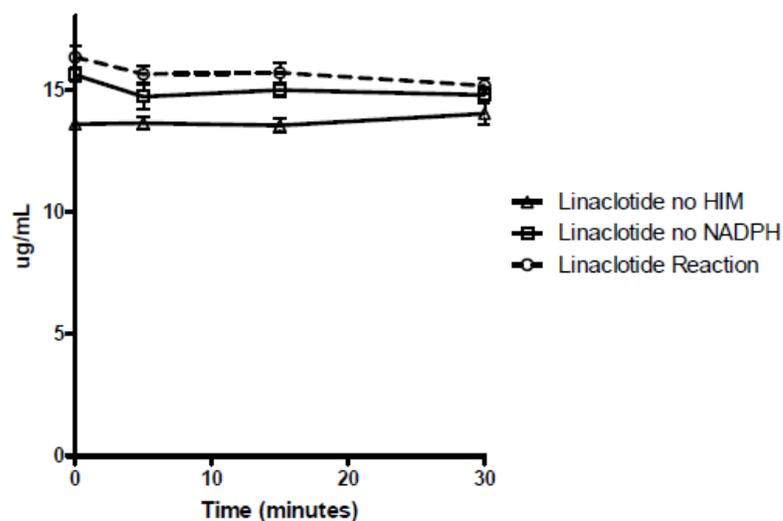
Results from the Caco-2 permeability studies are inconclusive with regard to permeability characteristics of linaclotide as two separate studies provided different results. Nevertheless, systemic concentrations of linaclotide after oral administration are low to negligible.

2.2.5.4 What are the characteristics of drug metabolism?

Study MDP-103-056 evaluated the metabolism potential of linaclotide in presence of human intestinal microsomes. In this study Linaclotide was incubated at 12 $\mu\text{g/mL}$ (7.9 μM) with 1 mg/mL of mixed gender human intestinal microsomes. Testosterone (10 μM) was used as a positive control.

For the active control agent, testosterone (10 μM) the time-dependent disappearance of testosterone and formation of its phase I metabolite, 6 β -hydroxytestosterone were noted in incubations of human intestinal microsomes, in presence of NADPH. For linaclotide, under the above conditions no metabolism was noted in human intestinal microsomes (HIM) with or without NADPH.

Figure 13: Absence of linaclotide metabolism in human intestinal microsomes

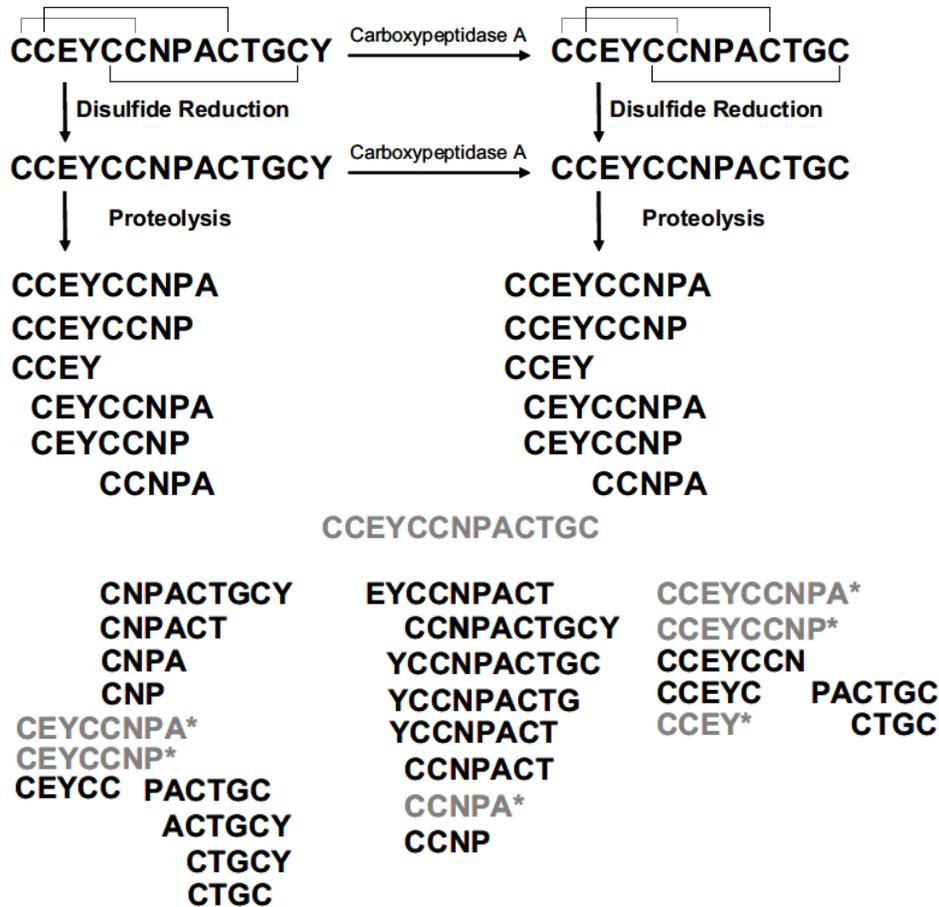


Data suggests that linacotide is not metabolized in presence of human intestinal microsomes and does not appear to be a substrate for CYP450 enzymes contained in these microsomes, in contrast to the active control testosterone. This was a non-GLP study.

Study MDP-103-041 evaluated the metabolism and digestion of linacotide in the luminal contents of the human intestine. Intestinal fluids obtained from cadaver donors within 1-5 hours of post-mortem were used in this assessment. Total thiol, activity levels of glutathione reductase and activity levels of glutaredoxin in these fluids were measured to determine whether the fluids can bring about the reduction of the three disulfide bridges, which is the first step in the breakdown of linacotide, before it can be acted upon by proteases. Linacotide was then incubated in the intestinal fluid for various times to determine using LC-MS/MS whether the peptide disappears with time, and whether the active metabolite (MM-419447) was formed over time. Dilutions of intestinal fluid were also used to slow down the rate of digestion of the peptide so that smaller fragments of the peptide if any can be isolated and characterized.

Fluid from the human intestinal lumen contained high thiol levels (3-8 mM) as well as glutathione reductase and glutaredoxin activities necessary for reducing the disulfide bridges. The initial concentration of linacotide was 100 $\mu\text{g/mL}$ (65,500 nM). Data suggests that linacotide when incubated in intestinal fluid almost completely disappeared within 24 hours. The active metabolite, formed by the loss of terminal tyrosine was formed over time at a maximum level of ~ 1 ng/mL reached within 2 hours after which it degraded. The average estimated half-life of linacotide in the human intestinal fluid from the three donors was 1.89 (0.29) hours. Incubation of linacotide in human intestinal fluid, which was diluted in buffer to slow the rate of digestion, resulted in the partial digestion of linacotide to small peptide fragments. These peptides were identified by LC/TOF-MS and ranged from 4 – 9 amino acids.

Figure 14: The purported metabolic pathway of linacotide



* Fragments detected after digestion with rat or human intestinal fluid

Findings from this study suggest that human intestinal fluid is capable of breaking down linaclotide into smaller peptide fragments. The intestinal fluids contain the necessary reducing agents to mediate the first step of the process, which is the cleavage of peptide-stabilizing disulfide bridges, following which the peptide is broken down into fragments by the action of proteases.

Coupled with findings from earlier study which shows that linaclotide is not metabolized in intestinal microsomes, thus suggesting the absence of a CYP450-mediated metabolism, information to date suggests that the primary pathway of metabolism of linaclotide is local within the intestinal fluid into smaller peptides.

2.2.5.5 What are the characteristics of drug excretion?

In vitro studies suggest that linaclotide is broken down locally in the intestinal fluids by the action of proteases into inactive peptide fragments and naturally occurring amino acids, which are available for absorption and incorporation into endogenous proteins.

