A Randomized, Double-blind, Placebo-controlled, 3 Dose Level, Parallel-group Study of the Efficacy and Safety of Plecanatide in Adolescents 12 to <18 Years of Age with Chronic Idiopathic Constipation (CIC)

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Sponsor Protocol No.:	SP304202-13
IND No.:	74883
Study Drug Name: Phase:	Plecanatide: Phase 2b
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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, 3 Dose Level, Parallel-group Study of the Efficacy and Safety of Plecanatide in Adolescents 12 to < 18 Years of Age with Chronic Idiopathic Constipation (CIC)

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.

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Declaration of the Investigator

Title: A Randomized, Double-blind, Placebo-controlled, 3 Dose Level, Parallel-group Study of the Efficacy and Safety of Plecanatide in Adolescents 12 to < 18 Years of Age with Chronic Idiopathic Constipation (CIC)

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 understood 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, 3 Dose Level, Parallel-group Study of the Efficacy and Safety of Plecanatide in Adolescents 12 to < 18 Years of Age with Chronic Idiopathic Constipation (CIC)
Sponsor	Synergy Pharmaceutical Inc.
Sponsor Study No.	SP304202-13
Phase	2b
Study Centers	The study will be conducted at approximately 40 clinical sites in the United States
Objectives	Primary objective: To evaluate the safety and efficacy of 3 different doses of oral plecanatide taken once daily for 8 weeks as treatment for CIC in adolescents 12 to less than 18 years of age Secondary objectives: • To identify the pharmacokinetic (PK) parameters of plecanatide in this patient population (to the extent possible since plecanatide is not expected to be absorbed) • To evaluate the effect of 3 selected dose levels of plecanatide on stool consistency and relief of CIC-related symptoms in adolescents 12 to less than 18 years of age • To assess the effect of 3 selected dose levels of plecanatide on the frequency of complete spontaneous bowel movements (CSBMs)
Study Design	This is a randomized, double-blind, placebo-controlled, multicenter, parallel-group study of the efficacy and safety of 3 dose levels of plecanatide (0.5, 1.0, or 1.5 mg) in adolescent patients 12 to less than 18 years of age with CIC. Patient randomization will focus on the balanced enrollment of males and females across the 4 treatment groups. This Phase 2b study will comprise a 28-day bowel habit Screening/Baseline Period, an 8-week Treatment Period, and a 2-week Posttreatment Follow-up Period. Patients will visit the clinic 5 times during the study. *Screening/Baseline Period*: Patients' legally authorized representatives (e.g., parent or guardian) will provide informed parental permission and patients will provide written 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 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 (using the Bristol Stool Form Scale [BSFS]), abdominal pain, abdominal discomfort, abdominal bloating, straining and pain when passing stools, fecal incontinence, excessive flatulence, and feeling of incomplete evacuation following bowel movement. 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 chronic constipation status with which the change from Baseline across and at the end of the 8-week treatment period will be compared. *Treatment Period*: Patients who meet all entry criteria will be randomly assigned to 1 of 4 treatment groups (3 active, 1 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 an oral dose of the study drug once daily for 8 weeks and continue to complete their daily electronic diaries with recording of

	BMs, rescue medication use for constipation, abdominal pain, and other symptoms. At Weeks 1, 4, and 8 of the Treatment Period, patients will return to the clinic to undergo safety and efficacy assessments. *Posttreatment 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 10. The planned duration of participation in this study will be approximately 14 weeks from signing of informed consent/assent through posttreatment.
Study Drug	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 be randomly assigned to 1 of 3 plecanatide doses (0.5, 1.0, or 1.5 mg) or to matching placebo. Study drug will be provided as a 4-week supply of tablets in blister packs. It will be distributed on Day 1 and replenished at Week 4. Patients will take the assigned dose of study drug each morning approximately at the same time for 8 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 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 120 adolescents with CIC will be randomized (~30 in each treatment group) into the study. Eligibility for study enrollment (2 or fewer SBMs per week, 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 adolescents 12 to less than 18 years of age diagnosed with CIC based on the Rome III criteria for child/adolescent functional constipation.
Key Exclusion Criteria (abbreviated)	• 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 eDiary and rescue medication use.
Criteria for Evaluation of Efficacy and Safety	<u>Efficacy endpoints</u> The primary efficacy endpoint is: Proportion of responders for the last 2 weeks of the Treatment Period (responder = a child who experiences >3 SBMs per week for each of the last two weeks of the treatment period) compared to placebo and across treatment groups. The key secondary efficacy endpoints are: • Change from Baseline in stool consistency (as assessed by BSFS) • Change from Baseline in frequency of SBMs • Change from Baseline in frequency of CSBMs Additional efficacy endpoints are: • Change from Baseline in CIC-related symptoms (abdominal pain, abdominal bloating, abdominal discomfort, straining with a bowel movement and pain with defecation) • Frequency/severity of excessive flatulence • Frequency of fecal incontinence • Patient Reported Global Assessments (PGA, PAC-SYM and PAC-QOL) <u>Pharmacokinetic endpoints</u> Single dose and steady state of plasma plecanatide (population modeling):

	• Plasma concentration of plecanatide (Note: measurable plecanatide levels have not been observed in the adult population) • PK parameters, including C_{max}, T_{max}, and C_{min} (only if a sufficient number of plecanatide levels can be measured to allow computation of these parameters) <u>Safety endpoints</u> • Frequency of treatment-emergent adverse events (TEAEs) • Significant shifts from Baseline values in laboratory analytes and vital signs
Statistical Methods	<u>Analysis Populations</u> Analysis populations include the Safety population, the Intent-to-treat (ITT) population, and the Per-Protocol (PP) population. The Safety population will include all patients who were randomly assigned to a treatment group and received at least 1 dose of study drug. The ITT population will include all patients who were randomized to receive study drug. The PP population will be used for sensitivity analyses, and will consist of all patients in the ITT population without any significant protocol violations. The ITT population will be the primary population for efficacy analyses. <u>Patient Characteristics and Disposition</u> Demographic characteristics (including age, gender, race, weight, height, and body mass index) and characteristics of patients' CIC history will be summarized by descriptive statistics for each treatment group. <u>Efficacy Analyses</u> *Primary Efficacy Analysis* The primary efficacy endpoint is the proportion of responders for the last 2 weeks of the Treatment Period (responder = a child who experiences >3 SBMs per week for each of the last two weeks of the treatment period) compared to placebo and across treatment groups. 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 plecanatide treatment group compared to placebo will be presented, with 95% confidence intervals and corresponding statistical p-values. *Secondary Efficacy Analysis* The secondary efficacy endpoints are: • Change from baseline in weekly average stool consistency (BSFS) score over the 8-week treatment period, and by study week • Change from baseline in the weekly rate of SBMs, by study week • Change from baseline in the weekly rate of CSBMs over the 8-week treatment period, and by study week 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. <u>Pharmacokinetic Analyses</u> Plasma plecanatide (and its major metabolite SP-338) concentrations will be assessed for all patients pre-dose on Day 1, and at Week 4 and Week 8 visits.

	Intensive PK sampling (30, 60, 90 and 120 min post-dose on Day 1) will be done in a subset of approximately 40 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 proportion of patients with abnormal clinical and vital sign results will be summarized.

LIST OF STUDY PERSONNEL

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADL	Activities of Daily Living
AE	Adverse event
AGA	American Gastroenterological Association
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
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)
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
IBS	Irritable Bowel Syndrome
ICF	Informed consent form
ICH	International Conference on Harmonization
IND	Investigational New Drug (application)
IRB	Institutional Review Board
ITT	Intent-to-Treat
IWRS	Interactive Web-based Response System
LAR	Legally authorized representative
LLN	Lower limit of normal
LOCF	Last observation carried forward
LS	Least Squares
MCG	Microgram
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MG	Milligram
mITT	Modified Intent-to-Treat
mL	Milliliter
PDUFA	Prescription Drug User Fee Act

PE	Physical examination
PGA	Patient Global Assessment
PI	Principal Investigator
PIN	Personal Identification Number
PP	Per-Protocol (Population)
PRO	Patient Recorded Outcome
PV	Pharmacovigilance
QD	Once daily
QoL	Quality of Life
RM	Rescue medication
RTSM	Randomization and Trial Supply Management
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
TEAE	Treatment emergent adverse event
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
US	United States
WHODD	World Health Organization Drug Dictionary

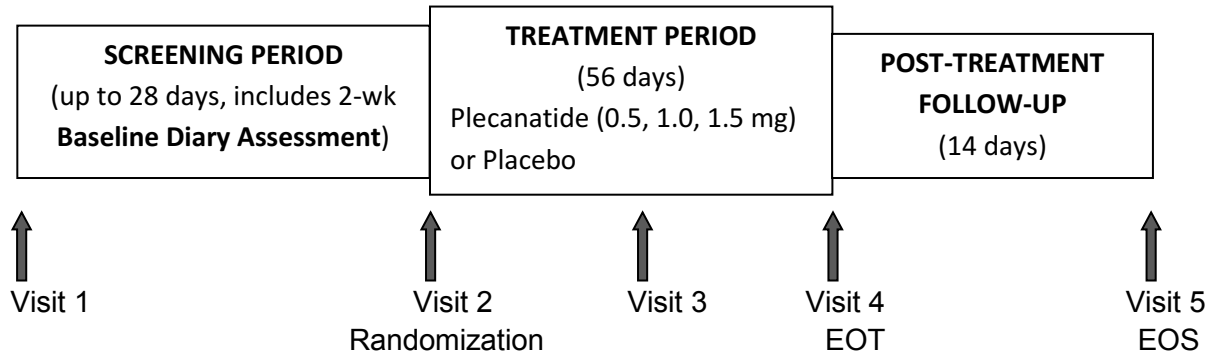
Definition of Terms

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 an exploratory, randomized, double-blind, placebo-controlled, multicenter, parallel-group study of the efficacy and safety of 3 dose levels of plecanatide (0.5, 1.0, or 1.5 mg) in adolescent patients 12 to less than 18 years of age with CIC. Approximately 120 patients will be enrolled at approximately 40 study sites in the United States. Patient randomization will focus on the balanced enrollment of males and females across the 4 treatment 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 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 CIC.

