COVER PAGE - **Protocol and SAP**

Title: A Randomized, Double-blind, Placebo-controlled, Dose Ranging, Parallel-Group Study of the Efficacy and Safety of Plecanatide in Children 6 to <18 Years of Age with Irritable Bowel Syndrome with Constipation (IBS-C)

NCT Number: 03596905

IRB Approval Date: 9/24/2018

[redacted]

A Randomized, Double-blind, Placebo-controlled, Dose Ranging, Parallel-group Study of the Efficacy and Safety of Plecanatide in Children 6 to <18 Years of Age with Irritable Bowel Syndrome with Constipation (IBS-C)

Sponsor: Bausch Health Americas, inc.	[redacted]
Clinical Research Organization:	[redacted]
Medical Monitor:	
Sponsor Protocol No.:	SP304202-14
IND No.:	115,118
Study Drug Name: Phase:	Plecanatide: Phase 2b
Date of Protocol:	August 6, 2018 (V2.0)
Date of Previous Protocol:	May 3, 2018 (V1.0)

The study will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP) and with other applicable regulatory requirements.

SIGNATURE PAGE

Declaration of Sponsor

Title: A Randomized, Double-blind, Placebo-controlled, Dose Ranging, Parallel-group Study of the Efficacy and Safety of Plecanatide in Children 6 to <18 Years of Age with Irritable Bowel Syndrome with Constipation (IBS-C)

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the guidelines on Good Clinical Practice.

[redacted]

Declaration of the Investigator

Title: A Randomized, Double-blind, Placebo-controlled, Dose Ranging, Parallel-group Study of the Efficacy and Safety of Plecanatide in Children 6 to < 18 Years of Age with Irritable Bowel Syndrome with Constipation (IBS-C)

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure, electronic Case Report Forms (eCRFs), and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB, except where necessary to eliminate an immediate hazard to the patients.

I have read and understand and agree to abide by all the conditions and instructions contained in this protocol.

Responsible Investigator of the local study center

Signature Date

Name (block letters)

Institution (block letters)

Phone number

PROTOCOL SYNOPSIS

Study Title	A Randomized, Double-blind, Placebo-controlled, Dose Ranging, Parallel-group Study of the Efficacy and Safety of Plecanatide in Children 6 to < 18 Years of Age with Irritable Bowel Syndrome with Constipation (IBS-C)
Sponsor	[redacted]
Sponsor Study No.	SP304202-14
Phase	2b
Study Centers	Approximately 20
Objectives	Primary objective: To evaluate the safety and efficacy of once daily oral plecanatide for 4 weeks as treatment for the relief of symptoms associated with IBS-C in children ages 6 to < 18 years. Secondary objective: To identify the pharmacokinetic (PK) parameters of plecanatide in this patient population (to the extent possible since plecanatide is not expected to be absorbed)
Study Design	This is a randomized, double-blind, placebo-controlled, parallel-group study of the efficacy and safety of 4 dose levels of plecanatide (0.5, 1.0, 2.0 or 3.0 mg) in children 6 to < 18 years of age with IBS-C. Enrollment will be stratified by age group (6 to 11 years and 12 to < 18 years). Randomization will be stratified by gender to ensure gender balance across treatment groups. The study will include a 28-day Screening/Baseline Period, a 4-week Treatment Period, and a 2-week Post-Treatment Follow-up Period. Patients/caregivers will visit the clinic 4 times during the study. *Screening/Baseline Period*: Patients' legally authorized representatives (e.g., parent/guardian/LAR) will provide informed consent and patients, as per IRB guidance, will provide written or verbal assent before they undergo any protocol-specified procedures or assessments. At the Screening visit, patients will undergo a review of medical history, a review of prior and concomitant medications, a brief dietary history, and a physical examination. During the 28-day Screening/Baseline Period prior to randomization, patients will record—through an electronic diary—daily assessments of bowel movements (BMs), stool consistency (Bristol Stool Form Scale (BSFS) or Modified Bristol Stool Form Scale for Children (mBSFS-C), and abdominal pain and other IBS-related symptoms. Data from the electronic diary will be used to confirm study eligibility immediately prior to the randomization visit, as well as to characterize the patient's baseline IBS-C status with which the change from Baseline across and at the end of the 4-week treatment period will be compared. *Treatment Period*: Patients 6 to 11 years of age (Group A) who meet all entry criteria will be randomly assigned to 1 of 3 age/dose groups (2 active treatment, 1 matching placebo) in a 1:1:1 ratio; patients 12 to < 18 years of age (Group B) will be randomly assigned to 1 of 4 age/dose groups (3 active treatment, 1 matching placebo) in a 1:1:1:1 ratio on Day 1 of the Treatment Period. Randomization will be stratified to ensure gender balance across treatment

	groups. Patients will take their daily oral dose of the study drug once daily for 4 weeks and continue to complete their daily electronic diaries recording BMs, rescue medication use for constipation, and abdominal pain and other symptoms. At Weeks 1 and 4 of the Treatment Period, patients will return to the clinic to undergo safety and efficacy assessments. *Post-treatment Follow-up Period*: For 2 weeks after the last dose of study drug, patients will continue to complete their daily electronic diaries. Patients will then return to the clinic for a final Follow-up visit at the end of Week 6. The planned duration of participation in this study will be approximately 10 weeks from signing of informed consent/assent through post-treatment.
Study Drug	Group A: Plecanatide 0.5 or 1.0 mg tablets and matching placebo for oral administration Group B: Plecanatide 0.5, 1.0, or 1.5 mg tablets and matching placebo for oral administration
Treatment	Following a 28-day Screening/Baseline Period, patients will take the assigned dose of study drug each morning at approximately the same time for 4 weeks. Dulcolax® will be provided as a rescue laxative that can be used if a patient has not had a bowel movement for at least 72 hours. Patients will be advised that it is preferred that they do NOT take rescue medication for the period from 24 hours before to 72 hours after Day 1 of dosing.
Number of Patients and Population	Approximately 210 children with IBS-C will be randomized (~30 in each age/dose group) into the double-blind portion of the study. Eligibility for study enrollment (< 3 Complete Spontaneous Bowel Movements [CSBMs] for each baseline diary week, ≤ 6 SBMs per week for each baseline diary week, a weekly average of abdominal pain score ≥ 3 on an 11-point [0-10] Numerical Pain Rating Scale or Wong-Baker Faces® Pain Scale for each baseline diary week or ≤ 2 days per week with abdominal pain score = 0 for each baseline diary week, ≤ 1 day in each baseline dairy week with BSFS = 6 for any SBM and no SBM with BSFS = 7 in either week for Group B or mBSFS-C = 4 for any SBM and no SBM with mBSFS-C = 5 in either week for Group A, adequate diary entry compliance, and proper use of rescue medication) will be confirmed by electronic diary entries made during each week of the two-week baseline diary assessment.
Key Inclusion Criteria	• Male or female child aged 6 to < 18; • Meets ROME IV for child/adolescent IBS-C defined as: o For at least 2 months before diagnosis the patient has had: ▪ Abdominal pain at least 4 days per month associated with one or more of the following: ▪ Related to defecation ▪ A change in frequency of stool ▪ A change in form (appearance) of stool; o The pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome);

	o After appropriate evaluation, the symptoms cannot be fully explained by another medical condition o More than one-fourth (25%) of bowel movements with Bristol stool form types 1 or 2 and less than one-fourth (25%) of bowel movements with Bristol stool form types 6 or 7 on the BSFS or 4 or 5 on the mBSFS-C.
Key Exclusion Criteria (abbreviated)	• Patient reports having more than 1 loose, mushy stool (eDiary-recorded stool consistency of 6 on the Bristol Stool Form Scale (BSFS or 4 on the modified Bristol Stool Form Scale for Children [mBSFS-C]) or any watery stool (eDiary-recorded stool consistency of 7 on the BSFS or 5 on the mBSFS-C) with any SBM that occurred in the absence of laxative use on the calendar day of the BM or the calendar day before the BM during the 14 days before the randomization day and up to the day of randomization; • Diagnosis of Irritable Bowel Syndrome with Diarrhea (IBS-D) or Irritable Bowel Syndrome Mixed (IBS-M); • Medical history or conditions that may be affecting GI motility or defecation; • Lack of willingness to use Dulcolax® as the only laxative; • Non-compliance with Diary and rescue medication use.
Criteria for Evaluation of Efficacy and Safety	<u>Primary Efficacy endpoint:</u> Change from baseline in weekly SBM frequency over the 4 Week Treatment Period compared to placebo and across treatment groups. <u>Secondary efficacy endpoints:</u> o Change from baseline in frequency and severity of abdominal pain and abdominal discomfort o Change from baseline in frequency of BMs, SBMs, and CSBMs o Time to First BM o Change from baseline in stool consistency (BSFS or mBSFS-C) o Use of Rescue Medication o Change from baseline in frequency of fecal incontinence o Change from baseline in frequency and severity of defecation pain o Change from baseline in frequency of large diameter stools <u>Pharmacokinetic endpoints:</u> Single dose and steady state of plasma plecanatide and its major metabolite SP-338 (population modeling): o Plasma concentration of plecanatide/SP-338 (Note: measurable plecanatide/SP-338 levels have not been observed in the adult population) o PK parameters, including Cmax, Tmax, and Cmin (only if a sufficient number of plecanatide/SP-338 levels can be measured to allow computation of these parameters) <u>Safety endpoints:</u> o Frequency of treatment-emergent adverse events (TEAEs) o Withdrawals due to adverse events and serious adverse events o Significant shifts from Baseline values in laboratory analytes and vital signs

Statistical Methods	Sample Size and Power Considerations Based on data from two phase 3 trials conducted by the sponsor in adults with IBS-C, consider the sample size necessary for observing a significant difference in the change from baseline in SBM frequency in Group B (the adolescents) since data from the adults is likely more applicable to Group B participants. Then, a study with a plecanatide group sample size of 102 and a placebo group sample size of 34 (representing 3:1 randomization, plecanatide to placebo) achieves 81.5% power at the 0.05% level of significance when the difference in mean change from baseline in weekly SBM frequency at four weeks is 1.5 and the placebo group and plecanatide group standard deviations are 2.3 and 3.5 respectively. Since study SP304202-14 is being conducted in children and adolescents with IBS-C, and the primary analysis of the primary endpoint will be an ANCOVA using a linear mixed-effects model, the sponsor believes that 30 patients per treatment group (placebo and 4 dose levels of plecanatide), yielding a planned target enrollment of a total of 210 patients (90 children and 120 adolescents), will prove sufficient for achieving the primary objective of this phase 2 study. Analysis Populations Analysis populations include the Full Analysis Set (FAS), the Per Protocol (PP) population, and the Safety Population (SP). The FAS will include all randomized and treated patients who had the baseline assessment and at least one post randomization assessment of the primary efficacy measure of weekly SBM frequency. The FAS will be the primary population for efficacy analyses. The SP will include all patients who were randomly assigned to a treatment group and received at least 1 dose of study drug. Patient Characteristics and Disposition Demographic characteristics (including age, gender, race, weight, height, and body mass index) and characteristics of patient's IBS history will be summarized by descriptive statistics for each treatment group. Efficacy Analyses • *Primary Efficacy Analysis* The weekly SBM rate for each patient will be computed for each week of the 4-week treatment period. The change from baseline in mean weekly SBM rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical p-values. The above analysis will be repeated for the PP Population. • *Secondary Efficacy Analysis* The mean in each period and the change from baseline will be summarized in each treatment group. Differences between treatment groups will be analyzed with an analysis of covariance, including fixed effects for gender (stratification variable) and treatment and corresponding baseline value as a covariate. A least

	squares mean with 95% confidence intervals will be presented for the difference between each plecanatide group and placebo. Pharmacokinetic Analyses Plasma plecanatide (and its major metabolite SP-338) concentrations will be assessed for all patient's pre-dose on Day 1 and at the Week 4 visit. Intensive PK sampling (30, 60, 90, and 120 minutes post dose on Day 1) will be done in a subset of approximately 70 patients at selected sites. Descriptive PK parameters will be presented and to the extent possible will be analyzed. Safety Analyses The Medical Dictionary for Regulatory Activities (Version 14.1 or higher) will be used to classify all AEs with respect to system organ class and preferred term. Adverse events, changes in clinical laboratory parameters, and vital signs will be analyzed descriptively. Shifts in key laboratory parameters will be summarized from Baseline to the end of the study. Similarly, shifts in toxicity of key laboratory parameters and the proportion of patients with abnormal clinical and vital sign results will be summarized.

LIST OF STUDY PERSONNEL

Sponsor:	[redacted]
Medical Monitor:	
SAE Reporting:	
Central Laboratory:	
Pharmacokinetic Laboratory	
Drug Manufacturer:	
Drug Packaging and Distribution:	

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADL	Activities of Daily Living
AE	Adverse Event
AGA	American Gastroenterological Association
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical [Classification System]
ß-HCG	Beta-Human Chorionic Gonadotropin
BM	Bowel Movement
BMI	Body Mass Index
BSFS	Bristol Stool Form Scale
CFR	Code of Federal Regulations
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CI	Confidence Interval
CIC	Chronic Idiopathic Constipation
cGMP	Cyclic Guanosine Monophosphate
CMH	Cochran-Mantel-Haenszel (Test)
CRF	Case Report Form
CRO	Contract Research Organization
CSBM	Complete Spontaneous Bowel Movement
CTCAE	Common Terminology Criteria for Adverse Events
DRE	Digital Rectal Examination
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOS	End of Study
EOT	End of Treatment
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GC-C	Guanylate Cyclase C
GCP	Good Clinical Practice
GI	Gastrointestinal
IB	Investigator Brochure
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome with Constipation
IBS-D	Irritable Bowel Syndrome with Diarrhea
IBS-M	Irritable Bowel Syndrome Mixed
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IND	Investigational New Drug (Application)
IRB	Institutional Review Board
IRT	Interactive Response Technology
IWRS	Interactive Web-Based Response System
LAR	Legally Authorized Representative
LLN	Lower Limit of Normal
LOCF	Last Observation Carried Forward
LS	Least Squares
mBSFS-C	Modified Bristol Stool Form Scale for Children

MCG	Microgram
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
mL	Milliliter
NPRS	Numeric Pain Rating Scale
PE	Physical Examination
PGA	Patient Global Assessment
PP	Per-Protocol (Population)
PRO	Patient Recorded Outcome
PV	Pharmacovigilance
QoL	Quality of Life
RM	Rescue Medication
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBM	Spontaneous Bowel Movement
SD	Standard Deviation
SOC	System Organ Class
SP-304	Previous Designation for Plecanatide
SP-338	Major Metabolite of Plecanatide
TEAE	Treatment Emergent Adverse Event
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal
US	United States
WAPI	Worst Abdominal Pain Intensity
WHODD	World Health Organization Drug Dictionary

Definition of Terms

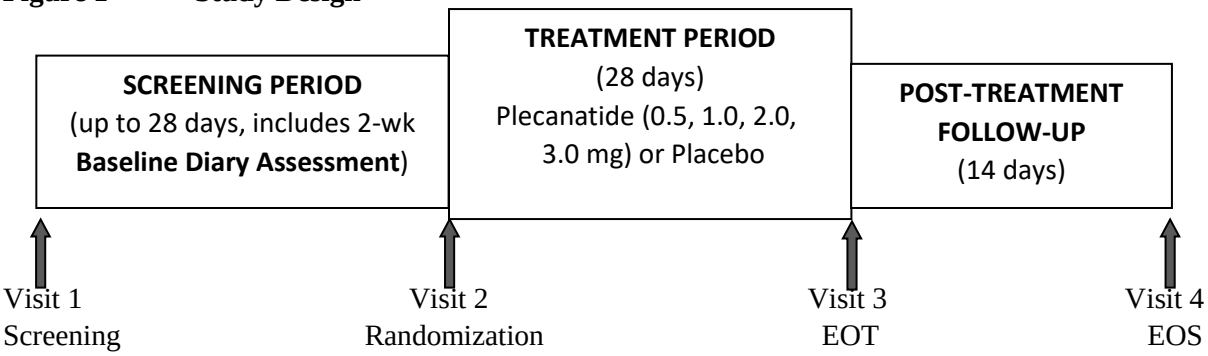
Spontaneous bowel movement (SBM)	A bowel movement that occurs in the absence of laxative use within the preceding 24 hours
Complete spontaneous bowel movement (CSBM)	A spontaneous bowel movement with the sense of complete evacuation

1. OVERALL DESIGN AND PLAN OF THE STUDY

This is a randomized, double-blind, placebo-controlled, multicenter, parallel-group study of the efficacy and safety of 2 dose levels of plecanatide (0.5 or 1.0 mg) in child patients 6 to 11 years of age with IBS-C and 3 dose levels of plecanatide (1.0, 2.0, or 3.0 mg) in adolescent patients 12 to less than 18 years of age with IBS-C. Approximately 210 patients will be enrolled at approximately 20 study sites in the United States. Patient randomization will focus on the balanced enrollment of males and females across the 7 age/dose groups.

The overall study plan is presented in Figure 1**.**

Figure 1 Study Design



After written informed consent/assent is obtained at Visit 1, patients will be screened for study eligibility. Qualifying patients and their caregivers (if applicable) will be instructed on the use of an electronic diary that will serve to record information on their daily bowel habits, rescue medication use, and symptoms associated with IBS-C.

Patients will begin diary entries following Visit 1 and continue daily diary entries for the remainder of the study. The information recorded by the patient in the diary during the 2 weeks just prior to Visit 2 will confirm study eligibility, confirm diary compliance and establish baseline values for primary and secondary efficacy endpoints. The interval between Visit 1 (Screening) and Visit 2 (Randomization) should ideally be 28 days to ensure 2 weeks of 'diary practice' experience and 2 weeks for baseline diary recording. A minimum of 1 week of 'diary practice' is required prior to the 2-week baseline diary recording.

At Visit 2 (Day 1 of Treatment Period), patients 6 – 11 years of age (Group A) will be randomly assigned to 1 of 2 plecanatide doses (0.5 or 1.0 mg) or matching placebo and patients 12 to less than 18 years of age (Group B) will be randomly assigned to 1 of 3 plecanatide doses (1.0, 2.0, or 3.0 mg) or matching placebo. The patients will take their first dose of study drug at the clinical site and continue to take their oral dose daily for 4 weeks. At Week 4, patients will return to the clinic to undergo safety and efficacy assessments and return all unused study drug.

For 2 weeks after the last dose of study drug, patients will continue to complete their daily electronic diaries. Patients will return to the clinic for the final Follow-up visit at the end of Week 6.

The planned duration of participation in this study will be approximately 10 weeks (70 days) from signing of informed consent/assent through post-treatment.

2. INTRODUCTION

2.1 BACKGROUND AND RATIONALE

2.1.1 Irritable Bowel Syndrome with Constipation (IBS-C in Adults)

Irritable Bowel Syndrome (IBS) is characterized by recurrent episodes of abdominal pain and discomfort with associated alterations in bowel habits. The altered bowel habits may include diarrhea, constipation or a mixed pattern of diarrhea and constipation. Abdominal discomfort or pain is a universal feature required for the diagnosis of IBS and the presence of the predominant abnormal bowel pattern leads to the subtyping of IBS: diarrhea-predominant (IBS-M), constipation-predominant (IBS-C), or mixed IBS (IBS-M). Of the approximately 30 million individuals in North America who meet the diagnostic criteria for IBS, approximately one-third experience constipation during episodes of disease activity (Drossman, Morris, Hu Y et al, 2005) [1]. The remaining patients are equally subdivided among the other two subtypes (Saito, Schoenfeld, Locke, 2002) [2]. IBS is a chronic condition and may start in adolescence with a 2-3:1 predominance in females as compared to males. The most widely accepted definition for the diagnosis of IBS is the Rome III criteria and includes recurrent abdominal pain or discomfort at least 3 days/month in the last 3 months associated with 2 or more of 1) improvement with defecation, 2) onset associated with a change in frequency of stool, and 3) onset associated with a change in form (appearance) of stool. The IBS-C subtype is further characterized by stool pattern such that ≥ 25% of defecations are hard or lumpy stools and ≤ 25% of defecations are loose or watery stool (Longstreth, Thompson, Chey, et al, 2006) [3].

Several underlying mechanisms have been implicated in the pathophysiology of IBS, although much remains poorly understood (Longstreth, Thompson, Chey, et al, 2006), (Drossman, Camilleri, Mayer, 2002) [3, 4]. The pain that patients with IBS experience is believed to be due to visceral hypersensitivity. In these patients, visceral stimuli which are usually normal or not bothersome (in non-IBS patients) are perceived as painful. Psychosocial stressors, genetic factors, altered intestinal microbiota, and altered brain-gut interaction have been theorized to exacerbate or lead to symptoms of IBS. Infection and post-infectious inflammation have also been postulated to lead to the development of IBS or symptoms of IBS (Powell, Fleming, Chapter 43) [5]. According to the Rome III criteria, a patient must have continuous or intermittent symptoms of abdominal discomfort for at least 6 months before the diagnosis of IBS can be considered (Drossman, Dumitrauscu, 2006) [6].

IBS-C is a multi-symptom disease and the goal of therapy is to provide treatment that alleviates its multiple symptoms. Currently, in clinical practice, treatments are typically focused on the treatment of the individual symptoms of IBS-C. If a patient has predominantly constipation symptoms, the patient is usually prescribed a fiber supplement or a laxative. If bloating and distension occur, the patient may be prescribed an antispasmodic or dietary modification. patient complains of abdominal pain, the patient may receive tricyclic anti-depressants although there is no FDA approved treatment for pain associated with IBS-C.

2.1.2 IBS-C in Children and Adolescents

IBS in children and adolescents shares the characteristics of recurrent episodes of abdominal pain or discomfort with associated alterations in bowel habits seen in adults. It can be debilitating and negatively impact young patients' quality of life, resulting in poorer school attendance and increased healthcare spending. The incidence and prevalence of IBS-associated abdominal pain in children and adolescents range from 8% to 17% and 13% to 38%, respectively (Saps, Seshadri, et al, 2009) [7].

Diagnosis in children and adolescents by ROME IV criteria differs slightly from adult diagnosis in that characteristic recurrent abdominal pain must be reported at least 4 days per month for at least 2 months prior to diagnosis while in adults it must be reported at least 1 day/week in the last 3 months with onset at least 6 months prior to diagnosis. As with adults, a variety of medications may be prescribed to address the child's or adolescent's predominant symptoms, such as dietary fiber, laxatives, antispasmodics, or antidepressants.

2.1.3 Plecanatide Mechanism of Action and Pharmacology

Plecanatide (SP-304) is a peptide discovered and synthesized by Synergy Pharmaceuticals Inc. (hereinafter referred to as Synergy).

Plecanatide binds to the GC-C receptor expressed on epithelial cells that line the intestinal lumen. This binding of drug to the GC-C receptor stimulates the intracellular production of cyclic guanosine monophosphate, resulting in decreased Na^+ reabsorption through Na^+/H^+ exchange and activation of Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) (Sindic, 2006)[8]. Activation of CFTR in turn enhances transepithelial efflux of chloride and bicarbonate ions and consequently fluid secretion into the intestinal lumen is stimulated. This fluid secretion is expected to facilitate BMs. Nonclinical data (in human colon carcinoma T84 cells and in animals) suggested potential therapeutic utility of orally dosed plecanatide in the treatment of CIC.

2.1.4 Clinical Experience in Adults

Plecanatide, marketed in the United States as Trulance®, received FDA approval for the treatment of adults with Chronic Idiopathic Constipation (CIC) in January 2017 and approval for the treatment of adults with IBS-C in January 2018. In clinical trials for CIC completed to date, plecanatide has been found to be safe and well-tolerated. Plecanatide has been shown to increase the frequency of bowel movements (BMs) in patients with Chronic Idiopathic Constipation (CIC); effects on stool consistency and reduction of the time to first bowel movement were also seen. Patients with IBS-C were shown to have ≥ 30% reduction in worst abdominal pain/discomfort and an increase of ≥ 1 complete spontaneous bowel movement (CSBM) over baseline, in the same week, for at least 50% of the 12 treatment weeks.

2.1.5 Clinical Experience in Adults with CIC and IBS-C

Plecanatide has been extensively evaluated in multiple clinical studies in adults with CIC and IBS-C. Two phase 1, single-dose studies in healthy volunteers have been completed; one with plecanatide doses ranging from 0.1 mg to 48.6 mg and a food-effect study with plecanatide 9 mg. The results showed plecanatide to be safe and well tolerated with minimal food effect. At very high doses, gastrointestinal (GI) adverse effects were observed. Three phase 2 dose-ranging studies were conducted in CIC and IBS-C patients looking at 0.3, 1, 3, and 9 mg plecanatide doses administered daily from 14 days to 12 weeks. Plecanatide continued to be safe and demonstrated statistically significant efficacy in durable overall CSBM responders (CIC) and overall responder rate (IBS-C) over placebo with maximum effect seen at the 3-mg plecanatide dose. Treatment-emergent AEs consisted of transient GI effects consistent with the drug's pharmacologic action. Phase 3 studies were conducted in two CIC and two IBS-C randomized, double-blind, placebo-controlled trials looking at plecanatide 3 and 6 mg administered daily for 12 weeks. These data showed a statistically significant CSBM durable overall responder rate (CIC) and overall responder rate (IBS-C) for both plecanatide doses over placebo and the doses were safe and well tolerated. Two open-label, long-term safety and tolerability studies were also completed with mean duration of study drug exposure of 167.4 days in the CIC population and 184.8 days in the IBS-C population. Treatment-emergent AEs both qualitatively and quantitatively were similar to those observed in prior double-blind 12-week plecanatide studies. A pediatric study in patients with CIC aged 12-18 years is currently underway.

On 19 January 2017 and 24 January 2018, the FDA approved plecanatide (Trulance®, 3 mg dosage strength) for the treatment of CIC and IBS-C, respectively in adults. Approval was based on efficacy and safety data from 3182 adult patients (1733 CIC and 1449 IBS-C) who were enrolled in four double-blind placebo-controlled clinical trials and received placebo or 3 mg Trulance once daily for 12 weeks. Additional safety data are available from a total of 9615 patients enrolled in these four studies and two longer-term chronic therapy studies of up to 72 weeks. The most common adverse reaction (reported in at least 2% of Trulance treated patients and at an incidence greater than placebo) was diarrhea 5.0% and 4.3% in the Trulance group (CIC and IBS-C, respectively) and 1% in the placebo group for each indication. The majority of reported cases of diarrhea occurred within 4 weeks of treatment initiation. In the CIC population, severe diarrhea

was reported in 0.6% of Trulance-treated patients compared to 0.3% of placebo-treated patients and was reported to occur within the first 3 days of treatment. In the IBS-C population, severe diarrhea was reported in 1.0% of Trulance treated patients compared to 0.1% of placebo treated patients and occurred within the first day of treatment.

Discontinuations due to adverse reactions occurred in 4.0% and 2.5% of Trulance-treated patients and 2.0% and 0.4% of placebo-treated patients; the most common adverse reaction leading to discontinuation was diarrhea in 2.0% and 1.2% of Trulance treated patients and 0.5% and 0% of placebo treated (CIC and IBS-C, respectively).

A description of the clinical trials, safety, and efficacy information can also be found in the Investigator's Brochure.

2.2 OBJECTIVES

2.2.1 Primary Objective

To evaluate the safety and efficacy of once daily oral plecanatide for 4 weeks as treatment for the relief of symptoms associated with IBS-C in children ages 6 to < 18 years.

2.2.2 Secondary Objective

To estimate the pharmacokinetic (PK) parameters of plecanatide in this patient population (to the extent possible, since plecanatide is not expected to be absorbed).

2.2.3 Rationale for Dose Selection

Safety and efficacy data with plecanatide in adult CIC and IBS-C clinical trials performed to date has defined a safe and effective dose roughly in the range of 0.05 mg/kg to 0.1 mg/kg. Data suggest that children aged 4 years through 16 years appear to have a GC-C receptor density more reflective of a "mature" intestine (Cohen, 1988) [9]. Although safety of a particular dose cannot be taken for granted, it is more likely that children in this age group will respond to a GC-C agonist similarly to adult patients. Thus, the expectation is that ≤ 0.05 mg/kg should represent safe and effective doses for treatment of IBS-C in children ages 6 to less than 18 years under study in this protocol. There are few data that might shed light on potential physiological differences between adults and children in terms of pharmacological dose-responsiveness to GC-C agonists.