In a non-GLP investigation conducted as part of the food-effect PD study, 24 hour stool collections were obtained to assess linaclotide and active metabolite concentrations (non-GLP stool assay with a LLOQ of 30 ng/g). Linaclotide was not present in the stools of subjects who fasted prior to receiving 300 mcg of linaclotide for 7 days, nor was it

present after receiving an additional 3000 mcg dose. All of the active peptide recovered in any of the stool samples was in the form of the primary metabolite, MM-419447, and ranged from 1.0 mcg to 47.7 mcg in seven subjects receiving daily doses of 300 mcg for seven days. The amount of MM-419447 recovered in the stool from eight fasted subjects receiving an additional single 3000 mcg dose on the eighth day ranged from 0 mcg to 315.9 mcg. The mean active peptide recoveries in fasted subjects were 4.89% (300 mcg for seven days) and 3.34% (300 mcg for seven days plus an additional 3000 mcg on Day 8), and the median values were 2.53% (300 mcg) and 2.78% (3000 mcg).

Subjects who were fed a high-fat meal prior to receiving 300 mcg doses of linaclotide for seven days did not have any linaclotide detectable in the stool; however, three of nine subjects had measurable quantities of linaclotide in their stools (4.9-23.3 mcg) following an additional 3000 mcg dose. Six of the nine fed subjects receiving 300 mcg doses for seven days had detectable levels of MM-419447 in their stools (2.0-25.1 mcg). The same number had detectable amounts of MM-419447 (19.1-515.3 mcg) following an additional 3000 mcg dose on the eighth day of dosing. The mean active peptide recoveries in fed subjects were 3.11% (300 mcg for seven days) and 3.80% (300 mcg for seven days plus an additional 3000 mcg on the eighth day), and the median active peptide recovery values were 2.04% (300 mcg) and 2.21% (3000 mcg).

2.2.6. Drug-drug interactions

Drug-drug interactions with oral linaclotide at the systemic level are in general unlikely to be a concern due to absence of systemic exposure of the peptide drug or its active metabolite following oral administration of clinically relevant doses. In addition, drug was found not to be a substrate of CYP450 enzymes and transporters and therefore is unlikely to be affected by inhibitors or inducers of these systems. Nevertheless to address the potential for linaclotide in drug-drug interactions with co-administered drugs via actions on CYP450 enzymes or transporters, sponsor has conducted several in vitro investigations incorporating gut level concentrations. Studies in general rule out potential for an interaction at local or systemic level following oral dosing of the drug.

CYP inhibitory potential of linaclotide and its main metabolite in vitro:

Study AML/21: In this study the extent of any inhibitory interactions of linaclotide and MM-419447 upon human hepatic CYP450 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 enzymes was determined. Linaclotide and MM-419447 were incubated separately at final incubation concentrations of 0, 0.05, 0.5, 5, 15 and 50 ng/mL in triplicate, with pooled human liver microsomes in the presence of CYP450-selective substrates. Although neither linaclotide nor its metabolite were detected in human plasma at the expected therapeutic doses, in a food effect study (Study number: MCP-103-103) following administration of a nominal 2897 µg dose (approximately 10 x the maximum therapeutic dose of 290 µg), linaclotide was barely detectable in plasma in 2 of 18 subjects, and MM-419447 was not detectable. The mean plasma C_{max} of linaclotide found in these two subjects was 474 pg/mL. Therefore, concentrations of linaclotide and MM-419447 to be tested in this study encompassed 0 – 50 ng/mL (approximately 100 x C_{max} at 10-fold therapeutic dose).

Table 15: Enzyme inhibition potential of linaclotide

Linaclotide concentrations (ng/mL)	% Inhibition of metabolite formation (mean of 3 incubations)								
	CYP 1A2	CYP 2B6	CYP 2C8	CYP 2C9	CYP 2C19	CYP 2D6	CYP 2E1	CYP 3A4 Testosterone	CYP 3A4 Mdz
0	-	-	-	-	-	-	-	-	-
0.05	0	17.1	0	0	1.54	4.06	3.77	4.09	0
0.5	0	7.18	0.31	2.88	0	2.58	2.12	0	0
5	0	5.33	2.78	8.33	1.93	7.05	3.06	0	0
15	0.87	0.45	4.27	16.2	2.06	5.69	4.36	0	0
50	5.69	4.52	0	12	0.25	5.31	5.06	0	0
<i>IC50 (ng/mL)</i>	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Positive Control Inhibitor	86.7	71.2	92.5	87.3	86.6	90.8	59.2	97.9	97.9

Table 16: Enzyme inhibition potential of active metabolite MM-419447

Linaclotide concentrations (ng/mL)	% Inhibition of metabolite formation (mean of 3 incubations)								
	CYP 1A2	CYP 2B6	CYP 2C8	CYP 2C9	CYP 2C19	CYP 2D6	CYP 2E1	CYP 3A4 Testosterone	CYP 3A4 Mdz
0	-	-	-	-	-	-	-	-	-
0.05	2.38	10.2	6.26	1.71	12.8	3.36	0	10.8	4.80
0.5	1.36	3.70	0	0	10.3	0	2.49	0	6.24
5	3.74	6.96	0	0	10.1	0.26	5.35	0	2.16
15	5.36	8.40	0	0	17.7	3.21	0.62	0	4.56
50	8.67	13.2	2.88	20.5	0	6.11	3.61	0	8.15
<i>IC50 (ng/mL)</i>	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50	> 50
Positive Control Inhibitor	86.7	71.2	92.5	87.3	86.6	90.8	59.2	97.9	97.9

Compared to the zero concentration samples, linaclotide concentrations ranging from 0.05 to 50 ng/mL appeared to have a small inhibitory effect on various CYP isozymes tested. However, the values were substantially below that of the positive control inhibitors included in the assay. In addition, IC₅₀ values (concentration of drug or metabolite causing 50 % inhibition of probe substrate metabolism; used to estimate relevance of in vitro inhibition as related to systemic or local concentrations) could not be determined in the study as such inhibition was not noted within the concentrations evaluated. Although only modest, an increasing inhibition of metabolite formation with increasing concentrations was often noted for the active metabolite but not for linaclotide.

Based on the values noted in this study, it appears that a modest effect (> 10 % inhibition at any of the concentrations tested) of linaclotide was noted on CYP2B6 and CYP2C9 and an effect of MM-419447 was noted on CYP2B6, 2C9, 2C19 and 3A4 mediated metabolism. However, based on the knowledge that systemic concentrations of linaclotide are not noted in most individuals or are minimal following oral administration, it is safe to conclude that at clinically relevant doses, the resulting systemic exposures noted for linaclotide or its metabolite cannot be expected to cause clinically meaningful inhibition of the metabolism of co-administered drugs.

Note: The effect of drug on inhibition of CYP enzymes CYP2C9 and CYP3A4 at concentrations likely to be encountered in the local GI tract [I_{gut}] were investigated in study MDP-103-066-IAR-01.

Study MDP-103-066-IAR-01: The aim of this study was to assess the potential of linaclotide to inhibit the catalytic activity associated with the formation of metabolites produced by cytochromes 2C9, 3A4 (midazolam), and 3A4 (testosterone). CYP-specific probe substrates were incubated with pooled human intestinal microsomes in the presence and absence of standard inhibitors or linaclotide (0, 0.1625, 0.325, 0.65, 1.3, 2.6, and 5.2 $\mu\text{g}/\text{mL}$). In addition, linaclotide (at concentrations previously noted) was pre-incubated for 30 minutes with pooled human intestinal microsomes before the addition of the CYP-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. When inhibition reached significant levels (>50%), IC50 values for both direct and time-dependent inhibition were determined.