Patient 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 who meet all study entry criteria will be randomly assigned to 1 of 4 treatment groups (3 active, 1 placebo) in a 1:1:1:1 ratio. The patients will take their first dose of study drug at the clinical site, and continue to take a single oral dose daily for 8 weeks. Supplies of the study drug will be replenished at the Week 4 Visit. At Weeks 4 and 8, patients will return to the clinic to undergo safety and efficacy assessments.

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 a final Follow-up visit at the end of Week 10.

The planned duration of participation in this study will be approximately 14 weeks (98 days) from signing of informed consent/assent through posttreatment.

2 INTRODUCTION

2.1 BACKGROUND AND RATIONALE

2.1.1 Chronic Idiopathic Constipation in Adults

Idiopathic or functional constipation is a common disorder, affecting approximately 15% of the adult population of the United States (US), depending on demographic factors and the definitions used (Higgins, 2004). Internationally, similar prevalence rates have been reported in most geographic areas (Suares, 2011; Pare, 2001; Peppas, 2008). Constipation is a symptom of many diseases and is a collective term used to imply infrequent stools, incomplete bowel movements (BMs), straining, bloating, and hard lumpy stool (Cash, 2007). Treatment for constipation is usually based on increased dietary fiber and supplementation with bulking agents; exercise; and habit training. However, often only partial relief is obtained, and many patients use non-bulking laxatives on a regular basis without medical supervision. Chronic use of laxatives is often inappropriate, and may lead to side effects, such as dependency and progressive tolerance, electrolyte imbalance, and, for the anthraquinones, melanosis coli. In addition, stimulant laxatives may damage the myenteric plexus, resulting in cathartic colon. Laxatives available over the counter are, in general, approved for episodic and not chronic use.

2.1.2 Chronic Idiopathic Constipation in Children

Although a lack of a uniform and consistent definition (or definitions) for "chronic constipation" make it difficult to estimate the prevalence of constipation, several published reports confirm that it is a prevalent condition worldwide in children as well as adults. It is important to recognize that while clinical trial populations are generally defined using Rome Criteria (**Appendix A**), it is not as common to apply them in clinical practice and therefore they are not used uniformly as the basis for epidemiology studies of chronic constipation across adult populations or studies among the various pediatric age groups.

A recent comprehensive review of the published literature on the epidemiology of pediatric constipation concludes that the median value of reported prevalence rates among 19 published articles was in a range from 0.7% to 29.6%, with a median of 12% (Mugie, 2011). No consistent gender differences for constipation in children have been noted. However, similar to adults, high body mass index (BMI) and low socioeconomic status appear to be associated with a higher prevalence of constipation in the pediatric population. A positive family history has been found in 28% to 50% of children with constipation and a higher incidence has been reported in monozygotic than in dizygotic twins. Approximately 40% of children with functional constipation develop symptoms during the first year of life (Davidson, 1963; Benninga, 1993) while 16% of parents of 22-month old children report constipation in their child (Cash, 2007). Peak incidence occurs at the time of toilet training (2-4 years of age), with an increased prevalence in boys.

Constipation in children is recognized as having a significant impact on the overall health care system, with constipation representing 3% to 5% of general pediatric outpatient visits and up to 25% of pediatric gastroenterology consultations (Partin, 1992; Caplan, 2005).

Two drugs are approved in the US for treatment of chronic idiopathic constipation in adults - linaclotide and lubiprostone. There is still unmet need for treatment of constipation in children.

Plecanatide as a member of the guanylate cyclase (GC-C) agonist class of oral therapeutic agents is expected to improve the current therapy for CIC.

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). Activation of CFTR in turn enhances transepithelial efflux of chloride and bicarbonate ions and as a consequence 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

In clinical trials 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.

SP304101-08 was a Phase 1 single-dose escalation study in 71 healthy adult subjects receiving doses of 0.1, 0.3, 0.9, 2.7, 5.4, 8.1, 16.2, 24.3, or 48.6 mg plecanatide (n=53) or placebo (n=18) (IB for Plecanatide). The study reported no systemic absorption at any dose and no serious adverse events (SAEs) or withdrawals due to adverse events (AEs). Diarrhea was the most common AE in the study, reported by 8 of 53 (15.1%) plecanatide subjects and 3 of 18 (16.7%) placebo subjects. The occurrence of diarrhea did not appear to be dose related. Other AEs occurring in 2 or more subjects included abdominal discomfort, nausea, and vomiting (Shailubhai, 2009).

SP304201-09 was a Phase 2a 14-day, repeat-dose, placebo-controlled, oral dose-escalation study to determine the safety of plecanatide in adult patients with CIC (Shailubhai, ACG 2010 Poster). Seventy-eight patients were enrolled in this study and randomized to receive 1 of 4 plecanatide doses (0.3 mg, 1.0 mg, 3.0 mg, or 9.0 mg) or placebo. Each dose cohort consisted of 20 patients (15 active; 5 placebo). After each cohort was completed, a safety review was conducted prior to initiating the next dose cohort. No SAEs or withdrawals due to AEs occurred for any of the patients who received plecanatide during this study. None of the 58 patients treated with plecanatide in this study had diarrhea. The majority of AEs were mild or moderate in severity and transient in nature.

SP304-20210 was a large, 12-week, double-blind, placebo-controlled, multicenter, dose-ranging study in adult patients with CIC (study data on file). A total of 951 patients were randomized at 113 clinical sites in the US. Randomization was 1:1:1:1 to plecanatide (0.3, 1.0, or 3.0 mg) or placebo. In this study, 948 patients received study drug (712 plecanatide) and 946 patients had at least 1 study assessment and were included in the modified Intent-to-Treat (ITT) population. In the high-dose group (3.0 mg, n=237), the proportion of patients who were overall responders

(19%) was statistically significantly greater than in patients treated with placebo (10.7%) (p=0.009).

Among secondary endpoints for Protocol SP304-20210, there were statistically significant changes from baseline (as compared to placebo) in the frequency of complete spontaneous bowel movements (CSBMs), stool consistency (higher Bristol Stool Form Scale [BSFS] score), and straining that were dose-related, with the greatest change and degree of statistical significance at the 3.0-mg dose group. The median times to first SBM were statistically significantly shorter for all doses of plecanatide compared to placebo (27.3 hours) with the shortest median time to first spontaneous bowel movement (SBM) of 12.5 hours at the 3.0-mg dose. In addition, the proportion of patients who had a SBM in the first 24 hours was 41.5% with placebo as compared to 55.7% (0.3 mg), 53.8% (1.0 mg), and 67.5% (3.0 mg) in plecanatide-treated patients. Statistically significantly different (from placebo) changes in patient global assessments and the PAC-SYM© and PAC-QOL© scales were also observed over the 12 weeks of treatment at the 1.0- and 3.0-mg doses. In general, plecanatide was safe and well tolerated.

Diarrhea was the most common AE, which increased with increasing dose to an incidence of 9.7% in the 3.0-mg dose group. Of the cases of diarrhea in patients treated with plecanatide, 0.7% overall were considered severe and in patients receiving 3.0 mg, only one case (0.4%) was considered severe. The other most common treatment-emergent AEs (TEAEs) occurring in at least 2% of patients were headache, abdominal pain, nausea, abdominal distension, urinary tract infection, flatulence, and upper respiratory infection. Serious AEs were uncommon, occurring in only 9 study patients, and none were considered to be related to study drug. Overall, approximately 5.5% of patients on 3.0 mg of plecanatide withdrew due to AEs compared to 3.4% on placebo. Three percent of patients at the 3.0-mg dose withdrew participation due to diarrhea compared to 0.4% on placebo. There were no clinically significant changes in laboratory tests, electrocardiograms (ECGs), or vital signs. Further details on plecanatide can be found in the investigator's brochure (IB).

The most recently completed clinical trials in population of patients with CIC were two Phase 3 studies SP304203-00 and SP304203-03. The objectives of these double-blind, placebo-controlled, parallel-group studies were to evaluate the safety and efficacy of 3 mg and 6 mg doses of plecanatide administered once daily for 12 weeks in a population of patients with CIC – male and female patients ages 18 to 80 years. Overall, safety findings were consistent with earlier observations from previous studies and indicate that 12 weeks of plecanatide administration at doses up to 6 mg are generally well tolerated by patients with CIC.

In the SP304203-00 study, a total of 1394 patients were randomized, and 1389 patients received at least one dose of study drug at 164 clinical sites in the US and Canada. The two doses of plecanatide (3 mg and 6 mg) that were evaluated over 12 weeks of treatment in CIC patients in this study were generally well tolerated and provided safety observations similar to those associated with exposure to placebo. The percentage of patients (34.3%) in the plecanatide groups reporting at least one TEAE was not substantially different from the percentage of patients in the placebo group (32.8%).

