STATISTICAL ANALYSIS PLAN: MCP-103-403-SAP-01

Final Version

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
Protocol Number:	MCP-103-403-P-04
Product Name:	Linaclotide
Sponsor:	Ironwood Pharmaceuticals, Inc. 301 Binney Street Cambridge, MA 02142
Final Date:	March 11, 2019

1. TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

2. LIST OF ABBREVIATIONS

Abbreviation/Term	Definition
ADO	adverse event leading to drop out
AE	adverse event
AIP	abdominal inductance plesthymography
ANCOVA	analysis of covariance
BM	bowel movement
BMI	body mass index
BSFS	Bristol Stool Form Scale
CMH	Cochran-Mantel-Haenszel
CSBM	complete spontaneous bowel movement
EOT	End-of-Treatment Period
HADs	Hospital Anxiety and Depression Scale
IBS-C	Irritable Bowel Syndrome with Constipation
IBS-SSS	Irritable Bowel Syndrome – Symptom Severity Scale
IPD	Important Protocol Deviations
ITT	Intent-to-Treat
LOCF	last observation carried forward
LS	least-squares
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
NRS	numerical rating scale
OC	observed case
PCS	potentially clinically significant
PT	preferred term
RM	rescue medication
SAE	serious adverse event
SAP	statistical analysis plan
SBM	spontaneous bowel movement
SD	standard deviation
SI	International System of Units

Abbreviation/Term	Definition
SOC	system organ class
TEAE	treatment emergent adverse event
ug	microgram

3. INTRODUCTION

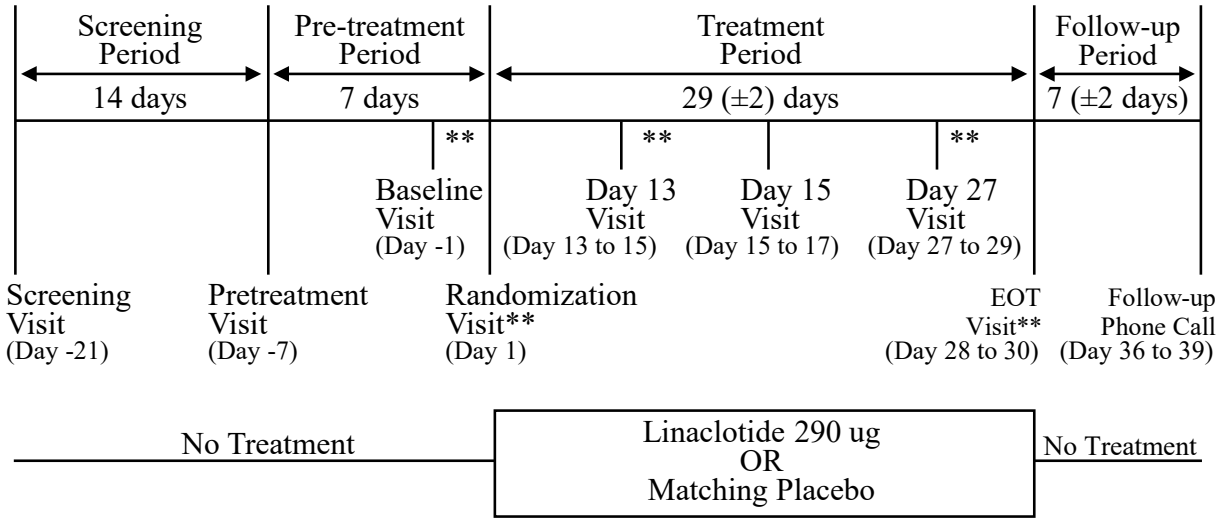
This statistical analysis plan (SAP) provides a more technical and detailed elaboration of the statistical analyses of the efficacy and safety data as outlined and/or specified in the final protocol of Study MCP-103-403-P-04 (Amendment 3, dated April 25, 2017). Specifications for the tables, figures, and data listings are contained in a separate document.

3.1 STUDY DESIGN SUMMARY

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 1. Study Schedule and Chronology



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

3.2 STUDY OBJECTIVES AND ENDPOINTS

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.