Tables 17 & 18: In vitro inhibitory potency of linaclotide (includes I_{gut} concentrations)

Direct Inhibition				Time-Dependent (MBI) Inhibition			
Linaclotide ($\mu\text{g}/\text{mL}$)	2C9	3A4 (1OHMDZ)	3A4 (6BT)	Linaclotide ($\mu\text{g}/\text{mL}$)	2C9	3A4 (1OHMDZ)	3A4 (6BT)
0	100	100	100	0	100	100	100
0.1625	105	106	108	0.1625	94.4	101	110
0.325	101	96.1	101	0.325	92.8	101	102
0.65	98.1	98.3	101	0.65	101	99.3	91.5
1.3	103	108	90.1	1.3	100	95.3	87.4
2.6	98.4	114	93.0	2.6	104	103	84.3
5.2	99.0	106	100	5.2	100	107	81.9
Positive Control	89.8	13.8	17.5	MBI Positive Control	43.5	4.4	7.4

Results suggest that positive control inhibitors for CYP3A4 affected activities as expected, confirming satisfactory incubation conditions for these assays. For the CYP2C9 inhibitor (positive control), 89.8 % of activity remained therefore the acceptability of the inhibition assay in this regard appears questionable. Linaclotide data suggests absence of significant inhibitory effects on 3A4 at concentrations encompassing local (I_{gut} ; 1.2 $\mu\text{g}/\text{mL}$) levels. Small decrease in CYP3A4 enzyme activity was noted when drug was pre-incubated for 30 minutes with human liver microsomes prior to addition of substrate testosterone. However, similar to other experiments involving direct or mechanism based inhibition, IC50 value could not be established in this study as it exceeded the maximum drug concentrations tested.

Overall data suggests that linaclotide doesn't cause direct or mechanism based inhibition of CYP3A4 at concentrations encompassing local (I_{gut}) levels.

Investigation of potential p-glycoprotein activity of linaclotide using caco-2 cell monolayers: P-gp substrate and/or inhibitory activity of linaclotide were investigated.

The bidirectional permeability of linaclotide was evaluated at concentrations of 0.12, 1.2 and 12 $\mu\text{g}/\text{mL}$ across the Caco-2 monocell layer. Permeability was also assessed at the highest concentration tested in presence of P-gp inhibitor cyclosporine. Labetalol was

included as the high permeability standard and mannitol as the low standard. At all concentrations of linaclotide, permeability values were lower than that of the high permeability marker.

The basolateral to apical (BL to AP) permeability values of linaclotide were lower than for AP to BL for all concentrations tested. This value paradoxically increased when the highest tested linaclotide concentration (12 µg/mL) was tested in presence of P-gp inhibitor drug cyclosporine.

Table 19: Caco-2 cell assay to evaluate P-gp substrate potential of linaclotide

Compound	Concentration (µg/mL)	P _{aap} AP-to-BL (n=6) (x10 ⁻⁷ cm/s)	P _{aap} BL-to-AP (n=6) (x10 ⁻⁷ cm/s)	Mass Balance (%)
Linaclotide	0.12	144.2 ± 12.0	1.88 ± 4.61	118.1 (AP-to-BL) 105.6 (BL-to-AP)
Linaclotide	1.2	13.4 ± 1.5	ND ¹	98.0 (AP-to-BL) ND ¹ (BL-to-AP)
Linaclotide	12	1.70 ± 1.05	0.23 ± 0.14	87.3 (AP-to-BL) 98.3 (BL-to-AP)
Linaclotide (+ 25 µM CsA)	12	1.79 ± 1.09	4.47 ± 7.74	89.3 (AP-to-BL) 98.2 (BL-to-AP)
Labetalol	100	334.1 ± 8.7	445.5 ± 7.0	87.5 (AP-to-BL) 95.4 (BL-to-AP)
Mannitol	0.36	6.54 ± 0.65	8.75 ± 7.56	94.7 (AP-to-BL) 102.1 (BL-to-AP)

¹ ND: The signal is below LOD

P-gp mediated efflux of digoxin was evidenced by differential permeability of digoxin across the Caco-2 monolayers, with higher values across the basolateral to apical direction (BL to AP). Thus the functional expression of P-gp has been adequately demonstrated in the cell line used in this study. The preferential permeability of digoxin across the BL to AP direction was inhibited significantly (from 11.3 to 1.3) when it was co-administered with cyclosporine, a known P-gp inhibitor. Addition of linaclotide to digoxin did not alter the preferential efflux of digoxin via P-gp as demonstrated by similar BL to AP/AP to BL permeability ratios in presence of 0.24, 2.4 and 24 µg/mL concentrations of linaclotide.

This information demonstrates that linaclotide does not inhibit P-gp mediated efflux of substrate drugs.

Table 20: Caco-2 cell assay to evaluate P-gp inhibitory potential for linaclotide

Treatment	Direction	$P_{app} \pm SD$ ($\times 10^6$ cm/s)	Ratio $\pm SD$ ($P_{app} \text{ BL-to-AP} / P_{app} \text{ AP-to-BL}$)	Mass Balance (%)
$[^3\text{H}]$ -Digoxin	AP-to-BL	2.8 ± 0.1	11.3 ± 0.6	106.3
	BL-to-AP	31.7 ± 1.2		104.1
$[^3\text{H}]$ -Digoxin + CsA (25 μM)	AP-to-BL	6.9 ± 0.4	1.3 ± 0.1	101.4
	BL-to-AP	9.3 ± 0.3		99.2
$[^3\text{H}]$ -Digoxin + Linaclotide (0.24 $\mu\text{g/mL}$)	AP-to-BL	3.4 ± 1.1	9.4 ± 3.0	105.5
	BL-to-AP	32.0 ± 0.5		107.5
$[^3\text{H}]$ -Digoxin + Linaclotide (2.4 $\mu\text{g/mL}$)	AP-to-BL	3.1 ± 0.8	9.9 ± 2.5	99.8
	BL-to-AP	30.6 ± 0.3		106.0
$[^3\text{H}]$ -Digoxin + Linaclotide (24 $\mu\text{g/mL}$)	AP-to-BL	3.5 ± 0.9	8.7 ± 2.3	102.7
	BL-to-AP	30.4 ± 1.4		108.9

Note: P_{app} values of $[^3\text{H}]$ -digoxin were measured in the absence or presence of linaclotide and inhibitor CsA (25 μM) in Caco-2 cell line. P_{app} values are presented as mean \pm SD (n = 6).

Conclusions: Overall, data doesn't suggest P-gp substrate potential for linaclotide as demonstrated by net flux ratios that were not greater than 2.0 and the absence of an expected effect of P-gp inhibitor cyclosporine on that ratio. Linaclotide didn't appear to be a P-gp inhibitor due to the absence of various concentrations of drug on the net flux ratio of P-gp substrate drug digoxin.

Study MDP-103-088: This in vitro study was conducted to evaluate the enzyme induction potential of linaclotide and its major metabolite. The positive controls included three mechanistically distinct and clinically relevant CYP inducers, namely omeprazole (an AhR activator and CYP1A2 inducer), phenobarbital (a CAR activator and CYP2B6 inducer), and rifampin (a PXR agonist and inducer of CYP3A4). Due to co-regulation with CYP2B6 and CYP3A4, CYP2C8, CYP2C9, and CYP2C19 were not tested in this study. Linaclotide or its metabolite are not detected in plasma at clinically relevant doses. Concentrations of linaclotide and MM-419447 that were tested in this study encompassed 0 – 50 ng/mL (approximately 100-fold the C_{max} at a 10-fold therapeutic dose encountered in a food-effect study). Maximum concentrations in the intestine from a 290 μg dose in 250 mL of water could be 1.2 $\mu\text{g/mL}$. Therefore, three additional concentrations were added to test CYP3A4 induction (0.625 $\mu\text{g/mL}$, 1.25 $\mu\text{g/mL}$, and 5 $\mu\text{g/mL}$).

