CLINICAL TRIAL PROTOCOL

PROTOCOL TITLE: A Phase IIa, Proof of Concept, Randomized, Double-Blind, Dose-Finding, Cross-Over Study of the Efficacy, Safety and Tolerability of a New Enteric-Coated Cholestyramine Capsule in Adult Short Bowel Syndrome Patients

PROTOCOL NUMBER: PMS-2018-002

STUDY PHASE: IIa

SPONSOR: Pharmascience Inc.
6111 Royalmount Ave,
Montréal (Quebec) H4P 2T4
CANADA

PROTOCOL DATE: February 16, 2021

PROTOCOL HISTORY:

Version	Date
3.0 (Amendment 04)	February 16, 2021
2.0 (Amendment 03)	November 6, 2019
1.2 (Administrative Amendment 02)	September 10, 2019
1.1 (Administrative Amendment 01)	August 15, 2019
1.0 (Original)	March 06, 2019

COMPLIANCE

The study will be conducted in accordance with standards of Good Clinical Practice, as defined by the International Conference on Harmonisation and all applicable federal and local regulations.

CONFIDENTIALITY STATEMENT

PROTOCOL AMENDMENT 04

Acknowledgment of Review

I have reviewed the clinical study protocol including the below-mentioned revisions, and I agree with the completeness and accuracy of its content.

_____ _____

PI
Protocol Amendment Author
Pharmascience Inc.

Date (dd/mm/yyyy)

_____ _____

PI
Sponsor's Representative
Pharmascience Inc.

Date (dd/mm/yyyy)

Rationale for Revisions Implemented in Protocol Amendment 04 (February 16, 2021):

The following substantial and minor revisions are to be submitted to the central ethics committee and national regulatory authority for approval prior to implementation:

1) Revision of Inclusion Criteria #5 to better reflect the patient population at the sites.

2) Replacement of section "Protocol Administrative Amendment 03" with section "Protocol Amendment 04" to document revisions in this version.

Text of Revisions:

1) **Synopsis, p. 10, Changed Inclusion Criteria #5 from:**

   Partial, Home Parenteral Nutrition and/or parenteral fluids are allowed, at a maximum frequency of 4 times a week throughout the trial

   To:

   Partial, Home Parenteral Nutrition and/or parenteral fluids are allowed, at a maximum frequency of 6 times a week throughout the trial, as long as the regimen has been stable for at least 2 weeks prior to screening and is expected to remain unchanged during the study

2) **Section 8.1 Inclusion Criteria, p. 32, Changed Inclusion Criteria #5 from:**

   Partial, Home Parenteral Nutrition and/or parenteral fluids are allowed, at a maximum frequency of 4 times a week throughout the trial

   To:

   Partial, Home Parenteral Nutrition and/or parenteral fluids are allowed, at a maximum frequency of 6 times a week throughout the trial, as long as the regimen has been stable for at least 2 weeks prior to screening and is expected to remain unchanged during the study

STUDY PERSONNEL AND SITES

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SPONSOR SIGNATURE PAGE

PRODUCT:	Enteric-coated cholestyramine 425 mg capsules (ECC)
PROTOCOL NUMBER:	PMS-2018-002
PROTOCOL DATE:	February 16, 2021
PROTOCOL VERSION:	3.0

A Phase IIa, Proof of Concept, Randomized, Double-Blind, Dose-Finding, Cross-Over Study of the Efficacy, Safety and Tolerability of a New Enteric-Coated Cholestyramine Capsule in Adult Short Bowel Syndrome Patients

I have reviewed the above-mentioned clinical study protocol and I agree with the completeness and accuracy of its content. On behalf of the Sponsor, I agree to comply with all of the procedures contained within this protocol.

_____ _____

PI

PI

Date (dd/mm/yyyy)

Sponsor's Representative
Pharmascience Inc.

INVESTIGATOR SIGNATURE PAGE

PRODUCT:	Enteric-coated cholestyramine 425 mg capsules (ECC)
PROTOCOL NUMBER:	PMS-2018-002
PROTOCOL DATE:	February 16, 2021
PROTOCOL VERSION:	3.0

A Phase IIa, Proof of Concept, Randomized, Double-Blind, Dose-Finding, Cross-Over Study of the Efficacy, Safety and Tolerability of a New Enteric-Coated Cholestyramine Capsule in Adult Short Bowel Syndrome Patients

I have read the protocol and by my signature below, I agree that it contains all necessary information required to conduct this trial. I agree to conduct the study in accordance with all stipulations of the protocol and in accordance with all applicable regulatory requirements, the current International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP), and with the principles of the most recent version of the Declaration of Helsinki.

I agree to ensure the confidentiality of my subjects; however, I agree to make available to the Contract Research Organization (CRO), the Sponsor of this clinical study or relevant regulatory authorities those sections of my subjects 'medical records which directly concern this study. I understand that the study may be terminated, or enrolment suspended at any time by Pharmascience Inc., or by me if it becomes necessary to protect the best interests of the study subjects.

_____ _____

Principal Investigator Date (dd/mm/yyyy)

1. SYNOPSIS

Name of Sponsor	Pharmascience Inc.
Name of Investigational Product	Enteric-coated Cholestyramine (ECC) Capsules, 425 mg
Study Title	A Phase IIa Proof of Concept, Randomized, Double-Blind, Dose Finding, Cross-Over Study of the Efficacy, Safety and Tolerability of a New Enteric-Coated Cholestyramine Capsule in Adult Short Bowel Syndrome Patients
Rationale	A new enteric-coated cholestyramine (ECC) capsule has been developed to manage diarrhea associated with Short Bowel Syndrome (SBS). SBS is usually caused by the significant resection or loss of function of the ileum, leading to reduced reabsorption of bile acids and subsequent osmotic diarrhea. The new ECC formulation could release cholestyramine in the remaining segment of the small intestine in SBS patients, delivering and binding bile acids before they induce diarrhea in the colon. The proposed advantages of this formulation are: a) to prevent drug-drug interactions in the proximal GI tract, b) to preserve the fat digestive properties of bile acids in the duodenum and 3) to offer a more palatable dosage form to patients. Moreover, since distal delivery of cholestyramine is expected to be more effective in diarrhea preventing/reduction in SBS, lower doses than the ones used with non-enteric coated cholestyramine may be sufficient. Two doses of ECC will be studied for efficacy, safety and tolerability in well-defined non colectomized, adult SBS patients suffering from diarrhea.
Phase of Development	Phase IIa
Objectives	The primary objective of this study is to investigate the clinical efficacy of new ECC capsules and select the most effective dose in adult patients with SBS. The secondary objectives of this study are to evaluate the safety and tolerability of new ECC capsules in adult patients with SBS, and to evaluate the patients' experience of related symptoms using a 11-point Visual Analog Scale (VAS).
Primary Efficacy Endpoint	Change in the weekly frequency of bowel movements measured between baseline and the second week of treatment (i.e. Days 8 to 14, and Days 36 to 42).
Secondary Endpoints	- Total number of bowel movements per week, after the first week of treatment (i.e. Days 1 to 7, and Days 29 to 35) - Total number of bowel movements for the whole 2-week treatment period (i.e. Days 1 to 14 and Days 29 to 42)