Overall, 469 of the 1389 (33.8%) patients in the Safety Population reported at least one TEAE. A total of 818 TEAEs were reported across all treatment groups. Of the 469 patients, 150 patients (32.8%) were in the placebo group, 168 patients (35.4%) were in the 3-mg plecanatide group,

and 151 patients (33.0%) were in the 6-mg plecanatide group. The highest rate of patients reporting a TEAE (35.4%) and the largest number of reported TEAEs (290) occurred in the 3-mg plecanatide group. The percentage of patients in the plecanatide groups reporting at least one TEAE, however, was not substantially different from the percentage of patients in the placebo group.

Treatment-emergent AEs experienced by ≥ 2.0% of patients (in any treatment group) included diarrhea (5.9%, 5.5% − 3-mg and 6-mg plecanatide groups, respectively); nasopharyngitis (2.4% − 6 mg plecanatide); sinusitis (2.1% − 3 mg plecanatide); and in the placebo group, urinary tract infection (2.2%), and back pain and headache (2.0% each). Diarrhea (59 [4.2%]) and nasopharyngitis (23 [1.7%]) were reported by the highest percentage of patients overall.

In the SP304203-03 study, a total of 1410 patients were randomized and 1402 patients received at least one dose of study drug at 162 clinical sites in the US. The two doses of plecanatide (3 mg and 6 mg) that were evaluated over 12 weeks of treatment in CIC patients in this study were generally well tolerated and provided safety observations similar to those associated with exposure to placebo. The percentage of patients (26.5%) in the plecanatide groups reporting at least one TEAE was not substantially different from the percentage of patients in the placebo group (24.7%).

Overall, safety findings were consistent with earlier observations from previous studies and from the other phase 3 study of identical design and indicate that 12 weeks of plecanatide administration at doses up to 6 mg are generally well tolerated by patients with CIC.

Overall, 372 of the 1402 (26.5%) patients in the Safety Population reported at least one TEAE. A total of 618 TEAEs were reported across all treatment groups. Of the 372 patients, 115 patients (24.7%) were in the placebo group, 120 patients (25.7%) were in the 3-mg plecanatide group, and 137 patients (29,2%) were in the 6-mg plecanatide group. The highest rate of patients reporting a TEAE (29.2%) and the largest number of reported TEAEs (226) occurred in the 6-mg plecanatide group. The percentage of patients in the plecanatide groups reporting at least one TEAE, however, was not substantially different from the percentage of patients in the placebo group.

Treatment-emergent AEs experienced by ≥ 2.0% of patients (in any treatment group) included diarrhea (3.2%, 4.5% − 3-mg and 6-mg plecanatide groups, respectively) and headache (2.1 % − 3 mg and 6 mg plecanatide groups each). The percentage of patients who reported diarrhea appeared to correlate with dose of plecanatide treatment.

2.2 OBJECTIVES

2.2.1 Primary Objective

The primary objective is to evaluate the safety and efficacy of 3 different doses of oral plecanatide taken once daily for 8 weeks as treatment for CIC in adolescents 12 to less than 18 years of age.

2.2.2 Secondary Objectives

Secondary objectives are:

- To estimate the pharmacokinetic (PK) parameters of plecanatide in this patient population (to the extent possible, since plecanatide is not expected to be absorbed)
- To evaluate the effect of 3 selected dose levels of plecanatide on stool consistency and relief of CIC-related symptoms in adolescents 12 to less than 18 years of age
- To assess the effect of 3 selected dose levels of plecanatide on the frequency of complete spontaneous bowel movements (CSBMs)

2.2.3 Rationale for Dose Selection

Safety and efficacy data with plecanatide in adult CIC 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). 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 CIC in adolescents ages 12 to 17 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. 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. 8.0 for Plecanatide, 1/2016. It is expected that the plecanatide safety profile observed in the 12 to < 18-year old pediatric population will be similar to that observed in adult patients with CIC.

The amount of blood to be drawn during the course of this study (approximately 57 mL for the majority of patients, and 81 mL for patients participating in the intensive PK analysis) is not considered to be a risk to 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 proportion of responders for the last 2 weeks of the Treatment Period (responder = a child who experiences >3 SBMs per week for each of the last two weeks of the treatment period) compared to placebo and across treatment groups.

2.4.1.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints are:

- Change from baseline in weekly average stool consistency (BSFS) score over the 8-week treatment period, and by study week
- Change from baseline in the weekly rate of SBMs, by study week
- Change from baseline in the weekly rate of CSBMs over the 8-week treatment period, and by study week

2.4.1.3 Additional Efficacy Endpoints

Additional efficacy endpoints related to CIC symptoms are:

- Change from baseline in abdominal pain, by study week
- Change from baseline in abdominal bloating, by study week
- Change from baseline in straining during a BM, by study week
- Change from baseline in pain with defecation, by study week Change from baseline in excessive flatulence, by study period
- Change from baseline in frequency of fecal incontinence, by study period

2.4.1.4 Patient Reported Outcomes

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

- PGA
- PAC-SYM
- PAC-QOL

2.4.1.5 Exploratory Efficacy Endpoints

The following exploratory endpoints will be assessed (see **Section 8.8.4** for definitions of these endpoints):

- 4/8 Week SBM 3 + 1 Responder
- 6/8 Week SBM 3 + 1 Responder
- 4/8 Week CSBM 3 + 1 Responder
- 6/8 Week CSBM 3 + 1 Responder

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, vital signs, and ECGs 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 8-week treatment in adolescent patients with CIC.

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 8 weeks, which is sufficient time to test the durability of response.

2.6 PLANNED SAMPLE SIZE AND NUMBER OF STUDY CENTERS

Approximately 120 patients (~ 30 patients per treatment group) will be recruited for this study at approximately 40 clinical sites in the USA. The patients will be enrolled in this multi-center outpatient study by gastroenterologists, internists, and general medicine practitioners 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 adolescents 12 to less than 18 years of age.
2. Diagnosed with CIC based on the Rome III criteria for child/adolescent functional constipation **(Appendix A).**
3. Patient is able to voluntarily provide written, signed, and dated (personally and via a legally authorized representative [LAR]) assent/informed consent as applicable to participate in the study.
4. Patient and patient's parent/LAR demonstrates an understanding, ability, and willingness to fully comply with study procedures (e.g., acceptance of venipuncture, patient willing and able to swallow tablets, acceptance of urine drug screen for opiates) and restrictions.

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. The patient, if female of childbearing potential (defined as postmenarche), does not agree to practice 1 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 weeks 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 protocol-prohibited medication prior to randomization.
16. The patient and his or her parent/guardian are unable to communicate well with the study staff and comply with the study requirements (restrictions, appointments, and examination schedule). (The patient must be able to complete all required Daily BM and Symptom electronic diary entries during the Screening/Baseline period and for the duration of the study. The patient must also agree to receive a reminder daily via phone should he or she not complete the daily electronic diary entries.)

Exclusion Criteria Based on Baseline Diary Entries

17. Completion of < 5 of the 7 required daily diary entries in each week of the 2-week baseline diary assessment.
18. Use of rescue medication (Dulcolax®, bisacodyl) for more than 2 days during either of the two weeks of the 2-week baseline diary assessment.
19. ≥ 3 SBM per week for either week of the 2-week baseline diary assessment.

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 date, stop date, 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 will be asked what medications they have taken during the last 30 days. At each subsequent study visit, patients will be asked what concomitant medications they are currently taking. Use of rescue medication 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 re-supplied at the study visits 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. As per Dulcolax® package insert (directions for use), Dulcolax® "may be taken 1 to 2 tablets as a single dose per day".

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.

Rescue medication usage will be recorded daily via the electronic diary. Sites will review rescue medication use with patients during their study 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® will be distributed to sites by Sharp Corporation. Sites may order additional bulk supplies of rescue medication by submitting a Dulcolax® Request Form. Supplies of Dulcolax® will not be reconciled at the completion of the study. Sites must track inventory of rescue medication supplies for the purpose of ensuring adequate quantities for re-supplying patients as needed. The site's supply of rescue medication will not be managed nor automatically re-supplied.

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 to allow 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 H_2 antagonists
- Antihistamines that have primarily anti H1 activity (eg, cetirizine, loratadine, and chlorpheniramine)
- Bulking agents (eg, 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 2-week Screening/Baseline Period and remains on this dose for the duration of participation in the study.

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 posttreatment). This includes the following:

- Lactulose
- Stimulant laxatives, including senna (Ex-Lax®) and sennosides (eg, Senokot®), cascara sagrada, anthraquinones, castor oil, aloe, or other
- Osmotic laxatives (eg, 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 (eg, 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, eg, Colace®)

3.4 DIETARY INTAKE

A stable dietary intake should be maintained including high fiber diet, fiber supplements, vitamins and minerals, probiotics, fish oil, 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

BMI will be calculated by measuring the patient's height (without patient wearing shoes) and weight and using these measurements (in meters and kilograms) in the formula: BMI (kg/m^2) = weight (kg) ÷ height^2 (m^2)

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 CIC 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 assure 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" calls should be made at the time of each bowel movement or use of rescue medication during the course of the day. End of Day calls 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 Posttreatment 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 constipation symptoms. Note that any data entered prior to the beginning of the 2-week baseline diary assessment 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 may report BMs and RM use as often as necessary);
- **End-of-Day daily reporting** constipation-related symptoms (and BM/RM if not already reported as an 'episodic report') at a specific time each evening. Patients who have not completed the prompted evening call will receive an automated reminder (via phone) each evening.