Table 21: In vitro enzyme induction potential assessment for linaclotide and metabolite

Treatment	Concentration	Percent positive control ^a		
		Phenacetin O-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Testosterone 6β-hydroxylation (CYP3A4/5)
DMSO	0.1% (v/v)	0 ± 0	0 ± 0	0 ± 0
Linaclotide	0.25 ng/mL	1.09 ± 0.89	-0.733 ± 0.865	-1.05 ± 4.87
Linaclotide	2.5 ng/mL	1.33 ± 0.57	0.00607 ± 0.64987	0.625 ± 1.576
Linaclotide	50 ng/mL	1.53 ± 0.74	-0.339 ± 0.597	-0.245 ± 3.211
Linaclotide	625 ng/mL	NA	NA	-0.587 ± 3.026
Linaclotide	1250 ng/mL	NA	NA	-0.736 ± 4.290
Linaclotide	5000 ng/mL	NA	NA	0.231 ± 4.276
MM-419447	0.25 ng/mL	1.81 ± 0.60	0.608 ± 0.473	0.375 ± 1.504
MM-419447	2.5 ng/mL	1.11 ± 0.47	-0.947 ± 0.873	2.23 ± 1.23
MM-419447	50 ng/mL	0.925 ± 0.306	-0.530 ± 1.459	2.71 ± 6.24
MM-419447	625 ng/mL	NA	NA	2.82 ± 2.57
MM-419447	1250 ng/mL	NA	NA	3.02 ± 3.49
MM-419447	5000 ng/mL	NA	NA	5.12 ± 1.96
Omeprazole	50 μM	100 ± 0	NA	NA
Phenobarbital	750 μM	NA	100 ± 0	NA
Rifampin	10 μM	NA	NA	100 ± 0

NA = Not applicable; DMSO = Dimethyl sulfoxide

^a Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H1026, H1029, and H1030).

CYP1A2: Treatment of cultured human hepatocytes with up to 50 ng/mL linaclotide or MM-419447 caused little or no change in CYP1A2 activity (< 2.0-fold increase and < 3% of the positive control, omeprazole).

CYP2B6: Treatment of cultured human hepatocytes with up to 50 ng/mL linaclotide or MM-419447 caused little or no change in CYP2B6 activity (< 2.0-fold increase and < 1% of the positive control, phenobarbital).

CYP3A4: Treatment of cultured human hepatocytes with up to 5000 ng/mL linaclotide or MM-419447 caused little or no change in CYP3A4/5 activity (< 2.0-fold increase and < 10% of the positive control, rifampin).

Conclusions: Neither linaclotide nor its primary metabolite induced CYP1A2, 2B6 or 3A4/5 in these in vitro investigations at the concentrations tested (up to 50 ng/mL for CYP1A2 and 2B6 and up to 5000 ng/mL for CYP3A4/5).

Study ^{(b) (4)} -03-30Sep2010: This study evaluated the in vitro Interaction Studies of Linaclotide and its Main Metabolite MM-419447 with human BCRP (ABCG2), MRP2 (ABCC2), MRP3 (ABCC3) and MRP4 (ABCC4) ABC (efflux) Transporters, and with human OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), OATP2B1 (OATP-B), PEPT1 and OCTN1 Uptake Transporters

Linaclotide doesn't cause inhibition of BCRP, MRP2 and MRP3 transporters at concentrations up to 10 μM. Some inhibition is noted on MRP4 at all concentrations of linaclotide tested (ranging from 18 %- 24 %). The active metabolite MM-419447 did not inhibit MRP 2/3/4 transporters at concentrations up to 10 μM but had a weak inhibitory effect (18 %) on BCRP transporter at the highest concentration.

BCRP and MRP2 are the efflux transporters expressed at the intestine. As IC50 values for inhibition of BCRP by the metabolite are > 10 μM and likely gut (local) concentrations of linaclotide (parent) are < 1 μM, this finding is unlikely to be of clinical relevance for drug interactions.

Linaclotide does not interact with the OATP1B1, OATP1B3, PEPT1 and OCTN1 uptake transporters at the highest tested concentration (10 μ M). A maximum inhibition of about 55 %, was observed in the case of linaclotide at 10 μ M on OATP2B1 uptake transporter. MM-419447 did not interact with OATP1B1, OATP1B3, OATP2B1, or OCTN1 uptake transporters at the highest tested concentration (10 μ M). However, this metabolite weakly inhibited (~18% - 21 %) the PEPT1-mediated uptake of glycylsarcosine at all investigated concentrations.

As systemic exposure potential of linaclotide and its major metabolite are negligible following clinically relevant doses, the observed inhibition of OATP2B1 (uptake transporter expressed at hepatocytes) at the highest concentration evaluated may not be clinically relevant. The modest effect of active metabolite on PEPT1 (uptake transporter at enterocyte) may not be relevant as IC₅₀ is > 10 μ M, which is 10 times the local (gut) concentrations of parent linaclotide at the maximum dose.

Sponsor has demonstrated in vitro that linaclotide doesn't inhibit CYP3A4 at high (local) concentrations encountered in the GI tract. Similarly, sponsor has demonstrated that linaclotide is not a substrate of P-gp nor is it an inhibitor of P-gp at these local concentrations. Similar investigations have not been conducted for the active metabolite MM-419447. Local (gut) concentrations of the active metabolite are unknown; additionally, it is not clear whether this metabolite is the major metabolite within the GI tract. Thus the design of these in vitro investigations as well as clinical relevance of findings from any such in vitro investigations is not clear. Additional investigations will not be requested from the sponsor at this time. These aspects were discussed in the Office of Clinical Pharmacology briefing on April 2, 2012 and have the concurrence of DCP3 management to not seek further in vitro studies in this regard for the active metabolite.

2.3 General Biopharmaceutics

2.3.1. What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The proposed to-be-marketed formulations were also used in the Phase 3 clinical trials. Hence no formal bioequivalence assessments are required. In the phase 2 dose-ranging trials a somewhat different formulation of linaclotide beads in capsules was used. However, due to the absence of significant systemic exposure of linaclotide or its active metabolite, relative bioavailability or bioequivalence determination for different formulations was not an option. However, sponsor has submitted dissolution profile comparisons for all formulations to provide support in this regard.

2.3.2 What is the effect of food on the bioavailability (BA) of the drug from the dosage form? What dosing recommendation should be made, if any, regarding administration of the product in relation to meals or meal types?

A food-effect pharmacodynamic and safety study was conducted using multiple daily doses of the linaclotide 300 μ g dose administered under fed or fasted conditions. Blood samples for assessing systemic concentrations of drug and active metabolite were also

collected after the last 300 µg dose in this study. The objective was evaluate systemic exposure (if any) using an improved LC-MS/MS method with a lower limit of quantitation of 0.2 ng/mL for linaclotide and 2 ng/mL for its active metabolite. PK was also evaluated in this study, after a single 3000 µg dose administered after the last dose of period 2 to improve the likelihood of identifying detectable plasma concentrations. Study and results are summarized briefly:

MCP-103-103: This was a randomized, open-label, two-period, two-sequence, crossover trial of oral linaclotide acetate administered to healthy volunteers under fed and fasting conditions. Subjects (18 – 65 years) received linaclotide 300 µg once daily for seven days during each of the treatment periods. In one of the periods, subjects were dosed under fasting conditions (10 h over night fast followed by 4h of fasting after dose); and in the other subjects were dosed under fed conditions (high fat breakfast). Each seven day treatment period was separated by a washout period of 21 days. After the second period, subjects received a single high dose of linaclotide (~ 3000 µg).

During the pre-treatment period and treatment period, subjects recorded their bowel movements (frequency, consistency, straining, complete evaluation) in their bowel habit diary for subsequent evaluation of PD effects of linaclotide treatment.

The primary endpoint was the change from pre-treatment phase to treatment phase (300 µg dose) for each Crossover Period in the weekly stool consistency score based on the BSFS rating. The primary endpoint analysis was the 90% confidence interval of the difference in the effect of linaclotide in a fasting versus a fed condition based on the change from pretreatment BSFS Scores. Equivalence margins of ± 0.6125 were used such that if the 90% confidence interval was contained within the equivalence margins, the study would demonstrate equivalence between the fasting and fed conditions relative to stool consistency.

Study findings:

Food-effect on PK: Upon administration of 300 µg of linaclotide for 7 days there were no quantifiable levels of linaclotide or its metabolite in any of the study subjects. Administration of the drug with food did not result in detectable concentrations.