<table>
<tr><td></td><td>
- Mean daily stool form score according to the Bristol Stool Form Scale (BSFS), measured during the first week of treatment (i.e. Days 1 to 7, and Days 29 to 35)
- Mean daily stool form score according to the BSFS, measured during the second week of treatment (i.e. Days 8-14, and Days 36-42)
- Mean daily stool form score according to the BSFS, measured during the whole 2-week treatment period (i.e. i.e. Days 1 to 14 and Days 29 to 42)
- Total number of bowel movements with a BSFS score ≥ 6 during the first week of treatment (i.e. Days 1 to 7, and Days 29 to 35)
- Total number of bowel movements with a BSFS score ≥ 6 during the whole 2-week treatment period (i.e. Days 1 to 14 and Days 29 to 42)
- Mean daily dose of loperamide in mg, if used, during the second week of treatment (i.e. Days 8-14, and Days 36-42)
- Mean Diarrhea Composite Index ([weekly bowel movement frequency X mean daily BSFS score] + loperamide use [weekly mg X 3]) during the second week of treatment (i.e. Days 8-14, and Days 36-42)
- Safety (to be evaluated through the assessment of adverse events (AE), laboratory tests, vital signs, ECG and physical examination)
- Tolerability (to be evaluated through assessment of TEAEs and TESAEs)
- Evaluation of severity of diarrhea, abdominal pain, urgency and bloating using an 11-point Visual Analog Scale (VAS) made on Days 0 (baseline), 15, 28 and 43
</td></tr>
<tr><td>Study Design and Procedures</td><td>
Multiple-center, randomized, double-blind, double dummy, 2-period, 2-sequence cross-over design. Eligible patients will be randomized to one of the following sequences:
<table>
<tr><th></th><th>Period 1</th><th>Period 2</th></tr>
<tr><td>Sequence AB (n=9)</td><td>Treatment A</td><td>Treatment B</td></tr>
<tr><td>Sequence BA (n=9)</td><td>Treatment B</td><td>Treatment A</td></tr>
</table>
- A 14-day washout period will separate the 2 periods. All subjects will first be enrolled in a 15-day screening phase during which SBS diagnosis will be confirmed and full study eligibility will be established. Daily number of bowel movements and daily BSFS scores will be measured during the last 14 days of the 15-day screening period (Day -14 to Day -1). Baseline safety parameters will also be assessed during screening. Treatment duration with ECC will be 14 consecutive days. The total number of bowel movements will be measured
</td></tr>
</table>

	during each of the 2 weeks of ECC treatment. The BSFS will be used daily during the whole study period, including the washout period. Patients' overall experience with related symptoms (abdominal pain, diarrhea, bloating, urgency) will be assessed using an 11-point VAS at the start and end of each period. Finally, safety will be assessed regularly during the trial, through physical exams, ECG, AEs, and adequate biochemical and hematological lab tests.
Study Population	Inclusion Criteria: 1. Adult, ambulatory male and female subjects 2. Provision of signed and dated informed consent form (ICF) 3. Age ≥ 18 years and ≤ 80 years 4. Stable SBS of: a. Non-surgical origin; OR b. Surgical origin where the last surgical ileal resection was performed at least 6 months prior to enrolment 5. Partial, Home Parenteral Nutrition and/or parenteral fluids are allowed, at a maximum frequency of 6 times a week throughout the trial, as long as the regimen has been stable for at least 2 weeks prior to screening and is expected to remain unchanged during the study 6. At least 50 % of the colon being intact 7. Intact duodenum 8. BMI ≥ 18 9. Presence of stable chronic diarrhea for at least 3 months prior to enrolment as evidenced by medical history 10. Presence of stable chronic diarrhea during the 2-week screening diary period before randomization, as evidenced by completion of a screening diary demonstrating: a. Mean daily production of at least 3 soft or watery stools (BSFS scores 6 or 7); **or** b. More than 3 bowel movements per day on average with >25% of them being BSFS type 6 or 7 11. Stated willingness and ability to comply with all study procedures, including daily recording of bowel movements and BSFS in the patient diaries, and availability for the duration of the study 12. Clinical laboratory values within the laboratory's stated normal range; if not within this range, they must be without clinical significance, as determined by the investigator 13. Female subjects must meet one of the following criteria: a) Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens from at least

	30 days prior to the first study treatment administration through to at least 30 days after the last dose of the study treatment. An acceptable method of contraception includes one of the following: a. Abstinence from heterosexual intercourse b. Systemic contraceptives (combined birth control pills, injectable/implant/insertable hormonal birth control products, transdermal patch) c. Intrauterine device (with or without hormones) d. Condom with spermicide b) Participants of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy, or bilateral oophorectomy) or is in a menopausal state (i.e. at least 1 year without menses prior to the first study drug administration) are eligible <u>Exclusion Criteria:</u> 1. Patients with known or suspected intestinal strictures of clinical relevance as judged by the Investigator 2. Active inflammatory bowel disease (IBD) or fistula during the screening period as judged by the Investigator 3. Crohn's disease patients not being in clinical remission for the last 12 weeks prior to randomization 4. Diarrhea caused by other causes than SBS 5. Presence of clinically significant steatorrhea, requiring pancreatic enzymes supplementation 6. Presence of complete biliary obstruction 7. Presence of active cancer (except resected cutaneous basal or squamous cell carcinoma and except *in situ* cervical cancer) and/or need to receive chemotherapy or radiotherapy during the study 8. History of allergic reaction to cholestyramine or any excipient of the investigational drug product or placebo, or packaging components 9. Females who are lactating at screening 10. Females who are pregnant according to the pregnancy test at screening or prior to the first study treatment administration 11. Significant history (at least 3 consecutive months in the year prior to Screening) of drug dependency or alcohol abuse (> 3 units of alcohol per day, intake of excessive alcohol, acute or chronic)