Based on considerable PK experience in the adult population it is known that plecanatide is essentially unabsorbed from the human GI tract in the dose range proven effective for the treatment of adult CIC and IBS-C. There is no expectation that pediatric patients will differ in this respect. Therefore, the doses selected for an adolescent population are anticipated to be safe and well tolerated.

2.3 RISK-BENEFIT ASSESSMENT

The most common TEAEs in adult patients treated with plecanatide have been diarrhea and other gastrointestinal (GI)-related symptoms such as abdominal cramps, gas, and bloating (Investigator Brochure (IB) Ed. 9.0 for Plecanatide, 02/28/2018). It is expected that the plecanatide safety profile observed in the 6 to < 18-year old pediatric population will be similar to that observed in adult patients with CIC and IBS-C.

The amount of blood to be drawn during the course of this study (approximately 42 mL for the majority of patients, and 66 mL for patients participating in the intensive PK analysis) is not considered to be a risk to child and adolescent patients qualified for enrollment in this study.

2.4 CRITERIA FOR EVALUATION OF THE STUDY

The efficacy and safety endpoints are described below. For information concerning the analyses of these endpoints, see **Section 8.8** and **Section 8.10** respectively.

2.4.1 Efficacy Endpoints

2.4.1.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in weekly SBM frequency over the 4 Week Treatment Period compared to placebo.

2.4.1.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are:

- Change from baseline in frequency and severity of abdominal pain and abdominal discomfort
- Change from baseline in frequency of BMs, SBMs, and CSBMs
- Time to First BM
- Change from baseline in stool consistency (BSFS or mBSFS-C)
- Use of Rescue Medication
- Change from baseline in frequency of fecal incontinence
- Change from baseline in frequency and severity of defecation pain
- Change from baseline in frequency of large diameter stools

2.4.1.3 Patient Reported Outcomes

The following patient reported outcomes will be assessed (see **Appendix I** for the assessment instruments used for these endpoints):

- Global IBS-Disease Severity
- Health-Related Quality of Life (KINDL® -QoL)
- Global Relief of IBS Symptoms
- Global Relief of Abdominal Pain
- Treatment Continuation Assessment
- Treatment Satisfaction Assessment

2.4.2 Pharmacokinetic Endpoints

Single dose and steady state PK of plecanatide and its major metabolite SP-338 (population modeling):

- Plasma concentration of plecanatide and SP-338 (Note: measurable plecanatide/SP-338 levels have not been observed in the adult population)
- PK parameters, including C_{max}, T_{max}, and C_{min} (only if sufficient number of plecanatide levels can be measured to allow computation of these parameters)

2.4.3 Safety Endpoints

The following safety endpoints will be assessed:

- Frequency of treatment-emergent adverse events (TEAEs)
- Withdrawals due to adverse events and serious adverse events

Clinically important changes in laboratory tests and vital signs will be presented.

2.5 JUSTIFICATION OF THE STUDY DESIGN

This is a randomized, double-blind, placebo-controlled, parallel group, Phase 2b study evaluating plecanatide 4-week treatment in child and adolescent patients with IBS-C.

Randomization and double-blind components of the study design are used to minimize bias in treatment group assignment and investigator/study personnel/patient bias, respectively, during the study. The fixed dose and time on study drug are the most objective ways to assess the therapeutic effect and safety of an investigational agent relative to placebo. The treatment period is 4 weeks, which is sufficient time to test the durability of response.

2.6 PLANNED SAMPLE SIZE AND NUMBER OF STUDY CENTERS

Approximately 210 patients (~ 30 patients per age/dose group) will be recruited for this study at approximately 20 clinical sites in the United States. The patients will be enrolled in this multi-center outpatient study by physicians who -have been qualified as Investigators.

3. STUDY POPULATION

Patients must meet all of the inclusion criteria and none of the exclusion criteria listed below to be enrolled in the study.

3.1 INCLUSION CRITERIA

A patient will be eligible for study participation if he or she meets all of the following criteria:

1. Male or female child or adolescent age 6 to < 18;
2. Meets ROME IV criteria for child/adolescent IBS-C defined as:
   For at least 2 months before diagnosis the patient has had:
   1. Abdominal pain at least 4 days per month associated with one or more of the following:
      a. Related to defecation
      b. A change in frequency of stool
      c. A change in form (appearance) of stool;
   2. The pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome);
   3. After appropriate evaluation, the symptoms cannot be fully explained by another medical condition
   4. More than one-fourth (25%) of bowel movements with Bristol stool form types 1 or 2 and less than one-fourth (25%) of bowel movements with Bristol stool form types 6 or 7 on the BSFS or 4 or 5 on the mBSFS-C;
3. Patient's parent/guardian/LAR is able to voluntarily provide written, signed, and dated consent and patient is able to voluntarily provide assent as per IRB guidance;
4. Patient and patient's parent/guardian/LAR demonstrates an understanding, ability, and willingness to fully comply with protocol requirements and study procedures (e.g., acceptance of venipuncture, acceptance of urine drug screen for opiates, visit schedule, complete daily electronic diary reporting).

3.2 EXCLUSION CRITERIA

A patient will be excluded from the study if he or she meets any of the following criteria:

1. The patient has a mental age <4 years in the investigator's opinion;
2. The patient has previously been diagnosed with anorectal malformations, neurological deficits, or anatomical anomalies that would constitute a predisposition to constipation;
3. The patient currently requires iron supplements, amitriptyline, or other tricyclic antidepressants for depression, opioid-containing medications or compounds for pain, or has other conditions that require medications known to cause constipation. A patient with an onset of constipation prior to the use of these medications and who has been on a stable dose for at least 8 weeks prior to Screening might be considered eligible for this study if the investigator deems these medications do not significantly contribute to the patient's constipation. Screening of these patients needs to be approved by the medical monitor and the sponsor;
4. The patient is pregnant or lactating;
5. Females age 12 to < 18 or females age 6 to 11 of childbearing potential (defined as post menarche) who does not agree to practice one of the following medically acceptable methods of birth control throughout the study;
   - Hormonal methods such as oral, implantable, injectable, vaginal ring, or transdermal contraceptives for a minimum of 1 full cycle (based on the patient's usual menstrual cycle period) before study drug administration.
   - Total abstinence from sexual intercourse (since the last menses before study drug administration
   - Intrauterine device.
   - Double-barrier method (condoms, sponge, or diaphragm with spermicidal jellies or cream.

6. The patient follows a diet not considered normal by the investigator for the patient's age, relative to variety of food, caloric content, and quantity. The patient must have been on a stable diet for at least 30 days prior to Screening;
7. The patient's mobility or normal exercise tolerance is compromised in the investigator's opinion;
8. The patient has a history of an eating disorder;
9. The patient has clinical or laboratory signs and symptoms of significant cerebral, respiratory, renal, hepatobiliary, pancreatic, intestinal (including acute appendicitis, inflammatory bowel disease, or undiagnosed abdominal pain), endocrinologic, or infectious disease that in the investigator's judgment could interfere with study assessments or completion of the study. (Note: A patient with a history of thyroid disease may be enrolled if he or she has normal T3 and T4 at Screening. If the patient is taking medication for active thyroid disease, his or her T3 and T4 level must be within normal limits and the dose of any medication used to treat it must be stable for at least 30 days prior to Screening);
10. The patient has any other medical condition or is receiving concomitant medication or therapy that would in the investigator's opinion compromise his or her safety or compliance with the study protocol or compromise data collection;
11. The patient has a history or evidence of drug or alcohol abuse in the 12 months before Screening;
12. The patient has a hypersensitivity, allergy, or contraindication to plecanatide;
13. The patient has received any experimental drug, including linaclotide and lubiprostone, or experimental therapy within 30 days of study start;
14. The patient is unable to tolerate protocol-prescribed rescue medication (Dulcolax®), or unwilling to use it as the only laxative for the duration of the trial;
15. The patient has taken a medication considered to be a protocol-defined prohibited prior or concomitant medication or supplement as defined in section 3.3.2;
16. The patient and his or her caregiver are unable to communicate well with the study staff and comply with the study requirements (restrictions, appointments, and examination schedule). (The patient/caregiver must be able to complete required Daily BM and Symptom diary entries during the Screening/Baseline period and for the duration of the study. The patient/caregiver must also agree to provide contact information to receive daily reminders should the patient not complete the daily electronic diary entries or require password resets);
17. The patient has been screened for or participated in this or another Synergy study in the past;
18. The patient has a sibling that is currently participating or has participated in another Synergy study.

Exclusion Criteria Based on Baseline Diary Entries

19. Patient reports having more than 1 loose, mushy stool (Diary-recorded stool consistency of 6 on the Bristol Stool Form Scale (BSFS or 4 on the modified Bristol Stool Form Scale for Children [mBSFS-C]) or any watery stool (Diary-recorded stool consistency of 7 on the BSFS or 5 on the mBSFS-C) with any SBM that occurred in the absence of laxative use on the calendar day of the BM or the calendar day before the BM during each 7-day (week) period before the randomization day and up to the day of randomization;
20. ≥ 3 CSBMs per week for either week of the 2-week baseline diary assessment immediately preceding the randomization visit;
21. > 6 SBMs per week for either week of the 2-week baseline diary assessment immediately preceding the randomization visit;
22. Patient reports worst abdominal pain intensity (WAPI) scores in the 2-week baseline diary that meet either of the following:
    a. WAPI score of 0 on the 11-point Numeric Rating Scale or Wong-Baker Faces® Pain Rating Scale for more than two days during each week of the 2-week baseline diary period.
    b. An average WAPI < 3 for either of the two weeks of the baseline diary;

23. Completion of < 5 of the 7 required daily diary entries in each week of the 2-week baseline diary assessment immediately preceding the randomization visit;
24. Use of rescue medication (Dulcolax®, bisacodyl) for more than 2 days during either of the two weeks of the 2-week baseline diary assessment immediately preceding the randomization visit.

3.3 PREVIOUS AND CONCOMITANT MEDICATIONS

Any medication the patient takes other than the study drug, including herbal and other non-traditional remedies, is considered a concomitant medication. The following information must be recorded in the eCRF for each concomitant medication: generic name, route of administration, start and stop dates (month and year at minimum), dosage, and indication. Any changes in the dose or regimen of a concomitant medication must also be recorded in the eCRF.

At the Screening visit, patients/caregivers will be asked what medications the patient has taken during the last 30 days. At each subsequent study visit, patients/caregivers will be asked what concomitant medications the patient is currently taking or has taken since their last visit.

Use of rescue medication (Dulcolax®) will be recorded daily in the BM Diary and should not be included under concomitant medications.

3.3.1 Permitted Concomitant Medications

Rescue Medication (RM) – Dulcolax®

Dulcolax® 5 mg tablets will be dispensed to patients at Visit 1 and resupplied as needed. From Visit 1 and for the duration of the trial, only protocol supplied Dulcolax® is to be used as the "rescue medication" (laxative) - no other laxatives are allowed. The study level allowable dosage for children age 6 to 12 years is one tablet (5 mg daily) and for children 12 years and older the dosage is 1 to 2 tablets daily (5 to 10 mg). Dulcolax® tablets must be swallowed whole.

Patients are instructed to take rescue medication only if it has been at least 72 hours since their last bowel movement. A patient is therefore expected to have no more than two days of RM use in any given week.

Each rescue medication usage will be recorded daily via the electronic diary. Clinical site personnel will review rescue medication use with patients/caregivers during their telephone contact visits and have the option to discontinue participation of patients who do not adhere to rescue medication guidelines.

Patients are advised NOT to take rescue medication for the period from 24 hours before to 72 hours after Day 1 of dosing. This avoids confounding the data collected in the first week of study drug administration and allows for accurate determination of the time to first BM. Dulcolax®. An initial supply of Rescue Medication will be provided to each site from [redacted]. For additional bulk supplies of rescue medication, the site will make a request through IWRS. Supplies of Dulcolax® will not be reconciled at the completion of the study. Sites must track inventory of rescue medication supplies as this will not be managed through IWRS.

3.3.2 Prohibited Prior and Concomitant Medications and Supplements

The following medications, laxatives, and supplements are **prohibited within 15 days** prior to the Screening visit and for the duration of the study, unless otherwise indicated:

- Oral anticholinergic agents (topical and inhaled anticholinergics are allowed)
- Drugs with activity at the 5-HT4, 5-HT3, and 5-HT2b receptors
- Antidiarrheal agents including Pepto Bismol™, kaolin, and opiates

- Drugs known to cause diarrhea such as orlistat, acarbose, misoprostol, and colchicine
- Bile acid sequestrants (cholestyramine and colestipol)
- Amitiza® (lubiprostone)
- Linzess®/Constella® (linaclotide)
- Resolor® (prucalopride) antibiotics, including rifaximin, and opioids, including tramadol or opiate antidiarrheals (diphenoxylate, and loperamide) are generally prohibited. However, short-term (< 15 days) use of opioids or antibiotics for the treatment of AEs or intercurrent illness may be administered during the Screening Period prior to Visit 2 (Day 1) or after randomization in the study as long as they are reported. If a patient needs to be started on an antibiotic or a narcotic during the Screening Period, the patient's Visit 2 may be delayed allowing for discontinuation of these medications at least 3 days before randomization.

Prohibited drugs when required and used to treat TEAEs are allowed. The following drugs are allowed only if the patient has been on a **stable dose for the 8 weeks** prior to the Screening/Baseline Period and the patient agrees to remain on this dose for the duration of the study.

- Anticonvulsants
- Antidepressants
- Calcium channel blockers
- Proton pump inhibitors and H2 antagonists
- Antihistamines that have primarily anti H1 activity (e.g., cetirizine, loratadine, and chlorpheniramine)
- Bulking agents (e.g., psyllium [Metamucil®] methylcellulose [Citrucel®], calcium polycarbophil)

Thyroid hormone supplementation: levothyroxine (T4) or natural desiccated thyroid hormone or liothyronine (T3) are allowed only if the patient has been on a stable dose for the 30 days prior to the 4-week Screening/Baseline Period and remains on this dose for the duration of participation in the study. TSH level will be determined at screening to determine eligibility (T3 and free T4 will only be tested if TSH is out of range).

Prohibited Laxatives

All laxatives (except for rescue medication as described in the following list) will be prohibited from the Screening Visit (Visit 1) and onward for the duration of the study (including 2 weeks post-treatment). This includes the following:

- Lactulose
- Stimulant laxatives, including senna (Ex-Lax®) and sennosides (e.g., Senokot®), cascara sagrada, anthraquinones, castor oil, aloe, or others (if unsure, clinical sites to discuss with medical monitor (MM).
- Osmotic laxatives (e.g., polyethylene glycol 3350 [MiraLAX®], magnesium hydroxide [Milk of Magnesia®], magnesium sulfate [Epsom Salts®] sodium biphosphate [Phospho-Soda®], saline laxatives [magnesium citrate], glycerine suppositories, glucitol [Sorbitol®] lactulose)
- Bisacodyl (e.g., Dulcolax®, Carter's Little Pills®, Alophen®, Correctol®) and other diphenylmethane laxatives (phenolphthalein). Note: See rescue medication exception in **Section 3.3.1**
- Stool softeners (docusate sodium, e.g., Colace®)

3.4 DIETARY INTAKE

Stable dietary intake should be maintained, including high fiber diet, fiber supplements, vitamins and minerals, probiotics, fish oil, etc. during the study. Patients must have been on a stable dietary regimen for at least 30 days before Visit 1, and are expected to remain on that diet, including all supplements, for the duration of the study.

4. VARIABLES AND METHODS OF ASSESSMENT

4.1 DEMOGRAPHICS AND BASELINE CHARACTERISTICS

4.1.1 Patient Demography

Patient demography consists of:

- Age at screening* (date of birth)
- Race
- Ethnicity
- Sex (Gender)
- Height (m), without shoes
- Weight (kg) and BMI

*If a patient is anticipated to turn 12 between screening and randomization, he/she will be randomized into the adolescent group. See section **9.6** for more information on age changes and informed consent/assent.

4.1.2 Disease and Medical History

A complete medical history, including history of GI diseases, should be recorded for all patients. Source documentation for diagnoses, previous treatments and interventions, where available, should be included in the patient's medical record to facilitate source documentation verification. Confirmation of diagnosis of IBS-C is required as part of evaluation for this study; if not previously documented in the patient's medical history, the diagnosis must be included in the patient's medical record for the purpose of source document verification.

4.1.3 Previous Medications and Diet

At the Screening Visit (Visit 1), the clinical site will ensure the patient is not or has not been on prohibited medications, supplements or investigational agents during the specified time (see **Section 3.3.2**). If the patient has used a prohibited medication within the proscribed time period prior to Visit 1, the site needs to ensure that the required washout period for the medication is completed before commencement of the 2-week baseline diary assessments. Previous medications will be documented as described in **Section 3.3.**

The diet history of each patient will be reviewed at Screening (Visit 1) to determine if the patient has been on a stable diet for the last 30 days, and whether the patient agrees to remain on that diet for the duration of the study.

4.2 EFFICACY VARIABLES

4.2.1 Data Collected via the Electronic Diary

At the Screening Visit (Visit 1), patients will be instructed on the use of the electronic diary and the importance of daily diary reports. Episodic or "real time" reporting should be made after each bowel movement or use of rescue medication during the course of the day. End of Day reporting should be made only once per day (between 7 pm to 11:59 pm each day). **Patients will begin using the electronic diary following training at Visit 1 and will continue using it through the Post-Treatment Period**.
The electronic diary will be activated at the Screening Visit (Visit 1) in order for the patient to become familiar with its daily use to record their BMs, rescue medication intake and IBS-C symptoms. Note that any data entered prior to the beginning of the 2-week baseline diary period will be utilized for assessment of compliance only and will not be saved.
The electronic diary is designed for:

- **Episodic reporting** of each bowel movement (BM) and rescue medication (RM) use in "real-time" as they occur throughout a 24-hour period (Patients/caregivers may report BMs and RM use as often as necessary);
- **End-of-Day daily reporting** of constipation-related symptoms (and BM/RM if not already reported as an 'episodic report') and fecal incontinence* at a specific time each evening. Patients who have not completed the prompted evening call will receive an automated reminder each evening.

Soiling of underwear in younger children may be indicative of Fecal Incontinence

To remain eligible for the study, patients must complete at least 5 of the 7 days of electronic End-of-Day diary entries for each of the 2 weeks of Baseline diary period.

Compliance with diary entries should be a focus for patient education at the telephone contact visits and as needed per the judgement of the clinical site staff.

The following endpoints will be assessed using data collected via the electronic diary in "real time":

- *Frequency of Bowel Movements*

  Patients/caregivers will be asked to report each BM experienced.
- *Stool Consistency*

  Patients/caregivers will be asked to rate the patient's stool consistency according to the BSFS (see **Appendix C)** or mBSFS-C (see **Appendix D**), which will be provided to them in the form of a laminated card (and will appear on the appropriate electronic device) at the Screening Visit (Visit 1), and may be referred to as often as necessary throughout the Screening, Treatment and Post-treatment Periods. The BSFS and mBSFS-C are validated measures of stool consistency commonly used in clinical trials.
- *Stool Diameter*

  Patients/caregivers will be asked to report the number of large diameter stools. A large diameter stool is defined as one that is large enough to clog the toilet.
- *Use of Rescue Medication (RM)*

  Patients/caregivers will report the use of provided rescue medication (Dulcolax®), including time of use and amount of rescue medication used. Use of rescue medication will determine whether a BM was spontaneous, but only this aspect of rescue medication use is part of the primary endpoint.

The following endpoints will be assessed using data collected via the electronic diary at the end of the day:

- *Fecal Incontinence*

  As part of the daily diary (end of day), the following question will be asked:

  How many episodes of fecal incontinence did you experience today?

  0 1 2 3 more than 3
- *IBS Symptoms*

  Patients/caregivers will record the frequency and severity of several IBS-related gastrointestinal symptoms in the electronic diary. These symptoms include abdominal pain, abdominal discomfort, and defecation pain. Each will be rated daily on an 11-point Numeric Pain Rating Scale from 0 (NO) to 10 (WORST POSSIBLE) for adolescents (age 12 to <18) (see **Appendix E**) or the Wong-Baker FACES® Pain Rating Scale for children (age 6 to 11) (see **Appendix F**). The exact descriptors for each symptom rating will be appropriate for each symptom (e.g. for abdominal pain and defecation pain 0 = no pain, 10 = worst possible pain).

- *Patient Global Questionnaires*

  In addition to the daily BM and gastrointestinal symptom entries via the e-diary, the following patient questionnaires will also be administered during the study visits (prior to any laboratory blood draws):

  - Global IBS-Disease Severity,
  - KINDL® Health-Related Quality of Life
  - Global Relief of IBS Symptoms
  - Global Relief of Abdominal Pain
  - Treatment Continuation Assessment
  - Treatment Satisfaction Assessment

4.3 SAFETY VARIABLES

4.3.1 Collection of Adverse Events

It is the responsibility of the Investigator to ensure that all AEs are collected. This includes both serious and non-serious AEs derived by spontaneous, unsolicited reports of patients, by observation, and by routine open questionings e.g., "How have you felt since I last saw you?"

NOTE: See section 7.1 for additional details on the recording of specific gastrointestinal symptoms related to IBS-C.

For Definitions, Assessments of AEs, Causality, Severity, Recording and Reporting of Adverse Events and Follow Up, see **Section 7.**

4.3.2 Laboratory Variables

Laboratory assessments will be performed by a central laboratory, as identified in the List of Study Personnel. Please see the Laboratory Manual for detailed instructions regarding the collection, processing, and handling of laboratory samples.

The laboratory variables presented in **Table 1** will be assessed in accordance with the Schedule of Assessments (**Table 2**). Routine laboratory tests (serum chemistry [including electrolytes], hematology, and urinalysis) will be performed at Visit 1 (Screening), Visit 2, and Visit 3 (EOT). A 6- to 12-hour fast is suggested for Visit 1 and is highly recommended prior to all visits when blood sampling is scheduled. The timing of other safety tests (e.g., pregnancy tests and drug screen) is presented in the Schedule of Assessments (**Table 2**).

Table 1	**Laboratory Assessments**
Pregnancy test:	Urine pregnancy tests via dip sticks (performed on-site) at all visits with a scheduled pregnancy test
Hematology:	Erythrocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils, eosinophils, basophils, lymphocytes, monocytes, platelets, leukocytes, hemoglobin, and hematocrit
Urinalysis:	Specific gravity, pH, protein, glucose, ketones, blood, and microscopic examination of sediment

Table 1	**Laboratory Assessments**
Serum chemistry:	Alanine aminotransferase, aspartate aminotransferase, creatinine, alkaline phosphatase, total bilirubin, direct bilirubin, blood urea nitrogen, total protein, albumin, uric acid, glucose, and cholesterol.
Hormone:	TSH at screening to determine eligibility (T3 and free T4 if TSH is out of range)
Electrolytes:	Sodium, potassium, chloride, magnesium, phosphorus, calcium
PK:	Plecanatide (and SP-338) plasma assessment
Urine screen for select opioids:	Performed in the clinic for methadone, morphine, and oxycodone.

At Screening, any laboratory abnormality considered clinically significant by the Investigator or the Medical Monitor will render the patient ineligible for study enrollment.

Approximately 5.0 mL of blood will be drawn for each hematology sample and approximately 5.0 mL of blood will be drawn for each serum chemistry sample. The volume of blood that will be required for these laboratory tests during the duration of the study, not including possible repeat tests, will be approximately 30 mL.

4.3.3 Pharmacokinetic (PK) Assessments

Blood samples (6 mL each) for measurement of plecanatide and SP-338 concentration will be collected from all patients on Day 1 of the Treatment Period (pre-dose) and at the Week 4 visits. Intensive sampling for formal PK analysis will be conducted in a subgroup of approximately 70 patients (approximately 35 patients per age group) who consent to the additional blood sampling at 30, 60, 90, and 120 minutes following study drug administration on Day 1 of treatment. The actual sampling times will be recorded on the eCRF for each PK sample. The total blood volume collected for PK analysis will be about 12 mL for the majority of patients, and 36 mL for patients participating in the intensive PK analysis. Only samples from patients randomized to active treatment will be analyzed.

Orally delivered plecanatide is not absorbed in the adult population nor is it expected to be absorbed in the 6 to <18-year-old age group. However, should any measurable levels of plecanatide or its primary metabolite (SP-338) be observed, to the extent possible, pharmacokinetic parameter estimates (C_{max}, C_{min}, t_{max}) will be undertaken. Nominal sampling time will be used for all parameter estimation.

In order to maintain the double-blind status of the study, results of the pharmacokinetic assay will be blinded to the investigators, to the sponsor, and monitors during the course of the study.

The procedure for the collection, handling, storage, and shipment of the samples for PK analysis are specified in the site reference laboratory manual.

4.3.4 Vital Signs

The following vital signs will be assessed in accordance with the Schedule of Assessments (**Table 2**). Measurements should be performed when the patient is seated. A clinically significant abnormality at screening may result in the patient being excluded from the study.

- Blood pressure (systolic and diastolic mmHg)

- Heart rate (beats per minute)
- Oral body temperature (°C)
- Respiration rate (breaths per minute)

4.3.5 Electrocardiograms

A standard 12-lead ECG will be performed in accordance with the Schedule of Assessments (**Table 2**). A clinically significant abnormality at Screening, as determined by the PI, will result in the patient being excluded from the study.

4.3.6 Physical Examinations

Physical examinations (PEs) will be performed in accordance with the Schedule of Assessments (**Table 2**). A clinically significant abnormality at Screening may result in the patient being excluded from the study.

The PE at the Screening Visit will be based on the following body systems: general appearance, head (ear, eyes, nose, and throat), cardiovascular, respiratory system, abdomen, musculoskeletal, neurological, lymph nodes, and skin. All other PEs will be symptom-directed.

Neither a urogenital exam or a digital rectal examination is required for the study but should take place at Screening if the Investigator feels that there may be a confounding factor for constipation symptoms e.g., anal pathology, or a rectocele in a female patient.

NOTE: The presence of other alarm symptoms (lower GI bleeding, iron-deficiency anemia, unexplained clinically-significant weight loss and systemic signs of infection or colitis) preclude patient eligibility for this trial, unless the PI has adequately assessed each alarm symptom and has discussed their medical relevance with the Medical Monitor, including the potential need for colonoscopic evaluation of the symptoms.

Height and weight will be measured at the Screening Visit only. BMI calculation will use the height and weight recorded at Visit 1 (Screening Visit).

4.3.7 Concomitant Medications and Diet

Concomitant medications or vitamins/nutritional supplements will be reviewed and documented at each study visit to ensure prohibited/restricted substances are not being taken. Concomitant medication will be documented as described in **Section 3.3**.

Drugs required to be at a stable dose prior to screening including thyroid hormone replacement (30-day stabilization) should not be initiated or dose adjusted during the study.

4.3.8 Rescue Medication

Use of rescue medication will be reported in the electronic diary. Patients will be instructed to take rescue medication only if 72 hours have elapsed since their last BM and reminded of this instruction at each visit.

5. STUDY CONDUCT

5.1 SCHEDULE OF ASSESSMENTS

For the timing of assessments and procedures throughout the study, refer to the Schedule of Assessments **Table 2.** Throughout the study, every reasonable effort should be made by study personnel to follow the timing of assessments and procedures in the Schedule of Assessments for each patient. If a patient misses a study visit for any reason, the visit should be rescheduled as soon as possible.