The bioanalysis of 18 subjects who also received 3000 µg dose on the final day of the second period resulted in measurable drug concentrations at one or more time points in two individuals (both in the fasted condition). Linaclotide C_{max} (T_{max}) values in these individuals were 0.735 ng/mL (2h) and 0.212 ng/mL (0.5 h), respectively in the plasma. One subject had only a single sample with a measurable level (0.212 ng/mL), while another subject had measurable concentrations over five sampling time points. PK parameters (other than the observed C_{max} and T_{max}) were not calculated due to limited time points with quantifiable concentrations following a dose ten times the maximum clinical dose proposed. There were no quantifiable levels of the metabolite MM-419447 in the plasma from any subject.

Table 22: Low (< 1 ng/mL) systemic concentrations of linaclotide following a 10-fold higher dose (3000 µg) of drug during the food-effect study

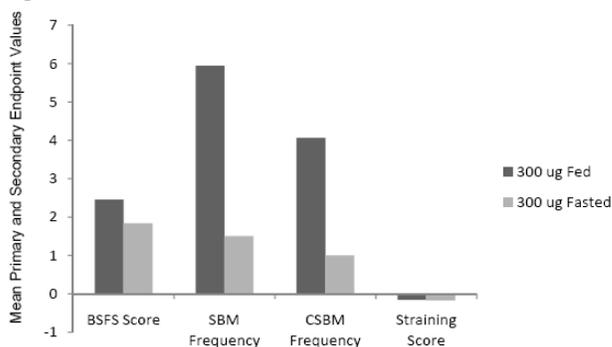
Subject Number (Treatment Sequence) Treatment Day (Hours Post-Dose)	Linaclootide Concentration	MM-419447 Concentration
001012 (Fed-Fasted)		
Day 50 (0.5 Hours)	0.27 ng/mL	< 2.00 ng/mL
Day 50 (1.0 Hours)	0.54 ng/mL	< 2.00 ng/mL
Day 50 (2.0 Hours)	0.735 ng/mL	< 2.00 ng/mL
Day 50 (3.0 Hours)	0.494 ng/mL	< 2.00 ng/mL
Day 50 (4.0 Hours)	0.432 ng/mL	< 2.00 ng/mL
001019 (Fed-Fasted)		
Day 50 (0.5 Hours)	0.212 ng/mL	< 2.00 ng/mL

Food-effect on PD: PD outcome was different across the fed and fasted conditions. Under fed conditions, the change from pre-treatment in primary and secondary endpoints was more pronounced than in fasted. Under fed conditions of dosing, the 300 µg dose of linaclotide produced a higher degree of PD effect as demonstrated by an increase in BSFS scores suggesting looser stools, increased frequency of stools, frequency of complete bowel evacuation from baseline. The 90% confidence interval was not contained within the pre-set equivalence margins of ± 0.6125 defined for the primary endpoint.

Table 23: Pharmacodynamic data from food-effect study

PD Score	Change from Pretreatment Mean (SEM)		Difference Mean and 90% CI	P-Value ^a
	Linaclootide (300 mcg) Fed	Linaclootide (300 mcg) Fasted	Fed-Fasted Difference	Fed vs Fasted
BSFS Score	2.45 (0.159)	1.84 (0.177)	0.61 (0.25, 0.98)	0.0092
SBM Frequency	5.94 (1.503)	1.50 (0.487)	4.44 (2.22, 6.67)	0.0031
CSBM Frequency	4.06 (1.181)	1.00 (0.443)	3.06 (0.90, 5.21)	0.0251
Straining Score	-0.16 (0.064)	-0.17 (0.057)	0.01 (-0.12, 0.14)	0.9387

Figure 15: Food-effect on PD



Dosing/Labeling considerations: Due to the findings of increased PD with food, during the Phase 2b and Phase 3 clinical trials of linaclotide in CC and IBS-C populations, dose was administered at least 30 minutes before breakfast. In the proposed labeling, it is noted that drug should be taken on an empty stomach. We recommend revision as shown: “Drug should be taken on an empty stomach at least 30 minutes prior to the first meal of the day”.

2.4 Analytical Section

An initial LC/MS/MS method with a lower limit of quantitation (LLOQ) of 3 ng/mL for both parent and metabolite was used in the two Phase 1 safety and tolerability studies; neither linaclotide nor MM-419447 were detected in the plasma of any subject in these studies. Subsequently, a more sensitive LC/MS/MS method with an LLOQ of 0.2 ng/mL for linaclotide and an LLOQ of 2.0 ng/mL for MM-419447 was developed and used in the analysis of PK samples from the Phase 1 food-effect study and for analysis of sparse samples in the Phase 3 clinical trials. Both LC/MS/MS methods for detecting and quantifying linaclotide and MM-419447 in human plasma were validated under Good Laboratory Practice (GLP) conditions.

Assay: Linaclotide, MM-419447, and the internal standard, MM-420026, are isolated from human plasma samples (0.4 mL) using a 96-well solid-phase extraction procedure. The sample extracts are then analyzed by electrospray liquid chromatography/tandem mass spectrometry (LC/MS/MS) in the positive ion mode.

Results of the validation runs:

Selectivity: The selectivity of the method was evaluated during each validation run to monitor for possible interfering peaks at the same chromatographic retention times as the analytes and IS. No significant chromatographic interferences were detected at either the retention times of the analytes or IS. Carryover was evaluated during each validation run by injecting at least one carryover blank (zero sample) after the ULOQ standards. Carryover was not observed for this assay.

Linearity/limit of quantitation: Acceptance criteria for precision (%CV) and accuracy (RE) were met for the calibration standard curves for the analytes in plasma. Limits of quantitation were from 0.2 to 10 ng/mL for linaclotide and from 2 to 10 ng/mL for MM-419447 and are the established linear ranges for this method in K2EDTA human plasma.

Precision and Accuracy: The intra- and inter-assay precision (CV) and accuracy (RE) of the method were assessed using six replicates each of human plasma LLOQ QC, QC1, QC2, and QC3 samples over three separate runs. The intra-assay and inter-assay precision and accuracy results for the determination of linaclotide and MM-419447 in human plasma met all acceptance criteria.

Sponsor reports that due to the high variability seen for MM-419447 response throughout this validation, they have adjusted the acceptance criteria for MM-419447 calibration standards and QC samples. Relative deviation from theoretical for these samples must be within $\pm 20\%$ ($\pm 25\%$ at the LLOQ).

Inter-lot matrix selectivity was also assessed by using multiple lots of matrix to determine whether any additional variability is observed. Control and zero samples were prepared in ten lots of control human plasma. The findings for inter-lot chromatographic selectivity experiments for both the analytes, as well as their inter-lot precision and

accuracy findings assessed by preparing LLOQ and ULOQ samples from 10 different human plasma lots were found acceptable.

Extraction Recovery: The extraction recovery (% Recovery) was determined by dividing the mean pre-extract values by the mean post-extract values and expressing the result as a percentage. Six replicates were used at each concentration. Recovery was found to be higher for the lowest QC samples, a finding which was replicated in a repeat analysis as well.

Reproducibility: The reproducibility of reinjecting processed human plasma samples was determined by reinjecting a set of previously assayed QC and calibration standard samples from an acceptable precision and accuracy run (resealed and stored at room temperature for 91 hours). The reinjection reproducibility was acceptable for linaclotide and MM-419447 after 91 hours.

Stability: Stability tests assessed using linaclotide and MM-419447 QC samples included processed sample stability, room temperature stability, freeze/thaw stability and storage stability.

Processed sample stability was evaluated after 91 hours of processed sample storage at room temperature. Linaclotide and MM-419447 were stable after standing for 24 hours at room temperature. The analytes were stable in human plasma subjected to four freeze/thaw cycles at approximately -70°C . The stability of linaclotide and MM-419447 in human plasma stored at approximately -70°C was demonstrated for 353 days. Linaclotide and MM-419447 was stable in whole blood samples after storage for one hour at room temperature and after storage for one hour in an ice bath.