	12. Subjects who took an Investigational Product (IP) in the 30 days prior to the first study drug administration 13. Any other clinically significant condition that is considered by the principal investigator as being susceptible to put the patient at greater safety risk, influence response to study product, or interfere with study assessments.
Number of Sites and Patients	3 clinical sites and 18 patients
Treatments	Product: ECC capsules, 425 mg (size 00) In order to fully blind the study, the double dummy technique will be used, i.e. the lowest dose (1.7 g daily) will be co-administered with an ECC matching placebo. Treatment A ("low" dose): 1.7 g total daily dose of ECC, BID + placebo Treatment B ("high" dose): 4.25 g total daily dose of ECC, BID Mode of administration: Oral, capsules taken at least 30 minutes before breakfast and the evening meal Manufacturer: Pharmascience Inc.
Concomitant Medications	Concomitant antidiarrheal drugs, such as loperamide, are allowed as long as their dose and dosing regimen remain stable at least 2 weeks before and for the total duration of the study. Antacid medications, such as H2 antagonists, and OTC products are prohibited. PPIs are allowed as long as their dose and dosing regimen are stable during the study. IBS-D medications, spasmolytics and any drug exerting significant effects of gastrointestinal transit are also prohibited. Gut trophic factors such as growth hormone and GLP-2 are also prohibited. Standard of Care, concomitant medications are allowed during the study only if judged essential by investigator and with a stable dose and dosing regimen for at least 2 months prior to enrolment and during the study. However, loperamide dose may be modified by the investigator if absolutely necessary. Loperamide doses will be recorded in the patient diary, on a daily basis.
Administration	In each of the study periods, patients will be instructed to take two doses of ECC capsules daily (BID regimen), in the morning, at least 30 minutes before breakfast and before evening meal, for 14 days. Patients randomized to Treatment A (Low dose, 1.7 g daily) will take 2 x 425 mg ECC capsules + 3 x placebo capsules in the morning and repeat in the evening. Patients randomized to Treatment B (High dose, 4.25 g daily) will take 5 x 425 mg ECC capsules in the morning and repeat in the evening. Capsules should be taken with water at ambient temperature. The capsules should be swallowed whole and not be chewed or crushed.

Dose Justification	Currently, at least 4 g daily is the dose recommended for regular, non-enteric coated cholestyramine. ECC may be as or more effective at a lower dose, i.e. 1.7 g daily.
Sample Size Justification	Using an alpha of 0.05 and beta of 0.2, a paired t-test, and assuming a standard deviation of 1.265 and a 20% attrition rate; 18 patients are required to show a minimal 25% difference between the two treatments in the reduction of the weekly number of bowel movements after 14 days of treatment (measured during the second week of treatment). Moreover, with 18 patients, we will have more than 80% power to detect a minimal reduction of 4 weekly bowel movements after 14 days of treatment with either 1.7 or 4.25 g of ECC per day (pooled data; assuming SD of 2).
Study Duration	12 - 16 months

2. SCHEDULE OF ACTIVITIES

All scheduled clinic visits and telephone contacts allow a +/- 1 day window.

	Screening and Study Start (Days -15 to 0)			Period 1 (Days 1 to 14)	Washout (Days 15 to 28)			Period 2 (Days 29 to 42)	End of Study (Day 43)
Days	**-15**	**-8**	**0**	**7**	**15**	**21**	**28**	**35**	**43**
Visits	**Visit 1**	**Phone 1 [6]**	**Visit 2**	**Phone 2 [6]**	**Visit 3**	**Phone 3 [6]**	**Visit 4**	**Phone 4 [6]**	**Visit 5**
Informed Consent [1]	X								
Eligibility criteria review	X		X						
Demographics	X								
Medical History	X								
Randomization (to sequence)			X						
Vital signs [2]	X		X		X		X		X
Phys. Exam., Body Weight, Height (Day -15 only), BMI	X				X		X		X
Clinical Laboratory Tests [3]	X				X		X		X
Urinalysis [4]	X				X		X		X
12-lead ECG	X								X
Pregnancy Test (females only)	X (serum)		X (urine)				X (urine)		X (urine)
Study Drug Dispensing			X				X		
Compliance check (study drug accountability)					X				X
BSFS and BM diary [5]	X	X	X	X	X	X	X	X	X
Visual Analog Scale (symptoms)[7]			X		X		X		X
Concomitant Medication [8]	X	X	X	X	X	X	X	X	X
AE Monitoring [9]	X	X	X	X	X	X	X	X	X

[1] The latest version of the consent form must be signed prior to subject's inclusion (prior to initiating any protocol procedure). The consent form can be signed up to 28 days before screening visit.

[2] Vital signs include pulse, blood pressure (diastolic-systolic) and body temperature.

[3] Clinical Laboratory safety tests include full clinical chemistry and hematology panels.

[4] Urinalysis includes nitrites, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin, leukocytes, glucose and appearance.

[5] BSFS: Bristol Stool Form Scale BM: bowel movement (defecation). Patients will use the 14-day diaries to record the frequency of BMs as well as stool morphology, throughout the study (ie. Diaries will be provided for Screening, Period 1, Washout and Period 2).

[6] Telephone contact will be established with patients at Days -8, 7, 21 and 35, to assess safety, report concomitant medication use, promote use of diary, and promote compliance.

[7] An 11-point Visual Analog Scale (VAS) will be used to assess the following symptoms: abdominal pain, diarrhea, bloating and urgency

[8] To be recorded as Prior Medication if taken before the first dose of study drug (i.e. during Screening). Only current medications to be reported (i.e. treatment ongoing at Day -15).

[9] From screening to the first dose of the study, AEs will be recorded as screening events or as part of the medical history, as applicable. Any serious adverse events spontaneously reported by subjects up to 30 days following the last day of dosing will be documented.

