Clinical Study Protocol: MCP-103-403-P-04

Final Version, 25 April 2017

Study Title:	A Phase 4, Single-centre, Randomised, Double-blind, Placebo-controlled, Parallel-group, Fixed-dose Study of the Effect of Linaclotide on Abdominal Girth in Participants with Irritable Bowel Syndrome with Constipation
Study Number:	MCP-103-403
Study Phase:	4
Product Name:	Linaclotide
Sponsor:	Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, Massachusetts 02142
Sponsor Contact:	[redacted]

Original Protocol Date:	14 October 2015
Protocol Amendment #1:	24 February 2016
Protocol Amendment #2:	26 July 2016
Protocol Amendment #3:	25 April 2017

[redacted]

SPONSOR SIGNATURE

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This clinical study protocol was subject to critical review and has been approved by the sponsor.

[redacted]

INVESTIGATOR'S SIGNATURE

Study Title:	A Phase 4, Single-centre, Randomised, Double-blind, Placebo-controlled, Parallel-group, Fixed-dose Study of the Effect of Linaclotide on Abdominal Girth in Participants with Irritable Bowel Syndrome with Constipation
Study Number:	MCP-103-403

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed [redacted]

SYNOPSIS

Study Number: MCP-103-403

Title of Study: A Phase 4, Single-centre, Randomised, Double-blind, Placebo-controlled, Parallel-group, Fixed-dose Study of the Effect of Linaclotide on Abdominal Girth in Participants with Irritable Bowel Syndrome with Constipation

Investigator: [redacted]

Study Centre: University of Manchester, Manchester, UK

Development Phase: Phase 4 (Collaborative Study)

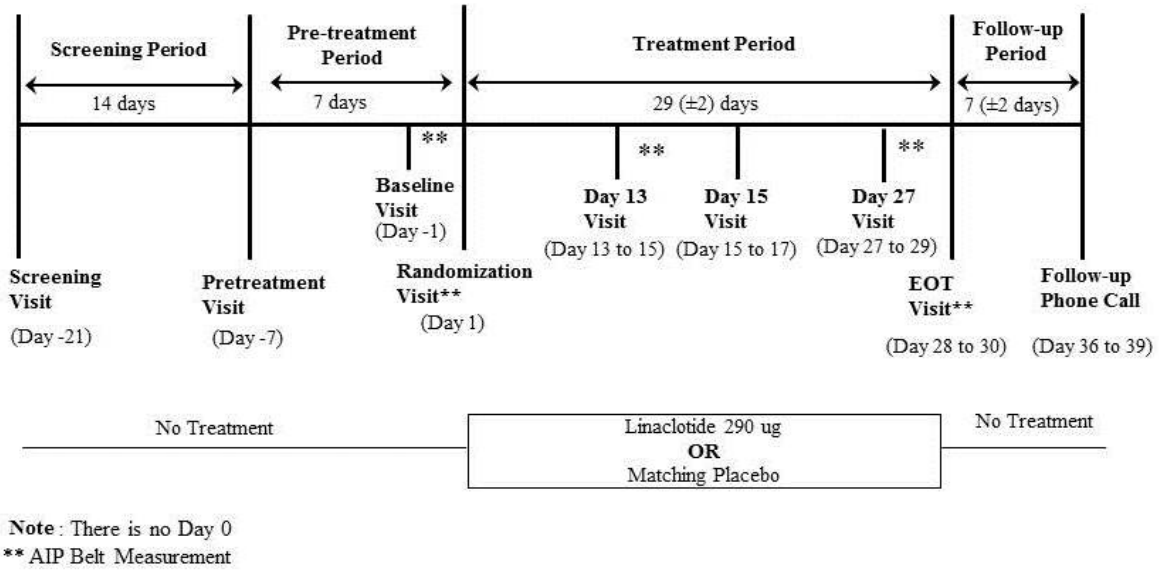
Objective:

To determine the effect of linaclotide on abdominal girth in irritable bowel syndrome with constipation (IBS-C) participants with the baseline symptoms of abdominal bloating and an increased abdominal girth.

Methodology:

This protocol describes a single-centre, randomised, double-blind, placebo-controlled, parallel-group, fixed-dose, 4-week study, consisting of 4 distinct periods. Participants attending the IBS clinic at the University Hospital of South Manchester or those held in the database of the Neurogastroenterology Unit should be eligible for the study as long as they have no concomitant exclusionary disease.

Figure S1. Study Design



Study Periods:

Screening Period (Day -21 through Day -8): The Screening Period starts with the signature of the informed consent form (ICF) and lasts for up to 14 calendar days. During this period, participant eligibility for entry into the Pretreatment Period will be determined. The end of the Screening Period coincides with the start of the Pretreatment Period, and the Screening Visit can occur on the same day as the Pretreatment Visit.

Pretreatment Period (Day -7 through Day -1): The Pretreatment Period is defined as the 7 calendar days immediately before randomisation. During this period, participants will record (via paper diary) Daily Bowel Habits, Daily Participant Symptom Severity Assessments scored on an 11-point numerical rating scale (NRS), and per protocol Rescue Medicine (bisacodyl tablets or suppositories) or any other laxative, suppository, or enema use. At the end of the Pretreatment Period, participants will be fitted with the abdominal inductance plethysmography (AIP) belt and their abdominal girth measured over the next 24 hours. During this time they will be asked to record their subjective symptoms of abdominal pain, discomfort, bloating, and distension on an hourly basis (during waking hours) via a Digestive Sensations Questionnaire. In addition, they will also be asked to empty their bladder on an hourly basis (during waking hours), or more frequently if they have the desire to go, to avoid confounding between symptoms of bladder fullness and IBS-C abdominal symptoms.

Treatment Period (Day 1 through Day 29): The Treatment Period begins with randomisation and lasts for 4 weeks. On return to the study centre for the Randomisation Visit (Day 1), the data from the AIP belt will be downloaded immediately and those exhibiting an increase in girth during the course of the previous day of >4 cm are eligible for the study. A participant whose girth does not increase by >4 cm will be refitted with the belt for another 24 hours. The abdominal girth requirement will not be disclosed to the participant. If they still do not reach the required girth measurement, they will be excluded from the study. Participants who meet all other entry criteria will be randomised (1:1) to once daily oral capsules containing 290 ug linaclotide or matching placebo. Participants will take their initial dose of study drug in the study centre during the Randomisation Visit; thereafter, study drug is to be taken once daily, in the morning, ≥ 30 minutes before breakfast. Participants will continue to record their daily diary assessments throughout the Treatment Period. Abdominal girth will be measured by the AIP belt, and the Digestive Sensations Questionnaire will be administered, for another 24 hours at the mid-point of the Treatment Period (Day 13 Visit) and again at the end of treatment (Day 27 Visit) to assess the effect of the treatment on abdominal girth and IBS-C symptoms.

Follow-up Period (Day 30 through Day 36): The Follow-up Period starts at the End of Treatment Visit (Visit 8) and finishes with the Follow-up Telephone Call on Day 36, one week after the End of Treatment Visit (Visit 8). During this call, participants will be asked to report any adverse events (AEs) since the End of Treatment Visit (Visit 8) and detail any other symptoms or comments they may have.

Note: End of Study will be defined as the last participant Follow-up Telephone Call (Day 36 ± 2).

For details regarding assessments during each study period, see the Schedule of Evaluations.

Ethical Considerations:

The study will be submitted to the Greater Manchester Research Ethics Committee (REC) for approval.

Concomitant and Rescue Medicine:

At the Screening Visit, all ongoing medicines taken by the participant will be recorded. Any over-the-counter or prescription laxatives, suppositories, or enemas used to treat IBS-C may not be used on the calendar day before the Pretreatment Visit. Other prohibited medicines may not be used during the 14 calendar days before the Pretreatment Visit.

A complete list of drugs that are conditionally allowed as concomitant medicines for either episodic or chronic use or as Rescue Medicine is provided in Appendix 1. Prohibited concomitant medicines are also included in Appendix 1.

During the Pretreatment and Treatment Periods, participants may use dispensed, protocol-defined laxatives (bisacodyl tablets or suppositories) as Rescue Medicine when at least 72 hours have passed since their previous BM or when their symptoms become intolerable. In order to qualify for randomisation into the Treatment Period, participants must not have used Rescue Medicine on the calendar day before the Randomisation Visit and on the day of the Randomisation Visit up until the time of the clinic visit. Participants must agree to refrain from using Rescue Medicine from the time of the clinic visit through the calendar day after randomisation. Furthermore, participants will be asked to refrain from using Rescue Medicine on the day before, day of, and day after each AIP measurement.

Any changes in concomitant medicines or new medicines added from the Screening Period through the Follow-up Period will be recorded on the CRF. Concomitant medicines will be recorded at all study visits throughout the entire study. Rescue Medicine will be documented by the participant on the Rescue Medicine record form.

Planned Number of Participants:

In order to ensure that 40 participants have completed the study as planned, up to 120 participants may need to be screened.

Diagnosis and Main Criteria for Inclusion:

Inclusion Criteria:

To be eligible to participate in the study, participants must meet all of the following criteria:

1. Participant has provided written informed consent before participating in the study after being given a full description of the study and prior to any study-specific procedures being performed.
2. Participant is a male or non-pregnant, non-breastfeeding female, and is age 18 years or older (no upper age limit).
3. Female participants of childbearing potential must complete a urine pregnancy test with negative results at the Screening Visit (Visit 1) and again at the Randomisation Visit (Visit 4) prior to dosing.

4. Female participants who are pre-menopausal should be enrolled between days 7 and 21 of their menstrual cycle.
5. Sexually active female participants of childbearing potential must agree to use one of the following methods of contraception from the date that they sign the ICF until the end of study:
    a) Hormonal contraception (i.e., oral contraceptive, contraceptive implant, or injectable hormonal contraceptive);
    b) Intra-uterine device (IUD);
    c) A barrier birth control (such as condoms or occlusive cap with spermicidal foam/gel/film/cream/suppository);
    d) Surgical sterilisation (i.e., bilateral oophorectomy, hysterectomy, or tubal ligation);
    e) Abstinence, when in the opinion of the Investigator, the participant's occupation or lifestyle gives sufficient evidence that abstinence will be maintained throughout the study and for 1 month thereafter.
6. Participant meets Rome III criteria for IBS-C, as well as abdominal pain score (average of ≥3 on 0-10 point numerical rating scale [NRS]) and abdominal bloating score (average of ≥5 on 0-10 point NRS) during the PreTreatment Period, and criterion for abdominal girth measurement (AIP increase of >4 cm) criteria over a 24-hour period.
7. Participant agrees to refrain from strenuous physical activity for 24 hours prior to each study visit and for all periods of AIP belt recordings from the time of fitting until belt removal.
8. Participant agrees to refrain from initiating any lifestyle or dietary changes.
9. Participant agrees to refrain from taking any probiotics from 14 days prior to enrollment in the study throughout the Treatment Period.
10. Participant is able to communicate well with the Investigator and to comply with the requirements for the entire study.
11. Participant has a body mass index (BMI) between 18.5 and 34.9 kg/m^2 (bounds included) at the Screening Visit.

Exclusion Criteria:

Participants who meet any of the following criteria will not be eligible to participate in the study:

1. Participant reports loose (mushy) or watery stools (BSFS score of 6 or 7) in the absence of any laxative, suppository, enema, or prohibited medicine (as described in Appendix 1) for >25% of BMs during the 12 weeks before the Screening Visit.