3.3 SCHEDULE OF EVALUATIONS

Table 1. Schedule of Evaluations

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		

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 ↓									
Daily diary recording		X	X	X	X	X	X	X	
Daily diary collection				X		X		X	
Digestive Sensations questionnaire given			X		X		X		
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

4. STATISTICAL METHODS, STUDY ENDPOINTS, AND DETERMINATION OF SAMPLE SIZE

4.1 STATISTICAL AND ANALYTICAL PLAN

Statistical analyses defined in the SAP will be performed using STATA Version 13 or newer, Rv3.2.4 or newer, and SAS version 9.4 or newer.

4.1.1 General Methods

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.

Percentages will be based on the total number of non-missing values. The number missing will be presented, but without a percentage.

Descriptive statistics will be presented as;

- Discrete data: number and percentage of participants in each category
- Ordinal categorical data: number and percentage of participants in each category, median, interquartiles, range
- Continuous data: number, mean, median, standard deviation, minimum, maximum

All confidence intervals reported will be two-sided and at the 95% confidence level. There is no adjustment for multiplicity.

If not otherwise specified, the baseline value is defined as the last nonmissing value measured before administration of study drug on Day 1.

4.1.1.1 Analysis Populations

Screening population: All participants who signed the consent form and had a Screening Visit.

Intent-to-Treat (ITT): All participants who were randomised to a treatment group at the randomisation visit (visit 4).

Safety Population: All randomised participants who received at least one dose of study drug.

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.

In the event a subject receives multiple randomization assignments, the subject will be analyzed in the ITT Population according to the first assignment they received; the subject will be analysed according to what they actually received in the Safety Population; the subject will not be included in the Evaluable Population as this is a major protocol deviation.

4.1.1.2 Study Medication

For the double-blind Treatment Period, participants will be supplied with identically appearing capsules containing 290 ug linaclotide, or matching placebo.

4.1.1.3 Statistical Methods

For descriptive summaries, N will represent the total number of patients within the summarized population and n will represent the number of non-missing patients for the summarized endpoint. All summary statistics will be presented by treatment and based on non-missing endpoint values, unless otherwise specified.

Table 2. Statistical Methods

Method	Definition
Categorical Summaries	• Number and percent of patients in each category • Percent based on number of non-missing values • Number of missing patients in each category; percent not presented.
Continuous Summaries	• Number of non-missing patients, mean, median, standard deviation, minimum, maximum

4.1.1.4 Missing Data

Table 3. Missing data handling conventions

Endpoint Type	Descriptions
24 hour recorded girth data	Linear interpolation

4.1.1.5 Study Periods

Table 4. Study Periods

Period	Start Date	End Date
Screening	Date of Signed ICF	PreTreatment Visit Date - 7
Pretreatment	PreTreatment Visit Date	Randomization Date (up to time of randomization)
Treatment	Date of Randomization	End of Treatment Visit Date
Follow-up Period	End of Treatment Visit Date	Follow-up Phone Call

4.1.2 Demographics

4.1.2.1 Disposition of Subjects

Table 5. Disposition Analyses

Analysis Population	Description	Summary Method
Screening, ITT, Safety, Evaluable	Overall	Counts
Screening	Reason for Screening and Pretreatment Failure	Categorical Summary
ITT, Safety	Premature Discontinuation • Completed Study • Discontinued Study • Reason for Discontinuation	Categorical Summary
ITT	Discontinuation by Time (Week) Time to D/C = 1 + <date of d/c> - <date of first dose>	Categorical Summary

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.

4.1.2.2 Protocol Deviations

Protocol deviations and Important Protocol Deviations (IPD) will be identified and documented for all randomized patients prior to unblinding through programmatic checks of the study data and select individual data reviews. IPDs will be determined based on review of all protocol deviations and protocol deviation categories (i.e., Use of Disallowed Medication) performed by members of the Ironwood Clinical Trials team, including but not limited to the Clinical Medical Researcher and study Biostatistician.