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LIST OF FIGURES

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4. GLOSSARY OF TERMS AND ABBREVIATIONS

AE: Adverse Event
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
AUC: Area Under the Curve
BAD: Bile Acid Diarrhea
BAM: Bile Acid Malabsorption
BID: *Bis In Die* (Latin), twice daily
BM: Bowel Movement
BMI: Body Mass Index
BSFS: Bristol Stool Form Scale
BW: Body Weight
CBC: Complete Blood Count
CRF: Case Report Form
CRO: Contract Research Organization
CV: Curriculum Vitae
DDI: Drug-drug Interaction
EC: Ethics Committee
ECC: Enteric-coated Cholestyramine
ECG: Electrocardiogram
GCP: Good Clinical Practice
GI: Gastrointestinal
GLP: Glucagon-like Peptide
GMP: Good Manufacturing Practice
HCTZ: Hydrochlorothiazide
HPN: Home Parenteral Nutrition
ICF: Informed Consent Form
IRB: Institutional Review Board
ITT: Intent-to-Treat
IV: Intravenous
IWRS: Interactive Web Response System
MedDRA: Medical Dictionary for Regulatory Activities
PK: Pharmacokinetic
PO: *per os* (Latin), by mouth, orally
PP: Per Protocol
PPI: Proton Pump Inhibitors
QD: *Quaque Die* (Latin), every day/daily
QTc: QT corrected for heart rate

RBC: Red Blood Cells
SAE: Serious Adverse Event
SAP: Statistical Analysis Plan
SD: Standard Deviation
SUSAR: Suspected Unexpected Serious Adverse Reactions
TEAE: Treatment Emergent Adverse Event
TMF: Trial Master File
TPN: Total Parenteral Nutrition
VAS: Visual Analog Scale

5. INTRODUCTION

5.1 Short Bowel Syndrome Overview

Short Bowel Syndrome (SBS) is a malabsorptive condition most often caused by significant resection of the small intestine. In patients with SBS, the small intestine absorptive surface area is insufficient and leads to malabsorption of nutrients and bile acids, diarrhea, steatorrhea, malnutrition, and electrolyte abnormalities. Some common causes of SBS in adults include Crohn's disease, radiation enteritis, trauma, mesenteric vascular accidents and recurrent intestinal obstruction. In the pediatric population, necrotizing enterocolitis, intestinal atresia, and intestinal volvulus are the most common etiologic factors (1).

The most important consequence of extensive intestinal resection is loss of absorptive surface area, which results in malabsorption of macro and micronutrients, electrolytes and water (2). Most macronutrients are absorbed in the proximal 100–150 cm of intestine. SBS almost always occurs when there is less than 200 cm of bowel remaining. SBS patients exposed to the greatest nutritional risk generally have a duodenostomy or a jejunoileal anastomosis with less than 35 cm of residual small intestine, jejunocolic or ileocoloic anastomosis with less than 60 cm of residual small intestine, or an end jejunostomy with less than 115 cm of residual small intestine (3).

Permanent total parenteral nutrition (TPN) support is usually needed in patients with less than 120 cm of intestine without colon in continuity and less than 60 cm with colonic continuity (4). Besides, malabsorption of macro- and micronutrients with a loss of intestinal absorptive surface area results in water and electrolyte malabsorption, which manifests as voluminous diarrhea, hypovolemia, hyponatremia and hypokalemia (5).

Gastrin, cholecystokinin, secretin, gastric inhibitory polypeptide and motilin are produced by endocrine cells in proximal gastrointestinal tract. In SBS, the status of these hormones remains intact. On the other hand, glucagon-like peptide (GLP) 1 and 2, neurotensin, and peptide YY are produced in ileum and proximal colon. In SBS, deficiency of these hormones is common, and this results in rapid gastric emptying, shortened intestinal transit and hypergastrinemia (6,7).

The small intestine is able to adapt to compensate for the reduction in absorptive surface area caused by intestinal resection. This process occurs in the first few years following resection. This progressive adaptive response results from changes in the intestinal structure, motility and function (8). The mechanism of intestinal adaptation is not entirely understood. The degree of intestinal adaptation is related to the extent and site of intestinal resection (9). Complete intestinal adaptation can be achieved in only several months if minimal small intestine resection is present, such as 100-150 cm.

5.2 Epidemiology of SBS

Challenges to estimating the prevalence of SBS include its multifactorial etiology, varying definitions, and difficulty in estimating intestinal length. Therefore, estimates of the incidence and prevalence of SBS are usually based on data from registries of patients on home parenteral nutrition (HPN), for which SBS is the most common indication. SBS occurs in about 15% of adult patients who undergo intestinal resection, with 3/4th of these cases resulting from massive intestinal resection and 1/4th from multiple sequential resections. About 70% of patients in whom SBS develops are discharged from the hospital and a similar percentage remain alive a year later. This improved survival rate has been achieved primarily by the ability to deliver long-term nutritional support (5).

The annual prevalence of HPN in the United States is approximately 120 per million population, of whom about 25%have SBS; this amounted to about 10,000 individuals (10).

5.3 Treatment of Short Bowel Syndrome

The early management of a patient with SBS is that of a critically ill surgical patient who has recently undergone intestinal resection and other concomitant procedures. Thus, control of sepsis, maintenance of fluid and electrolyte balance and initiation of nutritional support are important in the early management of these patients. For patients who have survived this early phase, the primary goals of management are to maintain adequate nutritional status and prevent development of complications related to both underlying pathophysiology and nutritional therapy.

5.3.1 Nutritional status maintenance

This is the primary goal in the management of SBS. Fluid and electrolyte losses from the gastrointestinal tract may be significant in the early postoperative period and must be monitored and replaced. TPN will be required in the early postoperative period and enteral nutrition should be initiated as soon as possible.

Patients with limited ileal resection (less than 100 cm) with or without right hemicolectomy can resume intake of solid food in late postoperative phase. These patients may develop diarrhea or steatorrhea with consumption of a regular diet due to fat malabsorption, which in turn can lead to deficiencies of fat soluble vitamins, vitamin B12, calcium and magnesium. Maintenance of nutritional status becomes all the more important in the setting of diarrhea, which is quite common in SBS and may be due to gastric acid hypersecretion, rapid intestinal transit time, fat malabsorption and poor water reabsorption. H2 blockers, antidiarrheals, cholestyramine and octreotide have all been used to control diarrhea.

5.3.2 Treatment of steatorrhea associated with ileal resection

Fat maldigestion due to bile salt malabsorption frequently occurs when more than 100 to 150 cm of terminal ileum has been resected. Various therapeutic options have been suggested for the treatment of the resulting steatorrhea. Use of bile salt replacement therapy with ox bile or a synthetic conjugated bile acid (cholesarcosine) has been reported (11). These patients may be put on low-fat high-carbohydrate diet. Low fat may decrease steatorrhea, but it also results in decreased energy intake which may worsen patient's energy balance. However, a high fat intake is associated with malabsorption of divalent cations, delayed gastric emptying, early satiety and increased water loss from colon.

Pharmacologic therapy for SBS is a rapidly expanding area of investigation. Recent evidence suggests that provision of appropriate diet, nutritional supplements such as glutamine and growth factors such as growth hormone improves intestinal absorption and perhaps modifies the adaptive response in patients with established SBS (5, 12,13). Currently GLP-2 appear to have the most promising results.