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 all study visits.

The following endpoints will be assessed using data collected via the electronic diary:

Frequency and Completeness of Bowel Movements

Patients will be asked to report in "real time" the numbers of BMs they experienced each day, the time of each BM, and the completeness of evacuation in the daily BM Diary. Frequency of BMs is one aspect of the primary endpoint for evaluation of study drug efficacy.

Stool Consistency—Bristol Stool Form Scale (BSFS)

Patients will be asked to rate their stool consistency according to the BSFS (see **Appendix C)**, which will be provided to them in the form of a laminated card (and will appear on the appropriate electronic hand-held device screen) at the Screening Visit (Visit 1), and may be referred to as often as necessary throughout the Screening, Treatment and Posttreatment Periods. The BSFS is a validated measure of stool consistency commonly used in clinical trials.

Use of Rescue Medication (RM)

As part of the daily diary, the patient will be questioned concerning the use of provided rescue medication (Dulcolax®), including time of use, frequency 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.

Constipation-related Symptoms

The patient will also record in the electronic diary the frequency and severity of several CIC-related gastrointestinal symptoms. These symptoms include abdominal pain, abdominal bloating, abdominal discomfort, straining during bowel movement and pain with defecation. Patients will be asked to rate their symptoms on an 11-point Numeric Rating Scale from 0 (NO) to 10 (WORST POSSIBLE) (See **Appendix D)**.

4.2.2 Variables Assessed by Questionnaires Administered at the Study Site

NOTE: Standard definitions for flatulence and fecal incontinence will be included in the study procedure manual

Excessive Flatulence

Excessive flatulence (NOTE: the term "excessive" is meant to be interpreted by each patient in terms of what he/she perceives as "more than normal" either for him or herself or as compared to his peers/friends) will be assessed via the IWRS at each study visit. Patients will be asked to rate their "excessive flatulence" using the IWRS on a scale from 0 to 4, where 0 = none, 1 = mild, 2 = moderate, 3 = severe, and 4 = very severe.

Fecal Incontinence

The following question will be asked at each study visit using the IWRS as follows:

Visit 1:

Have you ever experienced fecal incontinence? YES NO

If YES, Approximately how many episodes of fecal incontinence did you experience over the last two weeks?

0 1 2 3 more than 3

Visits 2 through 5:

Approximately how many episodes of fecal incontinence did you experience over the last two weeks?

0 1 2 3 more than 3

Three standardized patient questionnaires (see **Appendix E)** will also be administered during the scheduled study site visits to assess the severity of constipation and impact of the disease on the patient's quality of life.

Patient Assessment of Constipation—Symptoms (PAC-SYM)

The PAC-SYM is a previously validated questionnaire which is made up of 12 questions addressing specific symptoms of constipation. The patient will be asked to rate each symptom on a scale of 0 ("absent") to 4 ("very severe").

Patient Assessment of Constipation—Quality of Life (PAC-QOL)

The PAC-QOL is a previously validated questionnaire is made up of 28 questions which assess how the patient has been impacted by constipation over the specified period. The questions measure worries and concerns, physical discomfort, psychosocial discomfort, satisfaction, and overall effects on the patient's quality of life. Patients will be asked to give their response on a scale of 0 ("not at all" or "none of the time") to 4 ("extremely" or "all of the time").

Patient Global Assessment (PGA) Questionnaire

The Patient Global Assessment (PGA) questionnaire is designed to provide an assessment of constipation severity before, during, and after treatment. The PGA is reproduced in **Appendix E**.

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: Specific GI symptoms are recorded daily in the GI symptom diary (abdominal bloating, abdominal pain, abdominal discomfort, straining with a bowel movement and pain with defecation) in response to standardized questions. The frequency and severity ratings recorded for these symptoms are both an efficacy and safety record and therefore these events should generally NOT be recorded elsewhere as adverse events.

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 each study visit, from Screening to Posttreatment. A 6- to 12-hour fast is advised 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

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
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
Pregnancy test:	Urine pregnancy tests via dip sticks (performed on-site)
Immunogenicity:	Anti-plecanatide antibody serum assessment
PK:	Plecanatide (and SP-338) plasma assessment
Urine screen for select opioids:	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 2.0 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 28 mL.

4.3.3 Immunogenicity and Pharmacokinetic (PK) Assessments

Approximately 3.5 mL of blood for immunogenicity testing – measurement of serum concentration of anti-plecanatide antibodies, will be collected (and banked) from all patients, as specified in the Schedule of Assessments. The total volume of blood required for immunogenicity testing during the study will be approximately 11 mL.

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 (15 min prior to dosing) and at the Week 4 and Week 8 visits. Intensive sampling for formal PK analysis will be conducted in a subgroup of approximately 40 patients (10 patients per each treatment 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 18 mL for the majority of patients, and 42 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 12 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 and immunogenicity 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 after the patient has been seated for at least 5 minutes. 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

Standard 12-lead ECGs 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 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.

Neither a urogenital exam nor 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 will be measured at the Screening Visit only. Weight will be measured as part of all physical examinations; the same calibrated scale should be used for each measurement. BMI calculation will use the height and weight recorded at Visit 1 (Screening Visit).

4.3.7 Concomitant Medications and Diet

Concomitant medications or 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.

Diet will be reviewed at each study visit to ensure that each patient is maintaining an acceptable and stable diet throughout the study. Any change in diet considered clinically significant by the Investigator will be documented in the eCRF.

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

Patients will provide written informed consent before any study-related procedures are initiated, including the cessation of prohibited concomitant therapy.

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**	**Visit 3**	**Visit 4**	**Visit 5**
Study Week	**Week -4**	**Week 1**	**Week 4**	**Week 8(EOT) or EW**	**Week 10(EOS)**
Visit Day (Window)	**Day -28**	**Day 1**	**Day 28±3**	**Day 56 +3**	**Day 70 +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/Diet	X	X	X	X	X
Physical Examination [a]	X	X	X	X	X
Vital Signs [b]	X	X	X	X	X
Pregnancy Test (urine)[c]	X	X	X	X	X
Urine Drug Screen for Opioids [d]	X	X			
Serum Chemistry, Hematology, Urinalysis	X	X	X	X	
TSH (include T3 and T4 only if TSH is abnormal)	X				
Immunogenicity Test (serum collection)		X	X	X	
Plecanatide/SP-338 Concentration[e] (plasma collection)		X [e]	X	X	
PK Sampling (30, 60, 90 and 120 mins post-dose in a subset of patients)		X			
12-lead Electrocardiogram [f]	X	X*		X	
Electronic Diary Training and Activation [g]	X				
Diary Eligibility [h] / Compliance Check		X [h]	X	X	
Randomization		X			
Study Drug Dispensation and Administration[i]		X	X		

Study Period	Screening/ Baseline	Treatment			Post-treatment Follow-up
Visit Study Week	Visit 1 Week -4	Visit 2 Week 1	Visit 3 Week 4	Visit 4 Week 8(EOT) or EW	Visit 5 Week 10(EOS)
Visit Day (Window)	Day -28	Day 1	Day 28±3	Day 56 +3	Day 70 +3
At Visit PRO Assessments[j]	X	X	X	X	X
Study Drug Collection and Accountability			X	X	X
Rescue Medication Dispensation	X	X	X	X	
Adverse Events [k]	X	X	X	X	X

EOT = end of treatment; EW = early withdrawal; EOS = end of study; GI = gastrointestinal; PK = pharmacokinetic; TSH = thyroid-stimulating hormone.

a. Physical examination, including body weight measurement, will be performed at Visits 1 to 4 and EW; height is measured (and body mass index calculated) at the Screening Visit only.

b. Seated blood pressure (after patient has been seated for at least 5 minutes), heart rate, respiration, temperature.

c. Urine pregnancy test will be performed on-site for female patients; negative result must be confirmed at Screening and Day 1 prior to randomization.

d. 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.

e. 15 mins before study drug dosing.

f. The standard 12-lead ECG will be performed in the semi-recumbent or supine position. (*) At Visit 2 (Week 1/Day 1), ECG will be performed prior to study drug dosing and again one hour after the study drug administration.

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.

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 Assessments will be included in the Study Procedure Manual.

k. 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)

Patients are often pre-screened for the study. Pre-screening information is generally obtained through customary practice of routine medical intake for the patients. Of note, patients who have completed pre-screening are not pre-qualified for participation in the study, therefore the site is still responsible for completing all required screening assessments in accordance with the study protocol.