Table 2 Schedule of Assessments

Study Period	**Screening/ Baseline**		**Treatment**				**Post-Treatment Follow-up**
Visit	**Visit 1**	**Visit 2**	**TC 1[a]**	**TC 2[a]**	**TC 3[a]**	**Visit 3 Week 4 (EOT) or EW**	**Visit 4**
Study Week	**Week -4**	**Week 1**					**Week 6 (EOS)**
Visit Day (Window)	**Day -28**	**Day 1**	**Day 3 + 1**	**Day 7 +3**	**Day 25 +2**	**Day 28 +3**	**Day 42 +3**
Informed Consent/Assent	X						
Inclusion/Exclusion Criteria	X	X					
Demography	X						
Medical History (including GI and bowel habits)	X	X					
Prior and Concomitant Medications	X	X	X	X	X	X	X
Physical Examination[b]	X	X				X	X
12-lead Electrocardiogram[c]	X						
Vital Signs[d]	X	X				X	X
Pregnancy Test (urine)[e]	X	X				X	X
Urine Drug Screen for Opioids[f]	X	X					
Serum Chemistry, Hematology, Urinalysis	X	X				X	
TSH (include T3 and T4 only if TSH is abnormal)	X						
PK Sampling: pre-dose and at 28 days(30, 60, 90 and 120 mins post-dose in a subset of 70 patients)		X				X	
Electronic Diary Training and Activation[g]	X						
Diary Eligibility[h]		X					
Randomization		X					
Study Drug Dispensation and Administration[i]		X					
In clinic PRO Assessments[j]		X				X	X

Table 2 Schedule of Assessments

Study Period	Screening/ Baseline		Treatment				Post-Treatment Follow-up
Visit Study Week	Visit 1 Week -4	Visit 2 Week 1	TC 1[a]	TC 2[a]	TC 3[a]	Visit 3 Week 4 (EOT) or EW	Visit 4 Week 6 (EOS)
Visit Day (Window)	Day -28	Day 1	Day 3 + 1	Day 7 +3	Day 25 +2	Day 28 +3	Day 42 +3
Study Drug Collection and Accountability[k]						X	X
Rescue Medication Dispensation	X	X[l]				X[l]	
Adverse Events[m]	X	X	X	X	X	X	X

TC = Telephone Contact; EOT = end of treatment; EW = early withdrawal; EOS = end of study; GI = gastrointestinal; PK = pharmacokinetic; PRO = Patient reported outcome, TSH = thyroid-stimulating hormone.

a. In addition to on-site clinic visits, study sites will call patients/caregivers on Day 3, Day 7, and as needed to remind them to complete their daily diaries and on Day 25 to remind them to return all unused study drug at their EOT visit.
b. Physical examination (PE), including body weight measurement, will be performed at Visit 1. A symptom-directed PE will be performed as necessary at Visit 3 (EOT) or EW and Visit 4 (EOS); height is measured at the Screening Visit. Body mass index (BMI) will be calculated by EDC at the Screening Visit only. Height and body weight may be collected at Visit 2 if missed at Screening.
c. The standard 12-lead ECG will be performed in the semi-recumbent or supine position.
d. Seated blood pressure, heart rate, respiration, temperature.
e. Urine pregnancy tests on-site at clinic visits for female patients as per exclusion criteria 5
f. Urine drug screen for selected opioids (screen includes methadone, morphine and oxycodone) will be performed on-site; a negative result must be confirmed prior to dispensing the study medication.
g. Clinical site personnel will train patients on the use of electronic diaries at the Screening visit and remind them to make daily entries throughout the study, with retraining as needed. The interactive voice or web response system will provide daily reminders to improve compliance, and as per bullet a, study sites will call patients/caregivers on day 3, day 7, and weekly until Visit 3 (Week 4) to remind them to complete their daily diaries.
h. Baseline diary results will be reviewed programmatically and a "Diary Eligible" or "Not Diary Eligible" report will be provided to the site immediately prior to randomization.
i. Patients will take their first dose of study drug at the site on Day 1; it is recommended that for dosing at home, patients take it once daily at approximately the same time in the morning.
j. Details on timing of each PRO Assessment will be included in the Study Procedure Manual.
k. Study drug not returned at EOT must be returned at EOS
l. Only if needed
m. Adverse Event collection begins immediately after informed consent is signed. A symptom-directed physical examination should be performed as appropriate at discretion of the investigator.

5.2 PROCEDURES BY VISIT

5.2.1 Visit 1: Screening (Day −28)

The patient must be screened within 28 days before the randomization visit in the study. A 3-day window is allowed to complete all procedures of the Screening visit. The last 14 days prior to randomization will be used as the patient's baseline electronic diary assessment.
The following will be completed at the Screening Visit:

- Obtain informed consent/assent. The parent/guardian/LAR must provide written informed consent and the patient must provide written or verbal assent as required by the IRB providing oversight.
- Assign a unique patient number. Unique patient numbers will begin with the clinical site number, e.g., 001 followed by a 3-digit number. Unique patient numbers will be assigned sequentially by the IWRS system accessed by the clinical site personnel. This unique patient number will be kept for the duration of the study. Patients who discontinue from the study before randomization will retain their unique patient number (i.e., numbers from screen fail patients will NOT be reassigned).
- Assess willingness and ability to maintain a stable diet and fiber supplements (if applicable) for the study period and to comply with not using laxatives except for the rescue medication provided as part of the study.
- Determine eligibility, based on Rome IV criteria for child/adolescent IBS and IBS-C. (**Appendix A**)
- Review inclusion/exclusion criteria.
- Collect demographic information.
- Record medical history, including GI and bowel habit history.
- Record prior and concomitant medications, vitamins, nutritional supplements, and dietary history.
- Perform urine pregnancy test for females age 6 to 11 that are of child-bearing potential and all females 12 to < 18.
- Perform urine screen for opioids (see **Section 4.3.2** for details).
- Collect blood and urine samples for safety laboratory tests (serum chemistry, including thyroid-stimulating hormone [TSH], hematology, and urinalysis.
- Measure vital signs (body temperature and seated blood pressure, heart rate, respiration).
- Perform a physical examination, including measurement of body weight and height.
- Perform 12-lead ECG in the semi-recumbent or supine position.
- For a child patient (6 to 11 years of age), determine and document based on the child's level of understanding, whether the child or caregiver will be responsible for recording of diary responses.
- If applicable, document in a source document who the caregiver will be for the duration of the study.
- Train patients/caregivers how to use the electronic diary to record their responses about BM and IBS-C-related symptoms and activate the diary.
- If more than one person will act as the caregiver, e.g., both parents or a parent and an older sibling or grandparent, that person must be trained on diary usage.
- Distribute a laminated card with the BSFS or mBSFS-C to patients/caregivers and explain how to use it to record stool consistency in their daily electronic diaries.
- Ensure patients/caregivers understand the importance of entering data in their diaries daily for all questions.
- Dispense rescue medication to patients and instruct them on appropriate use.

Patients who fail to qualify for randomization during the Screening Period will be considered Screen Failures and the reason for failure will be documented.

Patients who screen-failed may be allowed to re-screen under certain circumstances upon review and approval by the Medical Monitor.

5.2.2 Visit 2: Randomization (Day 1)

Continued study eligibility must be confirmed ***prior to randomization,*** including the following:

- Verify Non-Diary eligibility:
  - Review inclusion and exclusion criteria
  - Confirm that patient has used only study-supplied rescue medication laxative (Dulcolax®) since Visit 1
  - Confirmation that patient is on a stable diet and has no significant changes in their consumption of liquids or fiber or their level of activity
  - Perform urine drug screen (a positive test results in automatic screen failure)
  - Perform urine pregnancy test, if applicable (a positive test results in automatic screen failure)
- Update medical history and concomitant medications, as necessary.
- Log into IWRS to obtain system-generated diary eligibility based on the previous 14 days of daily diary entries before randomization. (Only patients who demonstrated diary and RM compliance will be considered diary eligible. **The PI or site staff will <u>not</u> be responsible for evaluating diary data to make the diary-based eligibility determination.**
- Ineligible patients, as determined by the IWRS, will be screen-failed.
- For diary eligible patients, as determined by the IWRS, confirm patient continues to meet all other eligibility criteria and access IWRS to randomize the patient and obtain the study drug kit number.
- The following procedures will be performed ***prior to study drug dosing for patients eligible for randomization***:
- Symptom-directed physical examination and vital signs (body temperature and seated blood pressure, heart rate, respiration rate)
- Assessment of adverse events
- Patient/caregiver to complete all baseline PRO questionnaires (Per Study Procedure Manual) prior to any blood collections
- Collection of blood and urine samples for safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Collection of blood samples for plecanatide/SP-338 concentration assessments (see **Section 4.3.3** for details)
- Selection of drug kit(s) as per the IWRS-generated report
- Supervision of administration of first dose of study drug from the assigned study drug kit(s)
- Recording of time of dosing

The following procedures will be performed ***after study drug dosing***:

- At selected sites and in a subgroup of patients who consented to an additional PK analysis, blood samples will be obtained for PK assessment 30, 60, 90 and 120 minutes after study drug administration
- Dispense the assigned study drug kit(s) (4-week supply) from which the first dose was administered with the instructions for use and storage (as described in **Section 6.4**)
- Instruct patients/caregivers to save all unused study drug and drug packaging for return at Visit 3/Week 4 or EW/ET.
- Distribute additional rescue medication (Dulcolax® tablets), if needed
- Re-emphasize the need for diary compliance, as necessary

- Confirm the next visit and explain the importance of meeting the 3-day window for completion of study visits

5.2.3 Telephone Contact 1 (TC 1): Day 3 to 4

Site personnel will contact patients/caregivers by phone on Day 3 to 4 to review diary compliance ask the following which will be documented in the patient's source record:

- Is the patient taking the study drug as prescribed?
- How is your child feeling?
- Is the daily diary information being entered as instructed?
- Are you/your child experiencing any difficulties with daily diary entry?
- Have there been any changes in concomitant medications?

5.2.4 Telephone Contact 2 (TC 2): Day 7 to 10

Site personnel will contact patients/caregivers by phone on Day 7 to 10 to review diary compliance ask following which will be documented in the patient's source record:

- Is the patient taking the study drug as prescribed?
- How is your child t feeling?
- Is the daily diary information being entered as instructed?
- Are you/your child experiencing any difficulties with daily diary entry?
- Have there been any changes in concomitant medications?

5.2.5 Telephone Contact 3 (TC 3): Day 25 to 27

Site personnel will contact patients/caregivers by phone on Day 25 to 27 to review diary compliance ask the following which will be documented in the patient's source record:

- Is the patient taking the study drug as prescribed?
- How is your child feeling?
- Have there been any changes in concomitant medications?
- Remind participants/caregivers to bring all unused study drug to their next visit.

5.2.6 Visit 3: End of Treatment (EOT) Week 4, Day 28 (+3 days)

The following will be completed at Visit 3:

- Collect/record unused study drug from the supply dispensed at the previous visit
- Review the electronic diary compliance reports and re-emphasize the need for diary compliance throughout the follow-up period
- Have the patient complete the PRO questionnaires (per Study Procedure Manual)
- Assess/record changes in concomitant medications and diet
- Symptoms directed physical examination and vital signs (body temperature and seated blood pressure, heart rate, respiration rate)
- Safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Urine pregnancy test, if applicable
- Collect blood samples for plecanatide/SP-338 concentration assessments
- Assess adverse events
- Distribute additional rescue medication (Dulcolax® tablets), if needed

5.2.7 Visit 4: Week 6, Day 42 (+ 3 days) – End of Study (EOS)

The following will be completed at Visit 4:

- Assess/record changes in physical examination, vital signs, concomitant medications and diet
- Perform urine pregnancy test, if applicable
- Assess adverse events
- Collect/record unused study drug, if not collected (per protocol) at Visit 4
- Have the patient/caregiver complete the PRO questionnaires (per Study Procedure Manual)

5.2.8 Early Withdrawal/Early Termination Visit (EW/ET)

Patients are free to withdraw from participation in the study at any time. Investigators may choose to discontinue a patient's participation in the study if they believe it is in the patient's best interest clinically. If a patient's post-randomization compliance is of concern, contact the medical monitor to discuss. If the patient has an intervening illness that requires discontinuation of study participation, the patient and Investigator will follow procedures for early withdrawal. The following are examples of AEs that qualify for Early Withdrawal (EW):

- A positive pregnancy test will require discontinuation from the study (see **Section 7.7**).
- Changes in laboratory values, PE findings, or other assessments considered by the Investigator (or designee) to be clinically significant will require discontinuation from the study.
- Clinically significant TEAEs, including clinically significant laboratory test abnormalities or SAEs regardless of relatedness to study treatment that cause the patient, investigator, or Sponsor to feel it is not in the patient's best interest to continue.

A patient may also be withdrawn from study drug/study by the Sponsor, regulatory authorities, or the institutional review board (IRB). Patients will also be withdrawn if the entire study is terminated prematurely as described in **Section 9.9**.

Patients who discontinue early from the study should, if possible, have an Early Withdrawal Visit. The Investigator must make every effort (with proper documentation) to have the patient complete this final visit. This visit should take place as soon as possible (and within 5 days) after the patient stops taking study drug.

The following procedures will be performed at the Early Withdrawal Visit:

- Collect/record unused study drug from the supply dispensed at the previous visit
- Have the patient/caregiver complete the PRO questionnaires (see Study Procedure Manual)
- Assess/record changes in concomitant medications and diet
- Symptoms directed physical examination and vital signs (seated blood pressure, heart rate, respiration, and body temperature)
- Safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Perform urine pregnancy test, if applicable
- Collect blood samples for plecanatide/SP-338 assessments
- Assess adverse events.

In all cases, a single, primary reason for withdrawal must be recorded on the eCRF.
Patients withdrawn after randomization will **not** be replaced.

6. STUDY DRUG

6.1 IDENTITY OF PLECANATIDE AND PLACEBO

Plecanatide is a synthetic hexadecapeptide that is an analog (identical, with the exception of a single amino acid) of uroguanylin, a natural hormone. The chemical name, molecular formula, molecular weight, and amino acid sequence of plecanatide can be found in the IB provided to each clinical site.

The drug product is a tablet comprised of plecanatide, microcrystalline cellulose, and magnesium stearate. Matching placebo composition is identical but does not contain plecanatide. Both plecanatide and placebo tablets are manufactured by UPM Pharmaceuticals (Bristol, TN) and packaged by Sharp Packaging Solutions (Allentown, PA). Study supplies will be retested as required by the sponsor.

6.2 ADMINISTRATION

Study drug will be administered for 4 consecutive weeks according to the randomization scheme displayed in **Table 3**. Each patient will take one or two tablets (based upon randomization into Group A or B) orally (by mouth) daily preferably at the same time each day—**in the morning**—with approximately 240 mL (~8 oz.) of liquid, with or without food*.

Patients in treatment arms 1, 2, and 3 (Group A) will be dispensed one drug kit and take one tablet daily. Patients in treatment arms 4, 5, 6, and 7 (Group B) will be dispensed two drug kits and take two tablets daily (one tablet from each drug kit).

For smaller children that are unable to swallow the plecanatide or placebo tablet, the parent/caregiver may crush the tablet into applesauce or water. Rescue medication (Dulcolax®) tablets are very small and coated so must be swallowed whole.

No dose adjustments will be allowed on this study.

Table 3 Treatment Arms

Treatment Arm (Age Dose Group)		Treatment	No. of Tablets to be Taken Daily	Dose Level	Number of Patients
1	(6 to 11 yrs. old)	0.5 mg plecanatide	1	0.5 mg plecanatide	30
2	(6 to11 yrs. old)	1.0 mg plecanatide	1	1.0 mg plecanatide	30
3	(6 to11 yrs. old)	Matching placebo	1	placebo	30
4	(12 to < 18 yrs. old)	0.5 mg plecanatide	2	1.0 mg plecanatide	30
5	(12 to < 18 yrs. old)	1.0 mg plecanatide	2	2.0 mg plecanatide	30
6	(12 to < 18 yrs. old)	1.5 mg plecanatide	2	3.0 mg plecanatide	30
7	(12 to < 18 yrs. old)	Matching placebo	2	placebo	30

6.2.1 Overdose

Plecanatide has minimal systemic absorption, therefore standard treatment measures for the symptomatology being exhibited should be provided. Notable, plecanatide was generally well-tolerated in adults after single doses up to 48.6 mg and multiple doses up to 9.0 mg for 12 weeks in prior studies.

6.3 STOPPING CRITERIA

Overall safety monitoring for the trial is the responsibility of the Medical Monitor (MM) who reviews adverse event listings and provides ongoing medical oversight of adverse event reports throughout the duration of the trial. Any interruption of study drug for an individual patient, or for the study, based on the established stopping criteria outlined below will be initiated by the Medical Monitor.

6.3.1 Individual Patient Stopping Criteria

Adverse events due to study drug that affect individual patient safety may necessitate dose interruptions or early discontinuation as described in this section.

Plecanatide therapy in adults with and IBS-C and CIC has caused diarrhea in 4% and 5% of patients respectively, with severe diarrhea occurring in 0.7% and 0.6% of patients respectively. Severe diarrhea may cause dehydration. Pediatric patients may be at increased risk for diarrhea and dehydration with plecanatide therapy.

In general, study drug dosing should not be modified for mild diarrhea (Grade 1: increase of < 4 stools per day over baseline) or mild dehydration (Grade 1: dry mucous membranes, diminished skin turgor) (*National Cancer Institute, Common Terminology Criteria for Adverse Events [CTCAE] v5.0, November 27, 2017*). If study drug is not well tolerated by an individual patient, treatment will be permanently discontinued for that patient.

The following guidelines should be used in the case of moderate or severe diarrhea or dehydration:

Table 4 Individual Patient Stopping Criteria

Worst Observed Toxicity Grade NCI Common Toxicity Criteria	**Dose Modification for Study Drug**
Diarrhea	
Grade 2: Increase of 4-6 stools per day over baseline	No intervention required
Grade 3: Increase of ≥ 7 stools per day over baseline, incontinence, hospitalization indicated	Interrupt study drug for < 7 days until resolved to less than Grade 2 severity. If diarrhea was study drug-related, permanently discontinue study drug. If diarrhea was not study drug-related, e.g., viral gastroenteritis, then consider resuming treatment after consultation with Sponsor's Medical Monitor
Grade 4: Life-threatening consequences, urgent intervention indicated	Permanently discontinue study drug
Dehydration	
Grade 2: IV fluids indicated < 24 hour	Interrupt study drug for < 7 days until resolved to less than Grade 2 severity. If dehydration was study drug-related, permanently discontinue study drug. If dehydration was not study drug-related, consider resuming treatment after consultation with Sponsor's Medical Monitor
Grade 3: IV fluids or hospitalization indicated	Permanently discontinue study drug

Table 4 Individual Patient Stopping Criteria	
Grade 4: Life-threatening consequences, urgent intervention indicated	Permanently discontinue study drug

6.3.2 Study Stopping Criteria

Dosing of all patients in the study will be interrupted for at least one week if ≥2 patients receiving study drug develop drug-related Grade 3 diarrhea and/or dehydration or if one patient develops drug-related Grade 4 diarrhea and/or dehydration. This will allow the Sponsor to assess whether the trial should be stopped or modified.

6.4 PACKAGING, LABELING AND STORAGE

All study centers will be provided with adequate supplies of study medication—plecanatide tablets at 3 tablet strengths (see **Table 3**) and identically appearing placebo tablets, by [redacted].

6.4.1 Packaging

Each investigational drug kit will be supplied in blister packaging. At the randomization visit (Day 1), eligible patients enrolled in treatment group A (6 to 11 years old) will receive one drug kit for the entire 4 weeks of dosing (Weeks 1, 2, 3, and 4) 28 days plus 4 additional tablets to allow for a +3-day window. Patients in treatment group B (12 to < 18 years old) will receive two drug kits and will be instructed to take one tablet from each drug kit (2 tablets daily) for the entire 4 weeks of dosing (56 tablets) plus 8 additional tablets to allow for a +3-day window. Patients will be instructed to bring their kits to the EOT or EW study visit for determination of compliance and reconciliation of supplies.

Each investigational study drug kit will contain 32 tablets. Individually blister-packaged tablets will be provided in four connected strips (of 8 tablets) as a 4-panel key-pack that folds into a carton. The two bottom panels fold up into the two top panels, nesting blister cavities. Instructions for releasing a tablet from the blister dome are printed on the outside of the carton.

Clinical sites will save all empty packaging or packaging containing unused tablets for final disposition instructions from the sponsor.

6.4.2 Label

Each of the investigational drug kits will have a unique label containing the following information: protocol number, drug kit number, contents, directions for use, storage conditions, Sponsor name, city, state, and zip code, and the statements: "Caution: New Drug — Limited by Federal Law to Investigational use. Investigational Drug – To be used by Qualified Investigator Only. For Clinical Trial Use Only. Keep Out of Reach of Children under 6 years".

The label will include the following fields to be completed by site personnel: Investigator name and phone number, patient number, initials, and treatment weeks. When dispensing kit(s) to a patient, complete and remove the tear off portion of the label and keep this with the source documents. At each monitoring visit, the source document records will be verified against the IWRS assignment.

There will also be a smaller label on the carton spine that identifies the study number and drug kit number and Sponsor.

6.4.3 Storage

The Investigator, or qualified designee, is responsible for the proper storage of the study medications according to the Sponsor's recommendations and all applicable federal/state regulatory guidelines. At the clinical site, plecanatide should be stored at controlled room temperature 20°C to 25°C (68°F to 77°F) in a secure area with Min / Max Temperature Recording with restricted access. Deviations from Sponsors recommendations / guidelines should be reported, as directed in the Site Level Temperature Excursion Reporting Form.

Patients will be instructed to store their study drug at room temperature defined for this study as 20°C to 25°C (68°F to 77°F).

The Investigator must agree not to dispense or store the investigational drug at any location other than that listed on the Form FDA 1572.

6.5 BLINDING AND BREAKING THE BLIND

The study will be performed in a double-blind manner. All study drugs will be supplied in identical blister packs and tablets will be similar in color, smell, taste, and appearance, thereby assuring double-blind conditions.

In the event of a need to break the blind, the Investigator at the clinical site will have the ability to break the treatment code using the IWRS. The blind should only be broken following discussion on a case-by-case basis, with the Sponsor/Medical Monitor (except in a medical emergency). The Sponsor's Medical Monitor will also be granted emergency code-break privileges using the IWRS. All un-blinding events will be documented. The Sponsor and CRO, as applicable, will specify who in each company is able to review unblinded treatment in a blinded trial.

In the event of a treatment code-break, the time, date, reason, name, and signature of the person responsible for breaking the code must be fully documented in the patient's source documents and any associated AE recorded. The breaking of the blind will result in the withdrawal of the patient from study participation, and the patient should follow the procedures detailed for Early Withdrawal Visit study assessment (see **Section 5.2.8**).

The overall randomization code for the study will be broken at study completion. This will occur once all final clinical data have been entered into the database, data queries have been resolved, and the assignment of patients to the analysis sets has been completed.

6.6 DRUG ACCOUNTABILITY

Acknowledgement of receipt of drug shipments and distribution of all investigational drug kits will be recorded using the IWRS. The drug inventory log can be generated from IWRS at the site level as a standard report.

In addition, accurate records of study drug dispensed to patients will be kept by the Investigator, or qualified designee in IWRS, specifying the kit number, the patient number assigned, the amount dispensed to each patient, and the date dispensed. This information will be available in the IWRS for the overall study. At Visit 3 (EOT) or EW, the patient is asked to return their previously dispensed drug kit and the number of remaining tablets is entered into the IWRS for drug accountability.

A drug accountability log can also be generated from IWRS and must be available for inspection at each monitoring visit and at the completion of the study. At the completion of the study, the Investigator will provide signed copies of this accountability log to the Sponsor.

The Investigator is responsible for the retrieval of all study supplies from patients. At the completion of the study, all unused investigational drug kits will be returned to a third party in accordance with the Sponsor's (or designee's) written instructions. The Investigator must verify that all unused or partially used study drug supplies have been returned by the patient and that no remaining supplies are in the Investigator's possession.

6.7 COMPLIANCE

Patients will be advised as to how to take their daily medication and will return all unused study drug to the clinical site at the end of the Treatment Period or Early Withdrawal Visit. The number of remaining tablets will be counted and entered into IWRS for that patient. Patients unwilling or unable to maintain compliance with study drug administration or procedures may be discontinued from the study at the discretion of the investigator in conjunction with the medical monitor.

7. ASSESSMENT, REPORTING, RECORDING AND FOLLOW UP OF ADVERSE EVENTS

7.1 DEFINITION

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered to be drug related. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product or study procedure whether or not considered related to the product or procedure.

NOTE: Specific gastrointestinal symptoms related to IBS-C are recorded daily in the electronic Diary in response to standardized questions and are BOTH efficacy AND safety response variables. These symptoms are abdominal pain, abdominal discomfort, and pain with defecation. The diary is a "real-time" patient reported outcome record that is based on "within 24 hours" patient recall. Because these symptoms are considered adequately recorded in the IBS-C Symptom Diary, they should generally not also be reported in the adverse event database that relies on a much longer patient recall period. To prevent double-recording of a single specific event, the daily IBS-C Symptom Diary record is considered the highest quality record of an event and should therefore be the only record of the same event.

All AEs, including intercurrent illnesses and regardless of the source of identification (e.g., physical examination, laboratory assessment, reported by patient), occurring during the study will be documented in the eCRF. Concomitant illnesses that existed before entry into the study will not be considered AEs unless they worsen during the treatment period.

Adverse events occurring from the time of signing the informed consent form (ICF) up to the intake of the first dose of study medication will be classified as pre-existing conditions for the study and therefore will be recorded in the Medical History page of the electronic case report form (eCRF). Serious adverse events (as defined below) occurring after the signing of informed consent but before randomization (i.e., during screening) will be collected and reported as such. A treatment emergent adverse event (TEAE) is defined as an AE that begins or worsens in frequency and/or severity after at least one dose of study drug has been administered.

Minor fluctuations in laboratory values for standard monitoring (abnormal values) that the investigator does not consider clinically significant or related to study drug will not be recorded as AEs. However, if the laboratory abnormality is associated with a diagnosis, then the AE term for that diagnosis will be reported.

7.2 EVENT REPORTS OF SPECIAL INTEREST – INCREASE IN BM FREQUENCY AND LOOSENING OF STOOL CONSISTENCY

An increase in the frequency of BMs and loosening of the stool consistency from baseline are expected pharmacodynamic effects of plecanatide. These same attributes (increased stool frequency and looser stool consistency) are often used to define "diarrhea', however, in light of significant inter-patient variability in bowel habit "phenotype" and wide differences in the perception of a "normal" bowel habit there is no standard definition of "diarrhea" for this patient population. An increase in frequency or consistency for one person is a welcome event while for another patient it might be bothersome "diarrhea". In all cases, as mentioned in the NOTE above, the frequency and consistency of each bowel movement is recorded in the electronic diary. However, this objective record often does not go far enough to characterize any change in bowel frequency or consistency as clinically important or impactful for the patient (whether beneficial or harmful).

One mechanism to introduce a level of consistency across patients, sites and the trial is to code an event as diarrhea only if it is characterized by a specific patient-reported and clinically relevant attribute such as "bothersomeness". Therefore, for this trial, for any event associated with a significant increase from baseline in BM frequency and/or loosening of BM consistency - that would otherwise be coded as diarrhea

- the site is instructed to record it as an AE only if the patient reports that it was bothersome (as defined by each patient). Sites may question the patient/caregiver to ascertain whether or not they thought their diarrhea was bothersome.

7.3 ASSESSMENT OF ADVERSE EVENTS

Each AE will be assessed by the Investigator with regard to the following categories.

7.3.1 Seriousness

An SAE is defined as any untoward medical occurrence that at any dose is one of the following:

- Results in death
- Is life threatening (an adverse event or suspected adverse reaction is considered "life threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Is a congenital anomaly or birth defect
- Is a medically important event (examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse)

The FDA has recently defined additional **"Medically Important Events"** that should also be reported as SAEs (Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation, FDA, July 2009). These include cases where:

- Aminotransferases (ALT or AST) are > 3 times the upper limit of normal (ULN) with an associated elevation of total bilirubin > 2 times ULN without evidence of hemolysis or with alkaline phosphatase < 2 times ULN or not available, or
- ALT or AST activity that is > 5 times ULN

All patients with the above abnormalities should return as soon as possible or within 48 hours to the site for further evaluation of the abnormalities, including repeat ALT and AST measurements; total and direct bilirubin; alkaline phosphatase; and related laboratory assessments of albumin, PT and PTT, creatinine kinase, and GGTP or 5'nucleotidase. Hepatitis A, B, and C, hemolysis, and biliary obstruction should be ruled out. A detailed medical and drug use history should be taken to exclude all potential causes. All such laboratory tests should be performed locally if they are not listed in **Section 4.3.2**; **Table 1**.

Medical and scientific judgment should be exercised in deciding whether a case is serious and whether expedited reporting is appropriate as in the example above for "**Medically Important Event**." The medical monitor should be consulted.

7.3.2 Intensity (Severity)

Investigators should assess the severity of AEs according to the following general categorical descriptors:

Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated

Moderate:	Minimal, local, or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL) (instrumental ADL includes dressing, grooming, hygiene, home skills, school skills, using the telephone, managing money, etc.)
Severe:	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (self-care ADL includes bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden)

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity, whereas seriousness is a regulatory definition as per **Section 7.3.1**. An AE of severe intensity may not necessarily be considered serious (by definition) or a mild AE (mild stroke) may be considered an SAE.