5.3.3 Home parenteral nutrition and complications of SBS

Home parenteral nutrition (HPN) is an option for patients who require long-term TPN. To prepare the patient for HPN, the regime should be compressed gradually in 2 to 4 h daily increments so that the total volume can be infused over a 10–12 h period, typically over night. The HPN infusion is generally tapered off over a 30–60 min period to

avoid hypoglycemia. Gastric hypersecretion can be a serious problem in SBS and is due to parietal cell hyperplasia and hypergastrinemia. In addition to malabsorption and diarrhea, gastric hypersecretion can cause or flare up peptic ulcer disease. H2 receptor antagonists or PPI can be tried with good results (14,15).

5.4 SBS Associated Bile Acid Diarrhea

During normal digestion, bile acids are secreted into the intestines and are then re-absorbed from the intestinal tract and returned to the liver via the enterohepatic circulation. In SBS and other medical conditions, bile acids are insufficiently re-absorbed from the intestinal tract and make their way to the colon, giving rise to bile acid diarrhea (BAD). For instance, patients with an ileal resection of 30 cm or more may develop malabsorption of bile acids, resulting in their increased passage into the colon, where dihydroxy bile acid induces the secretion of salt and water and hence diarrhea (15,16).

A small proportion of the secreted bile acids are not reabsorbed in the ileum and reach the colon. Here bacterial action results in deconjugation (removal of the taurine or glycine) and dehydroxylation (removal of the 7-hydroxy group), producing the secondary bile acids, deoxycholate and lithocholate. Bile acids in the colon, in particular the dihydroxylated bile acids chenodeoxycholate and deoxycholate, stimulate electrolyte and water secretion (15). This condition is now better understood and has also been called bile salt or bile acid malabsorption (BAM). The usually effective enterohepatic circulation of bile salts is most obviously disturbed in ileal disease. Following ileal resection, typically for Crohn's disease, bile acids are not absorbed efficiently, resulting in clear-cut BAM. This was called cholegenic diarrhoea or cholerheic enteropathy when first described by Alan Hofmann in 1967 (15).

Cholestyramine is a quarternary ammonium anion exchange resin with a polystyrene polymer skeleton. As the chloride salt, it binds bile acids both *in vitro* and *in vivo*, exchanging chloride for bile acid. During normal digestion, bile acids are secreted into the intestines. A major portion of the bile acids is absorbed from the intestinal tract and returned to the liver via the enterohepatic circulation. Only very small amounts of bile acids are found in normal serum. Cholestyramine resin absorbs and combines with the bile acids in the intestine to form an insoluble complex which is excreted in the feces. This results in a partial removal of bile acids from the enterohepatic circulation by preventing their absorption. The increased fecal loss of bile acids due to cholestyramine resin administration leads to an increased oxidation of cholesterol to bile acids, a decrease in beta lipoprotein or low density lipoprotein plasma levels and a decrease in serum cholesterol levels. Although in man cholestyramine resin produces an increase in hepatic synthesis of cholesterol, plasma cholesterol levels fall (17,18).

Cholestyramine is indicated as adjunctive therapy to diet and exercise for the reduction of elevated serum cholesterol in patients with primary hypercholesterolemia. Cholestyramine may also be useful in lowering elevated cholesterol in patients with combined hypercholesterolemia and hypertriglyceridemia but it is not indicated where hypertriglyceridemia is the abnormality of most concern. In Canada, cholestyramine is also indicated as a symptomatic control of bile acid induced diarrhea due to SBS and for the relief of pruritus associated with partial biliary obstruction (19). The recommended adult dose is 4 g of cholestyramine resin, one to six times daily.

Management of SBS associated BAD generally relies on binding (or sequestering) excess bile salts in order to prevent their secretory actions. Because of its proven bile acids binding capacity, cholestyramine has been given for many years for diarrhea in patients with ileal resection (20). The principle for its use in diarrhea has been to prevent free bile acids from stimulating secretion in the colon. They are effective and most patients with abnormal SeHCAT values will respond. Subjects with lower SeHCAT values (less than 5%) are more likely to respond than those with higher

values (below 15%) (21). Another bile acid sequestrant, colesevelam, available in a tablet form, has been used initially for hyperlipidaemia (22). Colesevelam was shown to be effective at doses between 1.25 and 3.75 g/day and was well-tolerated (21).

The efficacy of cholestyramine as a treatment for diarrhea in patients with ileal resection was examined by inpatient and outpatient trials as early as in 1969, by Hofmann *et al*. Ten (10) of 12 patients having less than 100 cm of distal ileum resected and some remaining ascending colon responded to cholestyramine, 16 g/day, by a significant decrease in fecal frequency or fecal weight or both. By contrast, none of eight patients having resections of more than 100 cm of ileum responded. The degree of steatorrhea was also of predictive value since all eight patients with fecal fat excretion less than 20 g/day responded, whereas none of five patients with greater steatorrhea responded. Cholestyramine is useful in the symptomatic treatment of diarrhea in patients with small ileal resections and mild steatorrhea (16).

Cholestyramine may be of particular benefit in a select group of patients with ileal resection less than 150 cm and colon in continuity. Since bile salts are absorbed primarily in the distal ileum, these patients may experience spillover of bile salts into the colon, where they are metabolized by bacteria to lithocholic acid and deoxycholic acid and induce a secretory diarrhea. Bile acid-binding resins form insoluble complexes with bile salts and reduce diarrhea in these patients. However, more extensive ileal resection is more common. As noted above, patients with extensive ileal resection experience a decrease in bile acid pool and use of a conventional bile acid-binding resin may worsen fat malabsorption. Therefore, conventional cholestyramine should be used with caution in patients with SBS and clinicians and patients should monitor for steatorrhea, fat-soluble vitamin deficiencies, and decreased efficacy of concomitant medications that result from impaired absorption (13,14,16,23).

As an anion exchanging resin, cholestyramine may also bind other drugs that are administered concurrently. Cholestyramine resin may delay or reduce the absorption of concomitant oral medications such as thyroid and thyroxine preparations, warfarin, hydrochlorothiazide (acidic), phenylbutazone, phenobarbital, tetracycline, penicillin G, and digitalis, as well as therapeutic bile acids such as ursodiol and obeticholic acid. These medications are mostly absorbed in the proximal portion of the small intestine, such as the duodenum and the proximal jejunum. This is where a complexation with cholestyramine may occur. Thus, patients are recommended to take other drugs either one hour before, or 4-6 hours after, taking cholestyramine which is an inconvenience for most patients (13, 19, 20).