The patient must be screened, ideally, within 28 days before the randomization visit in the study. A 3-day window is allowed to complete the Day 1 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 patient must provide written assent and parent or caregiver must provide written informed consent.
- Assign a unique patient number. Unique patient numbers will begin with the clinical site number, eg, 001 followed by a 3-digit number. Unique patient numbers will be assigned sequentially by clinical site personnel accessing the EDC system. 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 (ie, 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 with the exception of the rescue medication provided as part of the study.
- Determine eligibility, based on Rome III criteria for child/adolescent functional constipation (**Appendix A**)
- Verify inclusion/exclusion criteria.
- Collect demographic information.
- Record medical history, including GI and bowel habit history.
- Record prior and concomitant medications and dietary history.
- Perform a physical examination, including measurement of body weight and height.
- Measure vital signs (seated blood pressure, heart rate, respiration, and body temperature).
- Perform urine screen for opioids (see **Section 4.3.2** for details).
- Perform a urine pregnancy test for female patients (a negative result must be confirmed at Screening and on Day 1 prior to randomization).
- Collect blood and urine samples for safety laboratory tests (serum chemistry, including thyroid-stimulating hormone [TSH], hematology, and urinalysis).
- Perform 12-lead ECG in the semi-recumbent position.

- Have the patient complete the Excessive Flatulence and Fecal Incontinence questionnaires (Per Study Procedure Manual).
- Train patients how to use the electronic diary to record their responses about BM and CIC-related symptoms and activate the diary.
- Distribute a laminated card with the BSFS to patients and explain how to use it to record stool consistency in their daily electronic diaries.
- Ensure patients understand the importance of entering data in their diaries on daily basis for all questions.
- Dispense rescue medication to patients and instruct them on the 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 of Treatment Period

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

- Confirm that patient has used only study-supplied rescue medication laxative (Dulcolax®) since Visit 1
- Urine drug screen (see **Section 4.3.2** for details)
- Urine pregnancy test, if applicable
- Update medical history and concomitant medications, as necessary

The last 14 days of daily diary entries prior to randomization will be used for determination of baseline data. Only patients who meet the protocol criteria for confirmation of CIC and demonstrate diary and RM compliance are eligible for randomization. This eligibility determination will include:

- A review of the baseline diary entries to ensure patients have completed at least 5 of the 7 days of diary entries in each of the 2 weeks of baseline assessments
- A review of the BM frequency data to ensure < 3 SBMs in each of the 2 weeks of the baseline diary assessment period
- A review of rescue medication use to ensure patients have not used RM for more than 2 days in each baseline week

The eligibility determination based on these criteria will be programmed into the IWRS and the system will report out eligibility (the PI or site staff will not be responsible for evaluating diary data to make the diary-based eligibility determination).

The following procedures will be performed ***only for patients eligible for randomization***:

Procedures ***prior to study drug dosing***:

- Physical examination (including body weight) and vital signs (seated blood pressure, heart rate, respiration, and body temperature)
- 12-lead ECG (semi-recumbent)
- Assess adverse events
- Safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Confirm that patient is on a stable diet and has no significant changes in their consumption of liquids or fiber or their level of activity
- Blood samples for plecanatide/SP-338 concentration and immunogenicity assessments (see **Section 4.3.3** for details)
- Have the patient complete all baseline PRO questionnaires (Per Study Procedure Manual).

The first dose of study drug will be administered at the clinical site under supervision. The time of dosing will be recorded.

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
- At one hour post dosing, the patient will have a standard 12-lead ECG performed; clinically significant findings will be assessed as an adverse event
- Dispense 4-week supply of study drug with the instructions for use and storage (as described in **Section 6.3**)
- 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 Visit 3: Week 4, Day 28 (± 3 days) of Treatment Period

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, as necessary
- Have the patient complete the PRO questionnaires (per Study Procedure Manual)

- Assess/record changes in concomitant medications and diet
- Physical examination (including body weight) and vital signs (seated blood pressure, heart rate, respiration, and body temperature)
- Safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Collect blood samples for immunogenicity assessments
- Urine pregnancy test, if applicable
- Assess adverse events
- Dispense 4-week supply of study drug
- Distribute additional rescue medication (Dulcolax® tablets), if needed

5.2.4 Visit 4: Week 8, Day 56 (+ 3 days) of Treatment Period

The following will be completed at Visit 4:

- 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, as necessary
- Have the patient complete the PRO questionnaires (per Study Procedure Manual)
- Assess/record changes in concomitant medications and diet
- Physical examination (including body weight) and vital signs (seated blood pressure, heart rate, respiration, and body temperature)
- 12-lead ECG (semi-recumbent)
- Safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Urine pregnancy test, if applicable
- Collect blood samples for plecanatide/SP-338 concentration and immunogenicity assessments
- Assess adverse events
- Distribute additional rescue medication (Dulcolax® tablets), if needed

5.2.5 Visit 5: Week 10, Day 70 (+ 3 days) – Posttreatment Follow-up (End of Study)

The following will be completed at Visit 5:

- 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 complete the PRO questionnaires ((per Study Procedure Manual)

5.2.6 Early Withdrawal Visit (EW)

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 9.10**.

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
- Review the electronic diary compliance reports and re-emphasize the need for diary compliance, as necessary
- Have the patient complete the PRO questionnaires (see Study Procedure Manual)
- Assess/record changes in concomitant medications and diet
- Physical examination (including body weight) and vital signs (seated blood pressure, heart rate, respiration, and body temperature)
- 12-lead ECG (semi-recumbent)
- Safety laboratory assessments (hematology, serum chemistry, urinalysis)
- Perform urine pregnancy test, if applicable
- Collect blood samples for plecanatide/SP-338 and immunogenicity assessments
- Assess adverse events.
- Distribute additional rescue medication (Dulcolax® tablets), if needed

In all cases, a single, primary reason for withdrawal must be recorded on the eCRF.

The End of Study assessments should be performed 2 weeks following the last study drug administration. 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 8 consecutive weeks according to the randomization scheme displayed in **Table 3**. Each patient will take one tablet 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.

No dose adjustments will be allowed on this study.

Table 3 Treatment Arms

Table 3: Treatment Arms

Treatment Arm	Treatment	Number of Patients
1	0.5 mg plecanatide	30
2	1.0 mg plecanatide	30
3	1.5 mg plecanatide	30
4	Matching placebo	30

Overdose

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

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 CIC has caused diarrhea in 5% of patients with severe diarrhea occurring in 0.6% of patients. 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) (Guidance for Industry, FDA, 2007). 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 (Guidance for Industry, FDA, 2007)	**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, eg, 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
Grade 4: Life-threatening consequences, urgent intervention indicated	Permanently discontinue study drug

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.

6.3 PACKAGING, LABELING AND STORAGE

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

6.3.1 Packaging

Each investigational drug kit will contain a 4-week supply in blister packaging—a total of 32 tablets, including 4 extra tablets to allow for a 3-day window extension for a study visit. Individually blister packaged tablets will be provided in four connected strips (each per 1 week) 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.

At each dispensing visit, each patient will be given a numbered drug kit corresponding to the treatment group into which the patient was randomized. At the randomization visit (Day 1 of the Treatment Period), the eligible patient will receive one drug kit for the first 4 weeks of dosing (weeks 1, 2, 3 and 4). At the Week 4 Visit, the patient will receive a new drug kit for their weeks 5, 6, 7 and 8 of dosing. Patients are instructed to bring their kits at the following study visit to allow for determination of compliance and reconciliation of supplies.

Save all empty packaging or packaging containing unused tablets for final disposition by the sponsor or contract pharmacy.

6.3.2 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, address 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 the age of 12".

The label will include the following fields to be completed by site personnel: Investigator name and phone number, patient number and treatment weeks. When dispensing a kit to a patient, complete and remove the tear off portion of the label and keep this in 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.

6.3.3 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 to 25°C (68 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 to 25°C (68 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.

6.4 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 telephone calls resulting in an un-blinding event 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 5.2.6**).

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.

6.5 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, specifying the kit number, the patient number assigned, and the amount dispensed to each patient and the date dispensed. This information will be available in the IWRS and the drug accountability log for the overall study. At each visit after Visit X, the patient is asked to return their previously dispensed drug kit and the number of remaining tablets is entered into the eCRF for drug accountability.

A blinded 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 copies of this accountability log to the Sponsor.

The Investigator is responsible for assuring 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.

6.6 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 each visit during the Treatment Period. The number of remaining tablets will be counted and entered into the appropriate eCRF page 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 CIC are recorded daily in the electronic Diary in response to standardized questions, and are BOTH efficacy AND safety response variables. These symptoms are abdominal bloating, abdominal pain, abdominal discomfort, straining with bowel movement 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 CIC Symptom Diary, they should generally not also be reported in the adverse event database that relies on a much longer patient recall period. In order to prevent double-recording of a single specific event, the daily CIC 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, electrocardiogram (ECG), 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 OF SPECIAL INTEREST – DIARRHEA

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).

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, 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 subjects 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 preparing meals, shopping for groceries or clothes, 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 Posttreatment 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 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.

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 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) within 24 hours of becoming aware of the event:

SAE REPORTING SAFETY CONTACT INFORMATION

Chiltern Safety and Pharmacovigilance

[redacted]

For any questions related to the reporting of an SAE, sites should call the PV Hotline and include the following information: Sponsor (Synergy Pharmaceuticals), protocol number (SP304202-13), 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 Chiltern Safety.