7.3.3 Causality

The investigator will assess the causality/relationship between the study drug, or study procedure, and the AE and record that assessment in the eCRF.

The causal relationship of the AE to study drug will be described in terms of:

Reasonable Possibility:	There is evidence to suggest a causal relationship between the drug and the AE (e.g., the AE is uncommon and known to be strongly associated with drug exposure or is uncommon in the study population but not commonly associated with drug exposure)
No Reasonable Possibility:	There is no evidence to suggest a causal relationship between the drug and the AE

The study conduct relatedness for AEs and SAEs will also be assessed and documented. The most likely cause of an AE or SAE (e.g., disease under treatment, concomitant disease, concomitant medication, other) will be indicated in the eCRF system with details of the concomitant disease or medication or other cause.

7.4 RECORDING ADVERSE EVENTS

Adverse event assessment and reporting will extend from signing of ICF until completion of the final visit (End of the Post-Treatment Period). Adverse events occurring from the time of ICF signing up to the intake of the first dose of study medication will be classified as pre-existing conditions for this study and therefore will be recorded in the Medical History page of the eCRF. If, however, an event is considered a serious adverse event (as defined below) and occurs after the signing of informed consent but before randomization (i.e., during screening), this will be collected and reported as non-treatment-emergent Serious Adverse Events and will be recorded in the Adverse Event page of the eCRF. Events that occur from time of first dose of study drug until completion of the final study visit will be considered Treatment-Emergent Adverse Events and will be recorded on the AE page of the eCRF. Adverse events occurring after the end of the study should be reported to the Sponsor by the Investigator only if the event meets at least one seriousness criterion and the Investigator considers the causal relationship to the study drug as ‘reasonably possible’.

All AE reports should contain a brief description of the event, date of onset, date of resolution, intensity, treatment required, relationship to study drug, action taken with the study drug, outcome, and whether the event is classified as serious.

All AEs experienced by patients who are randomized to treatment, regardless of the relationship to study drug, will be recorded in the eCRF. For patients who are screen failures, only SAEs (if any) will be reported on the AE page of the eCRF.

See Section 7.6 on requirements and expectations for follow up of Adverse Events.

7.5 REPORTING SERIOUS ADVERSE EVENTS

All SAEs that occur during the study, as defined by the protocol, must be reported by the Investigator to the designated SAE Safety Contact by submitting (faxing or emailing) the SAE Form **within 24 hours** from the point in time when the Investigator becomes aware of the SAE. In addition, all SAEs including any deaths, which occur up to and including 30 days after the administration of the last dose of the study medication, must be reported to the designated Safety Contact **within 24 hours.**

All SAEs will be collected and reported from signing of informed consent/assent until the end of the study. All SAEs that occur from signing of the ICF/assent to 30 days after the last dose of study medication must be reported whether or not considered causally related to the study medication. Any SAE that occurs beyond 30 days after last dose of study medication must be reported to the designated safety contact only if the event is considered (in the opinion of the Investigator) causally related to the study drug.

SAE forms will be provided to each clinical site. The information collected will include a minimum of the following: protocol number, Investigator information, patient number, event term, a narrative description of the event including its start date, and an assessment by the Investigator as to the intensity and possible relatedness to study medication. A sample of the SAE form can be found in the study manual. The Medical Monitor or Synergy may request follow up information regarding the SAE.

The Investigator will prepare or directly oversee preparation of each SAE report and the Medical Monitor will evaluate and confirm the seriousness and the causal relationship of the event to study treatment. In addition, the Investigator and Medical Monitor will evaluate the expectedness according to the reference document (Investigator's Brochure). Based on the Medical Monitor's and Sponsor's assessment of the event, a decision will be made concerning the need for further action.

The initial SAE Report Form must be faxed or emailed by the Investigator to the designated Safety Contact (below) <u>within 24 hours</u> of becoming aware of the event:

[redacted]

For any questions related to the reporting of an SAE, sites should call the Safety Contact and include the following information: Sponsor ([redacted]), protocol number (SP304202-14), and contact information.

The minimum information required for an initial report is:

- Name of person sending the report (i.e., name and address of Investigator)
- Patient identification (site number, patient number, initials, NOT patient name)
- Protocol number
- Description of SAE
- Causality assessment, if possible

However, as far as possible all points on the SAE form should be covered in the initial report, or the completed SAE form itself must be faxed to the designated Safety Contact. In addition, the event must be documented in the eCRF.

After receipt of the initial report, the Safety Contact and safety center will review the information and, if necessary, contact the Investigator, to obtain further information for assessment of the event. Acknowledgement of receipt of the SAE report will be sent to the site within 24 hrs. In the event acknowledgement is not received, the site should contact Research Assist Inc.
All SAEs must be reported by the Investigator to their IRB/IEC in writing, as well as appropriate regulatory authorities, as required by law. The designated Synergy Safety Contact will be responsible for all information processing and reporting according to local legal requirements.

7.6 FOLLOW-UP OF ADVERSE EVENTS

All AEs experienced by a patient, irrespective of the suspected causality, will be monitored until the AE (including abnormal laboratory values) has resolved or has returned to baseline or stabilized at a level acceptable to the investigator and medical monitor; until there is a satisfactory explanation for the changes observed; until the patient is lost to follow-up; or until the patient has died. **The recording of a stop or stabilized date is required for all reported adverse events.** The handling of a missing AE stop date when a patient is lost to follow-up or when an adverse event is considered stable but ongoing at the end of study visit will be outlined prior to database lock.

7.7 PREGNANCY

Female patients enrolled in the study should make every effort to avoid becoming pregnant during their participation in the study. Female patients should be reminded at every visit to use appropriate birth control methods. However, if a female patient should become pregnant during the study (i.e., from the date the ICF was signed until the patient's last visit), the investigator (or authorized delegate) should notify the designated safety contact and [redacted], Inc., on the initial Pregnancy Report form within 24 hours of the investigator (or authorized delegate) first becoming aware of the pregnancy. Pregnancy itself is not an AE, unless there is suspicion that the investigational product may have interfered with the effectiveness of a contraceptive medication. Pregnancy reports, however, are tracked and reported in the safety (SAE) database but will be reported separately from SAEs. Pregnancy occurring in the patient between the date the ICF was signed until 30 days after the last dose of study medication will fall under the expedited reporting procedure for serious adverse events. The pregnant patient will be immediately discontinued from the study but will be followed for the duration of the pregnancy. Details of the outcome of the pregnancy (e.g., full term normal delivery, stillbirth, congenital anomalies, or miscarriage) will be collected and reported by the site.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. However, elective abortion without complications should not be handled as SAEs.

All outcomes of pregnancy, if known, must be reported by the Investigator to the Medical Monitor or designated safety contact on the pregnancy outcome report form within 30 days after he/she has gained knowledge of the facts. See **Section 11**, **Appendix B** for additional details on pregnancy reporting requirements.

8. STATISTICAL METHODS

The objectives of the study are to assess the safety and efficacy of plecanatide 0.5, 1.0, 2.0 and 3.0 mg dosed daily, compared with placebo.

Before database lock and un-blinding, a formal statistical analysis plan (SAP) will be written and will include details of all statistical methods to be used to analyze the efficacy and safety data. The SAP will supersede the protocol with respect to the analyses specified, although the primary analysis described in this section will remain unchanged.

8.1 RANDOMIZATION AND TREATMENT ASSIGNMENT

This is a double-blind, placebo-controlled study with seven treatment age/dose groups that will be conducted at up to approximately 20 clinical sites. Approximately 210 patients (90 children and 120 adolescents) will be randomized to receive plecanatide or matching placebo. Patients 6-11 years of age will be randomly assigned to 1 of 3 treatment groups (2 active treatments, 1 matching placebo) in a 1:1:1 ratio and patients 12 to < 18 years of age will be randomly assigned to 1 of 4 treatment groups (3 active treatments, 1 matching placebo) in a 1:1:1:1 ratio using an Interactive Web-based Response System (IWRS).

Central, block randomization will be performed with stratification by gender.

8.2 SAMPLE SIZE AND POWER CONSIDERATIONS

Based on data from two phase 3 trials conducted by the sponsor in adults with IBS-C, consider the sample size necessary for observing a significant difference in the change from baseline in SBM frequency in Group B (the adolescents) since data from the adults is likely more applicable to Group B participants. Then, a study with a plecanatide group sample size of 102 and a placebo group sample size of 34 (representing 3:1 randomization, plecanatide to placebo) achieves 81.5% power at the 0.05% level of significance when the difference in mean change from baseline in weekly SBM frequency at four weeks is 1.5 and the placebo group and plecanatide group standard deviations are 2.3 and 3.5 respectively. Since study SP304202-14 is being conducted in children and adolescents with IBS-C, and the primary analysis of the primary endpoint will be an ANCOVA using a linear mixed-effects model, the sponsor believes that 30 patients per treatment group (placebo and 3 dose levels of plecanatide), yielding a planned target enrollment of a total of 210 patients (90 children and 120 adolescents), will prove sufficient for achieving the primary objective of this phase 2 study.

8.3 ANALYSIS POPULATIONS

The following populations will be assessed:

Safety Population (SP):	All randomized patients who receive at least one dose of the study drug. Patients will be analyzed according to the treatment received. All safety analyses will be based on the Safety Population.
Full Analysis Set (FAS):	All randomized and treated patients who had the baseline assessment and at least one post randomization assessment of the primary efficacy measure of weekly SBM frequency. The FAS is the main population for assessments of efficacy.
Per Protocol (PP) Population:	All patients in the FAS population who complete the 4-week Treatment Period or discontinue from study treatment due to adverse event(s) or lack of efficacy, with the exception of major protocol violators. Criteria to identify

the PP population will be detailed in the SAP. Decisions regarding exclusion from the PP Population will be made prior to unblinding the database.

Efficacy analyses will be based on the FAS Population. Efficacy analyses will be repeated for the PP Population to assess the sensitivity of the analyses to the choice of analysis set.

Deviations from the study protocol, including violations of inclusion/exclusion criteria, will be assessed as "minor" or "major.". Major deviations from the protocol will lead to the exclusion of a patient from the PP Population and will be determined prior to unblinding the study database. Missed telephone contact (TC) visits will not be considered deviations from the study protocol.

8.4 GENERAL CONSIDERATIONS

The final data analysis will be performed after all patients have either completed or have been discontinued from the study. The study will be unblinded after patient membership in each of the various analysis populations has been determined at a blinded data review meeting and upon confirmation of database lock.

Three treatment groups are identified for all analyses cited in the following as "by treatment group": A (patients in Age Group A receiving plecanatide doses of 0.5 mg or 1.0 mg); B (patients in Age Group B receiving plecanatide doses of 1.0 mg, 2.0 mg, or 3.0 mg); Placebo (all patients randomized to receive placebo.)

Any deviations from the analyses presented here will be detailed in the clinical study report. All outputs will be produced using SAS® version 9.2 or later.

All efficacy data will be summarized and analyzed using the FAS and PP Populations unless otherwise noted. Safety data will be summarized using the Safety Population.

Imputation of missing data, where appropriate, will be performed as indicated for a given analysis. Any spurious or erroneous data will be queried and followed up until satisfactorily resolved; if not resolvable, the data will be set to missing. All data collected in the clinical database will be included in the data listings.

Summary statistics—number of patients (n), mean, standard deviation (SD), median, minimum, and maximum—will be presented for continuous variables. Frequencies and percentages will be presented for categorical variables. Unless otherwise noted, percentages will be calculated using the total number of patients in the appropriate population and/or subgroup and per treatment group where applicable. Where data are collected over time, both the observed data and change from the Screening Period (baseline) will be summarized at each time point.

Statistical testing including p-values and confidence intervals (CIs) will be presented as described in each section below. The Type I error rate (alpha) for the analysis of the primary efficacy endpoint is 0.05 (two-sided).

8.4.1 Missing Data Conventions

Missing values of safety parameters will not be imputed.
The weekly BM (SBM, CSBM) rate is the sum of the BMs (SBMs, CSBMs) recorded for the days in the week for which a BM response is recorded in the daily diary. Note that this is equivalent to a "worst case" analysis in that the BM (SBM, CSBM) rate for the week is the sum of the BMs (SBMs, CSBMs) recorded for the days in the week for which BMs were reported, plus 0 BMs (SBMs, CSBMs) for each day in the week for which the BM response is missing. In any week in which no BM response is recorded, the weekly BM (SBM, CSBM) rate will be set to 0 in any summary or linear mixed-effects model analysis involving

the weekly BM (SBM, CSBM) rate. In particular, for a randomized patient who withdraws from study participation prior to the end of the study, the patient is considered to have a weekly BM (SBM, CSBM) rate equal to 0 for any weeks remaining in the planned duration of the study for which the patient has no BM responses recorded.

The weekly average IBS-related daily abdominal pain score is the average of the non-missing, abdominal pain scores recorded in that week, i.e., the weekly average is the sum of the abdominal pain scores recorded in the diary in the given week, divided by the number of pain scores recorded in the week. In any week in which a patient has no diary days for which an abdominal pain score is recorded, the weekly average abdominal pain score will be set to missing. In any linear mixed-effects model analysis involving the weekly average abdominal pain score, if the average score is set to missing in a given week, it will be left as missing in the linear model. In particular, for a randomized patient who withdraws from study participation prior to the end of the study, the weekly average abdominal pain score will be set to missing for any weeks remaining in the planned duration of the study for which the patient has no abdominal pain data.

In any analysis of the frequency of fecal incontinence, the frequency of defecation pain, and the frequency of large diameter stools will follow the same methodology as described above for the determination and analysis of the weekly BM rate.

In any analysis involving the severity of defecation pain, the determination of weekly averages will follow the same methodology as described above for the abdominal pain score. That is, the weekly average item score is the sum of the non-missing item scores recorded during the week, divided by the total number of non-missing item scores recorded. In any week in which a patient has no non-missing item scores recorded, the weekly average score will be set to missing in any summary or linear mixed-effects model analysis involving the weekly average item score.

In any analysis involving stool consistency (mBSFS-C scores or BSFS scores), the determination of weekly averages will follow the same methodology as presented above for abdominal pain.
However, since stool consistency cannot be assessed if the patient does not have at least one SBM during the week in question (i.e., stool consistency is based on SBMs only), the weekly average will also be set to missing if no SBMs are recorded for any days in the week. Also, it is possible that a patient has no baseline weekly average stool consistency score because of the SBM criterion; in that case the patient will be excluded from any change from baseline analyses of stool consistency.

8.4.2 Diary Data Visit Windows

The daily electronic diary entries reported during the Treatment Period will be classified into 4 weeks as follows:

Day 1-7 = Week 1
Day 8-14 = Week 2
Day 15-21 = Week 3
Day 22-28 = Week 4

Non-diary data, such as vital signs, clinical laboratory assessments, will be displayed and analyzed according to the nominal visit date on the eCRF. Assessments taken outside of protocol allowable windows for nominal visits will be displayed according to the slotting scheme presented in the SAP for unscheduled or out-of-window visits.

8.4.3 Baseline Definition

For each safety parameter, the most recent valid assessment recorded before randomization will be used as the baseline for all analyses of that safety parameter.

For efficacy parameters, the baseline values will be derived from data collected in the 2-week electronic diary assessment at the end of the Screening Period (baseline). The last 14 days of diary entries prior to Day 1 will be the default standard for derivation of these values.

The baseline BM (SBM, CSBM) weekly rate will be the weekly average number of BMs (SBMs, CSBMs) recorded during the 2-week baseline assessment period; similarly, for frequency of abdominal pain and abdominal discomfort, frequency of fecal incontinence, frequency of defecation pain, and frequency of large diameter stools. Baseline stool consistency, severity of abdominal pain, abdominal discomfort, and severity of defecation pain will be calculated as follows: for each week of the baseline assessment, the weekly average is the sum of the non-missing scores recorded during the week divided by the total number of non-missing scores recorded during that week; the baseline is then equal to one-half of the sum of the two weekly average scores.

8.5 Disposition of Patients

Patient disposition and reasons for discontinuation from the Treatment Period of the study will be summarized by treatment group for all randomized patients.

8.6 Treatment Compliance

Dosing compliance will be defined by the dosing compliance ratio: the number of doses actually taken by the patient divided by the number of doses that were expected to be taken during the same period multiplied by 100. Treatment compliance is defined as taking equal to or greater than 80% of the drug dosage prescribed. Otherwise, the patient will be considered noncompliant with study treatment. Rates of treatment compliance as defined above will be compared between treatment groups using the Chi-square test.

Patient compliance with making daily electronic diary entries is monitored by the IWRS; patients non-compliant in making daily diary entries will be withdrawn from the study.

8.7 Demographics, Medical History, Baseline Characteristics, and Concomitant Medications

Demographic data and other baseline characteristics, medical history, and concomitant **medications will be summarized by means of descriptive statistics. Where applicable,** comparisons among treatment groups will be performed using an analysis of variance (ANOVA) model for continuous variables and a Cochran-Mantel-Haenszel (CMH) test for categorical variables.

8.8 Efficacy Analyses

8.8.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in weekly SBM rate over the 4-week treatment period.

8.8.1.1 Analysis of the Primary Efficacy Endpoint

The weekly SBM rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 4-week treatment period. The change from baseline in mean weekly SBM rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical *p*-values.

The above analysis will be repeated for the PP Population.

8.8.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints and methods of analysis are:

8.8.2.1 Change from Baseline in Frequency and Severity of Abdominal Pain and Abdominal Discomfort

The weekly abdominal pain and the weekly abdominal discomfort rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly abdominal pain and abdominal discomfort rate and change from baseline in mean weekly abdominal pain and abdominal discomfort rates, for each of the 6 study weeks will be presented. The change from baseline in mean weekly abdominal pain and abdominal discomfort rates will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical *p*-values.

The weekly average abdominal pain and abdominal discomfort scores will be determined using the approach defined in **Section 8.4.1** and presented for each week in the 4-week treatment period; the baseline weekly average is determined as described in **Section 8.4.3**. The change from baseline in the weekly averaged abdominal pain and abdominal discomfort scores will be analyzed in the FAS Population using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value, and a random intercept for patient. Least Squares (LS) means, with 95% confidence intervals and the corresponding statistical p-value, will be presented for each week, and across all 4 weeks, of the 4-week treatment period for the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo. The analysis of interest for this secondary endpoint is the difference in LS means between each plecanatide group (A, B, and A and B combined) versus placebo for the estimated overall average change from baseline in abdominal pain and abdominal discomfort scores across the 4-week treatment period.

8.8.2.2 Change from Baseline in Frequency of BMs, SBMs, and CSBMs, by Study Week

The weekly BM rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly BM rate, and change from baseline in mean weekly BM rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly BM rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical p-values.

Similarly, for the change from baseline in frequency of SBMs and CSBMs by study week.

8.8.2.3 Time to First BM

For the time to first BM endpoint, the distribution for each treatment arm will be estimated by the Kaplan-Meier method and will be compared by the Log-rank test. The median time along with the 95% confidence

limits for the median will be presented for each treatment arm. Simultaneous confidence bounds for the Kaplan-Meier curve will be computed for all treatment groups.

8.8.2.4 Change from Baseline in Stool Consistency (mBSFS-C or BSFS)

Stool consistency for a bowel movement is measured using the mBSFS-C for patients in Group A and the BSFS for patients in Group B. The weekly average stool consistency scores will be determined using the approach defined in **Section 8.4.1** and presented for each week in the 4-week treatment period; the baseline weekly average is determined as described in **Section 8.4.3**. The change from baseline in the average weekly stool consistency score will be analyzed in the FAS Population using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value, and a random intercept for patient. Least Squares (LS) means, with 95% confidence intervals and the corresponding statistical p-value, will be presented for each week, and across all 4 weeks, of the 4-week treatment period for the difference between the difference in LS means for each of treatment groups A and B versus Placebo. The analysis of interest for this secondary endpoint is the difference in LS means between each plecanatide group and placebo for the estimated overall average change from baseline across the 4-week treatment period.

The above analysis will be repeated for the PP Population.

8.8.2.5 Use of Rescue Medication

The number of tablets of rescue medication used per week, and overall, for the 4-week treatment period will be analyzed by treatment group. The Mann Whitney U Test (Wilcoxon Rank Sum Test) will be used to compare the difference between each treatment group, A, B, and A and B combined, versus Placebo at each week, and over all 4 weeks, of the 4-week treatment period.

8.8.2.6 Change from Baseline in Frequency of Fecal Incontinence

The weekly fecal incontinence rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly fecal incontinence rate, and change from baseline in mean weekly fecal incontinence rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly fecal incontinence rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical *p*-values.

8.8.2.7 Change from Baseline in Frequency and Severity of Defecation Pain

The weekly defecation pain rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly defecation pain rate, and change from baseline in mean weekly defecation pain rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly defecation pain rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical p-values.

The weekly average defecation pain score will be determined using the approach defined in **Section 8.4.1** and presented for each week in the 4-week treatment period; the baseline weekly average is determined as described in **Section 8.4.3**. The change from baseline in the weekly averaged defecation pain score will be analyzed in the FAS Population using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value, and a random intercept for patient. Least Squares (LS) means, with 95% confidence intervals and the corresponding statistical p-value, will be presented for each week, and across all 4 weeks, of the 4-week treatment period for the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo. The analysis of interest for this secondary endpoint is the difference in LS means between each plecanatide group (A, B, and A and B combined) versus placebo for the estimated overall average change from baseline in abdominal pain and abdominal discomfort scores across the 4-week treatment period.

8.8.2.8 Change from Baseline in Frequency of Large Diameter Stools

The weekly large diameter stools rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly large diameter stools rate, and change from baseline in mean weekly large diameter stools rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly large diameter stools rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for patient will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment group, and the difference in LS means for each treatment group, A, B, and A and B combined, versus Placebo, with 95% confidence intervals and corresponding statistical p-values.

8.8.3 Patient Reported Outcomes (PRO)

PRO questionnaire data for IBS Disease Severity, Health-Related Quality of Life, Patient Global Rating of Change—IBS Symptoms, Patient Global Rating of Change—IBS Abdominal Pain, Treatment Continuation and Treatment Satisfaction Assessments will be included in the patient listings **(Appendix I)**. Summary analyses of these data will also be provided as described in the SAP.

8.8.4 Adjustments for Multiple Comparisons

Since this is a Phase 2 clinical trial for exploratory purposes, no adjustments for multiple comparisons will be undertaken.

8.9 PHARMACOKINETIC ANALYSIS

Plasma concentrations of Plecanatide and its major metabolite (SP-338) will be listed by patient and summarized by treatment group.

8.10 SAFETY ANALYSES

Evaluation of the safety of once daily plecanatide over 4 weeks of dosing will be based on the occurrence of TEAEs, vital signs, and clinical laboratory assessments as compared with those noted in the placebo group.

The safety analyses will use the Safety Population defined in **Section 8.3.**

The frequency of SAEs, AEs leading to withdrawal from study participation, and TEAEs will be included in the primary safety tables.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) classifications with reference to system organ classes (SOCs) and preferred terms. Only TEAEs will be included in summary tables. Summaries of TEAEs will be presented by SOC and preferred term, by treatment. Summaries will include the frequency of TEAEs, SAEs, and AEs leading to withdrawal from study participation, by dose as well as by intensity and relationship to study drug.

Out of range laboratory tests and vital signs of potential clinical importance will also be summarized; a listing of patients with at least one out of range value will be presented by visit for that test.

Laboratory tests (hematology, serum chemistry, and urinalysis), and vital signs will be summarized as changes from baseline. Laboratory shift tables will also be produced, where applicable. Listings of laboratory tests and vital signs will also be provided.

Physical examination and medical history listings will be provided but not summarized.

Concomitant medications will be auto-encoded using the WHODD coding system with reference to Anatomical Therapeutic Classification (ATC) text and preferred terms. Summaries of concomitant medication will be presented by ATC text and preferred terms. Prior, concomitant, prior and concomitant, and post-treatment medications will be presented separately.

8.11 INTERIM ANALYSES

No interim analyses are planned.

9. ETHICAL, LEGAL, AND ADMINISTRATIVE ASPECTS

9.1 DATA QUALITY ASSURANCE

The sponsor or sponsor's designee will inform the investigator of the responsibilities and procedures for ensuring adequate and correct 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. All information recorded in the eCRF for this study must be consistent with the patients' source documentation (i.e., medical records and charts).

9.1.1 Database Management and Quality Control

Data will be captured electronically at each clinical site using eCRFs and an interactive web response system (IWRS) and paper CRF as needed. Patient diary and questionnaire data will be collected using ePRO, IWRS, or paper CRFs. Once the eCRF clinical data have been submitted, corrections to the data fields will be captured in an audit trail. The reason for change, the name of the person who performed the change, together with the time and date will be logged to provide an audit trail.

Computerized data check programs and manual checks will identify any clinical data discrepancies for resolution. If additional corrections are needed, the responsible monitor or data manger will raise a query in the electronic data capture (EDC) application. The appropriate staff at the clinical site will answer queries sent to the investigator. The name of the staff member responding to the query, and time and date stamp, will be captured to provide an audit trail. Once all source data verification is complete and all queries are closed, the monitor will update the eCRF page to prevent further changes.

The specific procedures to be used for data entry and query resolution using the EDC and IWRS will be provided to study sites in training manuals and/or guidelines. In addition, clinical site personnel will receive training on the EDC system, including the eCRF, and the IWRS systems.

9.2 ELECTRONIC CASE REPORT FORMS AND SOURCE DOCUMENTATION

All data obtained during this study should be entered into the EDC system promptly or entered by the patient or site in the IWRS interface. All source documents from which eCRF entries are derived should be placed in the patient's medical records/charts. Reports/documents for which source documents are usually available include laboratory assessments and ECG recordings.

The original eCRF/IWRS entries for each patient may be checked against source documents at the clinical site by the site monitor. Instances of missing or un-interpretable data will be discussed with the investigator for resolution.

9.2.1 Data Collection

The investigators (and appropriately authorized staff) will be given access to an online web-based EDC/IWRS system that is 21 Code of Federal Regulations (CFR) Part 11 compliant. This system is specifically designed for the collection of the clinical data in electronic format. Access and rights to the EDC/IWRS system will be carefully controlled and configured according to each individual's role and duration on the study. In general, only the investigator and authorized staff will be able to enter data and make corrections.

The eCRF should be completed for each patient included in the study and should reflect the latest observations on the patient's participating in the study. Therefore, the eCRFs are to be completed as soon

as possible during or immediately after the patient's visit or assessment. **The expectation is that study sites will complete data entry within 48 hours of a patient visit**. The investigator must ensure that all data entries in the eCRF are accurate and correct. If some assessments cannot be done, or if certain information is unavailable, not applicable, or unknown, the investigator should indicate this in the eCRF.

Computerized data-check programs and manual checks will identify any clinical data discrepancies for resolution. Corresponding queries will be loaded into the system and the clinical site will be informed about new issues to be resolved online. All discrepancies will be resolved online directly by the investigator or by authorized staff. **The expectation is that study sites will respond to queries within 72 hours of issue**. Data managers and clinical site monitors will be available to assist sites in resolving queries. Offline edit checks will be done to examine relationships over time and across panels to facilitate quality data.

After completion of data entry and cleaning, the investigator will be required to electronically sign off on the clinical data collected in the eCRF.

Data concerning all study drug dispensed to the patient will be tracked in the IWRS. Study drug return will be tracked in the IWRS as well.

9.3 ACCESS TO SOURCE DATA

During the study, a monitor will make clinical site visits to review protocol compliance, compare eCRF/IWRS entries and individual patient medical records, assess drug accountability, and ensure that the study is being conducted according to pertinent regulatory requirements. eCRF entries will be verified using source documentation. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

Checking of the eCRF/IWRS entries for completeness and clarity, and cross-checking with source documents, will be required to monitor the progress of the study. Moreover, regulatory authorities, IRBs, and/or the sponsor's clinical quality assurance group (or designee) may wish to carry out such source data checks and/or on-site audit inspections. Direct access to source data will be required for these inspections and audits; they will be carried out giving due consideration to data protection and medical confidentiality. The investigator and authorized site staff will assure that auditors or monitors and the sponsor have full access to study data and files and the necessary support of site personnel at all times.

9.4 DATA CODING

Previous and concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary (WHODD), which employs the anatomical therapeutic chemical (ATC) classification system. Medical history/current medical conditions, prior procedures, concurrent therapies, and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) terminology.

The versions of the coding dictionaries used will be provided in the statistical analysis plan and the clinical study report.