Table 24: Validation parameters of linaclotide and active metabolite in plasma

Analytical Standards:	Linaclotide MM-419447
Internal Standard (IS):	MM-420026
Calibration Range:	0.2 to 10 ng/mL, linaclotide 2.0 to 10 ng/mL MM-419447

Intra- and Inter-assay Precision (CV) and Accuracy (RE):	CV (%)	<u>Linaclootide</u> ≤8.5%	<u>MM-419447</u> ≤13.1%
	RE (%)	-3.7 to 13.8%	-7.3 to 15.3%
(Intra-and inter-assay results from LLOQ QC, QC1, QC2, and QC3 samples prepared at 0.2, 0.6, 5.5, and 8.5 ng/mL, respectively, for linaclootide and at 2, 4, 5.5 and 8.5 ng/mL, respectively, for MM-419447. All QC samples analyzed in replicates of 6 in each of three precision and accuracy runs. Dilution QC samples were not required for this method.			
		<u>Linaclootide</u>	<u>MM-419447</u>
Reproducibility of Sample Reinjection:		91 hours	91 hours
Processed Sample Stability (4 °C):		91 hours	Not established
Stability in Plasma:	Freeze/Thaw (-70 °C)	4 cycles	4 cycles
	Room Temperature	24 hours	24 hours
	Freezer (-70 °C) ¹	353 days	353 days
Stability in Whole Blood:	Room Temperature	1 hour	1 hour
	Ice-Bath	1 hour	1 hour

The development and validation efforts for linaclootide and its active metabolite have been duly noted and are considered acceptable per the bioanalytical method development and validation guidelines. Thus the use of these methodologies in analyses of study samples for NDA 202811 is considered acceptable.

3 Detailed Labeling Recommendations



(b) (4)

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	202811	Brand Name	Linzess [Proposed]
OCP Division (I, II, III, IV, V)	DCP3	Generic Name	Linaclotide
Medical Division	DGIEP	Drug Class	Guanylate Cyclase-C (GC-C) Receptor Agonist
OCP Reviewer	Sandhya Apparaju, Ph.D.	Indication(s)	Chronic Constipation (CC) and IBS-C
OCP Team Leader	Sue Chih Lee, Ph.D.	Dosage Form	Capsules with immediate release beads
Pharmacometrics Reviewer	N/A	Dosing Regimen	145 ug ^{(b) (4)} Once daily on an empty stomach in CC; 290 ug once daily on empty stomach in IBS-C
Date of Submission	08/09/2011	Route of Administration	Oral
Estimated Due Date of OCP Review		Sponsor	Ironwood Pharmaceuticals (with Forest Laboratories)
Medical Division Due Date		Priority Classification	Standard
PDUFA Due Date	06/09/2012		

Clinical Pharmacology and Biopharmaceutics Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X	22		11 clinical trials 8 in vitro ADME studies 3 method validation reports
Tabular Listing of All Human Studies	X	11		Phase 1 SD, MD PK/PD and food-effect studies; Phase 2 dose finding and phase 3 S & E trials in CC and IBS-C
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	3		Two different LC-MS/MS methods were validated during drug development; the second one with improved sensitivity was used in food effect, and phase 3 studies; a third non-GLP method for fecal material assay was also developed
I. Clinical Pharmacology				
Mass balance:	N/A			
Isozyme characterization:	N/A			Drug is a 14-amino acid peptide with three disulfide bonds; Drug was shown <u>not</u> to be metabolized in intestinal microsomes;
Blood/plasma ratio:				

Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				Two SD and MD PK studies, one food-effect study and sparse sampling in phase 3 were conducted. However, in >99 % of patients systemic exposure was absent, therefore no standard PK parameters could be generated
Healthy Volunteers-				
single dose:	X	1		No systemic exposure of the drug and active metabolite;
multiple dose:	X	1		No systemic exposure of drug and active metabolite
Patients-				
single dose:				
multiple dose:	X	4		Sparse sampling in four phase 3 studies; No systemic exposure in > 99 % patients.
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:	X	8		In vitro studies to characterize permeability, metabolism and drug interaction potential
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				Sponsor notes that due to absence of systemic exposure, renal or hepatic impairment studies aren't relevant; Metabolism occurs locally via proteolytic degradation of the peptide within the intestine.
hepatic impairment:				
PD -				Colonic transit time, stool consistency and frequency
Phase 1:	X	2		
Phase 2:	X	4		
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				No systemic exposure was noted in the 4 clinical trials with sparse sampling; no population analyses could be conducted
II. Biopharmaceutics				

Absolute bioavailability				
Relative bioavailability - solution as reference:				
alternate formulation as reference:				
Bioequivalence studies - traditional design; single / multi dose:				Proposed formulation was used in the four Phase 3 studies; due to absence of systemic exposure following dosing, in vitro dissolution comparisons for various formulations are provided
replicate design; single / multi dose:				
Food-drug interaction studies	X	1		No systemic exposure; Presence of high fat meal seems to increase PD effect
Bio-waiver request based on BCS				
BCS class	X			Claim of BCS class III High Solubility; Low Permeability
Dissolution study to evaluate alcohol induced dose-dumping	N/A			
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan	X			
Literature References	X			
Total Number of Studies		22	20	Phase 2a studies using solution formulation were not reviewed in detail.

On **initial** review of the NDA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?			X	
2	Has the applicant provided metabolism and drug-drug interaction information?	X			In vitro studies;
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			No systemic exposure
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	X			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	X			

10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	X			Standard PK data couldn't be generated as there was no systemic exposure
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	X			Dose ranging phase 2a and 2b studies for each indication
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?			X	No systemic exposure
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			X	No systemic exposure
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	X			
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

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

None

Sandhya Apparaju, Ph.D.

Reviewing Clinical Pharmacologist

Date

Sue Chih Lee, Ph.D.

Team Leader/Supervisor

Date

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

/s/

SANDHYA K APPARAJU
04/05/2012

SUE CHIH H LEE
04/06/2012

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA 202811**

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	202811	Brand Name	Linzess [Proposed]
OCP Division (I, II, III, IV, V)	DCP3	Generic Name	Linaclotide
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OCP Reviewer	Sandhya Apparaju, Ph.D.	Indication(s)	Chronic Constipation (CC) and IBS-C
OCP Team Leader	Sue Chih Lee, Ph.D.	Dosage Form	Capsules with immediate release beads
Pharmacometrics Reviewer	N/A	Dosing Regimen	145 ug (b) (4) Once daily on an empty stomach in CC; 290 ug once daily on empty stomach in IBS-C
Date of Submission	08/09/2011	Route of Administration	Oral
Estimated Due Date of OCP Review	04/05/2012	Sponsor	Ironwood Pharmaceuticals (with Forest Laboratories)
Medical Division Due Date	04/09/2012	Priority Classification	Standard
PDUFA Due Date	06/09/2012		

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	“X” if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
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Tabular Listing of All Human Studies	X	11		Phase 1 SD, MD PK/PD and food-effect studies; Phase 2 dose finding and phase 3 S & E trials in CC and IBS-C
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	3		Two different LC-MS/MS methods were validated during drug development; the second one with improved sensitivity was used in food effect, and phase 3 studies; a third non-GLP method for fecal material assay was also developed
I. Clinical Pharmacology				
Mass balance:	N/A			
Isozyme characterization:	N/A			Drug is a 14-amino acid peptide with three disulfide bonds; Drug was shown <u>not</u> to be metabolized in intestinal microsomes;

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA 202811**

Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				Two SD and MD PK studies, one food-effect study and sparse sampling in phase 3 were conducted. However, in >99 % of patients systemic exposure was absent, therefore no standard PK parameters could be generated
Healthy Volunteers-				
single dose:	X	1		No systemic exposure of the drug and active metabolite;
multiple dose:	X	1		No systemic exposure of drug and active metabolite
Patients-				
single dose:				
multiple dose:	X	4		Sparse sampling in four phase 3 studies; No systemic exposure in > 99 % patients.
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:	X	8		In vitro studies to characterize permeability, metabolism and drug interaction potential
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:				
hepatic impairment:				Sponsor notes that due to absence of systemic exposure, renal or hepatic impairment studies aren't relevant; Metabolism occurs locally via proteolytic degradation of the peptide within the intestine.
PD -				Colonic transit time, stool consistency and frequency
Phase 1:	X	2		
Phase 2:	X	4		
PK/PD -				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				No systemic exposure was noted in the 4 clinical trials with sparse sampling; no population analyses could be conducted
II. Biopharmaceutics				
Absolute bioavailability				