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 Chiltern 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 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 Synergy Pharmaceuticals, 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 of 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, and 1.5 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 four treatment arms that will be conducted at approximately 40 clinical sites. Approximately 120 patients will be randomized to receive plecanatide 0.5, 1.0, and 1.5 mg, or the placebo equivalent, 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 CIC, assume a mean change from baseline in weekly SBM rate for placebo-treated subjects of 1.2 with a standard deviation of 2.6; similarly, for plecanatide-treated subjects, assume a mean change from baseline of 3.1 with a standard deviation of 4.0. Then, a *t*-test for the difference in means of two independent samples with unequal variances, at a two-sided level of significance of 0.05 and 80% power to detect the assumed difference in means, would require 51 subjects per treatment group. Since study SP304202-13 is being conducted in adolescents with CIC, 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 120 subjects, 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:	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.
Intent-to-Treat (ITT) Population :	All patients randomized to study treatment. Patients will be analyzed according to the treatment to which they are randomized. The ITT Population is the main population for assessments of efficacy.
Per Protocol (PP) Population:	All patients in the ITT population who complete the 8-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 ITT 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" in cooperation with the Sponsor. 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.

8.4 GENERAL CONSIDERATIONS

Categorical variables will be summarized by the number and percentage of patients in each category level. Continuous variables will be summarized by number of observations, mean, standard deviation (SD), median, minimum, and maximum values. Where data are collected over time, both the observed data and change from Baseline will be summarized at each time point.

Summaries will be presented by treatment group (placebo, and plecanatide 0.5 mg, 1.0 mg, and 1.5 mg), and combined active doses. All data collected in the eCRF will be presented in listings.

All statistical tests will be two-sided at the 5% level of significance, unless stated otherwise.

No interim analyses are planned.

8.4.1 Missing Data Conventions

In general, data will not be imputed for safety analyses.

The weekly SBM (CSBM) rate is the sum of the 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 SBM (CSBM) rate for the week is the sum of the SBMs (CSBMs) recorded for the days in the week for which BMs were reported, plus 0 SBMs (0 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 SBM (CSBM) rate will be set to 0 in any summary or linear mixed-effects model analysis involving the weekly 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 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 CIC-related 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 involving abdominal bloating, abdominal discomfort, straining during a bowel movement or pain with defecation, 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 scores recorded during the week, divided by the total number of non-missing scores recorded. In any week in which a patient has no non-missing item score 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 (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.

For the exploratory Responder analyses, in any given week, a patient must have at least 4 compliant diary days in that week. A compliant diary day means that the patient has responded to the BM and rescue medication use questions in the daily diary for that day. Patients who have fewer than 4 compliant diary days in a given week will be considered "non-responders" for that week. Note that this implies that for any week for which a patient has no BM or rescue medication use data, the patient is considered a non-responder; in particular, for a randomized patient who withdraws from study participation prior to the end of the study, the patient is considered a non-responder for any weeks remaining in the planned duration of the study for which the patient has no BM or rescue medication use data.

8.4.2 Diary Data Visit Windows

The daily electronic diary entries reported during the Treatment Period will be classified into 8 weeks as follows:

Day 1-7 = Week 1
Day 8-14 = Week 2
Day 15-21 = Week 3
Day 22-28 = Week 4
Day 29-35 = Week 5
Day 36-42 = Week 6
Day 43-49 = Week 7
Day 50-56 = Week 8

Non-diary data, such as vital signs, clinical laboratory assessments, and ECGs, 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 SBM (CSBM) weekly rate will be the weekly average number of SBMs (CSBMs) recorded during the 2-week baseline assessment period. Baseline stool consistency, severity of straining during a BM, and abdominal symptoms 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 system; 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 proportion of responders for the last 2 weeks of the Treatment Period (responder = a child who experiences >3 SBMs per week for each of the last two weeks of the treatment period) compared to placebo and across treatment groups.

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 8-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 plecanatide treatment group compared to placebo will be presented, with 95% confidence intervals and corresponding statistical *p*-values.

8.8.2 Secondary Efficacy Endpoints

The secondary efficacy endpoints, and methods of analysis for each, are:

8.8.2.1 Change from Baseline in Weekly Average Stool Consistency (BSFS) Score Over the 8-week Treatment Period, and by Study Week

The weekly average stool consistency (BSFS) score for each patient will be computed, as described in **Section 8.4.1**, for each week of the 8-week treatment period. The change from baseline in mean weekly average BSFS score over the 8-week treatment period 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 plecanatide treatment group compared to placebo will be presented, with 95% confidence intervals and corresponding statistical *p*-values.

As a supplement to the above analysis, the weekly average stool consistency (BSFS) score for each patient will be computed, as described in **Section 8.4.1**, for each week of the 8-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly average BSFS score, and change from baseline in mean weekly average BSFS score, for each of the 10 study weeks will be presented. The change from baseline in mean weekly average BSFS scores 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 plecanatide treatment group compared to placebo will be presented, with 95% confidence interval and corresponding statistical *p*-value, for each week of the 8-week treatment period and for the two follow-up weeks.

8.8.2.2 Change from Baseline in the Weekly Rate of SBMs, by Study Week

The weekly SBM rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 8-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly SBM rate, and change from baseline in mean weekly SBM rate, for each of the 10 study weeks will be presented. 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 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 plecanatide treatment group compared to placebo will be presented, with 95% confidence interval and corresponding statistical *p*-value, for each week of the 8-week treatment period and for the two follow-up weeks.

8.8.2.3 Change from Baseline in Weekly Rate of CSBMs Over the 8-week Treatment Period, and by Study Week

The weekly CSBM rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 8-week treatment period. The change from baseline in mean weekly CSBM rate over the 8-week treatment period 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 plecanatide treatment group compared to placebo will be presented, with 95% confidence intervals and corresponding statistical *p*-values.

As a supplement to the above analysis, the weekly CSBM rate for each patient will be computed, as described in **Section 8.4.1**, for each week of the 8-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly CSBM rate, and change from baseline in mean weekly CSBM rate, for each of the 10 study weeks will be presented. The change from baseline in mean weekly CSBM 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 plecanatide treatment group compared to placebo will be presented, with 95% confidence interval and corresponding statistical *p*-value, for each week of the 8-week treatment period and for the two follow-up weeks.

8.8.3 Additional Efficacy Endpoints

Additional efficacy endpoints related to CIC symptoms are:

- Change from baseline in abdominal pain, by study week
- Change from baseline in abdominal bloating, by study week
- Change from baseline in abdominal discomfort, by study week
- Change from baseline in straining during a BM, by study week
- Change from baseline in pain with defecation, by study week

8.8.3.1 Analysis of the Additional Efficacy Endpoints

The weekly average of each additional efficacy endpoint score (abdominal pain, abdominal bloating, abdominal discomfort, straining during a bowel movement, or pain with defecation) for each patient will be computed, as described in Section 8.4.1, for each week of the 8-week treatment period and for the two weeks of the follow-up period. A summary, by treatment group, of the mean weekly average symptom score, and change from baseline in mean weekly average symptom score, for each of the 10 study weeks will be presented. The change from baseline in mean weekly average symptom score 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. For each abdominal symptom score, the least squares (LS) mean change from

baseline will be presented, and the difference in LS means for each plecanatide treatment group compared to placebo will be presented, with 95% confidence interval and corresponding statistical *p*-value, for each week of the 8-week treatment period and for the two follow-up weeks.

8.8.4 Exploratory Efficacy Endpoints

The following exploratory efficacy endpoints will be assessed:

- 4/8 Week SBM 3 + 1 Responder
- 6/8 Week SBM 3 + 1 Responder
- 4/8 Week CSBM 3 + 1 Responder
- 6/8 Week CSBM 3 + 1 Responder

8.8.4.1 Analysis of Exploratory Efficacy Endpoints

A weekly SBM (CSBM) 3 + 1 Responder is a patient who has at least 3 SBMs (CSBMs) in a given week and an increase of at least 1 SBM (CSBM) from baseline during that week. A 4/8 Week SBM (CSBM) 3 + 1 Responder meets these criteria for at least 4 of the 8 weeks of the treatment period; a 6/8 Week SBM (CSBM) 3 + 1 Responder meets these criteria for at least 6 of the 8 weeks of the treatment period.

The responder rate for each of the four exploratory efficacy endpoints will be analyzed. In each of these responder analyses, for each plecanatide group, the proportion of responders will be compared to the proportion of responders in the placebo group using the CMH test stratified by gender. The number and percentage of responders for each treatment group (and 95% confidence intervals), the difference in responder rates between each plecanatide group and the placebo group (and 95% confidence intervals), and the two-sided *p*-value associated with the CMH test will be presented.

8.8.5 Patient Reported Outcomes

PRO questionnaire data for fecal incontinence, excessive flatulence, PGA, PAC—SYM, and PAC—QOL will be included in the patient listings. Summary analyses of these data will also be provided as described in the SAP.

8.8.6 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 8 weeks of dosing will be based on the occurrence of TEAEs, vital signs, clinical laboratory assessments, and ECGs and 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, ECGs, 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), vital signs, and ECG will be summarized as changes from baseline. Laboratory shift tables will also be produced, where applicable. Listings of laboratory tests, vital signs, and ECGs 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

All data will be captured electronically at each clinical site using eCRFs and the interactive web response system (IWRS). Patient diary and questionnaire data will be collected using the IWRS. 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 freeze the eCRF page.

The specific procedures to be used for data entry and query resolution using the EDC and/or 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 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 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 system will be carefully controlled and configured according to each

individual's role throughout the study. In general, only the investigator and authorized staff will be able to enter data and make corrections in the eCRFs.

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. 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 system. Study drug return will be tracked in the eCRF.

9.3 ACCESS TO SOURCE DATA

During the study, a monitor will make clinical site visits to review protocol compliance, compare eCRF 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 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 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 Harmonisation (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 F)**.