5.5 Rationale for a New ECC Formulation

Although treatment with conventional bile acid binding resins such as cholestyramine is effective in 75-88% of SBS patients, there are several physiological disadvantages. Firstly, the jejunal concentration of non-sequestered bile acids is below the optimum for sufficient solubilization of the lipolytic products after meals and thus the result is malabsorption of fat, steatorrhea, and diarrhea induced by fatty acids. Secondly, malabsorption of bile acids is increased because sequestered bile acids are not available for small intestinal absorption. Moreover, the reduced reabsorption of bile acids leads to a decreased bile acids pool and synthesis. Recent studies have shown that cholestyramine can lower FGF19 levels, through binding of bile acids in the small bowel, which potentially decreases the ability of bile acids to bind to the ileal nuclear receptor Farnesoid X Receptor that controls transcription of FGF19. Lundasen *et al*. showed that administration of cholestyramine to healthy volunteers reduced FGF19 by 87% and increased C4 18-fold (24). Since FGF19 has beneficial effects on bile acid metabolism as well as hepatic

gluconeogenesis and lipid storage, the lowering of FGF19 by bile acid sequestrants may be undesirable. Therefore, it might be an advantage to deliver cholestyramine in a more distal portion of the small intestine, beyond the segments involved in fat digestion and concomitant drugs absorption. Consequently, administering a delayed-release capsule dosage form that releases the active substance in the terminal ileum or proximal colon, would represent a therapeutic advantage. Such a formulation would not influence the fat digestive properties of bile acids but would still prevent their osmotic, laxative effects.

In fact, this concept had originally been proposed by Jacobsen *et al.* (25) , who conducted a small study comprising 14 patients who had undergone ileal resection of 40-150 cm for Crohn's disease. This double-blind cross-over trial was performed with placebo and cholestyramine enterocoated with cellulose acetate phthalate. During treatment with cholestyramine the daily fecal output decreased, the number of defecations each week decreased, and the intestinal transit time increased. Acceptability of the tablets was high, in contrast with general clinical experience with cholestyramine powder. No change was observed in the total fecal output of bile acids or fat. Cholestyramine tablets caused a reduction in diarrhea without noticeably interfering with the metabolism of fat or bile acid.

Another main concern with the use of conventional resins (sequestrants) is poor tolerance, with discontinuation rates of over 40%. Many of these effects are due to palatability or upper abdominal symptoms. In addition, conventional sequestrants may interact with other drugs including fat soluble vitamins (6,12,19). As such, a novel formulation that could deliver cholestyramine to the lower intestine and colon may have several advantages including a more favourable patient acceptance profile, fewer drug interactions, no or minimal effect on FGF19 and a better tolerability.

A new enteric-coated cholestyramine capsule (ECC) has been developed to manage diarrhea associated with SBS in patients who still have their transverse and descending colon. SBS is usually caused by the significant resection or loss of function of the terminal ileum, leading to reduced reabsorption of bile acids and subsequent osmotic diarrhea. The new ECC capsule can release cholestyramine in a more distal segment of the intestinal tract, downstream to the duodenum, in order to bind excess bile acids before they induce diarrhea. Moreover, non-enteric coated cholestyramine can interact with several drugs such as hydrochlorothiazide in the duodenum, leading to decreased bioavailability and therapeutic response of hydrochlorothiazide (26,27). It is then hypothesized that delivering cholestyramine in a more distal intestinal segment will prevent such interaction.

Gastrointestinal Behavior of New ECC Formulation

The anatomical site of release of cholestyramine is of paramount importance for the proposed indication, since the drug must be available for non-reabsorbed bile acids binding prior to their passage into the colon. At the same time, a too proximal delivery of the resin should be avoided to prevent any potential drug interaction in the duodenum and to preserve the lipolytic activity of bile acids.

In this context, an open-label, single center, single-dose, non-randomized scintigraphy study evaluating gastrointestinal transit, site of disintegration, site of dispersion, and associated variability of a new ECC formulation was conducted in 8 healthy, adult male volunteers (28). Safety evaluations included assessment of physical examination, ECG, adverse events (AEs) and periodic monitoring of vital signs (heart rate, blood pressure) and clinical laboratory tests (including hematology, serum chemistry, and urinalysis). The dosing period was about 24 hours in

duration post-dose of the cholestyramine capsules. Follow-up procedures were performed within 14 days of the dosing period inclusive of the final day of the dosing period.

A study-specific batch of ECC capsules was manufactured for PMS-2017-001, incorporating non-radioactive samarium oxide as a surrogate marker in the cholestyramine blend prior to encapsulation. The non-radioactive isotope samarium-152 (Sm-152) was subsequently converted to the radioactive isotope Sm-153.

Previous *in vitro* disintegration studies had shown that ECC remains intact at acidic pH, with capsule burst at pH [redacted]. The Phase I study results demonstrated proof of concept that the enteric-coating of ECC functionally resists disintegration under acidic pH conditions *in vivo* and enables successful targeting and delayed-release of cholestyramine prior to and in the ileum. The formulation was considered robust with a high degree predictability of release into the mid- to distal small intestine.

Drug-Drug Interaction Study with New ECC Formulation

A second Phase I, single-dose, cross-over, drug-drug interaction (DDI) study was conducted to investigate the effect of ECC on plasma kinetics of hydrochlorothiazide (HCTZ), used as a victim drug, in healthy volunteers. Cholestyramine powder is known to substantially reduce bioavailability of HCTZ as measured by plasma levels and urinary excretion of HCTZ. The effects of this DDI have been shown to be time-dependent and heightened by multiple dosing. Results of the study showed that both formulations of cholestyramine (ECC capsule and powder) exhibit an effect on the fasting pharmacokinetics of HCTZ. However, the impact of ECC is notably lower than the impact of the cholestyramine powder on HCTZ and is therefore expected to be of minor clinical significance compared to that of the non-enteric-coated powder formulation. The results suggest that the delivery of cholestyramine in a more distal intestinal segment via an enteric-coated capsule, successfully reduces the magnitude of DDIs. Overall, ECC was generally safe and well-tolerated by the subjects in this study.

5.6 Safety of Cholestyramine

The adverse events (AEs) and safety profile of cholestyramine powder is very well known since it has been used for many decades globally (20). The most frequent AE is constipation. Predisposing factors for most complaints of constipation are high dose and increased age (more than 60 years old). Most instances of constipation are mild, transient, and controlled with conventional therapy. Some patients require a temporary decrease in dosage or discontinuation of therapy.