9.5 ARCHIVING STUDY RECORDS

According to International Council for Harmonization (ICH) guidelines, essential documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Ten years is the recommended storage duration to ensure compliance with ICH requirements. However, these documents should be retained for a longer period if required by applicable legal requirements.

9.6 GOOD CLINICAL PRACTICE

The procedures set out in the study protocol are designed to ensure that the sponsor and investigator abide by the principles of the Good Clinical Practice guidelines of the ICH. The study also will be carried out in keeping with local legal requirements **(Appendix J).**

Before each patient is admitted to the study, informed consent will be obtained from the patient (or his/her legally authorized representative (LAR)) according to the regulatory and legal requirements. The informed consent form (ICF) must be dated and retained by the investigator as part of the study records. The investigator will not undertake any investigation specifically required only for the clinical study until valid consent has been obtained. The date of the consent must also be documented in the EDC system.

For this study involving child and adolescent populations, the IRB that is providing oversight will determine which form, consent or assent, the patient needs to fulfill in addition to consent by his/her LAR. Furthermore, the age of the patient will be calculated using the date of Screening. If a patient turns a different age during the trial that impacts the informed consent/assent that has already been discussed/signed, the IRB will determine whether re-consent is required.

If child assent is not required of a particular age child, as determined by the IRB, it is still the obligation of the Investigator or his/her designee to provide study information to that child.

If a protocol amendment is required, the ICF may need to be revised to reflect the changes to the protocol. If the ICF is revised, it must be reviewed and approved by the appropriate IRB and signed by all patients subsequently screened for the study as well as those currently enrolled in the study.

9.7 PROTOCOL APPROVAL AND AMENDMENT

Before the start of the study, the study protocol and/or other relevant documents will be approved by the IRB in accordance with local legal requirements. The sponsor must ensure that all ethical and legal requirements have been met before the first patient is screened in the study.
The protocol is to be followed exactly. To alter the protocol, amendments must be written, receive approval from the appropriate personnel at [redacted] and receive IRB approval prior to implementation (if appropriate). Following approval, the protocol amendment(s) will be submitted to the Investigational New Drug (IND) application under which the study is being conducted.

Administrative changes (not affecting the patient benefit/risk ratio) may be made without the need for a formal amendment but must be submitted to the applicable IRB/ethics committee. All amendments will be distributed to all protocol recipients with appropriate instructions.

9.8 DURATION OF THE STUDY

For an individual patient, the planned duration of the study will be 70 days (including 28 days for screening, 28 days of treatment and 14 days of follow-up.

9.9 PREMATURE TERMINATION OF THE STUDY

If the investigator, the sponsor, or the medical monitor becomes aware of conditions or events that suggest a possible hazard to patients if the study continues, the study may be terminated after appropriate consultation between the relevant parties. The study may also be terminated early at the sponsor's discretion in the absence of such a finding.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study

- Failure to enroll patients at an acceptable rate
- A decision on the part of the sponsor to suspend or discontinue development of the study drug

9.10 CONFIDENTIALITY

All study findings and documents will be regarded as confidential. The investigator and members of his/her research team must not disclose such information without prior written approval from the sponsor.

The anonymity of participating patients must be maintained. Patients will be identified in eCRFs and other documents submitted to the sponsor and CRO by their patient number, initials, and/or birth date, not by name.

9.11 OTHER ETHICAL AND REGULATORY ISSUES

If a significant safety issue is identified, either from an individual case report or review of aggregate data, then the sponsor will issue prompt notification to all parties – regulatory authorities, investigators, and IRBs.

A significant safety issue is one that has a significant impact on the course of the clinical study or program (including the potential for suspension of the development program or amendments to protocols) or warrants an immediate update of informed consent form.

9.12 LIABILITY AND INSURANCE

The sponsor will take out reasonable third-party liability insurance coverage in accordance with all local legal requirements. The civil liability of the investigator, the persons instructed by him or her, and the hospital, practice, or institute in which they are employed and the liability of the sponsor with respect to medical costs for physical injury and other damage that may arise as a result of the execution of this study are governed by applicable law(s).

The sponsor will arrange for patients participating in this study to be insured against medical costs for physical injury caused by the pharmaceutical products being tested or by medical steps taken in the course of the study.

9.13 PUBLICATION POLICY

By signing the study protocol, the investigator agrees with the use of results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. If necessary, regulatory authorities will be notified of the investigator's name, address, qualifications, and extent of involvement. An Investigator shall not publish any data (poster, abstract, paper, etc.) without having consulted with the Sponsor in advance. Details are included in the Clinical Study agreement completed for each investigational site.

10. REFERENCE LIST

1. Drossman DA, Morris CB, Hu Y, et al. A prospective assessment of bowel habits in irritable bowel syndrome in women: defining an alternator. Gastroenterol 2005; 128: 580–9.

2. Saito YA, Schoenfeld P, Locke GR III. The epidemiology of irritable bowel syndrome in North America: a systematic review. Am J Gastroenterol 2002; 97: 1910–5.

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4. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. Gastroenterol 2002; 123: 2108–31.

5. Powell PH, Fleming VH. Chapter 43. Diarrhea, Constipation, and Irritable Bowel Syndrome. In: Talbert RL, DiPiro JT, Matzke GR, Posey LM, Wells BG, Yee GC, eds. Pharmacotherapy: A Pathophysiologic Approach. 8th ed. New York: McGraw-Hill; 2011http://www.accesspharmacy.com/content.aspx?aID=7978775. Accessed May 10, 2012.

6. Drossman DA, Dumitrascu DL. Rome III: New Standard for Functional Gastrointestinal Disorders. Gastrointestin Liver Dis 2006; 15: 237-241.

7. Saps M, Seshadri R, Sztainberg M, et al. A prospective school-based study of abdominal pain and other common somatic complaints in children. J Pediatr. 2009; 154: 322-326.

8. Sindic A, Schlatter E. Cellular effects of guanylin and uroguanylin. J Am Soc Nephrol. 2006 17: 607–616.

9. Cohen MB, Guarino A, Shukla R, and Gianella RA. Age-related differences in receptors for escherichia coli heat-stable enterotoxin in the small and large intestine of children. Gastroenterology. 1988; 94: 367-373.

11. APPENDICES

A. ROME IV DIAGNOSTIC CRITERIA* FOR IRRITABLE BOWEL SYNDROME IN CHILDREN

Must include all of the following:

1. Abdominal pain at least 4 days per month associated with one or more of the following:
    a) Related to defecation
    b) A change in frequency of stool
    c) A change in form (appearance) of stool
2. In children with constipation, the pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome)
3. After appropriate evaluation, the symptoms cannot be fully explained by another medical condition

*Criteria fulfilled for at least 2 months before diagnosis.

Rome IV Diagnostic Criteria for IBS with Predominant Constipation

1. More than one fourth (25%) of bowel movements with Bristol stool form types 1 or 2 and
2. Less than one-fourth (25%) of bowel movements with Bristol stool form types 6 or 7 in the BSFS= and types 4 or 5 in the mBSFS-C.

Alternative for epidemiology or clinical practice:
Patient reports that abnormal bowel movements are usually constipation (like type 1 or 2 in the picture of Bristol Stool Form Scale (BSFS).

B. PREGNANCY REPORTING

If a female patient should become pregnant during the course of the study (i.e., from the date the informed consent was signed until the patient's last visit), the Investigator (or authorized delegate) should notify the designated Safety Contact within 24 hours of the Investigator (or authorized delegate) first becoming aware of the pregnancy. The notification of pregnancy should be submitted using the initial Pregnancy Report Form. The initial Pregnancy Report Form should be completed with study patient's details (e.g., patient number, initials, date of birth, and investigational product information.). Whenever possible, the initial notification of pregnancy should include detailed information on the pregnancy, including last menstrual period and/or expected date of delivery. If pregnancy is to be terminated, the anticipated date of termination should be provided. If a maternal AE is reported during the initial notification of pregnancy, the details of the AE should also be described in the narrative field of the initial Pregnancy Report Form.

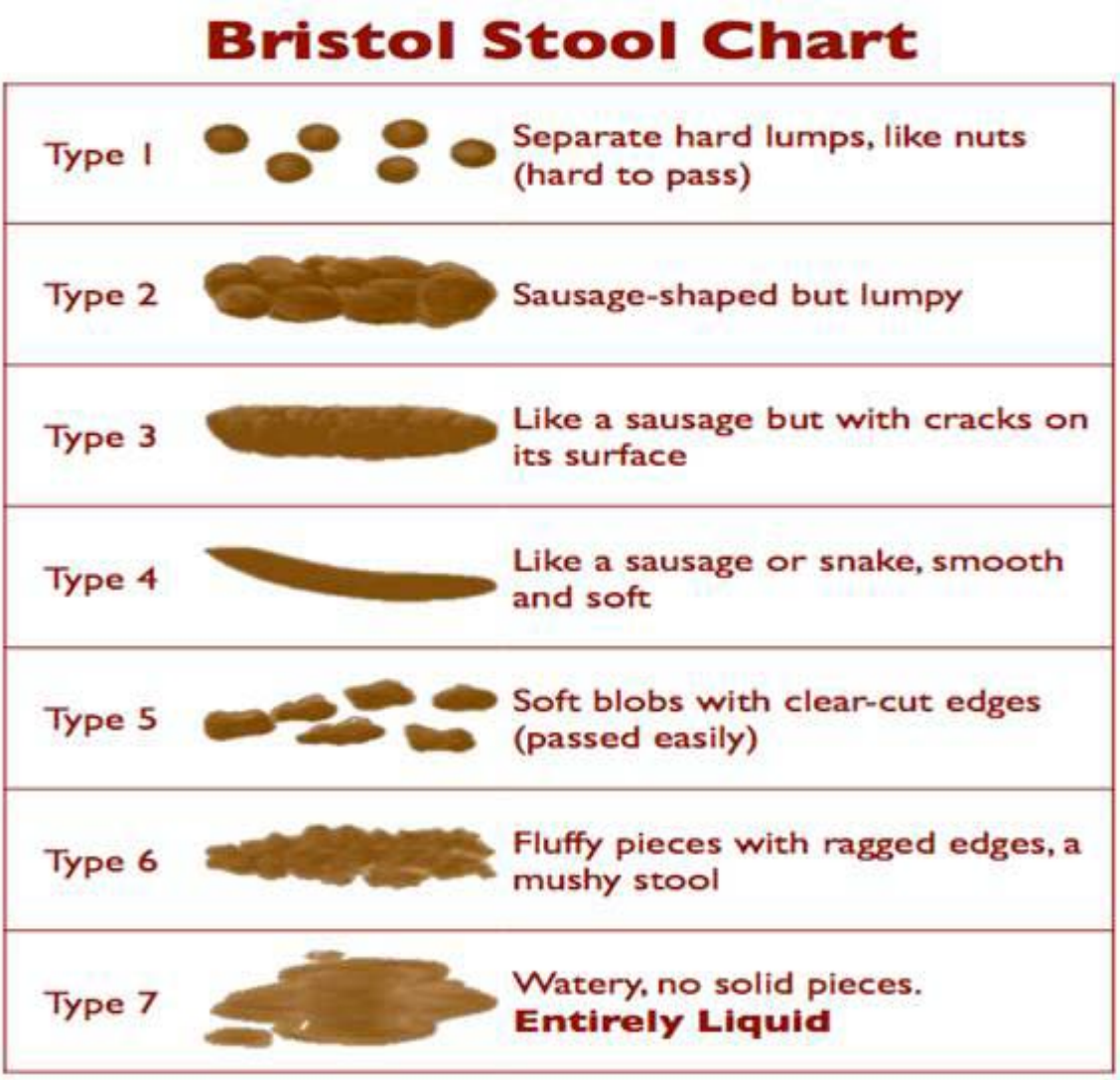
The Sponsor, Medical Monitor, or designated Safety Representative will request permission to follow the patient's progress with the doctor medically responsible for the pregnancy. If additional information on the progress of the pregnancy and/or any maternal AE is received "spontaneously" by the clinical site, the Investigator (or authorized delegate) should submit a follow-up Pregnancy Report Form to the Sponsor within 24 hours of becoming aware of the information.

If additional information on the outcome of the pregnancy and/or the details of the birth/delivery is received "spontaneously" by the clinical site, the Investigator (or authorized delegate) should also submit a pregnancy outcome report form to the designated Safety Contact within 24 hours of becoming aware of the information. If the outcome of the pregnancy is reported as premature birth, or as elective termination due to a medical reason or as spontaneous or accidental miscarriage, the details of the outcome should be described in the narrative section of the outcome Pregnancy Report Form. The pregnancy outcome will generally be reported as a follow-up report. Details of birth/delivery, including date of birth, weight, and sex of the fetus/newborn should also be described in the narrative field of the Pregnancy Report Form.

Complete a new SAE Report Form if the delivery outcome meets the criteria for a SAE (e.g., congenital anomaly/birth defect, still birth, some other sickness, etc.). The SAE Report Form should be completed with study patient's details (e.g., patient number, initials, date of birth, investigational product information, etc.) and the details of the fetal SAE should be described in the narrative field of the SAE Report Form.

C. BRISTOL STOOL FORM SCALE

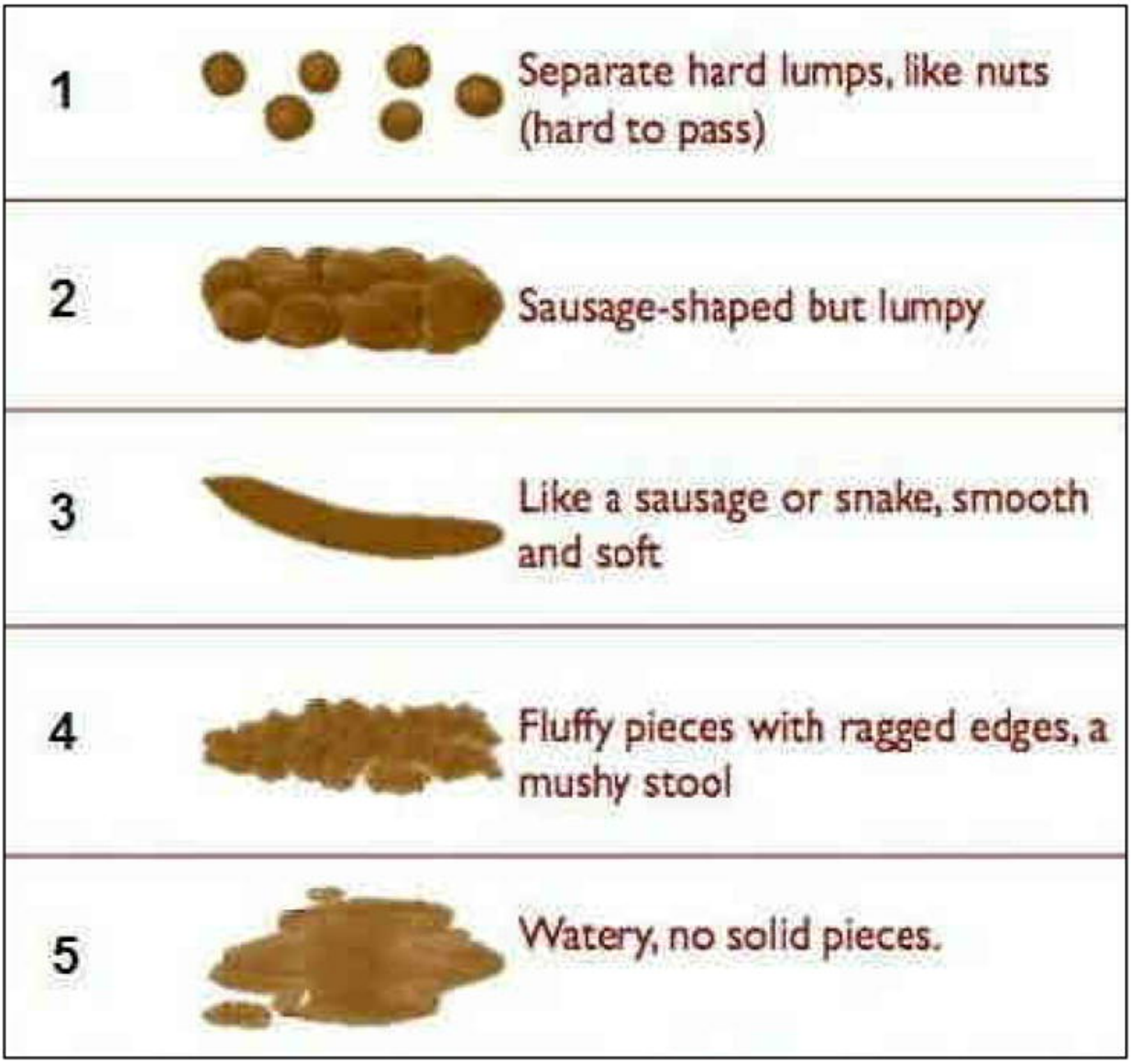
To be used for children in the 12 to < 18 age group.



D. MODIFIED BRISTOL STOOL FORM SCALE FOR CHILDREN

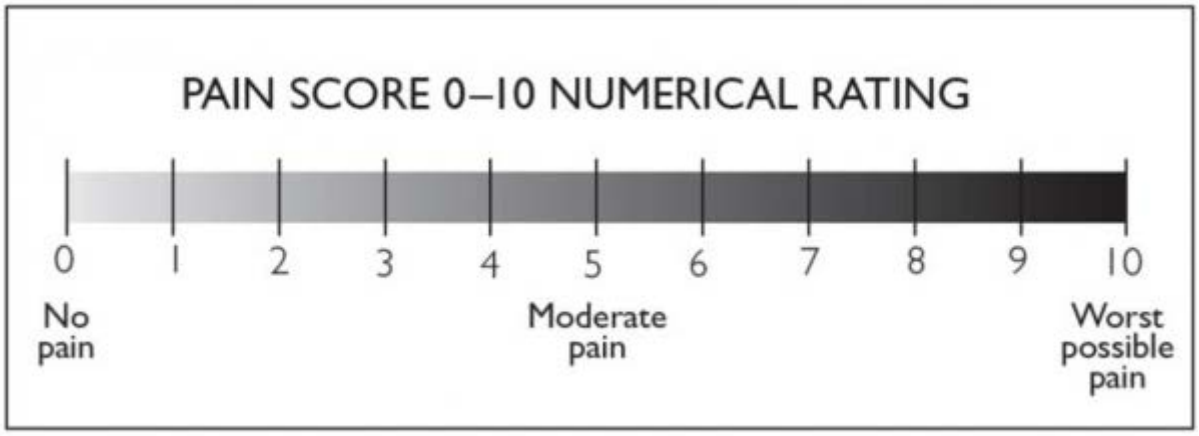
To be used for children in the 6 to 11 age group.

The modified Bristol Stool Form Scale for Children (mBSFS-C)



E. NUMERIC PAIN RATING SCALE

To be used for children for the 12 to < 18 age group.



F. WONG-BAKER FACES® PAIN RATING SCALE

To be used for children for the 6 to 11 age group.

G. ELECTRONIC DAILY DIARY FOR GROUP A (AGE 6 TO 11)

Note: The final text for the diary script will appear in the final Design Specifications for the electronic diary system.

[redacted]

H. ELECTRONIC DAILY DIARY FOR GROUP B (AGE 12 TO < 18)

I. PATIENT QUESTIONNAIRES

- **KINDL® Health-Related Quality of Life**
  - **Children age 6**
  - **Children age 7 to 13**
  - **Adolescents age 14 to 17**
- **Patient Global IBS-Disease Severity**
- **Global Relief of IBS Symptoms**
- **Global Relief of Abdominal Pain**
- **Treatment Satisfaction**
- **Treatment Continuation Assessment**

Kiddy-KINDL® Interview for Patients Age 6

[redacted]

[redacted]

Kid-KINDL® Interview for Patients Age 7 to 13

[redacted]

[redacted]

[redacted]

Kiddo-KINDL® Questionnaire for Adolescents Age 14 to 17

[redacted]

[redacted]

Patient Global IBS-Disease Severity

For the questions below, please choose the response that applies best to you

[redacted]

Patient Global Rating of Change – IBS Symptoms

For the questions below, please choose the response that applies best to you

[redacted]

Patient Global Rating of Change – Abdominal Pain

For the questions below, please choose the response that applies best to you

[redacted]

Patient Treatment Satisfaction Assessment

For the questions below, please choose the response that applies best to you

[redacted]

Patient Treatment Continuation Assessment

For the questions below, please choose the response that applies best to you

[redacted]

J. REGULATIONS AND GOOD CLINICAL PRACTICES

Regulations

Refer to the following United States Code of Federal Regulations (CFR):

- FDA Regulations 21 CFR, Parts 50.20 – 50.27
  Subpart B – Informed Consent of Human Subjects
- FDA Regulations 21 CFR, Parts 56.107 – 56.115
  Part 56 – Institutional Review Boards
  Subpart B – Organization and Personnel
  Subpart C – IRB Functions and Operations
  Subpart D – Records and Reports
- FDA Regulations 21 CFR, Parts 312.50 – 312.70
  Subpart D – Responsibilities of Sponsors and Investigators
- Good Clinical Practice Guidelines

  ICH GCP guidelines can be found at the following URL:

  http://www.ich.org/LOB/media/MEDIA482.pdf

STATISTICAL ANALYSIS PLAN

Study Title:	A Randomized, Double-blind, Placebo-controlled, Dose Ranging, Parallel group Study of the Efficacy and Safety of Plecanatide in Children 6 to <18 Years of Age with Irritable Bowel Syndrome with Constipation (IBS-C)
Phase:	Phase 2b
Protocol No.:	SP304202-14
Protocol Date(s):	August 6, 2018 (V2.0) May 3, 2018 (V1.0)
Analysis Plan Version and Date:	Final v2.0: March 02, 2023 v1.0: May 27, 2020
Prepared By:	[redacted]
Prepared For:	Bausch Health Companies Inc. 400 Somerset Corporate Blvd. Bridgewater, NJ 08807

CONFIDENTIAL AND PROPRIETARY INFORMATION

STATISTICAL ANALYSIS PLAN REVIEW AND APPROVAL

This Statistical Analysis Plan has been prepared in accordance with team reviewers' specifications.

Prepared by: [redacted]

Mar-21-2023 | 13:27 EDT

[redacted]
Date

[redacted]
Senior Biostatistician, MS
[redacted]

Review: [redacted]

Mar-22-2023 | 16:46 EDT

[redacted]
Date

[redacted]
Senior Director, Consulting Operations, Biostatistics
[redacted]

[redacted]

Mar-21-2023 | 10:43 PDT

[redacted]
Date

[redacted]
Sr. Director, Biostatistics
Bausch Health Americas, Inc.

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Mar-22-2023 | 09:20 EDT

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Date

Senior Manager, Nonclinical and Clinical Pharmacology
Bausch Health Americas, Inc.

TABLE OF CONTENTS

1. INTRODUCTION

This document describes the statistical methods and data presentations to be used in the summary and analysis of data from Protocol SP304202-14. Background information is provided for the overall study design and objectives. The reader is referred to the study protocol and case report forms (CRFs) for details of study conduct and data collection.

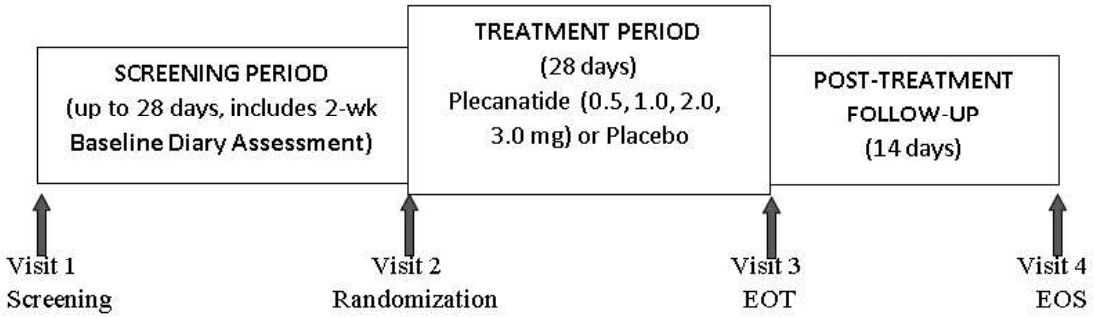
1.1. STUDY OVERVIEW

This is a randomized, double-blind, placebo-controlled, parallel-group study of the efficacy and safety of 4 dose levels of plecanatide (0.5, 1.0, 2.0 or 3.0 mg) in children 6 to < 18 years of age with Irritable Bowel Syndrome with Constipation (IBS-C). Enrollment will be stratified by age group (6 to 11 years in Group A and 12 to < 18 years in Group B). Randomization will be stratified in each Group by gender to ensure gender balance across treatment groups. The study will include a 28-day Screening/Baseline Period, a 4-week Treatment Period, and a 2-week Post-Treatment Follow-up Period. Subjects/caregivers will visit the clinic 4 times during the study.

If a subject is anticipated to turn 12 between screening and randomization, he/she will be randomized into the adolescent group. A subject who turns 12 during the treatment period or post-treatment follow-up period will remain in 6 to 11 years age group.

The overall study plan is presented in Figure 1.

Figure 1 Study Design



Screening/Baseline Period: Subjects' legally authorized representatives (e.g., parent/guardian/ legally authorized representative) will provide informed consent and subjects, as per IRB guidance, will provide written or verbal assent before they undergo any protocol-specified procedures or assessments. At the Screening visit, subjects will undergo a review of medical history, a review of prior and concomitant medications, a brief dietary history, and a physical examination. During the 28-day Screening/Baseline Period prior to randomization, subjects will record—through an electronic diary—daily assessments of bowel movements (BMs), stool consistency (Bristol Stool Form Scale (BSFS) or Modified Bristol Stool Form Scale for Children (mBSFS-C), and abdominal pain and other IBS-related symptoms. Data from the electronic diary will be used to confirm study

eligibility immediately prior to the randomization visit, as well as to characterize the subject's baseline IBS-C status with which the change from Baseline across and at the end of the 4-week treatment period will be compared.

Treatment Period: Subjects 6 to 11 years of age (Group A) who meet all entry criteria will be randomly assigned to 1 of 3 dose groups (2 active treatment, 1 matching placebo) in a 1:1:1 ratio stratified by gender; subjects 12 to < 18 years of age (Group B) will be randomly assigned to 1 of 4 dose groups (3 active treatment, 1 matching placebo) in a 1:1:1:1 ratio stratified by gender on Day 1 of the Treatment Period. Randomization will be stratified by gender to ensure gender balance across treatment groups within each age group. Subjects will take their daily oral dose of the study drug once daily for 4 weeks and continue to complete their daily electronic diaries recording BMs, rescue medication use for constipation, and abdominal pain and other symptoms. At Weeks 1 and 4 of the Treatment Period, subjects will return to the clinic to undergo safety and efficacy assessments.

Post-treatment Follow-up Period: For 2 weeks after the last dose of study drug, subjects will continue to complete their daily electronic diaries. Subjects will then return to the clinic for a final Follow-up visit at the end of Week 6.

The planned duration of participation in this study will be approximately 10 weeks from signing of informed consent/assent through post-treatment.