9.7 INFORMED CONSENT

Before each patient is admitted to the study, informed consent will be obtained from the patient (or his/her legally authorized representative) 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.

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.

9.8 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 Synergy Pharmaceuticals, 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.

9.9 DURATION OF THE STUDY

For an individual patient, the planned duration of the study will be 98 days (including 28 days for screening, 56 days of treatment and 14 days of follow-up.

9.10 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

9.11 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.

9.12 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.

9.13 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 carrying out 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.

9.14 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. Higgins PD, Johanson JF. Epidemiology of constipation in North America: a systematic review. Am J Gastroenterol. 2004 99:750-759.
2. Suares NC, Ford AC. Prevalence of, and Risk Factors for, Chronic Idiopathic Constipation in the Community: Systematic Review and Meta–analysis. Am J Gastroenterol. 2011 106:1582–1591.
3. Pare P, Ferrazzi S, Thompson WG, Irvine EJ, Rance L. An epidemiological survey of constipation in Canada: definitions, rates, demographics, and predictors of health care seeking. Am J Gastroenterol. 2001 96:3130-3137.
4. Peppas G, Alexiou VG, Mourtzoukou E, Falagas M. Epidemiology of constipation in Europe and Oceania: a systematic review. BMC Gastroenterol. 2008 8:5.
5. Cash BD, Chang L, Sabesin SM, Vitat P. Update on the management of adults with chronic idiopathic constipation. J Fam Pract. 2007 56: S13-19.
6. Mugie SM, Benninga MA, Di Lorenzo C. Epidemiology of constipation in children and adults: a systematic review. Best Pract Res Clin Gastroenterol. 2011 25:3-18.
7. Davidson M, Kugler MM, Bauer CH. Diagnosis and management in children with severe and protracted constipation and obstipation. J Pediatr. 1963 62:261-275.
8. Benninga MA, Büller HA, Taminiau JA. Biofeedback training in chronic constipation. Arch Dis Child. 1993 68:126-129.
9. Cash BD, Chang L, Sabesin SM, Vitat P (). Update on the management of adults with chronic idiopathic constipation. J Fam Pract. 2007 96: 513-519.
10. Partin JC, Hamill SK, Fischel JE, Partin JS. Painful defecation and fecal soiling in children. Pediatrics. 1992 89:1007-1009.
11. Caplan A, Walker L, Rasquin A. Validation of the pediatric Rome II criteria for functional gastrointestinal disorders using the questionnaire on pediatric gastrointestinal symptoms. J Pediatr Gastroenterol Nutr. 2005 41:305-316.
12. Sindic A, Schlatter E. Cellular effects of guanylin and uroguanylin. J Am Soc Nephrol. 2006 17:607–616.
13. Shailubhai K, Gerson W, Talluto C, Jacob G. SP 304 to Treat GI Disorders: Effects of a Single, Oral Dose of SP304 on Safety, Tolerability, Pharmacokinetics and Pharmacodynamics in Healthy Volunteers. Gastroenterology. 2009 136 (Issue 5 Supplement 1):A-641.
14. Shailubhai K, Talluto C, Comiskey S, Foss J, Joslyn A, Jacob G. A Ph IIa Randomized, Double-blind, Placebo-controlled, 14-day Repeat, Oral, Range-finding Study to Assess the Safety, Pharmacokinetic and Pharmacodynamic Effects of Plecanatide (SP 304) in Patients with CIC. American College of Gastroenterology (ACG) Annual Scientific Meeting. October 15-20, 2010 Poster no 762.
15. SP304-20210. A Randomized, 12-Week, Double-Blind, Placebo-Controlled, Repeat-Dose, Oral, Dose-Ranging Study to Assess the Safety and Efficacy of Plecanatide in Patients with Chronic Idiopathic Constipation. June 26, 2013 (data on file).

16. Investigator Brochure for Plecanatide: for Chronic Idiopathic Constipation (CIC) Program and for Irritable Bowel Syndrome with Constipation (IBS-C). Edition 8.0 dated January 21, 2016.
17. Cohen MB, Guarino A, Shukla R, and Gianella RA. 1988. Age-related differences in receptors for escherichia coli heat-stable enterotoxin in the small and large intestine of children. Gastroenterology 94:367-373.
18. US Department of Health and Human Services. Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials; 2007.
19. Guidance for Industry Safety Reporting Requirements for INDs and BA/BE Studies. DHHS FDA December 2012.
20. National Research Council: The Prevention and Treatment of Missing Data in Clinical Trials. Panel on Handling Missing Data in Clinical Trials. Committee on National Statistics, Division of Behavioral and Social Sciences and Education. Washington, DC: The National Academies Press; 2010.

11 APPENDICES

A. Rome III Diagnostic Criteria for Constipation in Child /Adolescent

B. Pregnancy Reporting

C. Bristol Stool Form Scale

D. Electronic Diary

E. Patient Questionnaires

F. Regulations and Good Clinical Practice Guidelines

A. ROME III DIAGNOSTIC CRITERIA FOR CONSTIPATION IN CHILD/ADOLESCENT

H3a. Functional Constipation

Diagnostic criteria*

Must include two or more of the following in a child with a developmental age of at least 4 years with insufficient criteria for diagnosis of IBS:

1. Two or fewer defecations in the toilet per week
2. At least one episode of fecal incontinence per week
3. History of retentive posturing or excessive volitional stool retention
4. History of painful or hard bowel movements
5. Presence of a large fecal mass in the rectum
6. History of large diameter stools which may obstruct the toilet

* Criteria fulfilled at least once per week for at least 2 months prior to diagnosis

B. PREGNANCY REPORTING

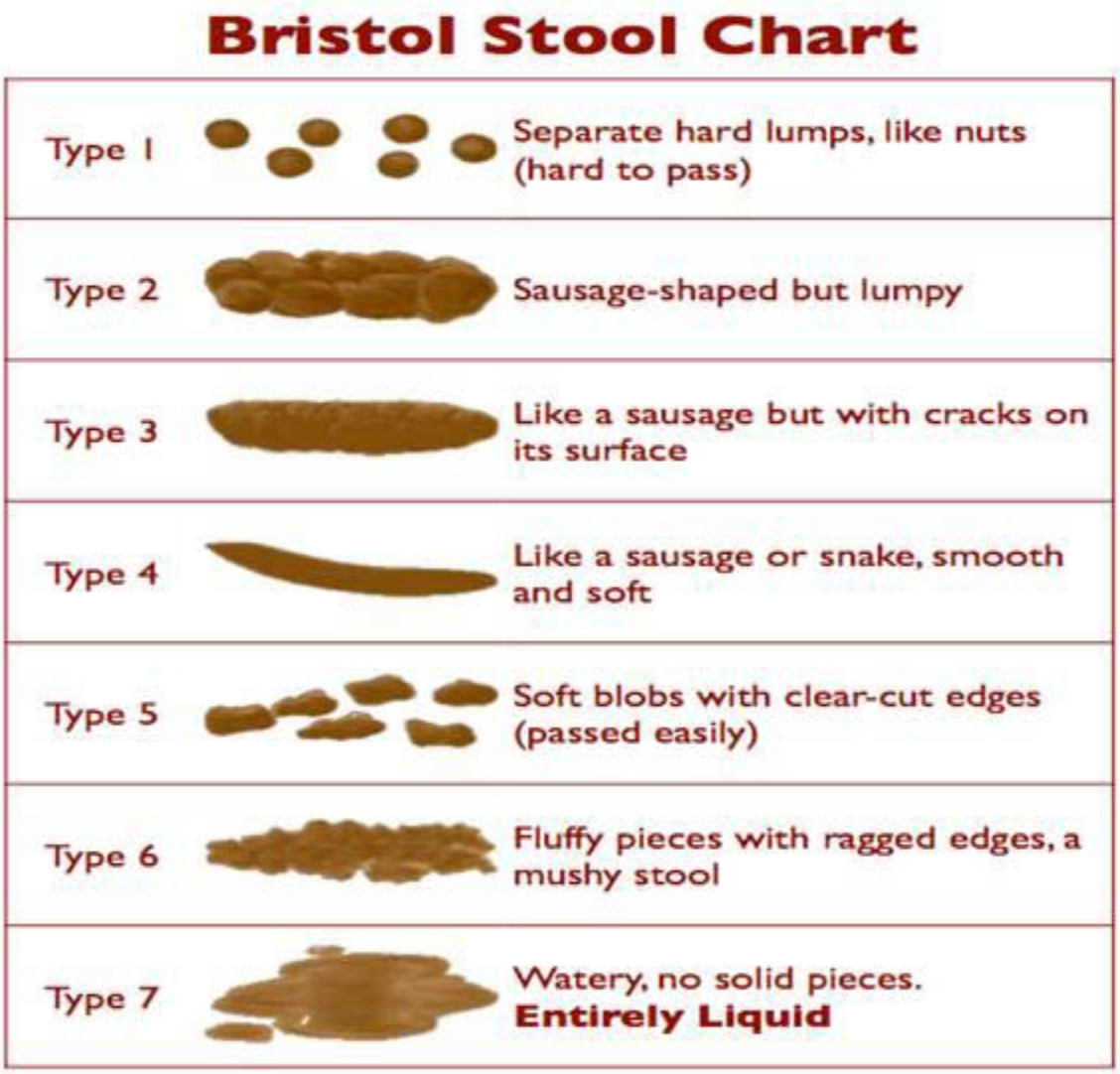
If a female patient should become pregnant during the course of the study (i.e., from the date the informed consent was signed until the patient's last visit), the Investigator (or authorized delegate) should notify the designated Safety Contact within 24 hours of the Investigator (or authorized delegate) 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 (eg, 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



D. ELECTRONIC DAILY DIARY

Note that the final text for the diary script will appear in the final Design Specifications for the electronic diary system and in the Study Procedure Manual.