Less frequent AEs include: abdominal discomfort and/or pain, flatulence, nausea, vomiting, diarrhea, heartburn, anorexia, dyspepsia and steatorrhea, bleeding tendencies due to hypoprothrombinemia (Vitamin K deficiency) as well as Vitamin A (one case of night blindness has been reported) and D deficiencies, hyperchloremic acidosis in children, osteoporosis, rash and irritation of the skin, tongue and perianal area.

5.7 Study Rationale and Risk/Benefit Assessment

The new ECC capsule has been developed to manage diarrhea associated with SBS. The formulation is expected to release cholestyramine in the remaining segment of the small intestine in SBS patients (mid-jejunum to terminal ileum), thus binding bile acids after fat digestion, but before induction of diarrhea in the colon. The delayed-release profile is also expected to help reduce the potential for DDIs occurring in the proximal small intestine.

Since delayed distal delivery of cholestyramine is expected to be more effective in prevention and reduction of diarrhea in SBS, compared to non-enteric coated cholestyramine powder, lower doses may be sufficient. The recommended adult dose of cholestyramine powder is 4 g, one to six times daily. Consequently, 2 doses of ECC were selected for this dose-finding study. The "high" dose will be 4.25 g (10 capsules) daily corresponding approximately to the recommended dose of cholestyramine powder. The "low" dose will be 1.7 g (4 capsules) daily. These doses will be evaluated for efficacy, safety, and tolerability in well-defined non fully colectomized SBS patients suffering from diarrhea.

To avoid any carry-over effect, a washout of 14 calendar days is planned between periods. Washout periods of a 7-14 day duration have been used in previous, published trials on cholestyramine and/or treatment of BAD.

The doses of ECC administered in this study are not anticipated to induce any potential risks to the patients and are well below the maximum doses of cholestyramine typically administered for other indications. The safety monitoring practices employed by this protocol (i.e. physical examination, vital signs, clinical laboratory assessments, and AE questioning) are adequate to protect the patients' safety and should detect all expected treatment-emergent AEs (TEAEs). The potential medical benefit from participation in this trial would be the expected reduction of diarrhea or bowel movements following treatment with "high" dose ECC, and potentially treatment with "low" dose ECC as well.

6. STUDY OBJECTIVES

6.1 Primary Objective

The primary objective of this study is to investigate the clinical efficacy of new ECC capsules and select the most effective dose in adult patients with SBS.

6.2 Secondary Objectives

The secondary objectives of this study are to evaluate the safety and tolerability of new ECC capsules in adult patients with SBS, and to evaluate patients' overall experience with related symptoms via an 11-point Visual Analog Scale (VAS).

7. STUDY DESIGN

7.1 Endpoints

7.1.1 Primary Endpoint

The primary endpoint will be the change in the weekly frequency of bowel movements, measured between baseline and the second week of treatment (ie. Days 8 to 14 and Days 36 to 42). Efficacy will be assessed as the overall difference vs baseline as well as the difference between the two treatment doses.

7.1.2 Justification for the Primary Endpoint

Several efficacy endpoints have been used in the study of the use of cholestyramine or other bile acids sequestrants in the management of bile acid diarrhea (BAD). These included fecal weight, fecal volume, weekly frequency of bowel movement (typically called ' number of stools' in the literature), mean stool type according to the Bristol Stool Form Scale (BSFS), loperamide use, etc. In a clinical trial in primary and secondary BAD, Walters *et al.* used an innovative

index taking into consideration the weekly stool frequency, the mean BSFS, as well as loperamide use (29). This index was: (weekly stool frequency X mean BSFS) + loperamide use. This index, while non-validated *per se*, was justified by the variable nature of BAD symptoms. All components of this index were influenced by the study treatment, obeticholic acid, in BAD patients.

Weekly stools (bowel movement [BM] frequency) is an objective endpoint that can be easily measured by patients, using a diary. BSFS can be more difficult to assess by study subjects, since it implies a visual evaluation of the stools and is consequently more subjective. Fecal weight and volume require regular stool collection, which is cumbersome.

In light of these considerations, the weekly number of bowel movements is proposed as the study primary efficacy endpoint. This endpoint is clinically relevant to the symptom of diarrhea, and an increased frequency translates to a decreased water reabsorption as well as an accelerated GI transit, both important pathogenic components of SBS. The data obtained from Walters and other authors show that bowel movement frequency (stool frequency) is a sensitive index for pharmacological intervention in SBS. While significant variability can be observed with this parameter, weekly bowel movement frequency can be greatly reduced by antidiarrheal drugs and bile acids modulating agents (e.g. obeticholic acid). Moreover, the second week of treatment with ECC will be used for this purpose, in order to reach an expected maximum and stable effect at each dose of the drug studied.

7.1.3 Secondary Endpoints

- Total number of bowel movements per week, after the first week of treatment (i.e. Days 1 to 7, and Days 29 to 35)
- Total number of bowel movements for the whole 2-week treatment period (i.e. Days 1 to 14 and Days 29 to 42)
- Mean daily stool form score according to the BSFS, measured during the first week of treatment (i.e. Days 1 to 7, and Days 29 to 35)
- Mean daily stool form score according to the Bristol Stool Form Scale (BSFS), measured during the second week of treatment (i.e. Days 8-14, and Days 36-42)
- Mean daily stool form score according to the BSFS, measured during the whole 2-week treatment period (i.e. i.e. Days 1 to 14 and Days 29 to 42)
- Total number of bowel movements with a BSFS score ≥ 6 during the first week of treatment (i.e. Days 1 to 7, and Days 29 to 35)
- Total number of bowel movements with a BSFS score ≥ 6 during the whole 2-week treatment period (i.e. Days 1 to 14 and Days 29 to 42)
- Mean daily dose of loperamide in mg, if used, during the second week of treatment (i.e. Days 8-14, and Days 36-42)
- Mean Diarrhea Composite Index ([weekly BM frequency X mean daily BSFS score] + loperamide use [weekly mg X 3]) during the second week of treatment (i.e. Days 8-14, and Days 36-42)
- Safety (to be evaluated through the assessment of AE, laboratory tests, vital signs, ECG and physical examination)
- Tolerability (to be evaluated through assessment of TEAEs and TESAEs)

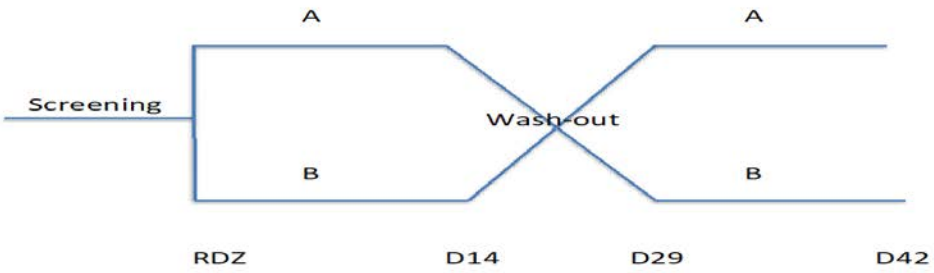
Overall evaluation of diarrhea, abdominal pain, urgency and bloating using an 11-point Visual Analog Scale (VAS) made on Days 0 (baseline), 15, 28 and 43