2. Participant has ever had a diagnosis of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or any other form of familial colorectal cancer, or inflammatory bowel disease.
3. Participant currently has both unexplained and clinically significant alarm symptoms (lower GI bleeding, iron-deficiency anaemia or any unexplained anaemia, or weight loss) or systemic signs of infection or colitis.
4. Participant currently has active peptic ulcer disease (i.e., disease that is not adequately treated or stable with therapy).
5. Participant has a history of any chronic GI condition (e.g., diverticulitis) that can be associated with abdominal pain or discomfort.
6. Participant has CNS disease or any organic condition associated with constipation.
7. Participant has had surgery that meets any of the following criteria:
    a. Has undergone any surgery within 6 months prior to the Screening Visit;
    b. Has had previous abdominal surgery except appendectomy, cholecystectomy, or hysterectomy.
8. Participant has diabetes.
9. Participant reports using a Prohibited Medicine (excluding laxatives, suppositories, and enemas) during the Pretreatment Period or is not willing or able to abide by the restrictions regarding use of Prohibited Medicines defined in Appendix 1. (Note: the use of fibre, bulk laxatives, or stool softeners [such as docusate] is acceptable provided that the participant has been on a stable dose during the 30 days before the Screening Visit and plans to continue on a stable dose throughout the study.)
10. Participant has taken commercially available linaclotide during the 30 days prior to the Screening Visit.
11. Participant is drinking alcohol above the recommended safe alcohol limit (< 21 units/week) or abusing drugs. In addition, participants will be asked to refrain from drinking alcohol for 24 hours before plethysmography recordings and until belt removal.
12. Participant has an acute or chronic condition that, in the Investigator's opinion, would limit the participant's ability to complete or participate in this clinical study.
13. Participant has lactose intolerance.
14. Participant is involved in this study as an Investigator, sub-Investigator, study coordinator, other study staff, or Sponsor member.
15. Participant has a known or suspected mechanical gastrointestinal obstruction.
16. Participant has a history of hypersensitivity to linaclotide or to any of the excipients contained in the study drug (active or placebo).

Test Product, Planned Doses, and Mode of Administration:

Linaclotide 290 ug once daily, oral administration. Should diarrhoea occur while taking the 290 ug dose, the daily dose may be temporarily suspended by the Investigator until resolution of the diarrhea, but participants must be taking study drug for 24 hours before AIP belt fittings.

Reference Therapy, Planned Dose, and Mode of Administration:

Placebo to match linaclotide 290 ug once daily, oral administration. Should diarrhoea occur while taking the 290 ug dose, the daily dose may be temporarily suspended by the Investigator until resolution of the diarrhea, but participants must be taking study drug for 24 hours before AIP belt fittings.

Duration of Treatment:

Total participation is expected to last for up to 57 days. There will be a Screening Period of up to 14 days, followed by a Pretreatment Period of 7 days, followed by a 29-day double-blind Treatment Period and a 7-day Follow-up Period.

Criteria for Evaluation:

Primary Outcome Measure:

Mean change from randomisation (Visit 4) to the end of the study (Visit 8) in abdominal girth (as measured by AUC) determined by 24-hour AIP between the linaclotide and placebo groups.

Secondary Outcome Measures:

Diary questions that determine the following:

- Daily participant assessment of abdominal pain, discomfort, bloating, and distension;
- Whether a BM is an SBM;
- Whether an SBM is a CSBM;
- Stool consistency (Bristol Stool Form Scale [BSFS]) with each BM.

Safety Measures: Adverse event recording, clinical laboratory measures, vital sign parameters, and physical examinations.

Statistical Methods:

Sample Size Determination:

To achieve 80% power to detect a difference of 2.2 cm or more (using a simple 2-sided t-test with estimated SD = 2.4 cm and the conventional 5% significance level) in the mean change in abdominal girth (as measured by AIP AUC) from the start to the end of the day, between the active and placebo groups, 20 participants per group (40 participants in all) are required. Assuming a 15% dropout rate, 47 participants would need to be randomised to yield 40 evaluable participants. Estimating a 60% screen/pretreatment failure rate (mostly from failure to meet distension criteria, but also pretreatment abdominal pain and abdominal bloating criteria), 120 participants would need to enter screening to achieve 40 randomised participants.

Analysis Populations:

ITT Population (all who were randomised to a treatment group at the randomisation visit), Safety Population (all randomised participants who received at least one dose of study drug) and Evaluable Population (all participants included in the Safety Population presenting no major protocol deviations with the potential to bias the criteria for evaluation and with full follow-up).

Efficacy Analyses:

Changes from Baseline in abdominal girth at 4 weeks (primary outcome) using analysis of covariance on AUC measures (24-hour AIP) and at 2 weeks as well as changes in maximal abdominal girth at both 2 and 4 weeks.

Change from Baseline in symptom severity and Bristol Stool Form Scale (diary data) using longitudinal regression analysis on weekly average scores.

Change from Baseline in digestive sensation at 2 and 4 weeks as measured by hourly scores, using analyses of covariance on AUC measures.

Safety Analyses:

Adverse events will be summarised by system organ class (SOC), preferred term (PT), and treatment group. Listings will be provided for severe AEs, drug-related AEs, SAEs, and AEs leading to study discontinuation. Descriptive statistics will be calculated on vital signs and clinical laboratory tests by treatment group.

Schedule of Evaluations									
	Screening Period (14 days)	**Pretreatment Period (7 days)**		**Treatment Period (29±2 days)**					**Follow-up Period (7± 2 days)**
Visit Name →	**Screening Visit**	**Pretreatment Visit**	**Baseline Visit**	**Randomisation Visit**	**Day 13 Visit**	**Day 15 Visit**	**Day 27 Visit**	**EOT Visit[a]**	**Follow-up**
Visit Number →	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Telephone Call**
Visit Day →	**Day -21**	**Day -7**	**Day -2**	**Day 1**	**Day 13±2**	**Day 15±2**	**Day 27±2**	**Day 28±2**	**Day 36 ± 2**
Study Procedure ↓									
Informed consent	X								
Inclusion and exclusion criteria verification	X			X					
Medical and surgical history	X			X					
Rome III status for IBS-C	X								
Physical examination[b]	X			X				X	
Body weight and height[c]	X			X		X		X	
Seated vital signs[d]	X			X		X		X	
Prior and concomitant medicines	X	X	X	X	X	X	X	X	
Clinical laboratory determinations[e]	X								
Pregnancy test[f]	X			X				X	
Completion of HADs and IBS-SSS	X							X	
Laxative, suppository, and enema washout instructions	X								
AE evaluations[g]		X	X	X	X	X	X	X	X
Rescue Medicine dispensed[h]		X		X		X			
Diary training, compliance verification, and reminder[i]		X	X		X		X		
Daily diary recording		X	X	X	X	X	X	X	
Daily diary collection				X		X		X	
Digestive Sensations questionnaire given			X		X		X		

Schedule of Evaluations									
	Screening Period (14 days)	Pretreatment Period (7 days)		Treatment Period (29±2 days)					Follow-up Period (7± 2 days)
Visit Name →	**Screening Visit**	**Pretreatment Visit**	**Baseline Visit**	**Randomisation Visit**	**Day 13 Visit**	**Day 15 Visit**	**Day 27 Visit**	**EOT Visit[a]**	**Follow-up**
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Visit Day →	**Day -21**	**Day -7**	**Day -2**	**Day 1**	**Day 13±2**	**Day 15±2**	**Day 27±2**	**Day 28±2**	**Day 36 ± 2**
Study Procedure ↓									
Digestive Sensations questionnaire collected				X		X		X	
AIP fitted[j]			X		X		X		
AIP Assessment				X		X		X	
Randomisation				X					
Study drug dispensed[k]				X					
Study drug accountability						X		X	
Follow-up phone call									X

a. Participants who are randomised but do not complete the Treatment Period (withdraw consent or are discontinued before they have completed 4 weeks of treatment) shall be considered Treatment-Period withdrawals and should complete the procedures required at the EOT Visit (even if out of window).
b. A physical examination should include the following: general appearance, HEENT (head, ears, eyes, nose, and throat), neck, cardiovascular, thorax/lungs, abdomen, musculoskeletal, lymph nodes, skin, neurologic, and mental status. Rectal examination is not required; however, sigmoidoscopy/colonoscopy may be performed at the discretion of the Investigator.
c. Height will only be measured at the Screening Visit (Visit 1)
d. Vital signs (oral temperature, respiratory rate, systolic and diastolic blood pressure, and pulse rate) must be obtained from participants who are in the seated position.
e. Complete hematology and chemistry will be required at the Screening Visit (Visit 1) if lab results obtained within the prior 30 days are not available.
f. To be eligible for the study, a negative urine pregnancy test must be documented at the Screening Visit (Visit 1) and Randomisation Visit (Visit 4); another urine pregnancy test will be performed at the EOT Visit (Visit 8).
g. All AEs occurring after the participant signs the ICF will be documented.
h. Rescue Medicine (bisacodyl tablets) will be supplied to participants at the Pretreatment Visit (Visit 2) and, if needed, at subsequent visits.
i. At the Pretreatment Visit (Visit 2) the Study Coordinator will instruct the participants about the use of the Daily Diary. At subsequent visits, the Study Coordinator will review the Daily Diary to verify participant compliance. After determining the participant's compliance, the Study Coordinator will remind participants to record their diary assessments daily.

j. Women who are actively menstruating during AIP belt placement should have their visits delayed for 48 hours. Participants who have taken Rescue Medicines within 24 hours of AIP belt placement should have their visits delayed until 24 hours have passed since the use of Rescue Medicine. Participants who have had study drug suspended due to diarrhea should have their AIP belt placement visits delayed until study drug has been resumed for 24 hours.
k. Study drug will be administered in the clinic at the Randomisation Visit (Visit 4). At this visit, study drug does not need to be taken in the morning before breakfast. On all other days, study drug will be taken once daily in the morning at least 30 minutes before breakfast.

AE = adverse event; AIP = abdominal inductance plethysmography; IBS-C = irritable bowel syndrome with constipation; EOT = end of treatment; HADs = Hospital Anxiety and Depression Scale; IBS-SSS= Irritable Bowel Syndrome Symptom Severity Score

SAFETY/MEDICAL MONITORING AND STUDY CONTACTS

A summary of key study participants is provided in Table 1.

Table 1. Key Study Participants

Role	Contact Information
Principal Investigator and Study Centre:	[redacted] University Hospital of South Manchester Southmoor Road Wythenshawe Manchester, UK M23 9LT [redacted]
Ironwood Contact:	[redacted] Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, MA 02142 [redacted]
Medical Monitor:	[redacted] Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, MA 02142 [redacted]
Safety Officer:	[redacted] Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, MA 02142 [redacted]
Serious Adverse Event (SAE) E-mail:	clinicalsafety@ironwoodpharma.com
Dedicated SAE Facsimile Number:	1-617-933-7688

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LIST OF ABBREVIATIONS

Abbreviation	Term
AE	adverse event
AIP	abdominal inductance plesthymography
AUC	area under the curve
BM	bowel movement
BMI	body mass index
BP	blood pressure
BSFS	Bristol Stool Form Scale
CFR	Code of Federal Regulations
cGMP	cyclic guanosine 3', 5'-monophosphate
CIC	Chronic idiopathic constipation
CNS	Central nervous system
CSBM	Complete spontaneous bowel movement
CT	Computed tomography
EOT	End of Treatment
FDA	Food and Drug Administration
GC-C	Guanylate cyclase-c
GCP	good clinical practice
GGT	gamma glutamyl transferase
IBS-C	Irritable Bowel Syndrome with Constipation
IBS-D	Irritable Bowel Syndrome with Diarrhoea
IBS-SSS	Irritable Bowel Syndrome – Syndrome Symptom Severity
ICF	informed consent form
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ITT	Intent to Treat
kg	kilogram
kg/m^2	kilograms/meters squared (body mass index)
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram

Abbreviation	Term
NRS	Numerical Rating Scale
PT	Preferred Term
REC	Research Ethics Committee
RW	Randomized Withdrawal
SAE	serious adverse event
SAP	Statistical Analysis Plan
SAS®	Statistical Analysis System
SBM	Spontaneous Bowel Movement
SD	Standard Deviation
SOC	system organ class
TEAE	treatment emergent adverse event
ug	microgram

1. ETHICAL CONSIDERATIONS

This clinical study is designed to comply with the International Conference on Harmonisation (ICH) Guidance on General Considerations for Clinical Trials (62 Code of Federal Regulations [CFR] 66113, December 17, 1997) and Good Clinical Practice (GCP): Consolidated Guidance (62 CFR 25692, May 9, 1997). The study will be conducted in full compliance with the UK guidelines on GCP and in accordance with the ethical principles that have their origins in the Declaration of Helsinki and 21 CFR § 312.120.