Daily Bowel Movement Diary

- Enter the number of bowel movements you wish to report at this time.
- Enter the time of the bowel movement in 12 hour format, entering 2 digits for the hours and 2 digits for the minutes.
- For AM press 1. For PM press 2.
- Did you feel like you completely emptied your bowels during this bowel movement? If yes, press 1, if no press 0.

Please refer to the Bristol Stool Scale diagram provided by your doctor's office. Please rate the form/consistency of this stool. For Type 1, press 1, For Type 2, press 2, For Type 3, press 3, For Type 4, press 4. For Type 5, press 5. For Type 6, press 6. For Type 7, press 7.

Rescue Medication usage

- Have you taken any Dulcolax® today? If yes, press 1, if no press 0.
- How many times have you taken Dulcolax® today?
- Enter the time you took Dulcolax® in 12 hour format, entering 2 digits for the hours and 2 digits for the minutes.
- For AM press 1. For PM press 2.
- Enter the number of tablets you took

Daily Symptom Diary

The patient will be asked to rate their symptoms on a scale of 0 to 10, where 0 is NO 'ABDOMINAL PAIN' and 10 is 'ABDOMINAL PAIN' AS BAD AS YOU CAN IMAGINE.

1. Abdominal Pain. Rate your abdominal pain at its worst on a scale of 0 to 10, where 0 is NO PAIN and 10 is PAIN AS BAD AS YOU CAN IMAGINE.
2. Abdominal Bloating. Rate your abdominal bloating at its worst on a scale of 0 to 10, where 0 is NO ABDOMINAL BLOATING and 10 is ABDOMINAL BLOATING AS BAD AS YOU CAN IMAGINE.
3. Abdominal Discomfort. Rate your abdominal discomfort at its worst on a scale of 0 to 10, where 0 is NO ABDOMINAL DISCOMFORT and 10 is ABDOMINAL PAIN AS BAD AS YOU CAN IMAGINE.
4. If you had one or more bowel movement today, rate your straining with a bowel movement at its worst on a scale of 0 to 10, where 0 is NO STRAINING WITH A BOWEL MOVEMENT and 10 is STRAIN WITH A BOWEL MOVEMENT AS BAD AS YOU CAN IMAGINE.
5. If you had one or more bowel movement today, rate your pain with defecation at its worst on a scale of 0 to 10, where 0 is NO PAIN WITH DEFECATION / WITH A BOWEL MOVEMENT and 10 is PAIN WITH DEFECATION / WITH A BOWEL MOVEMENT AS BAD AS YOU CAN IMAGINE

E. PATIENT QUESTIONNAIRES

Patient Assessment of Constipation -Quality of Life (PAC-QOL), Patient Assessment of Constipation -Symptoms (PAC-SYM), Patient Global Assessment (PGA) Questionnaires

PGA will be administered electronically via IWRS, while PAC-QOL and PAC-SYM are paper-based.

PAC-QOL ©

PATIENT ASSESSMENT OF CONSTIPATION

The following questions are designed to measure the impact constipation has had on your daily life over **the past 2 weeks**. For each question, please check one box.

The following questions ask about your symptoms related to constipation. During the past 2 weeks, to what extent or <u>intensity</u> have you...	**Not at all 0**	**A little bit 1**	**Moderately 2**	**Quite a bit 3**	**Extremely 4**
1. felt bloated to the point of bursting?	☐	☐	☐	☐	☐
2. felt heavy because of your constipation?	☐	☐	☐	☐	☐
The next few questions ask about how constipation affects your <u>daily life</u>. During the past 2 weeks, how much of the time have you...	**None of the time 0**	**A little of the time 1**	**Some of the time 2**	**Most of the time 3**	**All of the time 4**
3. felt any physical discomfort?	☐	☐	☐	☐	☐
4. felt the need to have a bowel movement but not been able to?	☐	☐	☐	☐	☐
5. been embarrassed to be with other people?	☐	☐	☐	☐	☐
6. been eating less and less because of not being able to have bowel movements?	☐	☐	☐	☐	☐

The next few questions ask about how constipation affects your daily life. During the past 2 weeks, to what extent or intensity have you...	**Not at all 0**	**A little bit 1**	**Moderately 2**	**Quite a bit 3**	**Extremely 4**
7. had to be careful about what you eat?	☐	☐	☐	☐	☐
8. had a decreased appetite?	☐	☐	☐	☐	☐
9. been worried about not being able to choose what you eat (for example, at a friend's house)?	☐	☐	☐	☐	☐
10. been embarrassed about staying in the bathroom for so long when you were away from home?	☐	☐	☐	☐	☐
11. been embarrassed about having to go to the bathroom so often when you were away from home?	☐	☐	☐	☐	☐
12. been worried about having to change your daily routine (for example, traveling, being away from home)?	☐	☐	☐	☐	☐
The next few questions ask about your feelings related to constipation. During the past 2 weeks, how much of the time have you...	**None of the time 0**	**A little of the time 1**	**Some of the time 2**	**Most of the time 3**	**All of the time 4**
13. felt irritable because of your condition?	☐	☐	☐	☐	☐
14. been upset by your condition?	☐	☐	☐	☐	☐
15. felt obsessed by your condition?	☐	☐	☐	☐	☐
16. felt stressed by your condition?	☐	☐	☐	☐	☐
17. felt less self-confident because of your condition?	☐	☐	☐	☐	☐
18. felt in control of your situation?	☐	☐	☐	☐	☐

The next questions ask about your feelings related to constipation. During the past 2 weeks, to what extent or intensity have you...	**Not at all 0**	**A little bit 1**	**Moderately 2**	**Quite a bit 3**	**Extremely 4**
19. been worried about not knowing when you are going to be able to have a bowel movement?	☐	☐	☐	☐	☐
20. been worried about not being able to have a bowel movement?	☐	☐	☐	☐	☐
21. been more and more bothered by not being able to have a bowel movement?	☐	☐	☐	☐	☐
The next questions ask about your life with constipation. During the past 2 weeks, how much of the time have you...	**None of the time 0**	**A little of the time 1**	**Some of the time 2**	**Most of the time 3**	**All of the time 4**
22. been worried that your condition will get worse?	☐	☐	☐	☐	☐
23. felt that your body was not working properly?	☐	☐	☐	☐	☐
24. had fewer bowel movements than you would like?	☐	☐	☐	☐	☐
The next questions ask about your degree of satisfaction related to constipation. During the past 2 weeks, to what extent or intensity have you been...	**Not at all 0**	**A little bit 1**	**Moderately 2**	**Quite a bit 3**	**Extremely 4**
25. satisfied with how often you have a bowel movement?	☐	☐	☐	☐	☐
26. satisfied with the regularity of your bowel movements?	☐	☐	☐	☐	☐
27. satisfied with the time it takes for food to pass through the intestines?	☐	☐	☐	☐	☐
28. satisfied with your treatment?	☐	☐	☐	☐	☐

PAC-SYM©

PATIENT ASSESSMENT OF CONSTIPATION©

This questionnaire asks you about your constipation in the **past 2 weeks.** Answer each question according to your symptoms, as accurately as possible. There are no right or wrong answers.

For each symptom below, please indicate **how severe** your symptoms have been during the **past 2 weeks.** If you have not had the symptoms during the past 2 weeks, check 0. If the symptom seemed mild, check 1. If the symptom seemed moderate, check 2. If the symptom seemed severe, check 3. If the symptom seemed very severe, check 4. Please be sure to answer every question.

How severe have each of these symptoms been in the last 2 weeks?	**Absent 0**	**Mild 1**	**Moderate 2**	**Severe 3**	**Very severe 4**
1. discomfort in your abdomen	☐	☐	☐	☐	☐
2. pain in your abdomen	☐	☐	☐	☐	☐
3. bloating in your abdomen	☐	☐	☐	☐	☐
4. stomach cramps	☐	☐	☐	☐	☐
5. painful bowel movements	☐	☐	☐	☐	☐
6. rectal burning during or after a bowel movement	☐	☐	☐	☐	☐
7. rectal bleeding or tearing during or after a bowel movement	☐	☐	☐	☐	☐
8. incomplete bowel movement, like you didn't "finish"	☐	☐	☐	☐	☐
9. bowel movements that were too hard	☐	☐	☐	☐	☐
10. bowel movements that were too small	☐	☐	☐	☐	☐
11. straining or squeezing to try to pass bowel movements	☐	☐	☐	☐	☐
12. feeling like you had to pass a bowel movement but you couldn't (false alarm)	☐	☐	☐	☐	☐

Patient Global Assessment Questionnaire

Directions to Patient: For the question below, please choose the response that applies best to you and circle the number of your response.

Constipation Severity
How would you rate the severity of your constipation at its worst in the past 24 hours? 1= none 2 = mild 3 = moderate 4 = severe 5 = very severe

F. REGULATIONS AND GOOD CLINICAL PRACTICE GUIDELINES

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