Table 6. Important Protocol Deviations

Analysis Population	Description	Summary Method
ITT	Important Protocol Deviations • those who entered the study even though they did not satisfy the entry criteria; • those who developed withdrawal criteria during the study but were not withdrawn; • those who received the wrong treatment or incorrect dose; • those who received an excluded concomitant treatment. • Other	Listing

4.1.2.3 Demographics and Baseline Characteristics

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 summarized by treatment group for the ITT population.

Table 7. Demographics

Endpoint	Description	Summary Method
Age	Age at informed consent date	Continuous Summary
Sex	Male Female	Categorical Summary
Race	Caucasian Non-Caucasian Black or African American Asian American Indian or Alaska Native Native Hawaiian or Pacific Islander Other	
Ethnicity	Hispanic or Latino Not Hispanic or Latino	

Table 8. Baseline Characteristics

Endpoint	Description	Summary Method
Weight	Weight (kg)	Continuous Summary
Height	Height (m)	
BMI	BMI (kg/m^2)	
Baseline Efficacy Endpoints	Abdominal girth (measured by AUC) CSBMs (standardized weekly rate) SBMs (standardized weekly rate) Abdominal pain Abdominal discomfort Abdominal bloating Abdominal distension BSFS *Note: include any measure that will be used as a baseline covariate in endpoint analyses*	

4.1.2.4 Medical History

Medical History, abnormalities and surgeries, reported prior to screening occurring prior to screening will be coded using MedDRA version 19.1 or newer. Summaries will be presented by

MedDRA system organ class (SOC) and preferred term by treatment arm and overall for the safety population. Patients will be counted once per SOC and PT.

Table 9. Medical History

Parameter	Description	Summary Method
Medical History	Surgeries and Abnormalities reported at Screening SOC PT	Categorical Summaries

4.1.2.5 Prior and Concomitant Medication

Reported medication will be coded using the World Health Organization (WHO) Drug Dictionary Enhanced B2, version dated December 2016 or newer to their Anatomical Therapeutical Chemical (ATC) class and PT. Summaries will be presented by ATC and PT for the Safety Population. Patients will be counted once per ATC and PT.

Table 10. Prior and Concomitant Medication

Parameter	Description	Summary
Prior Medications	Medication taken prior to first dose of study medication	Categorical Summary
Concomitant Medication	Medication taken on or after first dose of study medication.	

4.1.2.6 Drug compliance

Patient drug compliance over the double-blind treatment period will be calculated for each patient. Dosing compliance for the Treatment Period is defined as the number of capsules actually taken by a patient during the Treatment Period divided by the number of capsules that were expected to be taken during the Treatment Period, multiplied by 100. The total number of capsules actually taken will be calculated as the total number of capsules dispensed minus the total number of capsules returned. The total number of capsules expected to be taken during the Treatment Period is the number of days between the date of first dose taken and the date of last dose taken, inclusive (i.e., last dose date – first dose date + 1). Summaries will be presented by treatment arm and overall for the Safety Population.

Table 11. Study Drug Compliance

Parameter	Description	Summary Method
Overall Drug Compliance	Percent of drug actually taken relative to amount expected to be taken in defined interval	Continuous Summary

4.1.2.7 Diary Compliance

Diary compliance will be calculated for each patient. Summaries will be presented by treatment arm over the baseline and double-blind treatment periods, and weekly, for the ITT Population. A complete Diary is defined as having responses to each of the daily Diary questions asked during the daily report.

Table 12. Diary Compliance

Parameter	Description	Summary Method
Diary Compliance Baseline (Pretreatment Period) DB Treatment Period (Weeks 1-4) Week 1 Week 2 Week 3 Week 4	Percent of completed Diary days relative to number of days in reporting interval	Continuous Summary

4.1.3 Efficacy

4.1.3.1 Analysis of Primary Efficacy Endpoint

Baseline abdominal girth (as measured by AIP AUC) will be calculated using the first 24 hours of measurements from the belt following the Baseline Visit. If an AIP increase of >4 cm is not observed over the first 24-hour period, then baseline will be defined as the 24 hours leading up to the Randomization Visit on day 1. For subjects with an AIP increase of >4 cm in both 24 hour periods, the first 24-hour period will be used.