1.1 INSTITUTIONAL REVIEW BOARD/ETHICS COMMITTEES

Obtaining approval by the Research Ethics Committee (REC) prior to the start of the study will be the responsibility of the Investigator. A copy of the approval letter will be transmitted to the Sponsor or designee. During the course of the study, the Investigator will provide timely and accurate reports to the REC on the progress of the study, and will notify the REC and Sponsor of SAEs or other significant safety findings in a manner consistent with REC policies and ICH and GCP requirements. The study protocol (and any amendments), Informed Consent Form (ICF), and associated documentation will be approved by the REC prior to study initiation, in compliance with CFR, Title 21, Part 56.

1.2 PARTICIPANT INFORMATION AND INFORMED CONSENT

It is the responsibility of the Investigator (or qualified designee) to give each participant full and adequate information regarding the objectives and procedures of the study and the possible risks involved. The participants must be informed about their right to withdraw from the study at any time. Furthermore, it is the responsibility of the Investigator to obtain signed and dated written informed consent from all participants, and a dated signature from the persons conducting the informed consent discussion, before undertaking any study-related procedure. The written ICF must be approved by the REC for the purposes of obtaining and documenting consent. The Investigator must be available to answer all participants' questions regarding the study. The participant should receive a copy of the signed and dated ICF. The Investigator must retain each participant's original signed ICF. If the protocol is amended and the modifications necessitate a change to the ICF, then the ICF must be submitted to the REC for review and approval before initiation.

If new information becomes available that may be relevant to a participant's consent and willingness to participate in the study, the ICF will be revised. The revised consent form will be submitted to the REC for review and approval prior to its use.

2. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The Investigator at the study centre will be responsible for ensuring that the study is conducted according to the signed Clinical Trial Agreement, the protocol, REC requirements, and GCP guidelines. The Investigator will be responsible for the oversight of the study centre's conduct of the study, which will consist of completing all protocol assessments, maintaining the study file and the participant records, drug accountability, corresponding with the REC, and completing the CRFs.

3. INTRODUCTION

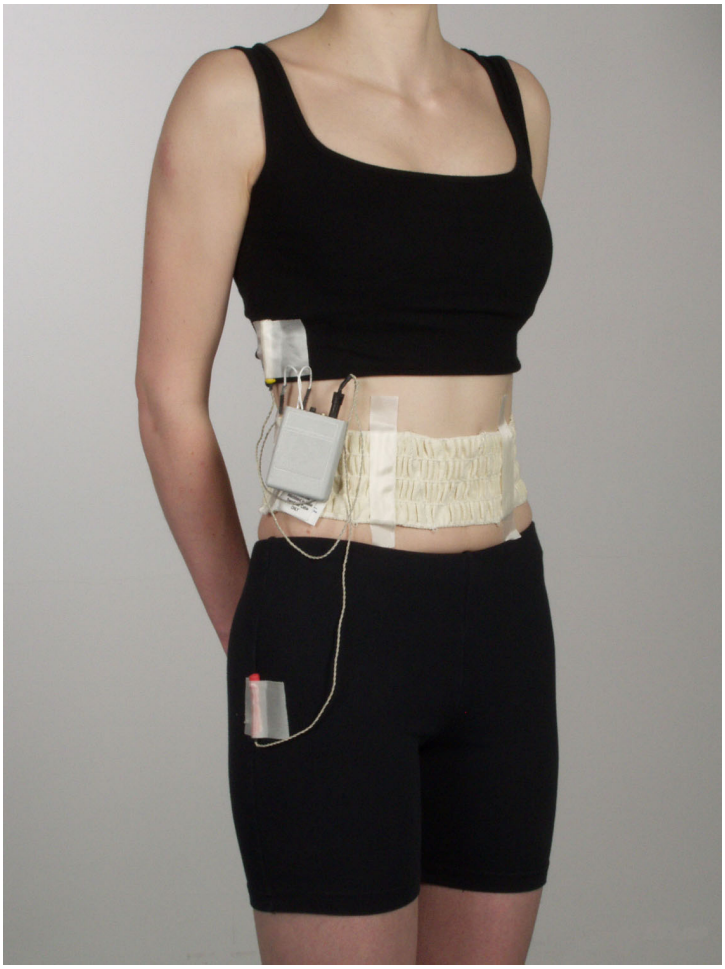
3.1 BACKGROUND

Abdominal bloating is an extremely common feature of irritable bowel syndrome (IBS) which many patients rank as their most intrusive symptom (1, 2). The exact pathophysiology of this problem is not fully understood, but its understanding has improved with the advent of various investigational techniques, such as abdominal inductance plethysmography (AIP), the gas infusion technique, and computed tomography (CT) scanning (3, 4). As a result of studies using these investigations, it is now appreciated that bloating can sometimes be accompanied by an actual increase in girth which, subjectively may be felt by the patient as abdominal distension. However, patients usually use the term bloating when describing this symptom, whether they feel bloated and/or whether it is accompanied by an actual increase in girth or neither.

In addition, it is now apparent that abdominal bloating and change in abdominal girth have somewhat different, but overlapping, underlying pathophysiologies. Bloating appears to be more common in patients with IBS with diarrhoea (IBS-D) and those who exhibit hypersensitivity of the rectal mucosa (5, 6). In contrast, an increase in abdominal girth appears to be more common in IBS with constipation (IBS-C) patients and those with slow transit (5, 7). Consequently, when contemplating the treatment of increased abdominal girth, relieving constipation or accelerating transit should lead to some improvement in patients with IBS-C. However, there has been little research on the possibility that relieving constipation brings about a reduction in abdominal girth.

One placebo-controlled trial (8) utilised AIP, which measures abdominal girth over a 24-hour period in an ambulatory fashion (see Figure 1) (9) and, therefore, offers the opportunity to assess whether a drug that relieves constipation also leads to a reduction in abdominal girth (9). This trial used a yoghurt containing bifidobacterium lactis DN-173 010, which has previously been shown to accelerate transit and improve the subjective feeling of bloating in patients with IBS-C (10, 11). A total of 34 IBS-C patients were randomised to ingest either an active yoghurt or a matching placebo yoghurt for 4 weeks. The active yoghurt led to an acceleration of both small and large bowel transit and a significant reduction in maximum abdominal girth compared with placebo (8).

Figure 1. Abdominal Inductance Plethysmography Belt



In addition, in recent years there have been a number of studies assessing the efficacy of potential new pharmacological treatments for constipation (12-16). In all of these studies, there has been evidence that the symptom of bloating has been improved, but girth was not objectively measured in any of these studies. However, it seems reasonable to assume that, as patients often describe an increase in abdominal girth as 'bloating,' that the latter may have been reduced in at least a proportion of these patients.

This study is designed to establish whether linaclotide reduces abdominal bloating and abdominal girth in patients with IBS-C.

3.2 INVESTIGATIONAL PRODUCT

Linaclotide is a minimally absorbed 14-amino-acid peptide, which binds to and activates guanylate cyclase C (GC-C) on the luminal surface of the intestinal epithelium. Activation of intestinal GC-C results in increased cyclic guanosine monophosphate (cGMP) production, which in turn causes chloride and bicarbonate to be secreted into the gastrointestinal lumen, with consequent increased fluid secretion and accelerated intestinal transit (17-19). Linaclotide reduces visceral hypersensitivity in animal models, and improves abdominal pain in patients with IBS-C; this effect may be related to cGMP modulation of afferent nerve activity (15, 20-24).

The clinical development program for linaclotide included 4 large double-blind, placebo-controlled registration trials and 2 large open-label safety studies. Two Phase 3 trials (LIN-MD-31 and MCP-103-302) were conducted in IBS-C patients (15, 23, 24) and two (LIN-MD-01 and MCP-103-303) were conducted in patients with chronic idiopathic constipation (CIC) (17). Two long-term safety studies (LIN-MD-02 and MCP-103-305), each with 78-week Treatment Periods, have also been completed. Linaclotide is approved in the US, Canada, and Mexico for the treatment of adults with IBS-C and CIC and approved in the EU and Switzerland for the treatment of adults with IBS-C.

The two Phase 3 IBS-C trials evaluated the safety and efficacy of 290 ug linaclotide administered as an oral capsule (15, 23, 24). The overall design of these two Phase 3 trials was identical through the first 12 weeks of the Treatment Period. One of the Phase 3 trials (LIN-MD-31) included a 4-week double-blind, Randomised Withdrawal (RW) Period immediately following the 12-week Treatment Period to assess the potential for rebound worsening of constipation or abdominal symptoms following discontinuation of linaclotide. The other trial (MCP-103-302) extended the double-blind Treatment Period an additional 14 weeks (26 weeks total treatment).

A total of 1606 patients were randomised to either to linaclotide 290 ug or matching placebo once daily. In both trials, linaclotide met all prespecified primary and secondary endpoints. For the FDA responder definition (one of 4 primary efficacy endpoints), a patient was required to have an increase of at least 1 complete spontaneous bowel movement (CSBM) from baseline, and a decrease of at least 30% in the mean abdominal pain score, during the same week, for at

least 6 of the first 12 weeks of the Treatment Period. For the MCP-103-302 trial, 33.7% of linaclotide-treated patients met this primary endpoint, compared with 13.9% of patients who received placebo. Similarly, in the LIN-MD-31 trial, 33.6% of linaclotide-treated patients met this primary endpoint, compared with 21.0% of patients who received placebo (for both trials, $p < 0.0001$ for linaclotide versus placebo).

Statistically significant improvements were demonstrated in patients' abdominal symptoms (including abdominal pain, discomfort and bloating) and bowel symptoms (including CSBM frequency, spontaneous bowel movement (SBM) frequency, stool consistency and straining). These improvements were sustained throughout 12 weeks of the Treatment Period of each of the two trials and for 26 weeks in Trial MCP-103-302. The results from the RW Period showed that there was no evidence of development of tolerance, nor was there evidence of rebound worsening of bowel or abdominal symptoms relative to baseline once linaclotide was discontinued.

Linaclotide was well tolerated in the IBS-C Phase 3 efficacy trials. Treatment-emergent adverse events (TEAEs) occurred in 54.9% of placebo-treated and 60.8% linaclotide-treated patients. Diarrhoea was the most frequent TEAE and was reported in 3.0% of placebo-treated patients and 19.8% of linaclotide-treated patients. Diarrhoea was generally mild to moderate in severity. Diarrhoea resulted in the discontinuation of 0.4% of patients treated with placebo and 5.3% treated with linaclotide. There were 9 (1.1%) SAEs in placebo-treated patients and 6 (0.7%) in linaclotide-treated patients. The SAEs were judged to be unlikely or unrelated to treatment by the Investigator, with the exception of 2 SAEs (pericarditis and pericardial effusion occurring in 1 patient treated with linaclotide) that were judged by the Investigator to be possibly related to treatment. There were no deaths. One patient died in the Screening Period but never received either linaclotide or placebo. Overall, there was no obvious pattern in the types of SAEs experienced in either the placebo or linaclotide group. There were no SAEs of diarrhoea.

Refer to Section 6 of the United States Package Insert (USPI) for a more detailed description of the safety of linaclotide, as it will serve as the Reference Safety Information for this study. Refer to the Investigator's Brochure for a more detailed description of the IBS-C and CIC clinical

trials, as well as the chemistry, pharmacology, and efficacy of linaclotide, based on studies conducted in animals, healthy volunteers, and in patients with CIC and IBS-C.

4. STUDY OBJECTIVES

To determine the effect of linaclotide on abdominal girth in irritable bowel syndrome with constipation (IBS-C) participants with baseline symptoms of abdominal bloating and an increased abdominal girth.