1.2. SCHEDULE OF EVENTS

Table 1 Schedule of Assessments

Study Period	Screening/ Baseline		Treatment				Post-Treatment Follow-up
Visit Study Week	Visit 1 Week -4	Visit 2 Week 1	TC 1[a]	TC 2[a]	TC 3[a]	Visit 3 Week 4 (EOT) or EW	Visit 4 Week 6 (EOS)
Visit Day (Window)	Day -28	Day 1	Day 3 + 1	Day 7 +3	Day 25 +2	Day 28 +3	Day 42 +3
Informed Consent/Assent	X						
Inclusion/Exclusion Criteria	X	X					
Demography	X						
Medical History (including GI and bowel habits)	X	X					
Prior and Concomitant Medications	X	X	X	X	X	X	X
Physical Examination[b]	X	X				X	X
12-lead Electrocardiogram[c]	X						
Vital Signs[d]	X	X				X	X
Pregnancy Test (urine)[e]	X	X				X	X
Urine Drug Screen for Opioids[f]	X	X					
Serum Chemistry, Hematology, Urinalysis	X	X				X	
TSH (include T3 and T4 only if TSH is abnormal)	X						

Study Period	Screening/ Baseline		Treatment				Post-Treatment Follow-up
Visit Study Week	Visit 1 Week -4	Visit 2 Week 1	TC 1[a]	TC 2[a]	TC 3[a]	Visit 3 Week 4 (EOT) or EW	Visit 4 Week 6 (EOS)
Visit Day (Window)	Day -28	Day 1	Day 3 + 1	Day 7 +3	Day 25 +2	Day 28 +3	Day 42 +3
PK Sampling: Pre-dose on Day 1 on Day 28		X				X	
Intensive PK Sampling: Pre-dose and at 30, 60, 90, and 120 min post-dose in a subset of 70 subjects on Day 1		X					
Electronic Diary Training and Activation[g]	X						
Diary Eligibility[h]		X					
Randomization		X					
Study Drug Dispensation and Administration[i]		X					
In clinic PRO Assessments[j]		X				X	X
Study Drug Collection and Accountability[k]						X	X
Rescue Medication Dispensation	X	X[l]				X[l]	
Adverse Events[m]	X	X	X	X	X	X	X

TC = Telephone Contact; EOT = end of treatment; EW = early withdrawal; EOS = end of study; GI = gastrointestinal; PK = pharmacokinetic; PRO = Patient reported outcome, TSH = thyroid-stimulating hormone.

a. In addition to on-site clinic visits, study sites will call subjects/caregivers on Day 3, Day 7, and as needed to remind them to complete their daily diaries and on Day 25 to remind them to return all unused study drug at their EOT visit.

b. Physical examination (PE), including body weight measurement, will be performed at Visit 1. A symptom-directed PE will be performed as necessary at Visit 3 (EOT) or EW and Visit 4 (EOS); height is measured at the Screening Visit. Body mass index (BMI) will be calculated by Electronic Data Capture (EDC) at the Screening Visit only. Height and body weight may be collected at Visit 2 if missed at Screening.
c. The standard 12-lead Electrocardiogram (ECG) will be performed in the semi-recumbent or supine position.
d. Seated blood pressure, heart rate, respiration, temperature.
e. Urine pregnancy tests on-site at clinic visits for female subjects as per exclusion criteria 5
f. Urine drug screen for selected opioids (screen includes methadone, morphine and oxycodone) will be performed on-site; a negative result must be confirmed prior to dispensing the study medication.
g. Clinical site personnel will train subjects on the use of electronic diaries at the Screening visit and remind them to make daily entries throughout the study, with retraining as needed. The interactive voice or web response system will provide daily reminders to improve compliance, and as per bullet a, study sites will call subjects/caregivers on day 3, day 7, and weekly until Visit 3 (Week 4) to remind them to complete their daily diaries.
h. Baseline diary results will be reviewed programmatically and a “Diary Eligible” or “Not Diary Eligible” report will be provided to the site immediately prior to randomization.
i. Subjects will take their first dose of study drug at the site on Day 1; it is recommended that for dosing at home, subjects take it once daily at approximately the same time in the morning.
j. Details on timing of each PRO Assessment will be included in the Study Procedure Manual.
k. Study drug not returned at EOT must be returned at EOS
l. Only if needed
m. Adverse Event collection begins immediately after informed consent is signed. A symptom-directed physical examination should be performed as appropriate at discretion of the investigator.

1.3. GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
ATC	Anatomical Therapeutic Chemical
BLQ	Below the Limit of Quantification
BM	Bowel Movement
BMI	Body Mass Index
BSFS	Bristol Stool Form Scale
CI	Confidence Interval
CMH	Cochran-Mantel-Haenszel (Test)
CRF	Case Report Form
CSBM	Complete Spontaneous Bowel Movement
CTCAE	Common Terminology Criteria for Adverse Events
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EOS	End of Study
EOT	End of Treatment
FAS	Full Analysis Set (Population)
FDA	Food and Drug Administration
GI	Gastrointestinal
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome with Constipation
IWRS	Interactive Web-Based Response System
LLOQ	Lower Limit of Quantification
LS	Least Squares
mBSFS-C	Modified Bristol Stool Form Scale for Children
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
PE	Physical Examination
PK	Pharmacokinetic
PP	Per-Protocol (Population)
PRO	Patient Recorded Outcome
RM	Rescue Medication
SAE	Serious Adverse Event
SBM	Spontaneous Bowel Movement
SD	Standard Deviation
SOC	System Organ Class
TC	Telephone Contact
TEAE	Treatment Emergent Adverse Event
TSH	Thyroid Stimulating Hormone
ULN	Upper Limit of Normal

2. OBJECTIVES

Primary Objective:

To evaluate the safety and efficacy of once daily oral plecanatide for 4 weeks as treatment for the relief of symptoms associated with Irritable Bowel Syndrome with Constipation (IBS-C) in children ages 6 to < 18 years.

Secondary Objective:

To estimate the pharmacokinetic (PK) parameters of plecanatide in this subject population (to the extent possible, since plecanatide is not expected to be absorbed).

3. GENERAL STATISTICAL CONSIDERATIONS

3.1. SAMPLE SIZE AND POWER

Based on data from two phase 3 trials conducted by the sponsor in adults with IBS-C, consider the sample size necessary for observing a significant difference in the change from baseline in SBM frequency in Group B (the adolescents) since data from the adults is likely more applicable to Group B participants. Then, a study with a plecanatide group sample size of 102 and a placebo group sample size of 34 (representing 3:1 randomization, plecanatide to placebo) achieves 81.5% power at the 0.05% level of significance when the difference in mean change from baseline in weekly SBM frequency at four weeks is 1.5 and the placebo group and plecanatide group standard deviations are 2.3 and 3.5 respectively. Since study SP304202-14 is being conducted in children and adolescents with IBS-C, and the primary analysis of the primary endpoint will be an ANCOVA using a linear mixed-effects model, the sponsor believes that 30 subjects per treatment group (placebo and 3 dose levels of plecanatide), yielding a planned target enrollment of a total of 210 subjects (90 children and 120 adolescents), will prove sufficient for achieving the primary objective of this phase 2 study.

3.2. RANDOMIZATION AND MASKING

This is a double-blind, placebo-controlled study with seven treatment age/dose groups that will be conducted at up to approximately 20 clinical sites. Subjects 6-11 years of age will be randomly assigned to 1 of 3 treatment groups (2 active treatments, 1 matching placebo) in a 1:1:1 ratio stratified by gender and subjects 12 to < 18 years of age will be randomly assigned to 1 of 4 treatment groups (3 active treatments, 1 matching placebo) in a 1:1:1:1 ratio stratified by gender using an Interactive Web-based Response System (IWRS).

Central, block randomization will be performed with stratification by gender.

Age Dose Group		Treatment		No. of Tablets to be Taken Daily	Dose Level	Number of Subjects
A	6 to 11 yrs. old	1	0.5 mg plecanatide	1	0.5 mg plecanatide	30
A	6 to 11 yrs. old	2	1.0 mg plecanatide	1	1.0 mg plecanatide	30

A	6 to 11 yrs. old	3	Matching placebo	1	placebo	30
B	12 to < 18 yrs. old	4	0.5 mg plecanatide	2	1.0 mg plecanatide	30
B	12 to < 18 yrs. old	5	1.0 mg plecanatide	2	2.0 mg plecanatide	30
B	12 to < 18 yrs. old	6	1.5 mg plecanatide	2	3.0 mg plecanatide	30
B	12 to < 18 yrs. old	7	Matching placebo	2	placebo	30

The study will be performed in a double-blind manner. All study drugs will be supplied in identical blister packs and tablets will be similar in color, smell, taste, and appearance, thereby assuring double-blind conditions.

The gender-stratified randomization schedule was prepared by Foehl Statistics & Analytics LLC.

3.3. HANDLING OF DATA

3.3.1. Strata and Covariates

Enrollment will be stratified by age group (6 to 11 years and 12 to < 18 years). Randomization will be stratified by gender to ensure gender balance across treatment groups.

3.3.2. Examination of Subject Subsets

No further examination of subject subsets will be performed.

3.3.3. Multiple Testing and Comparisons

Since this is a Phase 2 clinical trial for exploratory purposes, no adjustments for multiple comparisons will be undertaken.

3.3.4. Missing Data

Missing values of safety parameters will not be imputed.

For each day in the week for which the Bowel Movement (BM) (includes Spontaneous Bowel Movement [SBM] and Complete Spontaneous Bowel Movement [CSBM]) response is missing, 0 BMs (SBMs, CSBMs) will be used, and any week in which no BM response is recorded, the weekly BM (SBM, CSBM) rate will be set to 0. In particular, for a randomized subject who withdraws from study participation prior to the end of the study, the subject is considered to have a weekly BM (SBM, CSBM) rate equal to 0 for any weeks remaining in the planned duration of the study for which the subject has no BM responses recorded.

In any analysis of the frequency of abdominal pain (discomfort), use of rescue medication, the frequency of fecal incontinence, the frequency of defecation pain, the frequency of large diameter stools, missing values will be imputed to zero.

In any analysis involving the severity of abdominal pain and discomfort, the weekly average IBS-related daily abdominal pain (discomfort) score is the average of the non-missing, abdominal pain (discomfort) scores recorded in that week, i.e., the weekly average is the sum of the abdominal pain (discomfort)

scores recorded in the diary in the given week, divided by the number of pain (discomfort) scores recorded in the week. In any week in which a subject has no diary days for which an abdominal pain (discomfort) score is recorded, the weekly average abdominal pain (discomfort) score will be set to missing. In particular, for a randomized subject who withdraws from study participation prior to the end of the study, the weekly average abdominal pain (discomfort) score will be set to missing for any weeks remaining in the planned duration of the study for which the subject has no abdominal pain (discomfort) data.

In any analysis involving the severity of defecation pain, the weekly averages are the sum of the non-missing severity of defecation pain scores recorded during the week, divided by the total number of non-missing severity of defecation pain scores recorded. In any week in which a subject has no non-missing severity of defecation pain scores recorded, the weekly average score will be set to missing in any summary or linear mixed-effects model analysis involving the weekly average severity of defecation pain score.

In any analysis involving stool consistency (mBSFS-C scores or BSFS scores), the determination of weekly averages will follow the same methodology as presented above for abdominal pain. However, since stool consistency cannot be assessed if the subject does not have at least one SBM during the week in question (i.e., stool consistency is based on SBMs only), the weekly average will also be set to missing if no SBMs are recorded for any days in the week. Also, it is possible that a subject has no baseline weekly average stool consistency score because of the SBM criterion; in that case the subject will be excluded from any change from baseline analyses of stool consistency.

3.3.5. Imputation of Incomplete Dates

An incomplete date is any date for which either the day, month or year is unknown, but all three fields are not unknown. An incomplete date occurs when the exact date an event occurred or ended cannot be obtained from a subject. For many of the analyses, a complete date is necessary in order to determine if the event should be included in the analysis (i.e., if the event is treatment-emergent). In such cases, incomplete dates will be imputed but the collected date will be displayed on the listings.

For purposes of imputation, all events with an incomplete end date are assumed to have ended on or before the day the form was completed. In an effort to minimize bias, the project statistician will impute dates in a systematic, but reasonable manner, as described below.

Adverse Events

If the day portion of the start of the event is missing, then the start date will be set to the date of Day 1. If the day and month portion of the start of the event are missing, then these will be imputed as the day and month of Day 1. If the entire date is missing and the end date is unknown or on or after the treatment start date, then the date will be set to the treatment start date. If the month portion of the start of the event is missing and day and year are known, and day is on or after the day of the treatment start date, then month will be imputed as the month from the treatment start date. If month is missing, and day and year are known, and the day portion of the event is before the day of the treatment start date, then month will be imputed as the month after the treatment start date. If this produces an event with the end date before the start date, then the month will be imputed as the treatment start date month. If the year is missing and the day and month of the start of the event are before the day and month of enrollment, then the next year will be used (e.g. Date of enrollment = 13DEC2019, incomplete date = 03JANXXXX -> 03JAN2020). For nonexistent dates occurring at the end of a month created by this imputation method, the first date of the next month will be used (e.g. Day 1 = 31JAN2017, incomplete date = XXFEB2017 -> imputed date = 01MAR2017).

Concomitant medications

Since determining the concomitant status of medications relies primarily on the end date of the medication and study drug, only end dates will be imputed. If the day portion of the end of the medication date is missing, the day portion will be imputed as the end of the month in which it occurred. If the day and month portion of the end of the medication are missing, and the year is the same as or after the study drug treatment end date, the end of medication will be imputed as the study drug treatment end date. If the day and month portion of the end of the medication are missing, and the year is the before the study drug treatment end date, the day and month portion will be imputed as 31st of December. If the entire end date of the medication is missing (and not Ongoing), then the end date of the medication will be imputed as the study drug treatment end date. If day and year of the end of the medication are recorded and month is missing, month will be imputed as the month of the study drug end date (e.g. treatment end date = 15FEB2020, incomplete date =01XXX2020 ->01FEB2020). If the day and month of the medication are recorded and the year is missing, the year will be imputed as the year of the end date of medication.

3.3.6. Presentations by Study Visit

The daily electronic diary entries reported during the Treatment Period will be classified into 4 weeks – Week 1 (Day 1-7); Week 2 (Day 8-14); Week 3 (Day 15-21); Week 4 (Day 22-28); Week 5 (Day 29-35); Week 6 (Day 36-49).

Non-diary data, such as vital signs and clinical laboratory assessments, will be displayed and analyzed according to the nominal visit date on the eCRF unless the visit is an unscheduled visit. Unscheduled visit values will be used if the unscheduled visit falls in a visit window and the scheduled visit from the same visit window has a missing value. Visit windows will be calculated as follows for vital signs, urine pregnancy test, and physical examination:

Nominal Visit	Minimum Study Day	Maximum Study Day
Screening Week -4	-28	-1
Week 1	1	14
Week 4	15	35
Post-Treatment Week 6	36	49

Visit windows for serum chemistry, hematology, and urinalysis will be calculated as follow:

Nominal Visit	Minimum Study Day	Maximum Study Day
Screening Week -4	-28	-1
Week 1	1	14
Week 4	15	45

If assessments are collected multiple times within a given Study Visit window, the result closest to the scheduled visit date will be used for summary presentations. If two measurements have the same distance to the expected date, the latest result will be used. If a subject has multiple non-missing scheduled values on the same date, the latest result in terms of time will be used. All assessments will be presented in the listings.

3.3.7. Definitions and Terminology

Age
The age of a subject is defined as the number of whole years between the subject's birth date and the date of screening. If a subject is anticipated to turn 12 between screening and randomization, he/she will be randomized into the adolescent group but will use the Wong Baker and the mBSFS. A subject who turns 12 during the treatment period or post-treatment follow-up period will remain in 6 to 11 years age group and will use the Wong Baker and the mBSFS. The questionnaires will be provided based on the age at Screening.

All subjects in 6 to 11 years old age group are expected to get the parental oversight on eDiary records. All subjects in 12 to <18 years old age group can complete eDiary by themselves but should be discussed with parent.

Baseline Value
For purposes of safety analysis, the baseline value is defined as the last non-missing value obtained prior to the randomization.
For purposes of efficacy analysis, the baseline values will be derived from data collected in the 2-week electronic diary assessment at the end of the Screening Period (baseline). The last 14 days of diary entries prior to Day 1 will be the default standard for derivation of these values.

The baseline BM (SBM, CSBM) weekly rate will be the weekly average number of BMs (SBMs, CSBMs) recorded during the 2-week baseline assessment period; similarly, for frequency of abdominal pain and abdominal discomfort, frequency of fecal incontinence, frequency of defecation pain, and frequency of large diameter stools. Baseline stool consistency, severity of abdominal pain, abdominal discomfort, and severity of defecation pain will be calculated as follows: for each week of the baseline assessment, the weekly average is the sum of the non-missing scores recorded during the week divided by the total number of non-missing scores recorded during that week; the baseline is then equal to the average of the 2 weekly average scores defined as sum of the 2 weekly average scores divided by 2.

Day 1 (Baseline)
Day 1 is the earliest day that study drug is initiated.

Study Day
Study Day is defined relative to Baseline (Day 1). Thus, the study day of an event is calculated as:

Study Day = Event Start Date – Baseline Date + 1, for dates on or after Day 1;

Study Day = Event Start Date – Baseline Date, for dates before Day 1.

Study Visit
Study Visit is the nominal visit as recorded on the CRF.

Change from Baseline
Change from baseline for a given endpoint is defined as the Study Visit value minus the Baseline Value.

Last Dose of Plecanatide
Last Dose of Plecanatide is defined as the last date that the subject received Plecanatide as determined by last date of dosing as recorded on the Study Drug Administration CRF.

Duration of Plecanatide
Duration of Plecanatide is defined as the number of days from Day 1 to the date of Last Dose of Plecanatide.

Duration on Study
Duration on study is defined as the number of days from the date of screening to the date of last follow-up visit.

Dosing Compliance
Dosing compliance will be defined by the dosing compliance ratio: the number of doses actually taken by the subject divided by the number of doses that were expected to be taken during the same period multiplied by 100.

Treatment Compliance
Treatment compliance is defined as taking equal to or greater than 80% of the drug dosage prescribed. Otherwise, the subject will be considered noncompliant with study treatment.

Rescue Medication
Dulcolax® will be provided as a rescue laxative that can be used if a subject has not had a bowel movement for at least 72 hours. Subjects will be advised that it is preferred that they do NOT take rescue medication for the period from 24 hours before to 72 hours after Day 1 of dosing.

Adverse Event
An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered to be drug related. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product or study procedure whether or not considered related to the product or procedure.

Specific gastrointestinal symptoms related to IBS-C recorded daily in the electronic Diary will be summarized separately and generally not also recorded as Adverse events.

Treatment-emergent Adverse Event
A treatment emergent adverse event (TEAE) is defined as an AE that begins or worsens in frequency and/or severity after at least one dose of study drug has been administered. Additionally, it is assumed that an Adverse Event which was reported to have started on Day 1 without an associated onset time occurred after the initiation of study drug is treatment-emergent.

Treatment-emergent Laboratory Toxicities
A Treatment-emergent Laboratory Toxicity is defined as any post-baseline laboratory assessment occurring up to and including 14 days post treatment discontinuation representing an increase of 1 grade or more from the baseline toxicity value. If the baseline value is missing, any graded toxicity (grade 1 or higher) that occurs following initiation of treatment is considered treatment-emergent.

Spontaneous bowel movement (SBM)
A bowel movement that occurs in the absence of laxative use within the preceding 24 hours.

Complete spontaneous bowel movement (CSBM)
A spontaneous bowel movement with the sense of complete evacuation.

Weekly BM (SBM, CSBM) Rate
Weekly BM (SBM, CSBM) rate is the sum of the BMs (SBMs, CSBMs) recorded for the days in the week for which a BM (SBM, CSBM) response is recorded in the daily diary.

In any analysis of the frequency of abdominal pain, frequency of abdominal discomfort, frequency of fecal incontinence, the frequency of defecation pain, and the frequency of large diameter stools will follow the same methodology as described above for the determination and analysis of the weekly BM rate.

Weekly Average Abdominal Pain Score
Weekly average IBS-related daily abdominal pain score is the average of the non-missing, abdominal pain scores recorded in that week, i.e., the weekly average is the sum of the abdominal pain scores recorded in the diary in the given week, divided by the number of pain scores recorded in the week.

Weekly Average Defecation Pain Score
Weekly average item score is the sum of the non-missing defecation pain scores recorded during the week, divided by the total number of non-missing defecation pain scores recorded.

Weekly Average Stool Consistency
Stool consistency is the sum of the non-missing stool consistency scores recorded during the week, divided by the total number of non-missing stool consistency scores recorded. Note stool consistency cannot be assessed if the subject does not have at least one SBM during the week in question (i.e., stool consistency is based on SBMs only).

Concomitant Medications
Concomitant medications are those medications taken on or after the initiation of study drug. This definition includes medications started prior to the initiation of study drug but continuing concomitantly with study drug.

Prior Medications
Prior medications are those medications taken and ended prior to the initiation of study drug.

Post Medications
Post medications are those medications started after the last dose of study drug.

3.4. TIMING OF ANALYSES

The final data analysis will be performed after all subjects have either completed or have been discontinued from the study. The study will be unblinded after subject membership in each of the various analysis populations has been determined at a blinded data review meeting and upon confirmation of database lock.

No interim analyses are planned.

4. ANALYSIS POPULATIONS

The populations for analysis will include full analysis set (FAS) population, per protocol (PP) population, safety population, and intensive PK population.

4.1. FULL ANALYSIS SET (FAS)

All randomized and treated subjects who had the baseline assessment and at least one post randomization assessment of the primary efficacy measure of weekly SBM frequency. The FAS is the main population for assessments of efficacy. Subjects will be analyzed according to the treatment assigned

4.2. PER PROTOCOL (PP) POPULATION

All subjects in the FAS population who complete the 4-week Treatment Period or discontinue from study treatment due to adverse event(s) or lack of efficacy, with the exception of major protocol violators. If a diary compliance is < 70% or treatment compliance is < 80% then the subject is excluded from the PP Population. Subjects with Week 4 visit outside of the window 28 +/- 3 (study days 25 to 31) days will be excluded from the PP Population. Final decisions regarding exclusion from the PP Population will be made prior to unblinding the database.

4.3. SAFETY POPULATION

All randomized subjects who receive at least one dose of the study drug. Subjects will be analyzed according to the treatment received. All safety analyses will be based on the Safety Population.

4.4. INTENSIVE PK POPULATION

Approximately 70 subjects who enrolled for the intensive PK sampling (30, 60, 90, and 120 minutes post-dose on Day 1) at the selected cities.

5. STATISTICAL METHODS

Seven treatment groups are identified for all analyses cited in the following as "by treatment group": subjects in Age Group A receiving plecanatide doses of 0.5 mg, 1.0 mg, or placebo; subjects in Age Group B receiving plecanatide doses of 1.0 mg, 2.0 mg, 3.0 mg, or placebo. Following comparisons will be included:

1) For Age Group A:

Age: 6 to 11 years			
0.5 mg	1.0 mg	Active Doses Combined	Placebo

2) For Age Group B:

Age: 12 to < 18 years				
1.0 mg	2.0 mg	3.0 mg	Active Doses Combined	Placebo

3) Overall:

Overall	
Plecanatide	Placebo

Descriptive statistical methods will be used to summarize the data from this study, with hypothesis testing performed for the primary and secondary efficacy endpoints. Unless stated otherwise, the term "descriptive statistics" refers to number of subjects (n), mean, median, standard deviation (SD), minimum and maximum for continuous data and frequencies and percentages for categorical data. Unless otherwise

noted, percentages will be calculated using the total number of subjects in the appropriate population and/or subgroup and per treatment group where applicable. Where data are collected over time, both the observed data and change from the Screening Period (baseline) will be summarized at each time point.

Statistical testing including p-values and confidence intervals (CIs) will be presented as described in each section below. The Type I error rate (alpha) for the analysis of the primary efficacy endpoint is 0.05 (two-sided).

Where applicable**,** comparisons among treatment groups will be performed using an analysis of variance/covariance (ANOVA/ANCOVA) model for continuous variables and a Cochran-Mantel-Haenszel (CMH) test for categorical variables.

For all repeated measures efficacy analyses, the following covariance structures will be examined: compound symmetry, first order autoregressive, and unstructured. The covariance structure with the best model fit will be selected for each efficacy endpoint.

All data collected during the study will be included in data listings. Unless otherwise noted, the data will be sorted first by treatment group, subject number, and then by date within each subject number.

The statistical analyses will be conducted with the SAS® System version 9.4 or higher. All analyses will be subject to formal verification procedures. Specifically, results will be verified utilizing independent programming prior to issuance of the draft statistical report. All documents will be verified by the lead statistician to ensure accuracy and consistency of analyses.

5.1. SUBJECT DISPOSITION, DEMOGRAPHICS, EXPOSURE AND DEVIATIONS

5.1.1. Subject Disposition, Demographics and Baseline Characteristics

Subject disposition will be presented for all enrolled subjects. The number of screened subjects, screen failures, reason for screen failures, and randomized subjects will be presented by study population and treatment group. The number of subjects who completed the study and discontinued from the study will be provided. The primary reasons for discontinuation at any point also will be presented by treatment group. The reasons for discontinuation of study participation at any time point will also will be presented by treatment group. Additionally, days on study will be summarized by treatment group.

Demographic data and baseline characteristics including age at screening, race, ethnicity, gender, height, weight, and BMI at screening will be summarized using descriptive statistics for each population. Comparisons among treatment groups will be performed using an analysis of variance (ANOVA) model for continuous variables and a Cochran-Mantel-Haenszel (CMH) test for categorical variables.

A listing and descriptive summary of standard 12-lead ECG at screening will be provided. Also, a listing of medical history will be provided.

Additionally, a separate listing of discontinuation due to COVID-19 disruption will be provided.

5.1.2. Protocol Deviations

Deviations from the study protocol, including violations of inclusion/exclusion criteria, will be assessed as “minor” or “major”. A listing of protocol deviations and major protocol deviation will be provided. Additionally, a separate listing of inclusion criteria not met and exclusion criteria that were met will be provided.

5.1.3. Exposure and Compliance

Dosing compliance will be defined by the dosing compliance ratio: the number of doses actually taken by the subject divided by the number of doses that were expected to be taken during the same period multiplied by 100. Treatment compliance is defined as taking equal to or greater than 80% of the drug dosage prescribed. Otherwise, the subject will be considered noncompliant with study treatment. Rates of treatment compliance (<80%, ≥80%) as defined above will be compared between treatment groups using the Cochran–Mantel–Haenszel. A listing of study drug accountability will be provided.

5.2. EFFICACY ANALYSIS

5.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint is the change from baseline in weekly SBM frequency over the 4 Week Treatment Period compared to placebo across treatment groups.

5.2.2. Primary Efficacy Analysis

The weekly SBM rate for each subject will be computed for each week of the 4-week treatment period. The change from baseline in mean weekly SBM rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment groups, and the difference in LS means for each treatment group versus placebo and combined active doses within each age group versus placebo in a separate model for each age group, and overall active doses versus placebo in a separate model with 95% confidence intervals and corresponding statistical p-values.

The baseline SBM weekly rate will be the weekly average number of SBMs recorded during each of the 2 weeks preceding initiation of study drug.

The above analysis will be repeated for the PP Population.

5.2.3. Secondary Efficacy Endpoints

- Change from baseline in frequency and severity of abdominal pain and abdominal discomfort
- Change from baseline in frequency of BMs, SBMs, and CSBMs
- Time to First BM
- Change from baseline in stool consistency (BSFS or mBSFS-C)
- Use of Rescue Medication
- Change from baseline in frequency of fecal incontinence
- Change from baseline in frequency and severity of defecation pain
- Change from baseline in frequency of large diameter stools

5.2.4. Secondary Efficacy Analysis

The secondary efficacy endpoints and methods of analysis are:

5.2.4.1. Change from Baseline in Frequency and Severity of Abdominal Pain and Abdominal Discomfort

The weekly abdominal pain and the weekly abdominal discomfort rate for each subject will be computed, as described in Section 3.3.7, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly abdominal pain and abdominal discomfort rate and change from baseline in mean weekly abdominal pain and abdominal discomfort rates, for each of the 6 study weeks will be presented. The change from baseline in mean weekly abdominal pain and abdominal discomfort rates will be analyzed using a linear mixed-effects ANCOVA model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment groups, and the difference in LS means for each treatment group versus placebo and combined active doses within each age group versus placebo in a separate model for each age group, and overall active doses versus placebo in a separate model with 95% confidence intervals and corresponding statistical p-values.

The weekly average abdominal pain and abdominal discomfort scores will be determined using the approach defined in Section 3.3.7 with missing scores excluded as described in Section 3.3.4 and presented for each week in the 4-week treatment period. The change from baseline in the weekly averaged abdominal pain and abdominal discomfort scores will be analyzed in the FAS Population using a linear mixed-effects ANCOVA model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) means, with 95% confidence intervals and the corresponding statistical p-value, will be presented for each week, and across all 4 weeks, of the 4-week treatment period for the difference between the difference in LS means for each of treatment group versus placebo, combined active doses within each age group versus placebo, and overall active doses versus placebo. The analysis of interest for this secondary endpoint is the difference in LS means between each plecanatide group (each dose within each age group, combined active dose within each age group, and overall active dose) versus placebo for the estimated overall average change from baseline in abdominal pain and abdominal discomfort scores across the 4-week treatment period.

Baseline abdominal pain and abdominal discomfort will be calculated as the equally weighted average of the average abdominal pain (or abdominal discomfort) for each of the two weeks prior to initiation of study drug.

The above analysis will be repeated for the PP Population.