Table 13. Primary Efficacy Analysis

Endpoint	Description	Timing	Summary Method
Change from baseline in abdominal girth at Week 4	The mean change from randomization (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 be then 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.	Day -1 to Day 27	Continuous summary, 95% CI

4.1.3.2 Secondary and Additional Efficacy Analyses

Baseline score for abdominal symptoms will be calculated as the average of daily values reported over the Pretreatment Period.

A patient's daily Abdominal Score is calculated as the average of the daily abdominal pain, abdominal bloating, and abdominal discomfort assessments. If two or more of these individual daily abdominal symptoms are missing, then the Abdominal Score for that day will be missing.

A patient's daily Abdominal Score plus Distension is calculated as the average of the daily abdominal pain, abdominal bloating, abdominal discomfort, and abdominal distension assessments. If three or more of these individual daily abdominal symptoms are missing, then the Abdominal Score plus Distension for the day will be missing.

Table 14. Secondary and Additional Efficacy Endpoints

Endpoint	Description	Timing	Summary Method
Change from baseline in abdominal girth at Week 2	The mean change from baseline to the midpoint of treatment 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 be then individually standardised by dividing the total AUC over the period by that patient's number of hours of measurement included in the AUC.	Day -1 to Day 15	Continuous summary, 95% CI
Change from Baseline in Maximal Abdominal Girth 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.	1st hour, 2 hour to bedtime	Continuous summary, 95% CI

Table 14. Secondary and Additional Efficacy Endpoints

Endpoint	Description	Timing	Summary Method
Symptom Severity (weekly averages and change from baseline) Abdominal pain Abdominal discomfort Abdominal bloating Abdominal distension Multicomponent scores: Abdominal Score Abdominal Score + Distension	Each item assessed daily on 11-point NRS Weekly average scores (pain, discomfort, bloating, distension): 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) Abdominal Score: calculated weekly; the daily average of the following individual items (pain, discomfort, bloating) will be calculated, then the weekly average will be calculated as the mean of day 1 to day 7. Abdominal Score plus Distension: calculated weekly; the daily average of the following individual items (pain, discomfort, bloating, distension) will be calculated, then the weekly average will be calculated as the mean of day 1 to day 7. Change from baseline each post-baseline timepoint	Baseline, Week 1, Week 2, Week 3, Week 4; change from baseline at each post-baseline timepoint	Continuous summary, 95% CI
Change from Baseline in Digestive sensations	Change from baseline in digestive sensations (abdominal pain, discomfort, bloating and distension) at end of treatment (as measured by AUC of hourly digestive sensations)	Baseline, Week 2 (Visit 6), Week 4 (Visit 8)	Continuous summary, 95% CI
Weekly averages change from baseline in Bristol Stool Form Scale (BSFS)	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 2) Week 4=mean (day 22 to day 28) Change from baseline	Baseline, Week 1, Week 2, Week 3, Week 4, Change from baseline at each postbaseline timepoint	Continuous summary, 95% CI

4.2 SAMPLE SIZE JUSTIFICATION

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 distension criteria, but also pretreatment abdominal pain, abdominal bloating, and bowel movement criteria), 120 participants would need to enter screening to achieve 40 randomised participants.

Note: for a previous yogurt study, a mean difference in girth of 1.6 cm (SD = 2.4 cm) was observed

Sample size calculated using nQuery Advisor 3.0.

Note: Due to difficulties with enrollment, the study was terminated by the sponsor after 20 subjects were randomized.

4.3 SAFETY ANALYSIS

All safety analyses will be performed using the Safety Population.

4.3.1 Adverse Events

All adverse event reported during the study will be coded using MedDRA version 19.1. All summaries and listings will be presented of the safety population.