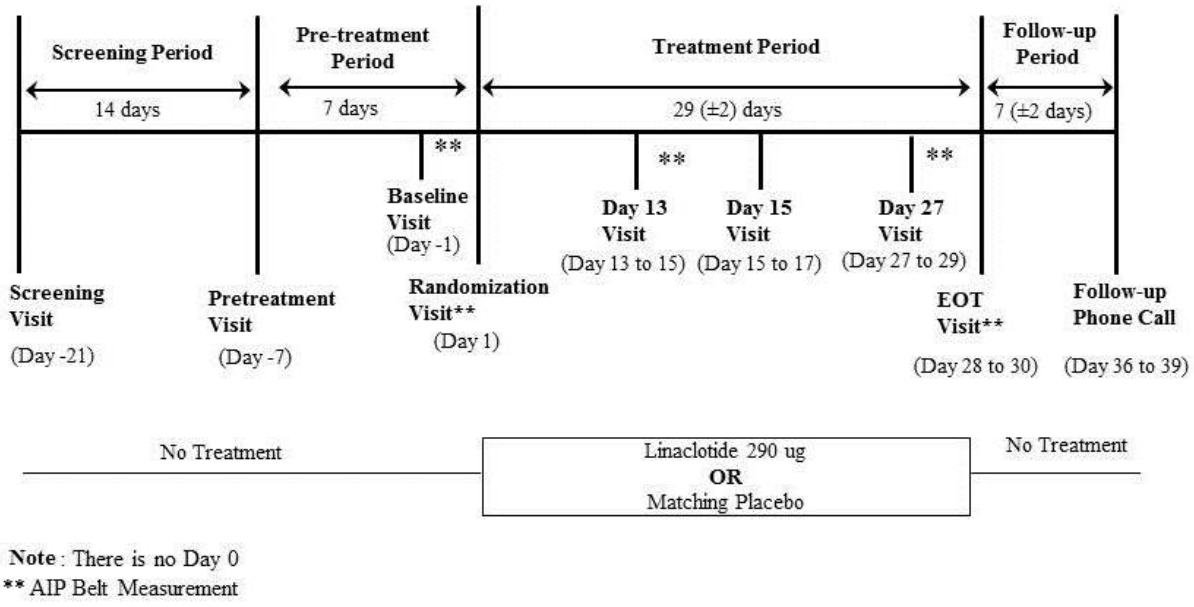
5. STUDY DESIGN

5.1 OVERALL STUDY DESIGN AND PLAN: DESCRIPTION

The study will be a single-centre, randomised, double-blind, placebo-controlled, parallel-group study comparing linaclotide to placebo. Approximately 40 participants with a diagnosis of IBS-C (Rome III criteria), who are shown to distend >4 cm over 24 hours during the Pretreatment Period, will be randomised.

The study will consist of up to 14 days of screening, 7 days of pretreatment, 29 (± 2) days of double-blind treatment, and 7 (± 2) days of follow-up. At the end of the Pretreatment Period, participants meeting the entry criteria for this study will be randomised (1:1) to one of two double-blind treatment groups: 290 ug linaclotide or matching placebo.

Figure 2. Study Schedule and Chronology



Note: There is no Day 0
** AIP Belt Measurement

5.1.1 Study Periods

5.1.1.1 Screening Period

The Screening Period starts with the signature of the ICF and lasts for up to 14 calendar days. During this period, participant eligibility for entry into the Pretreatment Period will be determined.

At the Screening Visit, all ongoing medicines taken by the participant will be recorded. Any over-the-counter or prescription laxatives, suppositories, or enemas used to treat IBS-C may not be used on the calendar day before the Pretreatment Visit; other prohibited medicines may not be used during the 14 calendar days before the Pretreatment Visit. The end of the Screening Period coincides with the start of the Pretreatment Period.

5.1.1.2 Pretreatment Period

The Pretreatment Period is defined as the 7 calendar days immediately before randomisation. During this period, participants will record (via paper diary) Daily Bowel Habits, Daily Participant Symptom Severity Assessments scored on an 11-point numerical rating scale (NRS), and per protocol Rescue Medicine (bisacodyl tablets or suppositories) or any other laxative, suppository, or enema use. At the end of the Pretreatment Period, participants will be fitted with the AIP belt in the morning and their abdominal girth measured over the next 24 hours. The AIP belt will be fitted prior to the dose of linaclotide and participants will be asked to record their subjective symptoms of abdominal pain, discomfort, bloating, and distension on an hourly basis (during waking hours) via a Digestive Sensations Questionnaire. In addition, they will also be asked to empty their bladder on an hourly basis (during waking hours), or more frequently if they have the desire to go, to avoid confounding between symptoms of bladder fullness and IBS-C abdominal symptoms.

5.1.1.3 Treatment Period

The Treatment Period begins with randomisation and lasts for 4 weeks. On return to the study centre for the Randomisation Visit (Day 1), the data from the AIP belt will be downloaded immediately and those exhibiting an increase in girth during the course of the previous day of >4 cm are eligible for the study. A participant whose girth does not increase by >4 cm will be

refitted with the belt for a further 24 hours. The abdominal girth requirement will not be disclosed to the participant. If they still do not reach the required girth measurement, they will be excluded from the study. Participants who meet all other entry criteria will be randomised (1:1) to once daily oral capsules containing 290 ug linaclotide or placebo. Participants will take their initial dose of study drug in the study centre during the Randomisation Visit; thereafter, study drug is to be taken once daily, in the morning, ≥30 minutes before breakfast. Participants will continue to record their daily diary assessments throughout the Treatment Period. Abdominal girth will be measured by the AIP belt, and the Digestive Sensations Questionnaire will be administered for another 24 hours at the mid-point of the Treatment Period (Day 13 Visit) and again at the end of the study (Day 27 Visit) to assess the effect of the treatment on abdominal girth and IBS-C symptoms.

5.1.1.4 Follow-up Period

The Follow-up Period starts at the End of Treatment Visit (Visit 8) and finishes with the Follow-up Telephone Call on Day 36 (± 2), one week after the End of Treatment Visit (Visit 8). During this call, participants will be asked to report any adverse events (AEs) since the End of Treatment Visit (Visit 8) and detail any other symptoms or comments they may have.

Note: End of Study will be defined as the last participant Follow-up Telephone Call (Day 36 ± 2).

5.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

A double-blind, placebo-controlled, parallel-group study design was chosen in accordance with the concepts in ICH E10, Choice of Control Groups and Related Issues in Clinical Trials (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, 2001), in order to provide comparable treatment groups and minimal chance of selection or investigator bias. This study has a 7-day Pretreatment Period to establish baseline without therapy, followed by randomisation and a 29-day Treatment Period, and a 7-day Follow-up Period.

6. STUDY POPULATION

6.1 SELECTION OF STUDY POPULATION

Participants attending the IBS clinic at the study centre or those held in the database of Neurogastroenterology Unit will be recruited for participation in the study. The study centre must have a full medical history from each subject's GP within the last 12 months, prior to enrollment in the study and to ensure that the participant does not have any concomitant exclusionary disease. Each subject's GP will be informed of their participation in the study.

6.2 INCLUSION CRITERIA

To be eligible to participate in the study, participants must meet all of the following criteria:

1. Participant has provided written informed consent before participating in the study after being given a full description of the study and prior to any study-specific procedures being performed.
2. Participant is a male or non-pregnant, non-breastfeeding female, and is age 18 years or older (no upper age limit).
3. Female participants of childbearing potential must complete a urine pregnancy test with negative results at the Screening Visit (Visit 1) and again at the Randomisation Visit (Visit 4) prior to dosing.
4. Female participants who are pre-menopausal should be enrolled between days 7 and 21 of their menstrual cycle.
5. Sexually active female participants of childbearing potential must agree to use one of the following methods of contraception from the date that they sign the ICF until the end of study:
    a. Hormonal contraception (i.e., oral contraceptive, contraceptive implant, or injectable hormonal contraceptive);
    b. Intra-uterine device (IUD);
    c. A barrier birth control (such as condoms or occlusive cap with spermicidal foam/gel/film/cream/suppository);
    d. Surgical sterilisation (i.e., bilateral oophorectomy, hysterectomy, or tubal ligation);
    e. Abstinence, when in the opinion of the Investigator, the participant's occupation or lifestyle gives sufficient evidence that abstinence will be maintained throughout the study and for 1 month thereafter.

6. Participant meets Rome III criteria for IBS-C, as well as abdominal pain score (average of ≥ 3 on 0-10 point numerical rating scale [NRS]), abdominal bloating score (average of ≥ 5 on 0-10 point NRS), and abdominal girth measurement (AIP increase of > 4 cm) criteria during the Pretreatment Period.
7. Participant agrees to refrain from strenuous physical activity for 24 hours prior to each study visit.
8. Participant agrees to refrain from initiating any lifestyle or dietary changes.
9. Participant agrees to refrain from taking any probiotics from 14 days prior to enrolment in the study throughout the Treatment Period.
10. Participant is able to communicate well with the Investigator and to comply with the requirements for the entire study.
11. Participant has a body mass index (BMI) between 18.5 and 34.9 kg/m^2 (bounds included) at the Screening Visit.

6.3 EXCLUSION CRITERIA

Participants who meet any of the following criteria will not be eligible to participate in the study:

1. Participant reports loose (mushy) or watery stools (BSFS score of 6 or 7) in the absence of any laxative, suppository, enema, or prohibited medicine (as described in Appendix 1) for > 25% of BMs during the 12 weeks before the Screening Visit.
2. Participant has ever had a diagnosis of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or any other form of familial colorectal cancer, or inflammatory bowel disease.
3. Participant currently has both unexplained and clinically significant alarm symptoms (lower GI bleeding, iron-deficiency anaemia or any unexplained anaemia, or weight loss) or systemic signs of infection or colitis.
4. Participant currently has active peptic ulcer disease (i.e., disease that is not adequately treated or stable with therapy).
5. Participant has a history of any chronic GI condition (e.g., diverticulitis) that can be associated with abdominal pain or discomfort.
6. Participant has CNS disease or any organic condition associated with constipation.
7. Participant has had surgery that meets any of the following criteria:
   a. Has undergone any surgery within 6 months prior to the Screening Visit;
   b. Has had previous abdominal surgery except appendectomy, cholecystectomy, or hysterectomy.

8. Participant has diabetes.
9. Participant reports using a Prohibited Medicine (excluding laxatives, suppositories, and enemas) during the Pretreatment Period or is not willing or able to abide by the restrictions regarding use of Prohibited Medicines defined in Appendix 1. (Note: the use of fibre, bulk laxatives, or stool softeners [such as docusate] is acceptable provided that the participant has been on a stable dose during the 30 days before the Screening Visit and plans to continue on a stable dose throughout the study.)
10. Participant has taken commercially available linaclotide during the 30 days prior to the Screening Visit.
11. Participant is drinking alcohol above the recommended safe alcohol limit (< 21 units/week) or abusing drugs. In addition, participants will be asked to refrain from drinking alcohol for 24 hours before plethysmography recordings until belt removal.
12. Participant has lactose intolerance.
13. Participant has an acute or chronic condition that, in the Investigator's opinion, would limit the participant's ability to complete or participate in this clinical study.
14. Participant is involved in this study as an Investigator, sub-Investigator, study coordinator, other study staff, or Sponsor member.
15. Participant has a known or suspected mechanical gastrointestinal obstruction.
16. Participant has a history of hypersensitivity to linaclotide or to any of the excipients contained in the study drug (active or placebo).

7. STUDY TREATMENTS

7.1 TREATMENTS ADMINISTERED

Study drug in the form of oral capsules will be provided by Ironwood or designee. For the double-blind Treatment Period, participants will be supplied with identically appearing capsules containing 290 ug linaclotide, or matching placebo.

7.2 IDENTITY OF INVESTIGATIONAL PRODUCT

All study drug will be supplied in bottles containing thirty-five (35) capsules of linaclotide 290 ug or matching placebo. Bottles will be uniquely numbered and labelled in a double-blind fashion that conforms to Regulatory requirements.