5.2.4.2. Change from Baseline in Frequency of BMs and CSBMs, by Study Week

The weekly BM (and CSBM) rate for each subject will be computed, as described in Section 3.3.7, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly BM (and CSBM) rate and change from baseline in mean weekly BM (and CSBM) rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly BM (and CSBM) rate will be analyzed using a linear mixed-effects ANCOVA model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment groups, and the difference in LS means for each treatment group versus placebo and combined active doses within each age group versus placebo in a separate model for each age group, and overall active doses versus placebo in a separate model with 95% confidence intervals and corresponding statistical p-values.

The baseline BM (and CSBM) rate will be calculated as the average BM (and CSBM) rate for each of the two weeks preceding initiation of study drug.

The above analysis will be repeated for the PP Population.

5.2.4.3. Time to First BM

For the time to first BM endpoint, the distribution for each treatment group within each age group, combined active doses within age group, and overall active doses will be estimated by the Kaplan-Meier method and will be compared by the Log-rank test. The median and quartile time along with the 95% confidence limits for the median will be presented for each treatment group.

Kaplan-Meier curves with 95% confidence limits will also be presented graphically by treatment group (Age group A and B with Placebo on one page, and Combined age group with Placebo on a second page).

The above analysis will be repeated for the PP Population.

5.2.4.4. Change from Baseline in Stool Consistency (mBSFS-C or BSFS)

Stool consistency for a bowel movement is measured using the mBSFS-C for subjects in Group A and the BSFS for subjects in Group B. The weekly average stool consistency scores will be determined using the approach defined in Section 3.3.7 with missing scores excluded as described in Section 3.3.4 and presented for each week in the 4-week treatment period. The change from baseline in the average weekly stool consistency score will be analyzed in the FAS Population using a linear mixed-effects ANCOVA model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. Least Squares (LS) means, with 95% confidence intervals and the corresponding statistical p-value, will be presented for each week, and across all 4 weeks, of the 4-week treatment period for the difference between the difference in LS means for each of treatment groups versus placebo, combined active doses within each age group versus placebo, and overall active doses versus placebo. The analysis of interest for this secondary endpoint is the difference in LS means between each plecanatide group and placebo for the estimated overall average change from baseline across the 4-week treatment period.

The baseline stool consistency (mBSFS-C or BSFS) rate will be calculated as the equally weighted average of the average stool consistency (mBSFS-C or BSFS) rate for each of the two weeks preceding initiation of study drug.

The above analysis will be repeated for the PP Population.

5.2.4.5. Use of Rescue Medication

The proportion of subjects who used at least one dose of rescue medication during the treatment period, will be summarized by treatment group. The number of tablets of rescue medication used per week, and overall, for the 4-week treatment period will be analyzed by treatment group. The Mann Whitney U Test (Wilcoxon Rank Sum Test) will be used to compare the difference between each treatment group, combined active doses within each age group, and overall active doses, versus Placebo at each week, and over all 4 weeks, of the 4-week treatment period.

The above analysis will be repeated for the PP Population.

5.2.4.6. Change from Baseline in Frequency of Fecal Incontinence

The weekly fecal incontinence rate for each subject will be computed, as described in Section 3.3.7, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly fecal incontinence rate, and change from baseline in mean weekly fecal incontinence rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly fecal incontinence rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment groups, and the difference in LS means for each treatment group versus placebo and combined active doses within each age group versus placebo in a separate model for each age group, and overall active doses versus placebo in a separate model with 95% confidence intervals and corresponding statistical p-values.

The baseline fecal incontinence rate will be calculated as the average fecal incontinence rate for each of the two weeks preceding initiation of study drug.

The above analysis will be repeated for the PP Population.

5.2.4.7. Change from Baseline in Frequency and Severity of Defecation Pain

The weekly defecation pain rate for each subject will be computed, as described in Section 3.3.7, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly defecation pain rate, and change from baseline in mean weekly defecation pain rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly defecation pain rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment groups, and the difference in LS means for each treatment group versus placebo and combined active doses within each age group versus placebo in a separate model for each age group, and overall active doses versus placebo in a separate model with 95% confidence intervals and corresponding statistical p-values.

The weekly average defecation pain score will be determined using the approach defined in Section 3.3.7 and presented for each week in the 4-week treatment period. The change from baseline in the weekly averaged defecation pain score will be analyzed in the FAS Population using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) means, with 95% confidence intervals and the corresponding statistical p-value, will be presented for each week, and across all 4 weeks, of the 4-week treatment period for the difference between the difference in LS means for each of treatment group versus placebo, combined active doses within each age group versus placebo, and overall active doses versus placebo. The analysis of interest for this secondary endpoint is the difference in LS means between each plecanatide group (each dose within each age group, combined active dose within each age group, and overall active dose) versus placebo for the estimated overall average change from baseline in defecation pain and defecation pain scores across the 4-week treatment period.

The baseline defecation pain score and rate will be calculated as the average defecation pain score and rate for each of the two weeks preceding initiation of study drug, respectively.

The above analysis will be repeated for the PP Population.

5.2.4.8. Change from Baseline in Frequency of Large Diameter Stools

The weekly large diameter stools rate for each subject will be computed, as described in Section 3.3.7, for each week of the 4-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly large diameter stools rate, and change from baseline in mean weekly large diameter stools rate, for each of the 6 study weeks will be presented. The change from baseline in mean weekly large diameter stools rate will be analyzed using a linear mixed-effects model with fixed effects for gender (stratification variable), treatment, week, the interaction of treatment and week, and the corresponding baseline value; a random intercept for subject will also be specified. The least squares (LS) mean change from baseline will be presented, by treatment groups, and the difference in LS means for each treatment group versus placebo and combined active doses within each age group versus placebo in a separate model for each age group, and overall active doses versus placebo in a separate model with 95% confidence intervals and corresponding statistical p-values.

The baseline large diameter stools rate will be calculated as the average large diameter stools rate for each of the two weeks preceding initiation of study drug.

The above analysis will be repeated for the PP Population.

5.3. SAFETY

Values for all safety variables will be listed by subject and visit (as applicable). All safety analyses will be conducted on the Safety Population.

5.3.1. Adverse Events

Adverse events will be mapped to a Medical Dictionary for Regulatory Activities (MedDRA) version 23.0 preferred term and system organ classification. If a subject experiences multiple events that map to a single preferred term, the greatest severity grade (mild, moderate, or severe), and strongest investigator assessment of relation (reasonable possibility, no reasonable possibility) to study medication will be assigned to the preferred term for the appropriate summaries. Should an event have a missing severity or relationship, it will be classified as having the highest severity and/or strongest relationship to study medication. Overall summary of TEAEs will be provided. The occurrence of treatment-emergent adverse events (TEAEs) will be summarized by treatment group using preferred terms, system organ classifications, and severity. Separate summaries of treatment-emergent serious adverse events (SAEs), TEAEs related to study drug, severe TEAEs, and TEAEs leading to the discontinuation of study will be generated using system organ class and preferred terms. All adverse events reported will be listed for individual subjects showing both verbatim and preferred terms. All adverse events that occurred prior to the initiation of study treatment will be excluded from the tables but will be included in the listings.

Missing onset dates will be imputed as previously outlined in Section 3.3.5 as required to determine treatment-emergent events.

5.3.2. Clinical Laboratory Assessments

Laboratory tests (hematology, serum chemistry, and urinalysis) will be summarized as changes from baseline. Laboratory shift tables from baseline to end of study will also be produced. Listings of laboratory tests will also be provided.

Toxicities for clinical laboratory will be characterized according to Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03. Out of range laboratory tests will also be summarized, and a listing of subjects with at least one out of range value will be presented by visit for that test.

Moreover, "Medically Important Events" recently defined by FDA will be reported as SAEs. These include cases where:

- Aminotransferases (ALT or AST) are > 3 times the upper limit of normal (ULN) with an associated elevation of total bilirubin > 2 times ULN without evidence of hemolysis or with alkaline phosphatase < 2 times ULN or not available, or
- ALT or AST activity that is > 5 times ULN

5.3.3. Vital Signs, Height, and Weight

Vital signs will be summarized as changes from baseline. Listings of vital signs will also be provided. A separate listing of subjects with at least one out of range value will be presented by visit for that assessment. The normal range of vital signs is summarized below:

Variable	Units	Normal Range
Systolic blood pressure	mmHg	≤ 120
Diastolic blood pressure	mmHg	≤ 80
Heart Rate	beats/minute	60 to 100
Respiratory Rate	breaths/min	12 to 25
Temperature	°C	< 38

5.3.4. Concomitant Medications

Concomitant medications will be coded using the WHODrug Global B3 March 1, 2020 with reference to Anatomical Therapeutic Classification (ATC) text and preferred terms. Summaries of concomitant medication will be presented by ATC text and preferred terms. Prior, concomitant, prior and concomitant, and post-treatment medications will be presented separately.

5.3.5. Other Safety Analyses

A listing of physical examination findings for all subjects will be provided (where available). Also, frequency summary of 12-lead electrocardiograms at screening for all subjects will be provided.

5.4. PATIENT RECORDED OUTCOME

PRO questionnaire data for Health-Related Quality of Life, IBS Disease Severity, Subject Global Rating of Change—IBS Symptoms, Subject Global Rating of Change—IBS Abdominal Pain, Treatment Continuation and Treatment Satisfaction Assessments will be included in the subject listings. Descriptive summaries of PRO questionnaire data and total score for each assessment will also be provided.

5.5. PHARMACOKINETIC ANALYSES

Plasma plecanatide (and its major metabolite SP-338) concentrations will be assessed for all subject's pre-dose on Day 1 and at the Week 4 visit. Intensive PK sampling (30, 60, 90, and 120 minutes post dose on Day 1) will be done in a subset of approximately 70 subjects at selected sites, which will be designated as belonging to the Intensive PK population. Plasma concentrations of Plecanatide and its major metabolite (SP-338) will be listed by subject for all subjects (Day 1 pre-dose and Week 4) and for the Intensive PK

population (Day 1 samples), and may be summarized using nominal sampling timepoints, if applicable, by gender and by treatment group. Listings will include both nominal and actual sampling times. If applicable, separate summaries will be presented for all subjects and for subjects in the Intensive PK population. For calculation of summary statistics, any samples below the limit of quantification (BLQ) reported before the first quantifiable timepoint will be assigned a value of "0". If 1 BLQ is reported between 2 quantifiable timepoints, the sample will be treated as missing. If 2 or more consecutive BLQ samples are reported after C_{max}, they will be set to ½ the lower limit of quantification (LLOQ). BLQ samples occurring after the last quantifiable timepoint that are not addressed by the previous statements should be treated as missing. Summarization of plasma concentrations will include N, quantifiable N, arithmetic mean, SD, CV%, median, minimum, and maximum. BLQ values set to "0" should be treated as such in the calculation of arithmetic means. Missing values should be excluded from all mean calculations. If values are excluded from any descriptive statistic, all reported descriptive statistics should reflect these exclusions.

PK parameters will only be presented for subjects in the Intensive PK population on Day 1. Actual sampling time will be used for all parameter estimation. C_{max}, C_{min}, and T_{max} will be listed by subject and may be summarized, if applicable, by gender and by treatment group. These parameters will be defined as follows for this study:

- C_{max} is the maximum concentration for the PK profile observed.
- T_{max} is the time of the maximum concentration for the PK profile observed.
- C_{min} is the minimum concentration for the PK profile observed.

For PK parameters, the summary of C_{max} and C_{min} will include N, quantifiable N, arithmetic mean, SD, CV%, median, minimum, maximum, geometric mean, 90% CI for geometric mean, and geometric CV%. When all samples for a subject have been set to "0", the C_{max} and C_{min} should be set to "0" and treated as such in the calculation of arithmetic means and should be set to ½ LLOQ for use in the calculation of geometric means. The summary of T_{max} will include N, reportable N, median, minimum, and maximum. For T_{max}, reportable N will consist of the number of subjects for which a quantifiable C_{max} occurred. When all samples for a subject have been set to "0", no T_{max} should be reported.

6. PROTOCOL DEVIATIONS

Deviations from the study protocol, including violations of inclusion/exclusion criteria, will be assessed as "minor" or "major". Major deviations from the protocol will lead to the exclusion of a subject from the PP Population and will be determined prior to unblinding the study database. Missed telephone contact (TC) visits will not be considered deviations from the study protocol.

Protocol deviations will be listed. A separate listing for major protocol deviation will be provided.

7. CHANGES IN THE PLANNED ANALYSES

In accordance with the FDA guidance (FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic, March 2020), processes were put in place to ensure that any impacted study assessments can be identified in the study database. Any impacted assessments for subjects participating in this study will be identified in the relevant individual subject listings. Any subject discontinued due to COVID-19 disruption, will be noted as such in the subject disposition summary and associated individual subject listing.

SAP Version 2.0 changes: For the Per Protocol population, the compliance threshold for eDiary was updated to eDiary compliance of at least 70% instead of at least 80%. Overall, this eDiary compliance threshold is considered adequate in this pediatric subject population and in the context of the COVID-19 pandemic.

The Per Protocol population was also updated to expand visit windowing for Visit 3/Week 4 from the protocol-specified window of 28 +3 days. to 28 days plus or minus 3 days. Protocol-specified visit window is 28 to 31 days, however it is expected that a subject with Week 4 completed in the 25 to 31 day window, with adequate treatment compliance and diary compliance as specified for the PP Population, is reflective of 4 weeks of study treatment, as intended by the study objectives.

Clarification was added for the pharmacokinetic analyses, utilizing actual sampling times for PK parameter estimation, and also to state the definitions for Cmax, Tmax, and Cmin.

8. PROGRAMMING CONVENTIONS

- Page orientation, margins, and fonts: Summary tables, listings, and figures will appear in landscape orientation. There should be a minimum of a 1" boundary on the upper (bound) edge, and a minimum of a 1.0" boundary on the remaining three edges. Output should be printed in Courier New with a point size of 8. Titles may be printed using a larger font (e.g., Arial point size 10).
- Identification of analysis population: Every summary table and figure should clearly specify the analysis population being summarized. Listings will be prepared for all subjects.
- Group headers: In the summary tables, the group headers will identify the summary group and the sample size for the indicated analysis population. Of note, the header's sample size does not necessarily equal the number of subjects actually summarized within any given summary module; some subjects in the analysis population may have missing values and thus may not be summarized.
- Suppression of percentages corresponding to null categories: When count data are presented as category frequencies and corresponding percentages, the percent should be suppressed when the count is zero in order to draw attention to the non-zero counts.
- Presentation of sample sizes: Summary modules should indicate, in one way or another, the number of subjects actually contributing to the summary statistics presented in any given summary module. As mentioned above, this may be less than the number of subjects in the analysis population due to missing data.
    - In the quantitative modules describing continuous variables (and thus presenting sample size, means, and standard deviations), the sample size should be the number of non-missing observations. The number of missing observations, if any, will be noted.
    - For categorical variables that are presented in frequency tables, the module should present the total count in addition to the count in each category. Percentages should be calculated using this total as the denominator, and the percentage corresponding to the sum itself (that is, 100%) should be presented so as to indicate clearly to a reviewer the method of calculation. The number of missing observations, if any, will be noted.
- Sorting: Listings will be sorted by treatment group, subject number and date, if applicable. If a listing is sorted in a different manner, a footnote will indicate as such.
- General formatting rules: Rounding for all variables will occur only as the last step, immediately prior to presentation in listings, tables, and figures. No intermediate rounding will be performed on derived variables. The standard rounding practice of rounding numbers ending in 0-4 down and numbers ending in 5-9 up will be employed.
- Numerical Values: The presentation of numerical values will adhere to the following guidelines:
    - Raw measurements will be reported to the number of significant digits as captured electronically or on the CRFs.
    - Standard deviations will be reported to one decimal place beyond the number of decimal places the original parameter is presented.
    - Means will be reported to the same number of significant digits as the parameter.
    - Calculated percentages will be reported with no decimals.
    - Dates will be formatted as DDMMMYYYY. Partial dates will be presented on data listings as recorded on CRFs. Time will be presented according to the 24-hour clock (HH:MM).

9. PROPOSED TABLES, LISTINGS, AND FIGURES

9.1. SUMMARY TABLES

Accountability and Baseline Characteristics

14.1.1.X	Subject Disposition and Termination from Study All Enrolled Subjects – <Age Group>
14.1.2.1.X	Demographic and Baseline Characteristics Safety Population – <Age Group>
14.1.2.2.X	Demographic and Baseline Characteristics FAS Population – <Age Group>
14.1.2.3.X	Demographic and Baseline Characteristics PP Population – <Age Group>
14.1.3.X	Summary of 12-Lead Electrocardiograms at Screening Safety Population – <Age Group>
14.1.4.1.X	Extent of Exposure and Treatment Compliance FAS Population – <Age Group>
14.1.4.2.X	Extent of Exposure and Treatment Compliance PP Population – <Age Group>
14.1.4.3.X	Extent of Exposure and Treatment Compliance Safety Population – <Age Group>

Efficacy

14.2.2.1.X	Summary of Weekly Spontaneous Bowel Movement Rates by Study Week FAS Population – <Age Group>
14.2.2.2.X	Summary of Weekly Spontaneous Bowel Movement Rates by Study Week PP Population – <Age Group>
14.2.3.1.1.X	Summary of Weekly Average Abdominal Pain Rates by Study Week FAS Population – <Age Group>
14.2.3.1.2.X	Summary of Weekly Average Abdominal Pain Rates by Study Week PP Population – <Age Group>
14.2.3.2.1.X	Summary of Weekly Average Abdominal Discomfort Rates by Study Week FAS Population – <Age Group>
14.2.3.2.2.X	Summary of Weekly Average Abdominal Discomfort Rates by Study Week PP Population – <Age Group>
14.2.3.3.1.X	Summary of Weekly Average Abdominal Pain Scores by Study Week FAS Population – <Age Group>
14.2.3.3.2.X	Summary of Weekly Average Abdominal Pain Scores by Study Week PP Population – <Age Group>
14.2.3.4.1.X	Summary of Weekly Average Abdominal Discomfort Scores by Study Week FAS Population – <Age Group>
14.2.3.4.2.X	Summary of Weekly Average Abdominal Discomfort Scores by Study Week PP Population – <Age Group>
14.2.4.1.1.X	Summary of Weekly Bowel Movement Rates by Study Week FAS Population – <Age Group>
14.2.4.1.2.X	Summary of Weekly Bowel Movement Rates by Study Week PP Population – <Age Group>
14.2.4.2.1.X	Summary of Weekly Complete Spontaneous Bowel Movement Rates by Study Week FAS Population – <Age Group>

14.2.4.2.2.X	Summary of Weekly Complete Spontaneous Bowel Movement Rates by Study Week PP Population – <Age Group>
14.2.5.1.X.1	Summary of Time to First Bowel Movement FAS Population – <Age Group>
14.2.5.2.X.1	Summary of Time to First Bowel Movement PP Population – <Age Group>
14.2.6.1.X	Summary of Weekly Stool Consistency (mBSFS-C) by Study Week FAS Population – <Age Group>
14.2.6.2.X	Summary of Weekly Stool Consistency (mBSFS-C) by Study Week PP Population – <Age Group>
14.2.7.1.X	Summary of Rescue Medication Use by Study Week FAS Population – <Age Group>
14.2.7.2.X	Summary of Rescue Medication Use by Study Week PP Population – <Age Group>
14.2.8.1.X	Summary of Weekly Fecal Incontinence Rates by Study Week FAS Population – <Age Group>
14.2.8.2.X	Summary of Weekly Fecal Incontinence Rates by Study Week PP Population – <Age Group>
14.2.9.1.1.X	Summary of Weekly Defecation Pain Rates by Study Week FAS Population – <Age Group>
14.2.9.1.2.X	Summary of Weekly Defecation Pain Rates by Study Week PP Population – <Age Group>
14.2.9.2.1.X	Summary of Weekly Defecation Pain Scores by Study Week FAS Population – <Age Group>
14.2.9.2.2.X	Summary of Weekly Defecation Pain Scores by Study Week PP Population – <Age Group>
14.2.10.1.X	Summary of Weekly Large Diameter Stool Rates by Study Week FAS Population – <Age Group>
14.2.10.2.X	Summary of Weekly Large Diameter Stool Rates by Study Week PP Population – <Age Group>
14.2.11.1	Summary of KINDL® Health-Related Quality of Life by Study Week FAS Population
14.2.11.2.X	Summary of Subject Global IBS-Disease Severity by Study Week FAS Population – <Age Group>
14.2.11.3.X	Summary of Global Relief of IBS Symptoms by Study Week FAS Population – <Age Group>
14.2.11.4.X	Summary of Global Relief of Abdominal Pain by Study Week FAS Population – <Age Group>
14.2.11.5.X	Summary of Global Relief of Treatment Satisfaction by Study Week FAS Population – <Age Group>
14.2.11.6.X	Summary of Global Relief of Treatment Continuation Assessment by Study Week FAS Population – <Age Group>

Safety

14.3.3.1.X	Summary of Treatment-Emergent Adverse Events Safety Population – <Age Group>
14.3.3.2.X	Treatment-Emergent Adverse Events by System Organ Class, Preferred Term, and Greatest Severity Safety Population – <Age Group>

14.3.3.3.X	Treatment-Emergent Serious Adverse Events by System Organ Class and Preferred Term Safety Population – <Age Group>
14.3.3.4.X	Treatment-Emergent Adverse Events Related to Study Drug by System Organ Class and Preferred Term Safety Population – <Age Group>
14.3.3.5.X	Treatment-Emergent Severe Adverse Events by System Organ Class and Preferred Term Safety Population – <Age Group>
14.3.3.6.X	Treatment-Emergent Adverse Events Leading to the Discontinuation of Study Drug by System Organ Class and Preferred Term Safety Population – <Age Group>
14.3.4.1.1.X	Summary of Quantitative Laboratory Values by Study Visit - Chemistry Safety Population – <Age Group>
14.3.4.1.2.X	Treatment-Emergent Abnormal Laboratory Toxicities - Chemistry Safety Population – <Age Group>
14.3.4.1.3.X	Laboratory Toxicity Shifts from Baseline to each Post-Baseline Visit - Chemistry Safety Population – <Age Group>
14.3.4.2.1.X	Summary of Quantitative Laboratory Values by Study Visit - Hematology Safety Population – <Age Group>
14.3.4.2.2.X	Treatment-Emergent Abnormal Laboratory Toxicities - Hematology Safety Population – <Age Group>
14.3.4.2.3.X	Laboratory Toxicity Shifts from Baseline to each Post-Baseline Visit - Hematology Safety Population – <Age Group>
14.3.4.3.1.X	Summary of Quantitative Laboratory Values by Study Visit - Urinalysis Safety Population – <Age Group>
14.3.4.3.2.X	Treatment-Emergent Abnormal Laboratory Toxicities - Urinalysis Safety Population – <Age Group>
14.3.4.3.3.X	Laboratory Toxicity Shifts from Baseline to each Post-Baseline Visit - Urinalysis Safety Population – <Age Group>
14.3.5.1.X	Summary of Vital Signs by Study Visit Safety Population – <Age Group>
14.3.7.1.X	Concomitant Medications by Drug Classification and Preferred Term Safety Population – <Age Group>
14.3.7.2.X	Prior Medications by Drug Classification and Preferred Term Safety Population – <Age Group>
14.3.7.3.X	Prior and Concomitant Medications by Drug Classification and Preferred Term Safety Population – <Age Group>
14.3.7.4.X	All Post-Treatment Medications by Drug Classification and Preferred Term Safety Population – <Age Group>
14.4.1.1.1.X	Plasma Concentrations of Plecanatide All Subjects – <Age Group>
14.4.1.1.2.X	Plasma Concentrations of SP-338 All Subjects – <Age Group>
14.4.1.2.1.X	Plasma Concentrations of Plecanatide by Gender All Subjects – <Age Group>
14.4.1.2.2.X	Plasma Concentrations of SP-338 by Gender All Subjects – <Age Group>
14.4.1.3.1.X	Plasma Concentrations of Plecanatide Intensive PK Population – <Age Group>
14.4.1.3.2.X	Plasma Concentrations of SP-338 Intensive PK Population – <Age Group>
14.4.1.4.1.X	Plasma Concentrations of Plecanatide by Gender Intensive PK Population – <Age Group>
14.4.1.4.2.X	Plasma Concentrations of SP-338 by Gender Intensive PK Population – <Age Group>

14.4.2.1.1.X	Plasma PK Parameters of Plecanatide Intensive PK Population – <Age Group>
14.4.2.1.2.X	Plasma PK Parameters of SP-338 Intensive PK Population – <Age Group>
14.4.2.2.1.X	Plasma PK Parameters of Plecanatide by Gender Intensive PK Population – <Age Group>
14.4.2.2.2.X	Plasma PK Parameters of SP-338 by Gender Intensive PK Population – <Age Group>

9.2. SUMMARY FIGURES

Efficacy

14.2.5.1.X.2	Kaplan-Meier Plot of First Time to Bowel Movement FAS Population – <Age Group>
14.2.5.2.X.2	Kaplan-Meier Plot of First Time to Bowel Movement PP Population – <Age Group>

9.3. DATA LISTINGS

14.3.2.1	Treatment-Emergent Adverse Events related to Study Drug All Subjects
14.3.2.2	Serious Treatment-Emergent Adverse Events All Subjects
14.3.2.3	Treatment-Emergent Adverse Events Leading to the Discontinuation of Study Drug All Subjects
16.1.7	Enrollment and Informed Consent All Subjects
16.2.1.1	Subject Dispositions All Subjects
16.2.1.2	Subject Discontinuations due to COVID-19 All Subjects
16.2.2.1	Protocol Deviations All Subjects
16.2.2.2	Major Protocol Deviations All Subjects
16.2.2.3	Inclusion/Exclusion Criteria at Screening All Subjects
16.2.4.1	Demographics All Subjects
16.2.4.2	Medical History All Subjects
16.2.4.3	Prior and Concomitant Medications All Subjects
16.2.4.4	Non-Drug Treatment/Therapies/Procedures All Subjects
16.2.5.1	Study Drug Accountability All Subjects
16.2.5.2	Treatment Compliance (Derived) All Subjects
16.2.5.3.1.1	Plasma Concentrations All Subjects
16.2.5.3.1.2	Plasma Concentrations Intensive PK Population
16.2.5.3.2	Plasma PK Parameters Intensive PK Population
16.2.6.1.1.X	Electronic Diary Results - Daily “Episodic” Bowel Movement Diary All Subjects – <Age Group>
16.2.6.1.2.X	Electronic Diary Results – Rescue Medication Usage All Subjects – <Age Group>
16.2.6.1.3.X	Electronic Diary Results – End of Day (Daily) Diary All Subjects – <Age Group>
16.2.6.2.1	Weekly Bowel Movement, Spontaneous Bowel Movement, and Complete Spontaneous Bowel Movement (Derived) All Subjects
16.2.6.2.2	Weekly Average Abdominal Pain, Abdominal Discomfort, Fecal Incontinence, and Defecation Pain (Derived) All Subjects
16.2.6.2.3	Weekly Average Stool Consistency Score, Large Diameter Stool Rates, and Use of Rescue Medication (Derived) All Subjects

10. APPENDICES

A. ROME IV DIAGNOSTIC CRITERIA* FOR IRRITABLE BOWEL SYNDROME IN CHILDREN

Must include all of the following:

1. Abdominal pain at least 4 days per month associated with one or more of the following:
    a) Related to defecation
    b) A change in frequency of stool
    c) A change in form (appearance) of stool
2. In children with constipation, the pain does not resolve with resolution of the constipation (children in whom the pain resolves have functional constipation, not irritable bowel syndrome)
3. After appropriate evaluation, the symptoms cannot be fully explained by another medical condition

*Criteria fulfilled for at least 2 months before diagnosis.

Rome IV Diagnostic Criteria for IBS with Predominant Constipation

1. More than one fourth (25%) of bowel movements with Bristol stool form types 1 or 2 and
2. Less than one-fourth (25%) of bowel movements with Bristol stool form types 6 or 7 in the BSFS= and types 4 or 5 in the mBSFS-C.

Alternative for epidemiology or clinical practice:
Patient reports that abnormal bowel movements are usually constipation (like type 1 or 2 in the picture of Bristol Stool Form Scale (BSFS).