Table 15. AE classification

AE classification	Definition
Treatment Emergent Adverse Event (TEAE)	TEAE if: • Treatment Start date/time <= AE Start Date/Time <=Date of Last Treatment +1 And • PT not previously reported prior to Treatment • PT reported prior to Treatment however increased in severity after start of treatment
Serious Adverse Event (SAE)	Any event meeting the following criteria: • Results in death • Is life-threatening • Requires inpatient hospitalization or prolongation of an existing hospitalization • Results in persistent or significant disability or incapacity • Results in a congenital anomaly or birth defect These criteria will identified by AE Serious criteria = Yes

Patients reporting multiple incidence of an adverse event mapping to the same PT will be counted only once overall, at the most severe severity level, and at the closest relationship to treatment. AEs will be summarized as described in Table 6.

Table 16. Adverse Events

Summaries	Description	Summary Method
Overall Summary	• TEAEs • Deaths • On-Therapy SAEs • Withdrawals Due to TEAEs (ADOs)	Categorical Summary
TEAEs	• At Least One AE • By SOC • By PT within SOC	
TEAEs By Severity		
TEAEs By Relationship		
Listings of All Adverse Event for Patients with: 1. SAEs 2. ADOs 3. Fatal	For Patients meeting the defined criteria, list all all the pateints Adverse Events	Data Listing – See Table Shell for variables to include

4.3.2 Clinical Laboratory Data

Clinical laboratory measure recorded over the course of the study will be summarized as defined in Table 17.

Table 17. Clinical Laboratory Summaries

Endpoint	Description	Timing	Summary Method
Descriptive	Summary of Laboratory Category and Parameter (SI Units) at Screening Visit	Screening	Continuous Summary

4.3.3 Vital Signs Parameters

Vital signs endpoints will be defined and summarized as described in Table 18.

Table 18. Vital Signs Summaries

Endpoint	Description	Timing	Summary Method
Descriptive	Summary Vital Sign measures at each timepoint measured plus Change from baseline	Day 1, Day 15, Day 28 (EOT)	Continuous Summary

4.3.4 Physical Examination

Clinically significant physical examination results for all subjects will be presented in a data listing for the Safety Population.

5. CHANGES FROM ANALYSES PLANNED IN THE PROTOCOL

The following analyses differ compared to those specified in the study protocol:

- All continuous data, regardless of normality, will be presented as number, mean, standard deviation, and range. Protocol had stated continuous, non-normally distributed data would be presented as number, median, interquartiles, and range.

- Section 11.3.4 of the Protocol states that change from baseline will be calculated for clinical laboratory evaluations. Clinical labs were only taken at screening and therefore, no change values will be calculated.

- The following secondary efficacy endpoints have been renamed:

    - 'Change from baseline in abdominal distension at week 2' will now be referred to as 'change from baseline in abdominal girth at week 2'

    - 'Change from baseline in maximal abdominal distension at week 4' will now be referred to as 'change from baseline in maximal abdominal girth at week 4'

    - 'Change from Baseline in digestive sensations at weeks 4 and 8' was an error as the study is only 4 weeks in duration. This should have been 'Change from baseline in digestive sensations at week 2 (Visit 6) and week 4 (Visit 8)'.

    - 'Change from baseline in Bristol Stool Form Scale at weeks 4 and 8' was an error as the study is only 4 weeks in duration. This should have been 'Change from baseline in Bristol stool form scale at week 2 (Visit 6) and week 4 (Visit 8)'.

    - Change from baseline in Bristol Stool Form Scale at Weekk 2 and 4 will be analyzed using linear mixed models with appropriate contrasts. The Protocol had previously specified generalized estimating equations (GEE) would be used for analysis.

- The HADS and IBS-SSS were assessed at Screening and EOT. These values will not be analyzed.

- Section 11.3.3.5 of the Protocol reports additional analysis that will be conducted. Specifically, it states, "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." This analysis will no longer be conducted.

- Given that the study was terminated by the sponsor after only 20 patients were randomized, there will be no formal statistical modeling (ANCOVA, MMRM) and descriptive statistics and confidence intervals will be presented only.

6. REFERENCES

1. nQuery (2017). Sample Size and Power Calculation. “Statsols” (Statistical Solutions Ltd). Cork, Ireland