All study drug will be provided by Ironwood or designee. Study drug will be stored at the study centre in an appropriate secure, temperature controlled area at 77°F (25°C), excursions permitted to 59-86°F (15-30°C). Any deviations from the storage conditions must be reported to Ironwood and use of the study drug suspended until authorisation for its continued use has been provided by Ironwood.

The Investigator must ensure that the receipt and use of all study drug supplied is recorded and supervise the storage and allocation of these supplies. All study drug supplies must be retained in a locked room that may only be accessed by the Investigator or other duly designated persons. Study drug must not be used outside the context of this protocol and under no circumstances should the Investigator or study centre personnel allow the supplies to be used other than as directed by this protocol without prior authorisation from Ironwood. All unused study drug must be returned to Ironwood's designee. Whenever study drug is returned, unit counts must be performed by study centre staff to ensure reliable drug accountability. Drug unit counts are documented on the appropriate CRF form. Prior to the end of the study, instructions for the return of all unused capsules and study drug bottles will be provided.

7.3 METHOD OF ASSIGNING PARTICIPANT TO TREATMENT GROUPS

Participants will be allocated to treatment using a computer-generated simple block randomisation method (with block sizes randomly varying between 2 and 8). The randomisation list will be derived prior to the start of the study and held by a person who will be independent of

the main study research group. Consecutively numbered opaque envelopes containing the appropriate allocation will be prepared in advance and used by the researcher in charge of recruitment.

7.4 SELECTION AND TIMING OF DOSE FOR EACH PARTICIPANT

All study drug will be administered orally as a single daily dose. Participants who meet all eligibility criteria at screening and pretreatment will be randomised to treatment at the Randomisation Visit and dispensed one bottle containing 35 capsules. Participants will be instructed to take one capsule in the morning at least 30 minutes before breakfast. Participants will take their initial dose of study drug (either one capsule of 290 ug linaclotide, or matching placebo) at the study centre during the Randomisation Visit. Should intolerable diarrhoea occur while taking study drug, the daily dose may be temporarily suspended until such time as the diarrhea resolves.

7.5 BLINDING

Both the participants and the research team will be blinded to randomisation and allocation of study drug/placebo.

In the scenario that the Investigator must unblind a participant, the Investigator will have access to individual sealed unblinding envelopes, which can be opened to identify the treatment assignment for an individual participant in an emergency. The reason for breaking the blind must be documented in the participant's CRF.

The Investigator, all other study personnel not previously noted, and the participant will remain blinded to the participant's treatment assignment throughout the study.

7.6 CONCOMITANT MEDICINES

At the Screening Visit, all ongoing medicines taken by the participant will be recorded. Any over-the-counter or prescription laxatives, suppositories, or enemas used to treat IBS-C may not be used on the calendar day before the Pretreatment Visit. Other prohibited medicines may not be used during the 14 calendar days before the Pretreatment Visit (refer to Appendix 1).

A complete list of drugs that are conditionally allowed as concomitant medicines for either episodic or chronic use or as Rescue Medicine is provided in Appendix 1. Prohibited concomitant medicines are also included in Appendix 1.

During the Pretreatment and Treatment Periods, participants may only use dispensed, protocol-defined laxatives (bisacodyl tablets or suppositories) as Rescue Medicine when at least 72 hours have passed since their previous BM or when their symptoms become intolerable. In order to qualify for randomisation into the Treatment Period, participants must not have used Rescue Medicine on the calendar day before the Randomisation Visit and on the day of the Randomisation Visit up until the time of the clinic arrival. Participants must agree to refrain from using Rescue Medicine from the time of the clinic arrival through the calendar day after randomisation. Furthermore, participants will be asked to refrain from using Rescue Medicine on the day before, day of, and day after each AIP measurement.

Any changes in concomitant medicines or new medicines added from the Screening Period through the Follow-up Period will be recorded on the CRF. Concomitant medicines will be recorded at all study visits throughout the entire study. Rescue Medicine will be documented by the participant on the Rescue Medicine record form.

7.7 TREATMENT COMPLIANCE

Study drug will be administered to the participant by study centre staff at the Randomisation Visit. For the other days in the Treatment Period, study drug will be taken by the participant once daily in the morning at least 30 minutes before breakfast. Study drug compliance by the participant will be closely monitored during the Treatment Period by counting the number of capsules returned and recording that information on the CRF. Every effort will be made to collect all unused study drug.

8. PROCEDURES

8.1 SCREENING PERIOD (DAY -21 TO DAY -8)

The Screening Period will begin with the signature of the ICF and will last for up to 14 calendar days. The end of the Screening Period will coincide with the beginning of the Pretreatment Period.

8.1.1 Screening Visit (Visit 1)

The Screening Visit will occur on Day -21. Participants will undergo the following assessments:

- Informed consent
- Check of inclusion and exclusion criteria
- Medical and surgical history
- Rome III status for IBS-C
- Physical examination
  - A physical examination should include the following: general appearance, HEENT (head, ears, eyes, nose and throat), neck, cardiovascular, thorax/lungs, abdomen, musculoskeletal, lymph nodes, skin, neurologic, and mental status). Rectal examination is not required; however, sigmoidoscopy/colonoscopy may be performed at the discretion of the Investigator.
- Body weight and height
- Seated vital signs
- Documentation of prior and concomitant medicines
- Collection of blood samples for clinical laboratory determinations (complete blood count, chemistry), if lab results obtained within 30 days prior to Visit 1 are not available.
- Urine pregnancy test (result must be confirmed as negative in order to qualify for study participation)
- Completion of the HADS (Appendix 3) and IBS-SSS (Appendix 4)
- Instructions for participants to discontinue use of all laxatives, suppositories, and/or enemas at least one calendar day before the first day of the Pretreatment Period, and all other restriction and prohibited medicines as defined in Appendix 1

8.2 PRETREATMENT PERIOD (DAY -7 TO DAY -1)

The Pretreatment Period will be the 7 days immediately before the Randomisation Visit. The purposes of this period are to collect data to determine whether the participant is eligible to continue into the Treatment Period of the study, to provide the participant with experience using the data collection methods employed during the study (e.g., daily diary, Digestive Sensations Questionnaire), and to provide baseline data for comparison with data collected during the Treatment Period.

8.2.1 Pretreatment Visit (Visit 2)

The Pretreatment Visit will occur on Day -7. Participants will undergo the following assessments:

- Documentation of concomitant medicines
- Review of AEs, from the signing of the ICF to Visit 2
- Rescue Medicine dispensed; review with participant how Rescue Medicine should be used
- Delivery of daily diary (vol. 1); diary training and start of daily recording

8.2.2 Baseline Visit (Visit 3)

The Baseline Visit will occur on Day -1. Participants will undergo the following assessments:

- Documentation of concomitant medicines
- Review of AEs
- Diary compliance check and continued daily recording
- Distribution of Digestive Sensations Questionnaire and instructions for completion
- Participants fitted with the AIP belt (the schedule of this visit should be adjusted in the following instances):
    - Women who are actively menstruating during AIP belt placement should have their visits delayed for 48 hours.
    - Participants who have taken Rescue Medicines within 24 hours of AIP belt placement should have their visits delayed until 24 hours have passed since the use of Rescue Medicine.

- Participants who have had study drug suspended due to diarrhea should have their AIP belt placement visits delayed until study drug has been resumed for 24 hours.

8.3 TREATMENT PERIOD (DAY 1 TO DAY 29)

The Treatment Period begins with randomisation on Day 1 and lasts for 29 (±2) days, through the End of Treatment Visit (Visit 8) on Day 27 (±2). The end of the Treatment Period will coincide with the beginning of the Follow-up Period.

8.3.1 Randomisation Visit (Visit 4)

The Randomisation Visit will occur on Day 1. Participants will undergo the following assessments:

- Verification of inclusion and exclusion criteria
- Medical and surgical history
- Physical examination:
  - A physical examination should include all assessments: general appearance, HEENT, neck, cardiovascular, thorax/lungs, abdomen, musculoskeletal, lymph nodes, skin, neurologic, and mental status. Rectal examination is not required; however, sigmoidoscopy/colonoscopy may be performed at the discretion of the Investigator.
- Body weight
- Seated vital signs
- Documentation of concomitant medicines
- Urine pregnancy test (result must be confirmed as negative in order to qualify for study participation)
- Review of AEs
- Rescue Medicine dispensed, as needed; review with participant how Rescue Medicine should be used
- Diary collection (vol. 1), delivery of daily diary (vol. 2), and continued daily recording
- Collection of Digestive Sensations Questionnaire
- Data from the AIP belt downloaded
- Randomisation

- Delivery and administration of study drug

8.3.2 Day 13 Visit (Visit 5)

The Day 13 Visit will occur on Day 13 (± 2 days). Participants will undergo the following assessments:

- Documentation of concomitant medicines
- Review of AEs
- Diary compliance check and continued daily recording
- Distribution of Digestive Sensations Questionnaire and instructions for completion
- Participants fitted with the AIP belt: (the schedule of this visit should be adjusted in the following instances):
  - Women who are actively menstruating during AIP belt placement should have their visits delayed for 48 hours.
  - Participants who have taken Rescue Medicines within 24 hours of AIP belt placement should have their visits delayed until 24 hours have passed since the use of Rescue Medicine.

8.3.3 Day 15 Visit (Visit 6)

The Day 15 Visit will occur on Day 15 (± 2 days). Participants will undergo the following assessments:

- Body weight
- Seated vital signs
- Documentation of concomitant medicines
- Review of AEs
- Rescue Medicine dispensed, as needed; review with participant how Rescue Medicine should be used
- Diary collection (vol. 2), delivery of daily diary (vol. 3), and continued daily recording
- Collection of Digestive Sensations Questionnaire

- Data from the AIP belt downloaded
- Study drug accountability

8.3.4 Day 27 Visit (Visit 7)

The Day 27 Visit (Visit 7) will occur on Day 27 (± 2 days). Participants will undergo the following assessments:

- Documentation of concomitant medicines
- Review of AEs
- Diary compliance check and continued daily recording
- Distribution of Digestive Sensations Questionnaire and instructions for completion
- Participants fitted with the AIP belt: (the schedule of this visit should be adjusted in the following instances):
  - Women who are actively menstruating during AIP belt placement should have their visits delayed for 48 hours.
  - Participants who have taken Rescue Medicines within 24 hours of AIP belt placement should have their visits delayed until 24 hours have passed since the use of Rescue Medicine.

8.3.5 End of Treatment Visit (Visit 8)

The End of Treatment Visit (Visit 8) will occur on Day 28 (± 2 days). Participants will undergo the following assessments:

- Physical examination
  - A physical examination should include all assessments: general appearance, HEENT, neck, cardiovascular, thorax/lungs, abdomen, musculoskeletal, lymph nodes, skin, neurologic, and mental status. Rectal examination is not required; however, sigmoidoscopy/colonoscopy may be performed at the discretion of the Investigator.
- Body weight
- Seated vital signs
- Documentation of concomitant medicines

- Urine pregnancy test
- Review of AEs
- Diary daily recording and collection (vol. 3)
- Collection of Digestive Sensations Questionnaire
- Data from the AIP belt downloaded
- Study drug accountability

8.4 FOLLOW-UP PERIOD (DAY 29 TO DAY 35)

The Follow-up Period starts at the end of the End of Treatment Visit (Visit 8) and finishes on Day 36 (± 2), one week after the End of Treatment Visit.

8.4.1 Follow-up (Telephone Call)

The Follow-up Telephone Call will occur on Day 36 (± 2). During this call, participants will be asked to report any AEs since the End of Treatment Visit (Visit 8) and detail any other symptoms or comments they may have.

9. STUDY OUTCOME ASSESSMENTS

9.1 EFFICACY MEASUREMENTS

9.1.1 Primary Efficacy Assessment

Mean change from randomisation (Visit 4) to the end of the study (Visit 8) in abdominal girth (as measured by AUC) determined by 24-hour AIP between the linaclotide and placebo groups.