B. PREGNANCY REPORTING

If a female patient should become pregnant during the course of the study (i.e., from the date the informed consent was signed until the patient's last visit), the Investigator (or authorized delegate) should notify the designated Safety Contact within 24 hours of the Investigator (or authorized delegate) first becoming aware of the pregnancy. The notification of pregnancy should be submitted using the initial Pregnancy Report Form. The initial Pregnancy Report Form should be completed with study patient's details (e.g., patient number, initials, date of birth, and investigational product information.). Whenever possible, the initial notification of pregnancy should include detailed information on the pregnancy, including last menstrual period and/or expected date of delivery. If pregnancy is to be terminated, the anticipated date of termination should be provided. If a maternal AE is reported during the initial notification of pregnancy, the details of the AE should also be described in the narrative field of the initial Pregnancy Report Form.

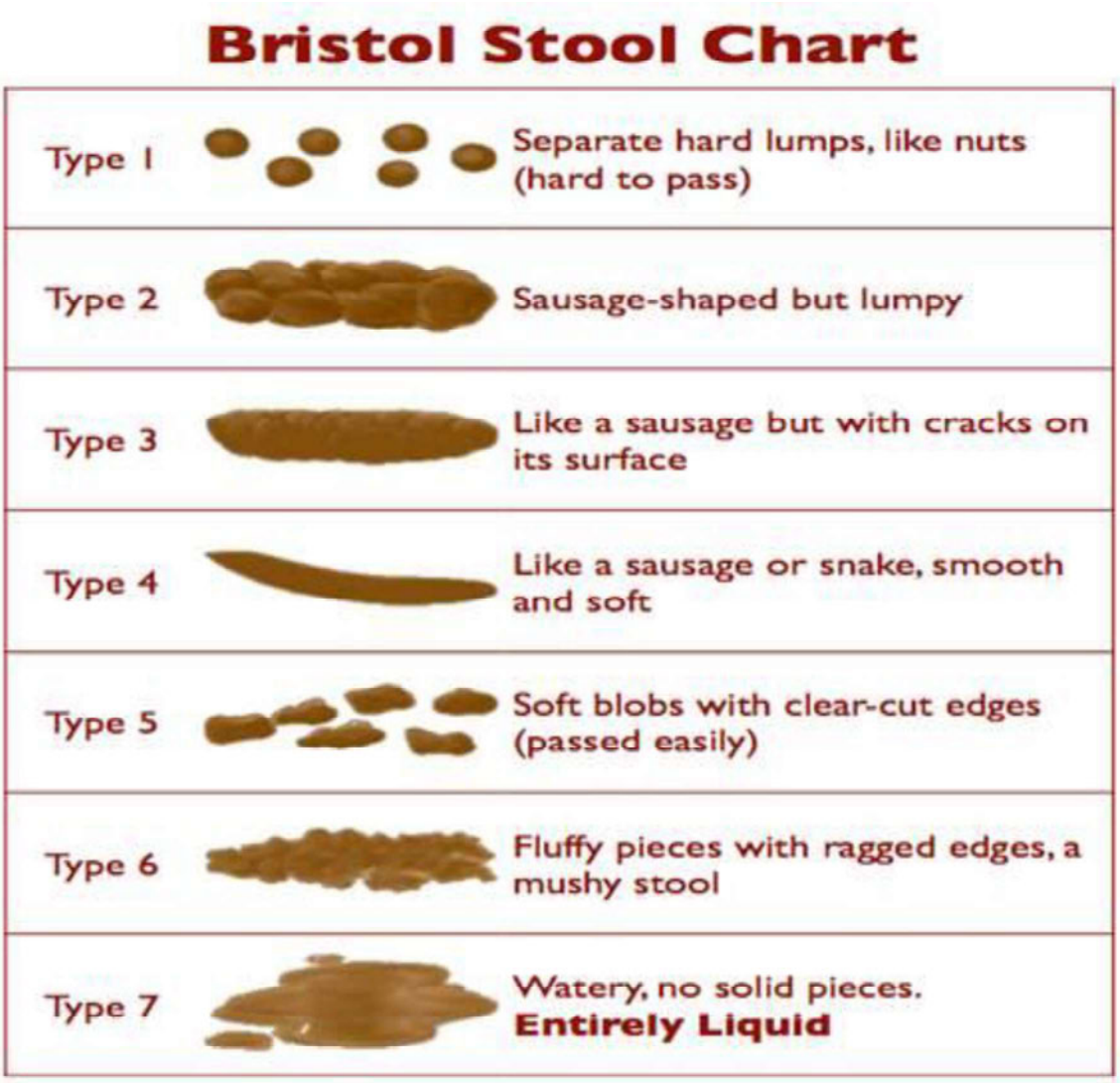
The Sponsor, Medical Monitor, or designated Safety Representative will request permission to follow the patient's progress with the doctor medically responsible for the pregnancy. If additional information on the progress of the pregnancy and/or any maternal AE is received "spontaneously" by the clinical site, the Investigator (or authorized delegate) should submit a follow-up Pregnancy Report Form to the Sponsor within 24 hours of becoming aware of the information.

If additional information on the outcome of the pregnancy and/or the details of the birth/delivery is received "spontaneously" by the clinical site, the Investigator (or authorized delegate) should also submit a pregnancy outcome report form to the designated Safety Contact within 24 hours of becoming aware of the information. If the outcome of the pregnancy is reported as premature birth, or as elective termination due to a medical reason or as spontaneous or accidental miscarriage, the details of the outcome should be described in the narrative section of the outcome Pregnancy Report Form. The pregnancy outcome will generally be reported as a follow-up report. Details of birth/delivery, including date of birth, weight, and sex of the fetus/newborn should also be described in the narrative field of the Pregnancy Report Form.

Complete a new SAE Report Form if the delivery outcome meets the criteria for a SAE (e.g., congenital anomaly/birth defect, still birth, some other sickness, etc.). The SAE Report Form should be completed with study patient's details (e.g., patient number, initials, date of birth, investigational product information, etc.) and the details of the fetal SAE should be described in the narrative field of the SAE Report Form.

C. BRISTOL STOOL FORM SCALE

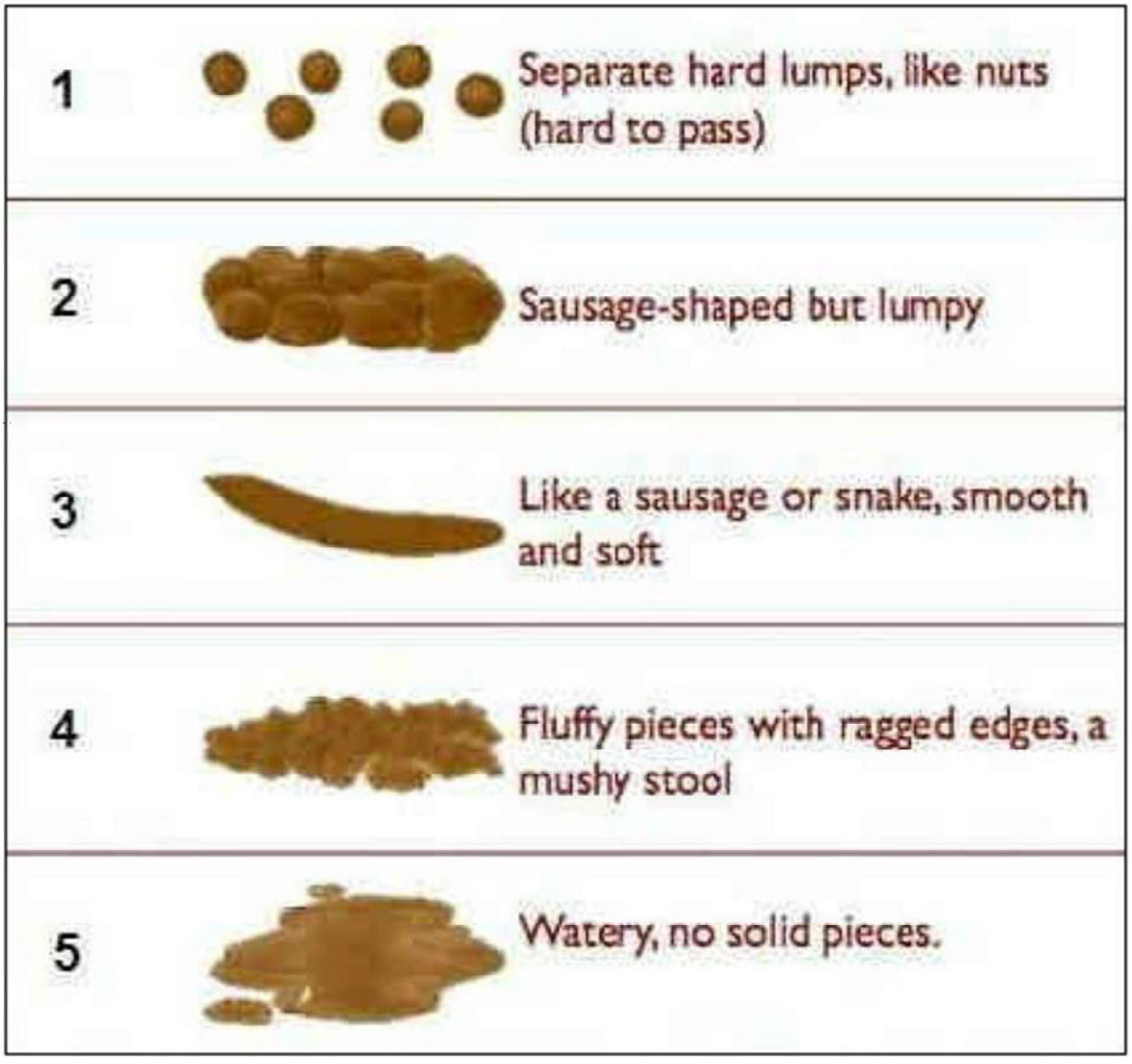
To be used for children in the 12 to < 18 age group.



D. MODIFIED BRISTOL STOOL FORM SCALE FOR CHILDREN

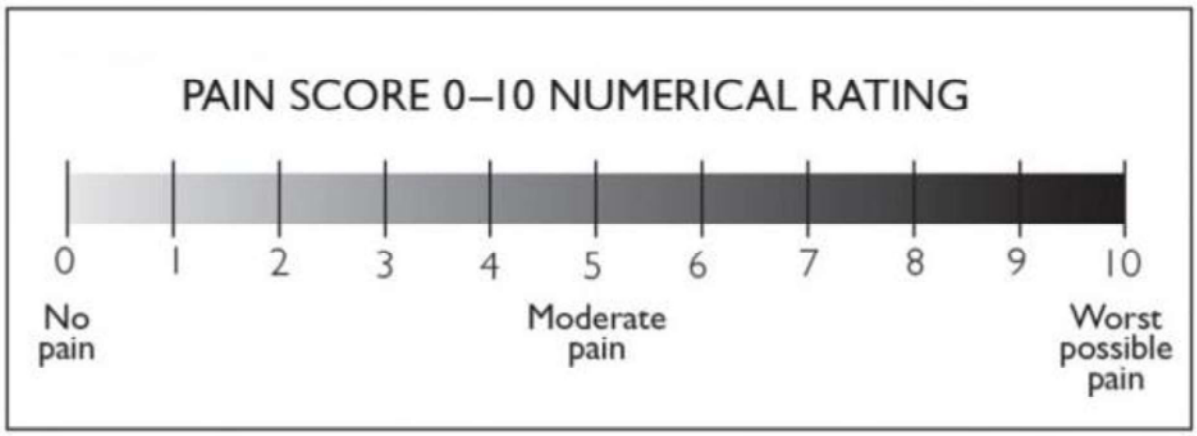
To be used for children in the 6 to 11 age group.

The modified Bristol Stool Form Scale for Children (mBSFS-C)



E. NUMERIC PAIN RATING SCALE

To be used for children for the 12 to < 18 age group.

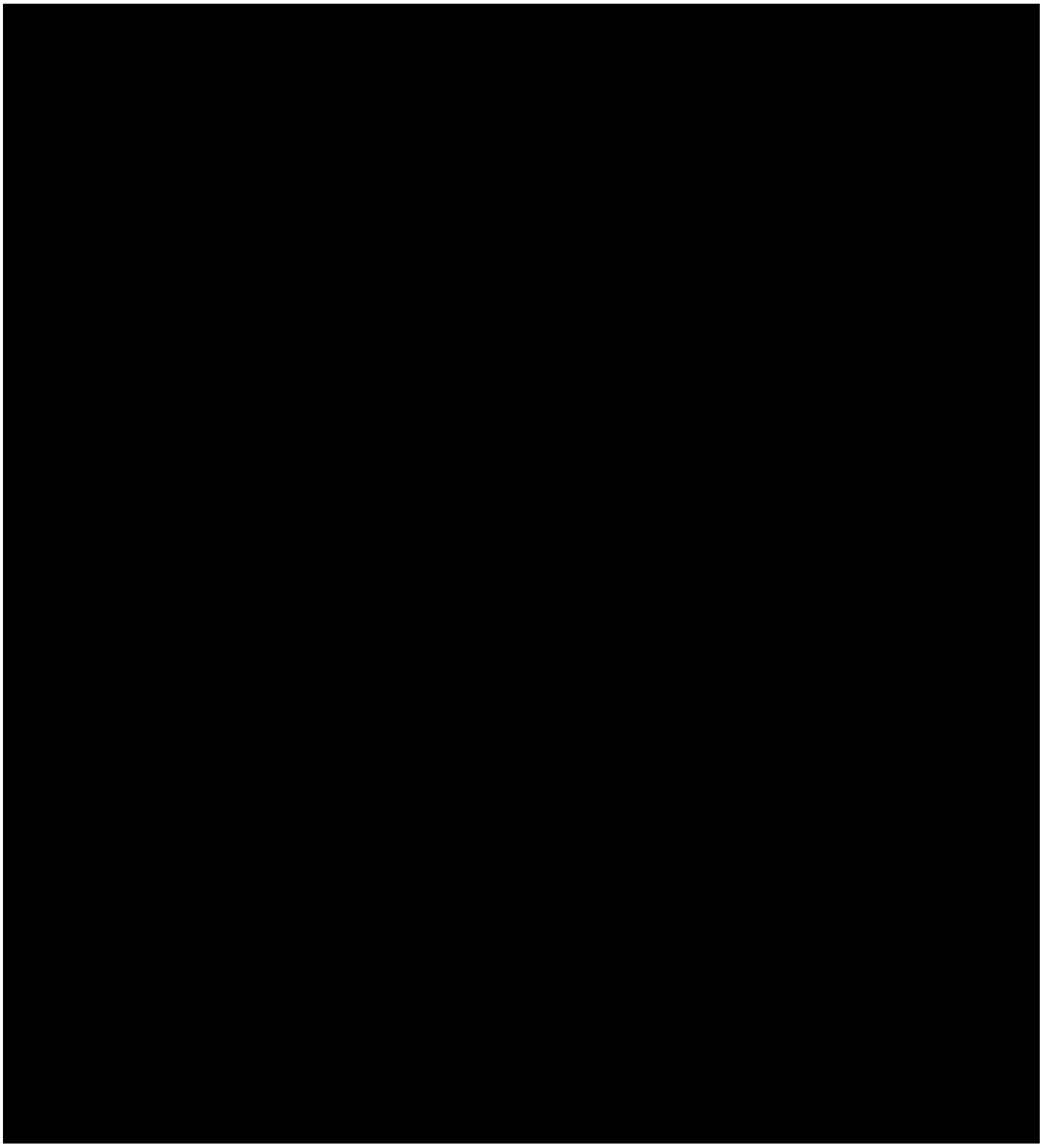


F. WONG-BAKER FACES® PAIN RATING SCALE

To be used for children for the 6 to 11 age group.

G. ELECTRONIC DAILY DIARY FOR GROUP A (AGE 6 TO 11)

Note: The final text for the diary script will appear in the final Design Specifications for the electronic diary system.



H. ELECTRONIC DAILY DIARY FOR GROUP B (AGE 12 TO < 18)

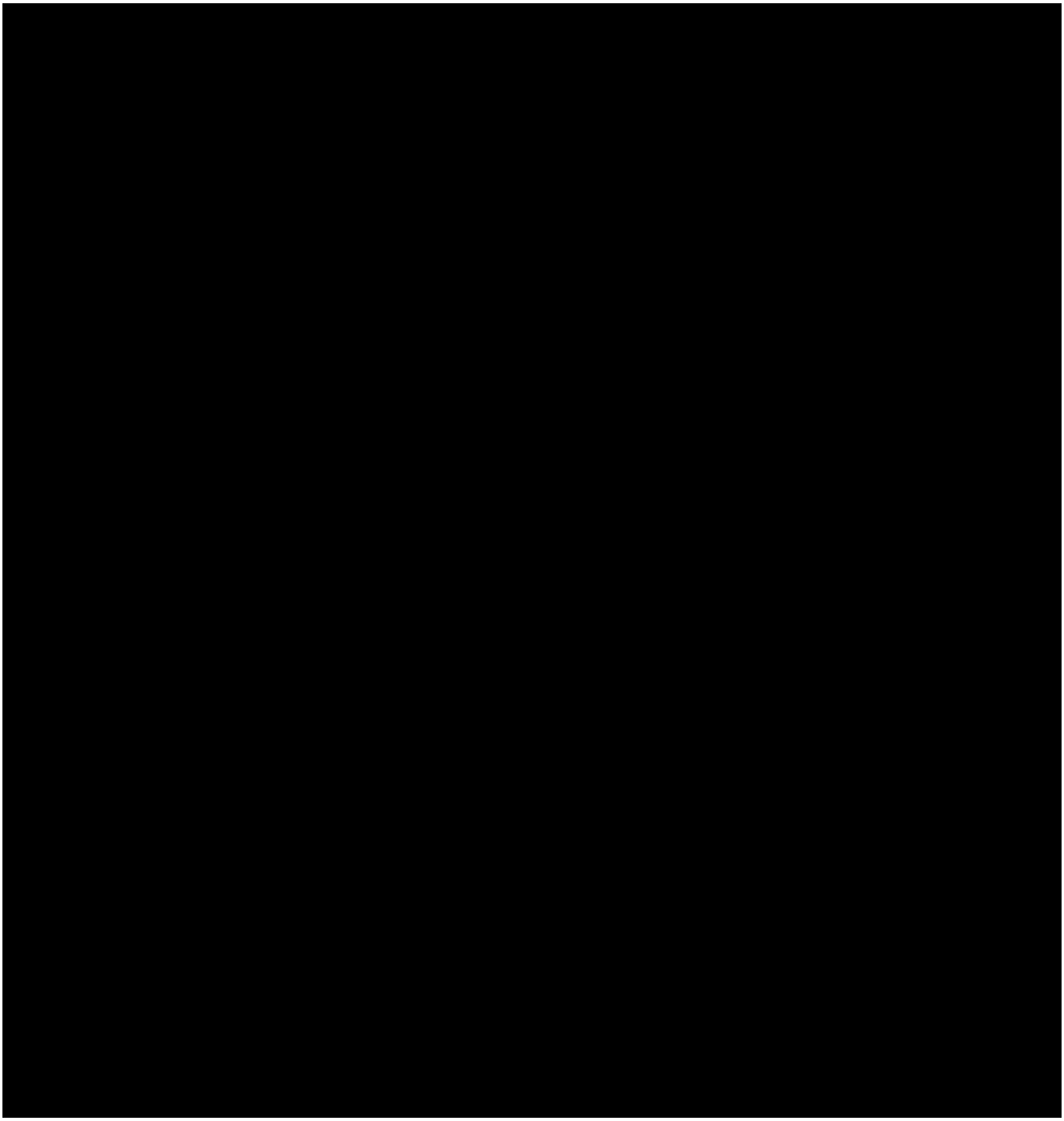
Note: The final text for the diary script will appear in the final Design Specifications for the electronic diary system.

I. PATIENT QUESTIONNAIRES

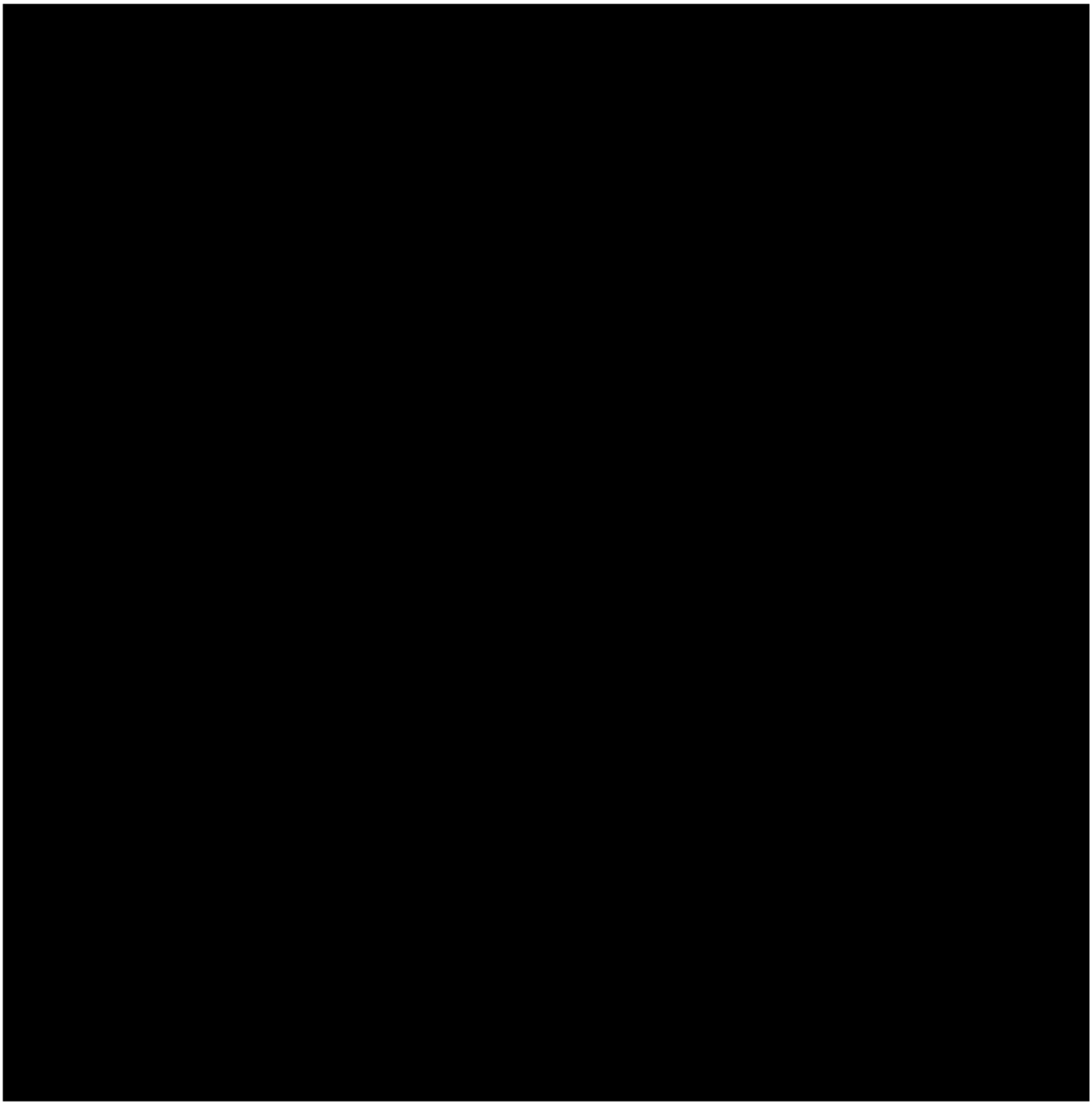
- **KINDL® Health-Related Quality of Life**
  - **Children age 6**
  - **Children age 7 to 13**
  - **Adolescents age 14 to 17**
- **Patient Global IBS-Disease Severity**
- **Global Relief of IBS Symptoms**
- **Global Relief of Abdominal Pain**
- **Treatment Satisfaction**
- **Treatment Continuation Assessment**

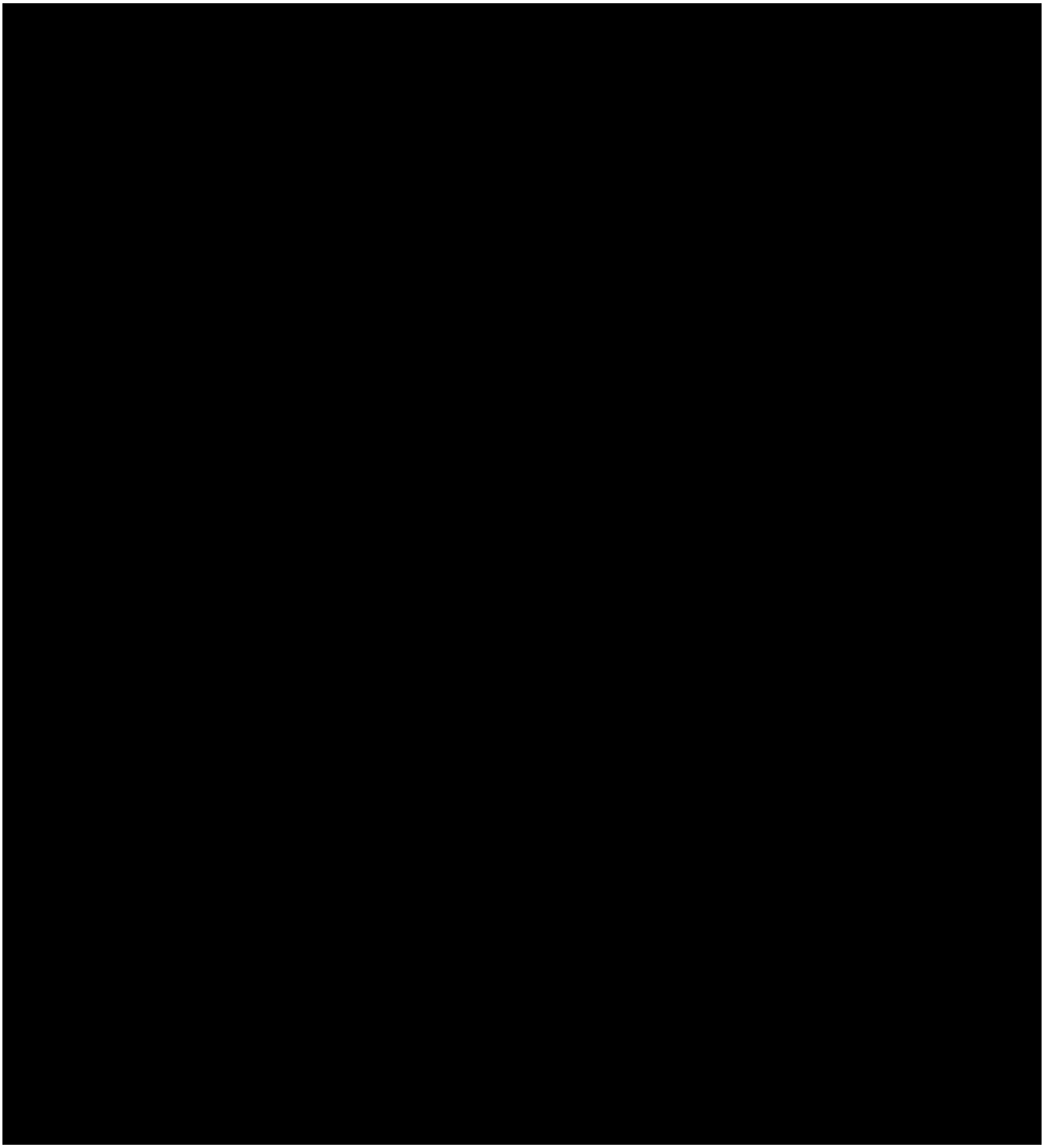
Kiddy-KINDL® Interview for Patients Age 6

Kid-KINDL® Interview for Patients Age 7 to 13



Kiddo-KINDL® Questionnaire for Adolescents Age 14 to 17





Patient Global IBS-Disease Severity

For the questions below, please choose the response that applies best to you

IBS-DISEASE SEVERITY
[redacted]

Patient Global Rating of Change – IBS Symptoms

For the questions below, please choose the response that applies best to you

RELIEF OF IBS SYMPTOMS
[redacted]

Patient Global Rating of Change – Abdominal Pain

For the questions below, please choose the response that applies best to you

RELIEF OF ABDOMINAL PAIN
[redacted]

Patient Treatment Satisfaction Assessment

For the questions below, please choose the response that applies best to you

TREATMENT SATISFACTION
[redacted]

Patient Treatment Continuation Assessment

For the questions below, please choose the response that applies best to you

TREATMENT CONTINUATION