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA 202811**

Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				Proposed formulation was used in the four Phase 3 studies; due to absence of systemic exposure following dosing, in vitro dissolution comparisons for various formulations are provided
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies	X	1		No systemic exposure; Presence of high fat meal seems to increase PD effect
Bio-waiver request based on BCS				
BCS class	X			BCS class III High Solubility; Low Permeability
Dissolution study to evaluate alcohol induced dose-dumping	N/A			
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan	X			
Literature References	X			
Total Number of Studies		22		

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2	Has the applicant provided metabolism and drug-drug interaction information?	X			In vitro studies;
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	X			No systemic exposure
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	X			
5	Has a rationale for dose selection been submitted?	X			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	X			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	X			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	X			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission	X			

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA 202811**

	discussions, submitted in the appropriate format (e.g., CDISC)?				
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			X	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	X			Standard PK data couldn't be generated as there was no systemic exposure
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	X			Dose ranging phase 2a and 2b studies for each indication
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?			X	No systemic exposure
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?			X	No systemic exposure
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			X	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			X	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	X			
General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	X			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			X	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

Yes

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

None

Sandhya Apparaju, Ph.D.

Reviewing Clinical Pharmacologist

Date

Sue Chih Lee, Ph.D.

Team Leader/Supervisor

Date

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA 202811

Tabulated list of studies for review:

<i>Type of Study</i>	<i>Study Identifier</i>	<i>Objective(s) of the Study</i>	<i>Study Design and Type of Control</i>	<i>Test Product(s)^a; Dosage Regimen; Route of Administration</i>	<i>Number of Subjects^b</i>	<i>Healthy Subjects or Diagnosis of Patients</i>	<i>Duration of Treatment</i>	<i>Study Status; Type of Report</i>
<i>Biopharmaceutic Studies</i>								
Safety, PK, and PD	MCP-103-103-CSR-01	Evaluation of safety and PD effect of multiple doses of Lin on fed and fasting subjects	Phase 1, R, OL, CO (F/F)	290, 2897 ug Lin; once daily; multiple oral dose (capsule)	19	Healthy subjects	15 days (14 days Lin 290 ug + 1 day Lin 2897 ug)	Complete; Full CSR
Method Development History	BAS-103-005-MRQ-02	Summarize Lin bioanalytical method development	--	--	--	--	--	Complete; Method Development Report
Method Validation	MNP-103-004-MVR-01	Validate human plasma assay method used for analyzing human samples (from MCP-103-001 and MCP-103-002)	LC/MS/MS Assay	--	--	--	--	Complete; Method Development Report
Method Validation	MNP-103-043-MVR-03	Validate human plasma assay method used for analyzing human samples (from MCP-103-302, LIN-MD-01, and LIN-MD-31)	LC/MS/MS Assay	--	--	--	--	Complete; Method Development Report

<i>Type of Study</i>	<i>Study Identifier</i>	<i>Objective(s) of the Study</i>	<i>Study Design and Type of Control</i>	<i>Test Product(s)^a; Dosage Regimen; Route of Administration</i>	<i>Number of Subjects^b</i>	<i>Healthy Subjects or Diagnosis of Patients</i>	<i>Duration of Treatment</i>	<i>Study Status; Type of Report</i>
<i>Studies Pertinent to Pharmacokinetics Using Human Biomaterials</i>								
Metabolism	MDP-103-056-IAR-02	Assessment of Lin stability in human intestinal microsomes	--	12 ug/mL	--	--	--	Complete; Ironwood Departmental Report
Absorption	PRD-RPT-EXP-00031	Assessment of ability of Lin to permeate intestinal epithelial cells	--	0.24, 2.4, 24 ug/mL	--	--	--	Complete; Forest Departmental Report
Metabolism	MDP-103-041-IAR-01	Lin metabolism and degradation in the luminal contents of the human intestine	--	100 ug/mL	--	--	--	Complete; Ironwood Departmental Report
Drug-Drug Interactions	MDP-103-066-IAR-01	Assessment of the human intestinal CYP450 inhibition potential of Lin	--	0.1625 – 5.2 ug/mL	--	--	--	Complete; Ironwood Departmental Report
Drug-Drug Interactions	MDP-103-057-IAR-01	Determination of potential P-gp substrate or inhibitory activity of Lin	--	0.12 – 24 ug/mL	--	--	--	Complete; Forest Departmental Report
Drug-Drug Interactions	MDP-103-088-IAR-01	Assessment of human liver CYP450 induction potential of Lin and MM-419447	--	0.25 – 5000 ug/mL	--	--	--	Complete; CRO Report

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA 202811

Type of Study	Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) ^a ; Dosage Regimen; Route of Administration	Number of Subjects ^b	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
Drug-Drug Interactions	(b) (4) (b) (4)3-30Sep2010	Assessment of potential for Lin and MM-419447 to exhibit efflux and uptake transporters	--	Lin: 0.153 – 15.3 ug/mL MM-419447: 0.136 – 13.6 ug/mL	--	--	--	Complete; CRO Report
Drug-Drug Interactions	(b) (4) 21-IAR-02	Assessment of human liver CYP450 inhibition potential of Lin and MM-419447	--	0.00005 – 0.05 ug/mL	--	--	--	Complete; CRO Report
<i>Human Pharmacokinetics Studies</i>								
Safety, PK, and PD	MCP-103-001-CSR-03	Evaluation of safety, tolerability, PK, and PD of single ascending dose on fasting subjects	Phase 1, R, DB, PC	29, 97, 290, 966, 2897 ug Lin, or PBO; ascending single oral dose (liquid solution)	30 (4 Lin 29 ug, 8 Lin 97 ug, 4 Lin 290 ug, 4 Lin 966 ug, 4 Lin 2987 ug, 6 PBO)	Healthy subjects	1 day	Complete; Full CSR
Safety, PK, and PD	MCP-103-002-CSR-02	Evaluation of safety, tolerability, PK, and PD of multiple ascending dose	Phase 1, R, DB, PC	29, 97, 290, 966 ug Lin, or PBO; once daily; ascending multiple oral dose (liquid solution)	48 (8 Lin 29 ug, 8 Lin 97 ug, 8 Lin 290 ug, 8 Lin 966 ug, 16 PBO)	Healthy subjects	7 days	Complete; Full CSR
<i>Efficacy and Safety Studies</i>								
Safety and PD	MCP-103-004-CSR-01	Evaluation of safety and PD of multiple doses of Lin	Phase 2a, R, DB, PC, PG	97, 290, 966 ug Lin, or PBO; once daily; multiple oral dose (liquid solution)	42 (12 Lin 97 ug, 10 Lin 290 ug, 10 Lin 966 ug, 10 PBO)	Patients with CC	14 days	Complete; Full CSR
Safety, Efficacy, and Dose Response	MCP-103-201-CSR-01	Evaluation of dose-ranging safety, efficacy, and dose response of multiple doses of Lin	Phase 2b, R, DB, PC, DRF, PG	72, 145, 290, 579 ug Lin, or PBO; once daily; multiple oral dose (capsule)	309 (59 Lin 72 ug, 56 Lin 145 ug, 62 Lin 290 ug, 63 Lin 579 ug, 69 PBO)	Patients with CC	28 days	Complete; Full CSR
Efficacy and Safety	MCP-103-303-CSR-01	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	145, 290 ug Lin, or PBO; once daily; multiple oral dose (capsule) with RW	643 (217 Lin 145 ug, 217 Lin 290 ug, 209 PBO)	Patients with CC	16 weeks (12 weeks DB + 4 weeks RW)	Complete; Full CSR
Efficacy and Safety	LIN-MD-01	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	145, 290 ug Lin, or PBO; once daily; multiple oral dose (capsule)	633 (213 Lin 145 ug, 205 Lin 290 ug, 215 PBO)	Patients with CC	12 weeks	Complete; Full CSR