Data on abdominal girth will be downloaded and analysed from the data logger by a blinded member of the research team when participants return with the belt at the Randomisation, Day 15, and End of Treatment Visits.

Comparisons of the mean change in abdominal girth over 24 hours (as measured by AUC) will be made between the baseline and mid-study, and baseline and end-of-treatment assessments.

9.1.2 Secondary Efficacy Assessments and Additional Efficacy Assessments

Daily Diary

The secondary efficacy assessments are the diary questions that determine the following:

- Daily participant assessment of abdominal pain, discomfort, bloating, and distension;
- Whether a BM is an SBM;
- Whether an SBM is a CSBM;
- Stool consistency (Bristol Stool Form Scale [BSFS]) with each BM.

To determine these secondary outcome measures, participants will be asked to complete paper daily diaries in the evening (or approximately the same time each day) recording:

- Daily Participant Assessment of Bowel Habits:

  "Have you taken any laxatives, suppositories, or enemas since yesterday?"

  1=Yes

  2=No

  "If yes, which rescue medicine did you take?"

  1=Oral Bisacodyl

  2=Bisacodyl suppository

  3=Other laxatives, suppositories, or enemas

  "How many bowel movements did you have since yesterday?"

  "Did you feel like you completely emptied your bowels?"

  1=Yes

  2=No

  "Please refer to the laminated Bristol Stool Form Scale given to you. Please describe the consistency of the bowel movement using the following scale where:"

  1=Separate hard lumps like nuts (difficult to pass)

  2=Sausage shaped but lumpy

  3=Like a sausage but with cracks on surface

  4=Like a sausage or snake, smooth and soft

  5=Soft blobs with clear-cut edges (passed easily)

  6=Fluffy pieces with ragged edges, a mushy stool

  7=Watery, no solid pieces (entirely liquid)

- Daily Participant Symptom Severity Assessments

  "How would you rate your abdominal pain at its worst over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal pain and 10 represents very severe abdominal pain."

"How would you rate your abdominal discomfort at its worst over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal discomfort and 10 represents very severe abdominal discomfort."

"How would you rate your abdominal bloating at its worst over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal bloating and 10 represents very severe abdominal bloating."

"How would you rate your abdominal distension at its worst over the last 24 hours? Enter a number from 0 to 10, where 0 represents no abdominal distension and 10 represents very severe abdominal distension."

Digestive Sensations Questionnaire

A Digestive Sensations Questionnaire (Appendix 2) will be used to record abdominal pain, discomfort, bloating, distension, and overall IBS symptoms on an hourly basis (waking hours only) during the 24 hours the participants are fitted with the AIP belt, using an 11-point NRS. Participants will be asked to record the following:

"How would you rate your abdominal pain over the last hour? Circle a number from 0 to 10, where 0 represents no abdominal pain and 10 represents very severe abdominal pain."

"How would you rate your abdominal discomfort over the last hour? Circle a number from 0 to 10, where 0 represents no abdominal discomfort and 10 represents very severe abdominal discomfort."

"How would you rate your abdominal bloating over the last hour? Circle a number from 0 to 10, where 0 represents no abdominal bloating and 10 represents very severe abdominal bloating."

"How would you rate your abdominal distension over the last hour? Circle a number from 0 to 10, where 0 represents no abdominal distension and 10 represents very severe abdominal distension."

"How would you rate your overall IBS symptoms over the last hour? Circle a number from 0 to 10, where 0 represents no IBS symptoms and 10 represents very severe IBS symptoms."

9.2 SAFETY OUTCOME ASSESSMENTS

9.2.1 Adverse Events

All participants will be monitored for AEs and use of concomitant medicines throughout the study. All AEs and concomitant medicines will be recorded in accordance with the procedures outlined in Section 10.

9.2.2 Clinical Laboratory Determinations

Clinical laboratory tests will be performed at the Screening Visit if lab results obtained within the prior 30 days are not available.

The following clinical laboratory tests will be performed:

- **Haematology:** Absolute white blood cell count, erythrocyte count, haemoglobin, haematocrit, platelet count, and red blood cell indices (mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration)
- **Chemistry:** Sodium, magnesium, potassium, calcium, chloride, glucose, blood urea nitrogen, creatinine, total protein, alkaline phosphatase, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, bicarbonate, phosphate, and cholesterol
- **Pregnancy test:** Urine human chorionic gonadotropin pregnancy test will be conducted for all females at the Screening Visit and other visits specified in the Schedule of Evaluations. A negative urine pregnancy test for females of childbearing potential must be documented at the Randomisation Visit for the participant to be eligible for randomisation and dosing with study drug. Positive results on the pregnancy test will exclude a participant from participating or continuing in the study.

9.2.3 Vital Signs

Vital sign measurements will be performed at the Screening Visit and other visits defined in the Schedule of Evaluations with the participants in the seated position and documented. The parameters are oral temperature, pulse rate, respiratory rate, and systolic and diastolic blood

pressure (BP). Pulse and BP readings will be taken after the participant has been sitting for five minutes.

9.2.4 Physical Examination

A complete physical examination will be performed at the Screening Visit and other visits defined in the Schedule of Evaluations by the Investigator or a licensed health professional. Any physical examination abnormality that the Investigator considers to be potentially clinically significant and changed from the baseline will be reported as an AE.

9.2.5 Medical History

A complete medical history will be provided by the participant at the Screening Visit and the Randomisation Visit.

9.2.6 Appropriateness of Measurements

The safety assessments to be conducted during this study, including monitoring for AEs, physical examination findings, vital signs parameters, and clinical laboratory measures, are widely used and generally recognised as reliable, accurate, and relevant.

9.3 CONCOMITANT MEDICINES

Restrictions regarding concomitant medicines are described in Section 6.3. All concomitant medicines taken from the time of informed consent at the Screening Visit through the End of Treatment Visit will be recorded on the appropriate concomitant medicine page of the participant's CRF.

10. ADVERSE EVENTS

10.1 DEFINITIONS

10.1.1 Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigational subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE, therefore, can be any unfavourable and unintended sign (including a clinically significant abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE includes, but is not limited to, the following:

- Any unfavourable changes in general condition;
- Any clinically significant worsening of a preexisting condition;
- Any intercurrent diseases and accidents.

NOTE: A procedure is not an AE, but rather the reason for the procedure is the AE.

10.1.2 Serious Adverse Event

An SAE is defined as any AE occurring at any dose that results in any of the following outcomes:

- Death;
- Life-threatening: the patient was at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction that hypothetically might have caused death if it had occurred in a more severe form);
- Hospitalisation or prolongation of existing hospitalisation;
- Persistent or significant disability/incapacity: a substantial disruption of a person's ability to conduct normal daily functions;
- Congenital anomaly/birth defect;

- Important medical events: events that may not result in death, be life threatening, or require hospitalisation. Such an event may be considered serious when, based on appropriate medical judgement, it may jeopardise the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency department or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalisation, or the development of drug dependency or drug abuse.

Clarification should be made between the terms "serious" and "severe" since these 2 terms ARE NOT synonymous. The term "severe" is used to describe the *intensity* (severity) of a specific event (as in mild, moderate, or severe myocardial infarction) (see Section 10.4.1); the event itself, however, may be of relatively minor medical significance (such as a severe headache). "Serious" is based on the patient/event *outcome or action* criteria described above and is usually associated with events that pose a threat to a patient's life or functioning. A **severe** AE does not necessarily need to be considered **serious**. For example, persistent nausea of several hours duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

10.2 PROCEDURES FOR RECORDING ADVERSE EVENTS

Adverse events will be collected and recorded from the time the participant signs the ICF at the Screening Visit through completion of the Follow-up Telephone Call. All AEs, regardless of the assumption of a causal relationship with study procedures or study drug, must be recorded on the appropriate AE page of the participant's CRF. This record includes AEs the participant reports spontaneously, those observed by the Investigator, and those elicited by the Investigator in response to open-ended questions such as, "Have you had any unusual signs or symptoms since your last visit?" during the study.

Any medical condition that is present when a participant is screened and does not deteriorate in severity and/or frequency should be reported as Medical History and not as an AE. However, if the condition does deteriorate in severity and/or frequency at any time during the study, it should be reported as an AE.

All AEs will be followed until resolution, until the event has stabilised, or until the participant is lost to follow-up.

10.3 PROCEDURES FOR REPORTING SERIOUS ADVERSE EVENTS

An AE that meets any of the serious criteria must be reported to Ironwood within 24 hours from the time that study centre personnel first learn of the event, using the SAE Report form provided for the study. Regardless of causality, all SAEs must be reported and will be collected and recorded from the time the participant signs ICF at the Screening Visit until the Follow-up Telephone Call. All SAEs must also be recorded on the AE page of the participant's CRF.

The initial report should include at least the following information:

- Participant's study number;
- Description and onset of the event;
- Serious criteria;
- Causality assessment to study drug.

Special Situation: Exposure to Study Drug during Pregnancy

In the event that a pregnancy occurs in a participant, the study drug must be stopped at once, and study personnel must report the pregnancy as soon as possible (within 24 hours after notification) on the pregnancy notification form provided for this study. The study personnel must follow the pregnancy until the end and report the pregnancy outcome on the pregnancy outcome form provided for this study. If the pregnancy is associated with an SAE (e.g., if the mother is hospitalised for dehydration), a separate SAE form must be completed.

All relevant SAE or pregnancy information should be emailed to Ironwood Drug Safety and Pharmacovigilance.

All SAE Report Forms should be emailed to:

Clinical Drug Safety & Pharmacovigilance
Ironwood Pharmaceuticals, Inc.
clinicalsafety@ironwoodpharma.com
Fax: 1-617-933-7688

If follow-up is obtained, or requested by Ironwood, the additional information should be emailed on a SAE Report Form to Ironwood, in a timely manner according to the procedures outlined above. Copies of discharge summaries, consultant reports, autopsy reports, and any other relevant documents may also be requested.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgement to treat the SAE. These measures and the participant's response to these measures should be recorded. All SAEs regardless of relationship to study drug will be followed by the Investigator until satisfactory resolution, until the Investigator deems the SAE to be chronic or stable, or until the participant is lost to follow-up. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

The Investigator will be responsible for reporting all SAEs to Ironwood within 24 hours of learning of the event. The Investigator will provide timely and accurate reports to the REC on the progress of the study, and will notify the REC and the Sponsor of SAEs or other significant safety findings in a manner consistent with REC policies and ICH and GCP requirements. Ironwood shall keep detailed records of all SAEs that are reported by the investigator for future queries, if requested. Ironwood will also be responsible for reporting any suspected unexpected serious adverse reactions as soon as possible (but within a maximum of 7 days for suspected unexpected serious adverse reactions that are fatal or life-threatening, and 15 days for all other suspected unexpected serious adverse reactions) to the regulatory authorities and ensuring all SAEs are reported to the REC.

10.4 RECORDING REQUIREMENTS

10.4.1 Severity

The Investigator will provide an assessment of the severity of each AE by recording a severity rating on the AE page of the participant's CRF. *Severity,* which is a description of the intensity of manifestation of the AE, is distinct from *seriousness,* which implies a participant outcome or AE-required treatment measure associated with a threat to life or functionality. Severity will be assessed according to the following scale:

Mild: A type of AE that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

Moderate: A type of AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.

Severe: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

10.4.2 Causality

For all AEs, the Investigator must provide an assessment of causal relationship to study drug. The causality assessment must be recorded on the AE page of the participant's CRF. Causal relationship must be assessed according to the following:

- **Related**: An event where there is a reasonable possibility of a causal relationship between the event and the study drug;
- **Unrelated:** Any other event.

10.4.3 Laboratory Abnormalities

All of the following laboratory abnormalities should be captured as AEs:

- Any laboratory test result that meets criteria for an SAE;
- Any laboratory abnormality that resulted in study discontinuation;

- Any laboratory abnormality that required the participant to receive specific corrective therapy;
- Any laboratory abnormality that the Investigator considers to be clinically significant.

Ongoing abnormal laboratory values/conditions that are being treated at baseline will be captured as an AE if the abnormality increases in severity and/or frequency while on study or if the abnormality requires more frequent treatment. If a participant is treated for an abnormal laboratory value just before the Screening Visit, then the medical history should reflect the severity of the abnormality before treatment.

10.5 UNBLINDING

In the scenario that the Investigator must unblind a participant, the Investigator will have access to individual sealed unblinding envelopes, which can be opened to identify the treatment assignment for an individual participant in an emergency. The reason for breaking the blind must be documented in the participant's CRF.

The Investigator, all other study personnel not previously noted, and the participant will remain blinded to the participant's treatment assignment throughout the study.

10.6 TERMINATION OF PARTICIPANTS

Participants will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The Investigator will make reasonable efforts to keep each participant in the study. However, if the Investigator removes a participant from the study or if the participant declines further participation, the evaluations required at the EOT Visits should be performed if possible. All evaluations and observations, together with the description of the reason(s) for study withdrawal, must be recorded on the appropriate page of the participant's CRF.

The following are justifiable reasons for the Investigator to withdraw a participant from the study:

- The occurrence of an AE;
- Withdrawal of consent;
- Unforeseen events: any event that, based on the Investigator's judgement, makes further treatment inadvisable;
- Serious violation of the study (including persistent participant attendance failure and non-compliance).

An effort must be made to determine why a participant fails to return for the necessary visits or is dropped from the study. Regardless of the reason for termination, all data available for the participant at the time of discontinuation of follow-up should be recorded where applicable on the CRF. All reasons for discontinuation of treatment should be documented.

11. STATISTICAL METHODS

Details regarding the statistical methods will be provided in the Statistical Analysis Plan (SAP), to be finalised before unblinding of the study.

11.1 DETERMINATION OF SAMPLE SIZE

A power calculation was used to determine how many participants will be required, as can be seen below. A total of 40 (20 in each group) participants are required to complete the study, with 120 needed to enter screening to account for ineligible participants and dropouts.

To achieve 80% power to detect a difference of 2.2 cm or more (using a simple 2-sided t-test with estimated SD = 2.4 cm and the conventional 5% significance level) in the mean change in abdominal girth (as measured by AIP AUC) from the start to the end of the day, between the active and placebo groups, 20 participants per group (40 participants in all) are required. Assuming a 15% dropout rate, 47 participants would need to be randomised to yield 40 evaluable participants. Estimating a 60% screen/pretreatment failure rate (mostly from failure to meet abdominal girth criteria, but also pretreatment abdominal pain and abdominal bloating criteria), 120 participants may need to enter screening to achieve 40 randomised participants.

11.2 ANALYSIS POPULATIONS

11.2.1 ITT Population

The Intent-to-treat (ITT) Population will consist of all participants randomised to a treatment group at the Randomisation visit (visit 4).

11.2.2 Safety Population

The Safety Population will consist of all randomised participants who received at least one dose of study drug.

11.2.3 Evaluable Population

The Evaluable Population will consist of all participants included in the Safety population presenting no major protocol deviations with the potential to bias the criteria for evaluation and with full follow-up.

11.3 STATISTICAL ANALYSES

11.3.1 General Considerations

A more detailed description of the statistical methodology will be given in the Statistical Analayis Plan which will be finalised before unblinding of data.

Distribution of study parameters and covariates will be summarised depending on the type of variable with the number of subjects and the number of missing data (where relevant).

Verification of the normality of the distribution for continuous data will be carried out by graphical inspection and consideration of skewness and kurtosis measures.

Descriptive statistics will be presented as;

- Qualitative data: number and percentage of participants in each category
- Ordinal qualitative data: number and percentage of participants in each category, median, interquartiles, range
- Continuous normally distributed data: number, mean, standard deviation, range
- Continuous non-normally distributed data: number, median, interquartiles, range

All statistical comparison tests will be performed using the conventional two-sided significance level of 5%.

11.3.2 Disposition, Demographics, and Baseline Characteristics

The number of screen failures (i.e., participants who entered the Screening Period but not the Pretreatment Period) and the number of pretreatment failures (i.e., participants who entered the Pretreatment Period but were not randomised) will be tabulated by reason for failure.

The overall number of participants screened and the number of participants randomised to each treatment group will be presented. The number and percentage of participants included in each of the analysis populations (ITT, Safety, and Evaluable) will be presented by treatment group and overall. The number and percentage of participants who completed the study or discontinued early, including the reason for discontinuation, will be presented by treatment group and overall.

Participant demographics (age, sex, race, ethnicity, weight, height, and BMI [defined as weight in kg divided by height in meters squared]) and other relevant baseline characteristics will be summarised by treatment group for the ITT Population.

11.3.3 Efficacy Analyses

Assessment of efficacy of treatment will be based on changes between baseline data provided at the Randomisation Visit (Visit 4) and subsequent study visits. Linear interpolation (for the 24 hr recorded girth data) or multiple imputation methods will be used to account for missing outcome data where appropriate . All analyses will be carried out on the ITT, Safety and Evaluable Populations.

11.3.3.1 Primary Efficacy Endpoint

Change from Baseline in Abdominal Girth at Week 4

The mean change from randomisation (Visit 4) to the end of the study (Visit 8) in abdominal girth (as measured by AUC) determined by 24-hour AIP (with hourly averages), between the linaclotide and placebo groups.

The AUC will be calculated using the Trapezoidal method from the first reliable hour of measurement to last measurement (bedtime). The AUC for each patient will then be individually standardised by dividing the total AUC over the period by that patient's number of hours of measurement included in the AUC.

In case of missing data in the middle of the valid period of measurement, AUC is calculated with linear interpolation.

11.3.3.2 Primary Efficacy Analysis

Changes in girth from the first hour of the 24-hour AIP at Visit 8 will be compared between the linaclotide and placebo groups with the AUC measure, adjusting for the corresponding changes in girth at Visit 4 and any other important confounding factors (such as age and BMI), using analyses of covariance.

11.3.3.3 Secondary Efficacy Endpoints

The secondary efficacy endpoints are as follows:

Change from Baseline in Abdominal Distension at Week 2

The mean change from randomisation (Visit 4) to (Visit 6) in abdominal girth (as measured by AUC) determined by 24-hour AIP, between the linaclotide and placebo groups.

Change from Baseline in Maximal Abdominal Distension at Week 4

The maximum change in girth from the first hour, over the period from the 2nd hour to bedtime. The percentage change in maximum distension from baseline to 4 weeks will also be calculated.

Change from Baseline of symptom severity (abdominal pain, discomfort, bloating, and distension)

Daily diary scores for each of the digestive symptoms and a composite score of these parameters will be averaged to obtain 'weekly' scores as follows;

Baseline = mean (day -7 to day -1)
Week 1 = mean (day 1 to day 7)
Week 2 = mean (day 8 to day 14)
Week 3 = mean (day 15 to day 21)
Week 4 = mean (day 22 to day 28)

Change from Baseline in Digestive sensations (subjective bloating, abdominal discomfort, abdominal distension and abdominal pain) at Weeks 4 and 8

The mean change from randomisation (Visit 4) to Visit 6 and from randomisation to Visit 8 in

each of the hourly measures of digestive sensations (as measured by AUC), between the linaclotide and placebo groups.

Change from Baseline of Bristol Stool Form Scale at Weeks 4 and 8

Daily diary BSFS scores will be recoded to represent discrepancies from a 'normal' stool type 4 as follows:

- Type 1 – coded 3
- Type 2 – coded 2
- Type 3 – coded 1
- Type 4 – coded 0
- Type 5 – coded 1
- Type 6 – coded 2
- Type 7 – coded 3

Hence a higher score represents greater discrepancy from a 'normal' stool consistency.

Daily average recoded BSFS scores for each subject will be computed by averaging over the first 1, 2 or 3 stools as appropriate. For a subject with no stools on a particular day then their BSFS daily average BSS for that day is denoted as 'missing'.

The following means will be calculated for each subject:

- Baseline = mean (day -7 to day -1)
- Week 1 = mean (day 1 to day 7)
- Week 2 = mean (day 8 to day 14)
- Week 3 = mean (day 15 to day 21)
- Week 4 = mean (day 22 to day 28)

11.3.3.4 Secondary Efficacy Analyses

Change from Baseline in Abdominal Distension at Week 2

Changes in girth from the first hour of the 24-hour AIP at Visit 6 will be compared between the linaclotide and placebo groups with the AUC measure, adjusting for the corresponding changes

in girth at Visit 4 and any other important confounding factors (such as age and BMI), using analyses of covariance.

Change from Baseline in Maximal Abdominal Distension at Week 4

Changes in maximal abdominal girth will be compared between the linaclotide and placebo groups, adjusting for the corresponding changes in girth at Visit 4 and any other important confounding factors (such as age and BMI), using analyses of covariance.

The percentage change in maximal distension will be compared using a simple t-test or Mann-Whitney U-test as appropriate.

Change from Baseline of symptom severity (abdominal pain, discomfort, bloating, and distension)

Each of the digestive symptoms and the composite score will be compared between the linaclotide and placebo groups over the study period using longitudinal regression analysis with generalised estimating equations on the weekly scores.

Change from Baseline in Digestive sensations at Weeks 4 and 8

Changes in each of the digestive sensation scores at Visit 6 and Visit 8 separately, will be compared between the linaclotide and placebo groups with the AUC measure over the period hour 1 to bedtime, adjusting for the corresponding measures at Visit 4 and any other important confounding factors (such as age and BMI), using analyses of covariance.

Change from Baseline of Bristol Stool Form Scale at Weeks 4 and 8

The weekly average BSS recoded scores will be compared between the linaclotide and placebo groups over the study period using longitudinal regression analysis with generalised estimating equations on the weekly scores.

11.3.3.5 Additional Analysis

The relationship between the hourly digestive sensation scores for bloating and the corresponding hourly difference in girth measures from hour 1 over the relevant time period

during each of the two 24-hour periods at visit 4 and visit 8 will be assessed using longitudinal regression analysis.

11.3.4 Safety Analyses

Adverse events will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) available at the start of the study. TEAEs are those AEs that started or worsened in severity after the administration of study drug. TEAEs will be summarised by system organ class (SOC) and preferred term (PT) for each treatment group. In addition, listings of severe TEAEs, drug-related AEs, SAEs, AEs leading to study discontinuation, and AEs leading to death (if any) will be provided.

Vital signs and clinical laboratory evaluations at each assessment time point and the change from baseline at each post-dose time point will be summarised for each treatment group.

11.4 COMPUTER METHODS

Statistical analyses will be performed using SPSS, version 22.0 and STATA version 13 (or newer).

12. STUDY OPERATIONS

12.1 GOOD CLINICAL PRACTICE

This study will be conducted according to the protocol and in compliance with ICH GCP, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

The Investigator confirms the above by signing the protocol.

12.2 FINANCIAL DISCLOSURE

Relevant financial disclosures will be included in the submission to the ethical committee.

12.3 ESSENTIAL DOCUMENTS

1. Current, signed curricula vitae of the Investigator and all sub-Investigators;
2. Copy of current medical license of the Investigator and all sub-Investigators (as applicable);
3. Copy of REC approved Participant information sheet;
4. Evidence of Sponsor insurance or indemnity;
5. Copy of the REC approval letter for the protocol and informed consent;
6. Copy of the REC-approved informed consent document to be used;
7. Copy of the REC approval of recruitment advertising (if applicable);
8. A list of REC members and their qualifications, and a description of the committee's working procedures.