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA 202811

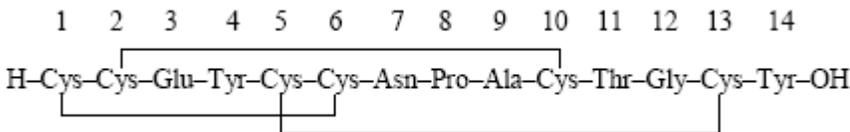
Type of Study	Study Identifier	Objective(s) of the Study	Study Design and Type of Control	Test Product(s) ^a ; Dosage Regimen; Route of Administration	Number of Subjects ^b	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status; Type of Report
PD	MCP-103-005-CSR-01	Evaluation of dose-ranging PD of multiple doses of Lin	Phase 2a, R, DB, PC, PG	97, 966 ug Lin, or PBO; once daily; multiple oral dose (liquid solution)	36 (12 Lin 97 ug, 12 Lin 966 ug, 12 PBO)	Patients with IBS-C	5 days	Complete; Abbreviated CSR
Safety, Efficacy, and Dose Response	MCP-103-202-CSR-01	Evaluation of dose-ranging safety, efficacy, and dose response of multiple doses of Lin	Phase 2b, R, DB, PC, DRF, PG	72, 145, 290, 579 ug Lin, or PBO; once daily; multiple oral dose (capsule)	420 (79 Lin 72 ug, 82 Lin 145 ug, 85 Lin 290 ug, 89 Lin 579 ug, 85 PBO)	Patients with IBS-C	12 weeks	Complete; Full CSR

Efficacy and Safety	MCP-103-302-CSR-01	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	290 ug Lin or PBO; once daily; multiple oral dose (capsule)	805 (402 Lin 290 ug, 403 PBO)	Patients with IBS-C	26 weeks	Complete; Full CSR
Efficacy and Safety	LIN-MD-31	Evaluation of efficacy and safety of multiple doses of Lin	Phase 3, R, DB, PC, PG	290 ug Lin or PBO; once daily; multiple oral dose (capsule) with RW	802 ^c (406 Lin 290 ug, 396 PBO)	Patients with IBS-C	16 weeks (12 weeks DB + 4 weeks RW)	Complete; Full CSR
Long-Term Safety	MCP-103-305	Evaluation of long-term safety and treatment satisfaction (additional) of multiple doses of Lin	OL, SA, LTS study	290 ug Lin (with option of reduction to 145 ug); once daily; multiple oral dose (capsule)	1725 ^d	Patients with CC or IBS-C	Up to 78 weeks (18 months)	Ongoing
Long-Term Safety	LIN-MD-02	Evaluation of long-term safety and treatment satisfaction (additional) of multiple doses of Lin	OL, SA, LTS study	290 ug Lin (with option of reduction to 145 ug); once daily; multiple oral dose (capsule)	1553 ^d	Patients with CC or IBS-C	Up to 78 weeks (18 months)	Ongoing

CC = chronic constipation; CO = crossover; CRO = contract research organization; CSR = clinical study report; DB = double-blind; DRF = dose-range-finding; F/F = fed/fasting; IBS-C = irritable bowel syndrome with constipation; LC/MS/MS = liquid chromatography/ tandem mass spectrometry; Lin = linaclotide; LTS = long-term safety; OL = open-label; PBO = placebo; PC = placebo-controlled; PD = pharmacodynamics; PG = parallel-group; P-gp = P-glycoprotein; PK = pharmacokinetics; R = randomized; RW = randomized withdrawal; SA = single-arm

- Updated dose-strength expression based on nominal linaclotide content; see Module 2.7.1 (Summary of Biopharmaceutic Studies and Associated Analytical Methods) for details regarding clinical dose expression changes
- Number of subjects in the Safety Population
- Includes 2 patients who were each randomized twice into the trial
- Includes rollover patients (i.e., patients who completed either a Phase 2b or Phase 3 study)

Structure of Linaclotide:



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

/s/

SANDHYA K APPARAJU
10/06/2011

SUE CHIH H LEE
10/07/2011

BIOPHARMACEUTICS FILING REVIEW
Office of New Drug Quality Assessment

Application No.:	NDA 202-811	Reviewer: Kareen Riviere, Ph.D.	
Submission Date:	8/9/2011		
Division:	Gastroenterology Products	Team Leader: Angelica Dorantes, Ph.D.	
Sponsor:	Ironwood Pharmaceuticals, Inc.	Secondary Signature: Sandra Suarez-Sharp, Ph.D.	
Trade Name:	Linzess	Date Assigned:	8/31/2011
Generic Name:	Linaclootide	Date of Review:	10/6/2011
Indication:	Treatment of constipation (b) (4) irritable bowel syndrome (IBS-C) and chronic constipation (CC).	Type of Submission: Original New Drug Application	
Formulation/strengths:	Capsules, 145 µg and 290 µg		
Route of Administration:	Oral		

SUBMISSION:

This is a 505(b)(1) New Drug Application for 145 µg and 290 µg capsules of Linzess (linaclootide) indicated for the treatment of constipation (b) (4) irritable bowel syndrome (IBS-C) and chronic constipation (CC).

BIOPHARMACEUTIC INFORMATION:

Linaclootide, a 14-amino acid synthetic peptide, is a minimally absorbed agonist of the guanylate cyclase type-C (GC-C) (b) (4), which lines the luminal surface of the intestine and is involved in the regulation of intestinal fluid homeostasis and bowel function.

During the development of linaclootide, three different clinical formulations were used. However, no relative bioavailability (BA) or bioequivalence (BE) studies were performed because concentrations of linaclootide and its metabolite are generally undetectable following oral administration and, therefore, standard pharmacokinetic (PK) parameters cannot be calculated. Although a BE study was not necessary because the proposed commercial formulation was used in the Phase 3 trials, comparability of the oral capsule formulations was established based on in vitro dissolution studies.

This submission includes a drug product development section, a dissolution development report with a proposed dissolution specification and acceptance criterion.

The proposed dissolution method:

Apparatus: (b) (4)
 Speed of Rotation: (b) (4)
 Media Volume: (b) (4)
 Dissolution Media: (b) (4)
 Sampling Times: (b) (4)
 Sampling: (b) (4)
 Filter: (b) (4)

The proposed acceptance criterion: Q = (b) (4) at (b) (4) minutes.

The Biopharmaceutics review for this NDA will be focused on the evaluation and acceptability of the proposed dissolution methodology and acceptance criterion as well as on the approval of a biowaiver for the 145 µg strength based on comparative dissolution data and other supporting information.

To aid in the review of the Applicant's submission, the following will be requested in the 74-day letter:

1. Provide information on the pH solubility profile of Linaclotide. The report should include solubility data for the drug substance covering the entire physiological pH range.
2. Conduct testing and provide data to demonstrate the discriminating capability of the selected dissolution method.
3. Submit the dissolution method report including the complete dissolution profile data (individual, mean, SD, profiles) collected during the development and validation of the proposed dissolution method.
4. Provide the complete dissolution profile data (raw data and mean values) from the clinical and primary stability batches supporting the selection of the dissolution acceptance criterion (i.e., specification-sampling time point and specification value).

RECOMMENDATION:

The ONDQA/Biopharmaceutics team has reviewed NDA 202-811 for filing purposes. We found this NDA **filable** from a Biopharmaceutics perspective. The Applicant has submitted a reviewable submission. The comments above should be conveyed to the Applicant as part of the 74-Day letter.

Kareen Riviere, Ph.D.

Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

Sandra Suarez-Sharp, Ph.D.

Senior Biopharmaceutics Reviewer
Office of New Drug Quality Assessment

cc: Angelica Dorantes, Ph.D.

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

/s/

KAREEN RIVIERE
10/07/2011

SANDRA SUAREZ
10/07/2011