During the study, the Investigator must maintain the following essential/administrative documents related to the study:

1. Copy of the signed Protocol Signature Page;
2. Copy of financial disclosure form(s) for the Investigator and all sub-Investigators;
3. Curricula vitae of any new Investigator(s) and/or sub-Investigators involved in the study;
4. SAE Reports;
5. Drug Inventory Forms (drug receipts, drug dispensing and inventory forms);
6. Name and address of local or central laboratory, list of normal laboratory values and units of measurements, as well as laboratory certification or hospital accreditation;
7. Updates of medical/laboratory/technical procedures/tests:

c. Normal value(s)/range(s),
d. Certification,
e. Accreditation,
f. Established quality control and/or external quality assessment,
g. Other validation (where required);

8. Record of retained body fluids/tissue samples (if any);
9. Correspondence with the Sponsor;
10. Responsibility Log;
11. Other logs (screening, enrolment, etc.);
12. Signed ICFs;
13. Participant diaries;
14. Validated questionnaires;
15. CRFs;
16. Master randomisation list.

12.4 GENERATION OF STUDY RECORDS

Ironwood, or its designated representative, will oversee the conduct of a Study Centre visit to verify the qualifications of each Investigator, inspect study centre facilities, and inform the Investigator of responsibilities and procedures for ensuring adequate and correct study documentation.

The Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. For this study, information recorded on the CRFs is considered to be the participant's source documentation.

12.5 CASE REPORT FORMS AND DATA MANAGEMENT

All data relating to the study will be recorded in the participant's CRF. The CRFs are to be completed at the time of the participant's visit, except for results of tests performed outside the Investigator's office. CRF data should indicate the participant's participation in the study and should document the dates and details of study procedures, AEs, all observations, and participant status. The Investigator is responsible for verifying that all data entries on the CRFs are accurate and correct and ensure that all data are entered in a timely manner, as soon as possible after information is collected. An explanation should be provided for all missing data. The Investigator must provide his or her formal approval of all the information on the CRFs and changes to the CRFs to endorse the final submitted data for the participants for which he or she is responsible.

The CRFs will be in the form of paper and will be stored initially in locked filing cabinets in the Neurogastoenterology unit and subsequently archived. A paper copy will be made of each CRF for retention by Ironwood.

A record of participant screen failures will be maintained for participants who do not qualify for enrolment, including the reason for the failure.

12.6 STUDY MONITORING

Ironwood, or its designee, may perform quality control and assurance checks as per the study monitoring plan. Before enrolling any participants into this study, Ironwood personnel, and/or a trained designated representative, will review the following with the Investigator: the protocol, the Investigator's Brochure, the United States Package Insert (USPI), the CRFs and instructions for their completion, the procedure for obtaining informed consent, and the procedure for reporting AEs and SAEs. The Clinical Site Monitor will ensure that the investigation is conducted according to the protocol and regulatory requirements and will maintain current personal knowledge of the study through observation, review of study records, and discussion of the conduct of the study with the Investigator, sub-Investigators, and staff.

All aspects of the study will be overseen by Ironwood, or its designee for compliance with applicable government regulations in respect to GCP and current standard operating procedures.

12.7 CHANGE IN INVESTIGATOR

If any Investigator retires, relocates, or otherwise withdraws from conducting the study, the responsibility for maintaining records may be transferred to another person (Ironwood, REC, or other Investigators) who will accept the responsibility. Ironwood must be notified of and agree to the change.

12.8 RECORD RETENTION AND ARCHIVING

Records and documents pertaining to the conduct of this study, including CRFs, consent forms, laboratory test results, and study drug inventory records, will be retained until Ironwood no longer need the data for any commercial purposes. No study records shall be destroyed without notifying Ironwood and giving Ironwood the opportunity to take such study records or authorising in writing the destruction of records after the required retention period.

12.9 REPORTING AND PUBLICATION POLICY

All data generated in this study will be the property of Ironwood. An integrated clinical and statistical report will be prepared at the completion of the study.

Publication of the results by the Investigator will be subject to mutual agreement between the Investigator and Ironwood.

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14. APPENDICES

APPENDIX 1 CONCOMITANT MEDICINES

Rescue Medicine

Rescue Medicine, which will be selected by the Investigator and dispensed to participants, will be a choice of 5-mg bisacodyl tablets or 10-mg bisacodyl suppositories. During the Pretreatment and Treatment Periods, participants may use dispensed, protocol-permitted laxatives (bisacodyl tablets or suppositories) as Rescue Medicine when at least 72 hours have passed since their previous BM or when their symptoms become intolerable. In order to qualify for randomisation into the Treatment Period, participants must have refrained from using Rescue Medicine on the calendar day before the Randomisation Visit and on the day of the Randomisation Visit up until the time of the clinic visit. Participants must agree to refrain from using Rescue Medicine from the time of the clinic visit through the calendar day after the Randomisation Visit and 24 hours prior to any visit where participants will be fitted with the AIP belt (Visit 3, Visit 5, and Visit 7). If a Rescue Medicine has been used within 24 hours of a scheduled AIP belt placement visit, the visit should be postponed until 24 hours after the administration of the rescue medicine.

Prohibited Medicines

All medicine listed in the sections below are excluded from the Screening Visit for the duration of the study. Please note the specific restriction windows for detailed instructions.

1. Drugs with known pharmacological activity at 5-hydroxytryptophan (HT)4, 5-HT2b or 5-HT3 receptors (e.g., cisapride, tegaserod, ondansetron, tropisetron, granisetron, dolasetron, and mirtazapine).
2. Any treatment specifically taken for IBS-C or CIC alone or in combination, including lubiprostone, an approved chloride channel activator that enhances intestinal fluid secretion, colchicine, and misoprostol.
3. Prokinetic agents (e.g., metoclopramide, itopride, and domperidone).
4. Anti-cholinergic agents (e.g., dicyclomine, flavoxate, scopolamine, hyoscyamine, propantheline, oxybutynin, tolterodine, solefenacin, darifenacin, and trospium). Note: inhaled ipratropium and tiotropium are permitted.
5. Bile acid sequestrants (e.g., cholestyramine and colestipol).
6. Cholinomimetic agents (e.g., bethanechol, pyridostigmine, tacrine, and physostigmine). Note: intraocular cholinomimetic agents (e.g., pilocarpine) are permitted.

7. Antipsychotic agents (e.g., risperidone, haloperidol, droperidol, chlorpromazine, perphenazine, all phenothiazines, quetiapine, olanzapine, and clozapine) unless the participant has been on a stable dose for 30 days before the Screening Visit and there is no plan to change the dose after the Screening Visit. Note: paliperidone is permitted without restriction.
8. Antidepressants unless the participant has been on a stable dose for at least 3 months before the Screening Visit and there is no plan to change the dose after the Screening Visit. Specifically included are the following:
   - Tricyclic antidepressants (e.g., amitriptyline, imipramine, and nortriptyline)
   - Monoamine oxidase inhibitors (e.g., furazolidone, isocarboxazid, pargyline, phenelzine, and selegiline tranylcypromine)
   - Selective serotonin reuptake inhibitors (e.g., fluoxetine, sertraline, paroxetine, and citalopram)
   - Serotonin and norepinephrine reuptake inhibitors (e.g., venlafaxine and desvenlafaxine succinate)
   - Others (e.g., trazodone, and bupropion)
9. Calcium channel blocker verapamil, unless the participant has been on a stable dose for 30 days before the Screening Visit and there is no plan to change the dose after the Screening Visit. Note: all other calcium channel blockers (e.g., nifedipine, diltiazem, amlodipine, felodipine, nicardipine, nimodipine, nisoldipine, etc.) are permitted and may be used without restriction.
10. Oral and parenteral antibiotics within 60 days of the Screening Visit.
11. Any investigational drugs.
12. All narcotics either alone or in combination (e.g., tramadol, codeine, morphine, propoxyphene, loperamide, diphenoxylate, and paregoric). Note: narcotics used as anaesthesia for a colonoscopy require a 5 calendar day wash-out prior to the participant entering into the Pretreatment Period.
13. Any medicine taken for the purpose of losing weight (e.g., orlistat, phentermine, phendimetrazine, diethylpropion, benzphetamine, and sibutramine).
14. Any medicine that is known to cause diarrhoea (e.g., acarbose).
15. Proton pump inhibitors (e.g., omeprazole, lansoprazole, esomeprazole, pantoprazole, rabeprazole) unless the participant has been on a stable dose for at least 3 months before the Screening Visit and there is no plan to change the dose after the Screening Visit.
16. Others: barbiturates (e.g., butalbital and phenobarbital) and chronic oral or parenteral glucocorticoids (which must be discontinued at least 3 months before the Screening Visit; however, one 10-day course of oral or 1 injection of parenteral glucocorticoids is permitted).

Pregabalin is acceptable, provided the participant has been on a stable dose during the 30 days before the Screening Visit and plans to continue stable dosing throughout the study.

17. Thyroxin, unless the participant has been on a stable dose for at least three months before the Screening Visit and there is no plan to change the dose after the screening visit.
18. Participant is taking analgesic drugs, except paracetamol.
19. Long-term oral iron preparations.

1 Day Prior to the Pretreatment Visit and Visits 3, 5, and 7

1. Any over-the-counter or prescription laxative, suppository, or enema (e.g., polyethylene glycol, lactulose, Fleet's). Note: The use of fibre, bulk laxatives, stool softeners (surfactants such as docusate), and probiotics is acceptable, provided the participant has been on a stable dose during the 30 days before the Screening Visit and plans to continue stable dosing throughout the study.
2. Any medicine used to treat diarrhoea (e.g., bismuth subsalicylate, kaolin).
3. NSAIDs if taken for abdominal pain or discomfort.

APPENDIX 2 DIGESTIVE SENSATIONS QUESTIONNAIRE

APPENDIX 3 HOSPITAL ANXIETY AND DEPRESSION SCALE (HADS)

Hospital Anxiety and Depression Scale (HADS)

GL assessment
the measure of potential

Name: _____ Date:_____

FOLD HERE

Clinicians are aware that emotions play an important part in most illnesses. If your clinician knows about these feelings he or she will be able to help you more.

This questionnaire is designed to help your clinician to know how you feel. Read each item below and **underline the reply** which comes closest to how you have been feeling in the past week. Ignore the numbers printed at the edge of the questionnaire.

Don't take too long over your replies, your immediate reaction to each item will probably be more accurate than a long, thought-out response.

A	D			A	D
		I feel tense or "wound up"	**I feel as if I am slowed down**		
3		Most of the time	Nearly all the time		3
2		A lot of the time	Very often		2
1		From time to time, occasionally	Sometimes		1
0		Never	Never		0
		I enjoy the things I used to enjoy	**I get a sort of anxious feeling like "butterflies" in the stomach**		
	0	Definitely	Never	0	
	1	Not quite so much	Occasionally	1	
	2	Only a little	Often	2	
	3	Hardly at all	Very often	3	
		I get a sort of frightened feeling as if something awful is about to happen	**I have lost interest in my appearance**		
3		Very definitely and fairly badly	Definitely		3
2		Yes, but not too badly	Often I don't take as much care as I should		2
1		Sometimes, but it doesn't worry me	Sometimes I don't take as much care as I should		1
0		Never	I take just as much care as ever		0
		I can laugh and see the funny side of things	**I feel restless as if I have to be on the move**		
	0	As much as I always could	Definitely	3	
	1	Not quite so much now	Quite a lot	2	
	2	Definitely not so much now	Not very much	1	
	3	Never	Never	0	
		Worrying thoughts go through my mind	**I look forward with enjoyment to things**		
3		A great deal of the time	As much as I ever have		0
2		A lot of the time	Somewhat less than I used to		1
1		Not too often	Much less than I used to		2
0		Almost never	Rarely		3
		I feel cheerful	**I get sudden feelings of panic**		
	3	Never	Very often	3	
	2	Not often	Often	2	
	1	Sometimes	Not very often	1	
	0	Most of the time	Never	0	
		I can sit at ease and feel relaxed	**I can enjoy a good book, radio or television program**		
0		Always	Often		0
1		Usually	Sometimes		1
2		Not often	Not often		2
3		Never	Very seldom		3

FOLD HERE

Please make sure you have answered all the questions.

	A	D
TOTAL		

APPENDIX 4 IRRITABLE BOWEL SYNDROME SYMPTOM SEVERITY SCORE (IBS-SSS)