7.2 Study Overview

7.2.1 Study Design

This is a multiple center, randomized, double-blind, double dummy, 2-period, 2-sequence cross-over study design (Figure 1). The study will be conducted across three study centers. A total of 18 patients will be selected to participate in the study. All 18 patients will receive treatment with ECC during each study period. Patients will be randomized to either "low" dose ECC (1.7 g with matching placebo BID), or "high" dose ECC (4.25 g BID) daily for 14 days, followed by a 14-day washout, and then allocated to the other treatment for the second period.

Figure 1 - Study Design



A: ECC 1.7 g BID
B: ECC 4.25 g BID
D: Day
Screening: 15-day duration

The duration of the clinical portion of this study (excluding the screening period) is expected to be approximately 43 days. The actual overall study duration may vary.

The end of the study is defined as completion of the last visit or study procedure, as shown in the schedule of activities) for the trial, globally.

7.2.2. Study/Treatment Arm Stopping Rule Guideline

The following recommendations are proposed for stopping the enrolment and/or further drug administration in a given treatment arm. An agreement will be reached with the Study Medical Monitor and the Sponsor on making such decisions. The following guidelines will be followed:

- Occurrence of any case of death that may be attributed to ECC (in such a case, the blind will have to be broken and allocated treatment dose identified);

- Occurrence of a significant number of unexpected SAEs that could be attributable to the drug (at least *probable* causality relationship) and that raise Investigators/Sponsor concerns about patients' safety;
- Increased frequency and/or severity of expected cholestyramine-related AEs, that in the judgment of the Investigators/Sponsor, will result in the advice to discontinue ECC treatment.

7.2.3 Individual Patient Stopping Rules

The following should be used as a guide for the Investigator to determine if the study drug administration is to be interrupted:

- A subject develops a medical condition that requires prolonged concomitant therapy with a prohibited medication or prolonged interruption of the study drug (more than 2 consecutive days)
- Occurrence of an AE or SAE that could be attributable to the drug (at least *probably* causality relationship) or not, and causes discontinuation of study drug for more than 2 days;
- High and unexpected frequency and/or severity of AEs (at least *probably* causality relationship), serious or not, that in the judgment of the Investigator, requires withdrawal of the patient from the study.

7.2.4 Treatment Arm Dose Reduction Guideline

No ECC treatment dose reduction will be permitted during the study, within each treatment arm.

7.3 Blinding and Randomization

This study will be fully blinded, using the double dummy technique (combination of active ECC capsules and matching placebo in the low dose treatment arm). Subjects will be randomized to one of the following 2 treatments per period (Table 1):

- **Treatment A**: ECC at the 1.7 g daily dose, administered BID as 2 capsules of ECC, plus 3 capsules of placebo, at least 30 minutes before breakfast and 2 capsules of ECC, plus 3 capsules of placebo at least 30 minutes before evening meal.
- **Treatment B**: ECC at the 4.25 g daily dose, administered BID as 5 capsules of ECC at least 30 minutes before breakfast and 5 capsules of ECC at least 30 minutes before evening meal.

The capsules should be swallowed whole and not be chewed or crushed. All 18 patients will receive both ECC doses by the end of the study.

If the patient misses the morning dose, they should take the dose at least 30 minutes before lunch. If the patient misses the evening dose, they should skip that dose. Doses should not be doubled. Patients should note the times that they take the study medication on their bowel movement diaries.

Table 1 - Randomization

	Period 1	**Period 2**
Sequence AB (n=9)	Treatment A	Treatment B

Sequence BA (n=9)	Treatment B	Treatment A

JSS Medical Research will develop the computer-generated randomization sequences to be programmed into the Interactive Web Response System (IWRS), according to the study design, the number of subjects and the sequence of treatment administration. The IWRS will assign a randomization number to each patient. The random allocation of each sequence of drug administration to each subject is done in such a way that the study is balanced. Once generated, the randomization code will be final and will not be modified.

7.4 Procedure for Unblinding

This is a double-blind study. Patients, investigators and site staff, Sponsor and CRO personnel, and data managers will be kept blinded to treatment assignments until the end of the study, except for emergency unblinding as described below.

Only the Packaging/Distribution provider's unblinded staff and CRO staff administering the IWRS or performing the statistical analyses will be knowledgeable of individual treatment assignments. As well, in the case of emergency unblinding as described below, some additional people will become aware of treatment assignment for specific patient(s).

The study blind may be broken for an individual patient or several patients in the event of an emergency in which knowledge of the treatment assignment is needed for the safety of the patient(s) and/or for medical decision-making or as required by local regulatory authority. Unless the event for which the blind needs breaking is life-threatening, the investigator must first contact the study's medical monitor (or designee) prior to breaking the blind. The investigator will obtain unblinding information from the staff administering the IWRS. The reason and justification for breaking the blind must be fully documented in the source documentation and captured on the patient(s) electronic case report form (e-CRF).

8. SELECTION AND WITHDRAWAL OF PATIENTS

Subjects who meet all the inclusion criteria and none of the exclusion criteria at the screening visit will be eligible for participation in this study. Continued eligibility will be assessed upon baseline visit at start of Period 1, prior to the first study drug administration.

An effort will be made to include similar proportions of males and females in the study.

8.1 Inclusion Criteria

1. Adult, ambulatory male and female subjects
2. Provision of signed and dated informed consent form (ICF)
3. Age ≥ 18 years and ≤ 80 years
4. Stable SBS, of:
    a. Non-surgical origin; OR
    b. Surgical origin where the last surgical ileal resection was performed at least 6 months prior to enrolment

5. Partial, Home Parenteral Nutrition and/or parenteral fluids are allowed, at a maximum frequency of 6 times a week throughout the trial, as long as the regimen has been stable for at least 2 weeks prior to screening and is expected to remain unchanged during the study
6. At least 50 % of the colon being intact
7. Intact duodenum
8. BMI ≥ 18
9. Presence of stable chronic diarrhea for at least 3 months prior to enrolment as evidenced by medical history
10. Presence of stable chronic diarrhea during the 2-week screening diary period before randomization, as evidenced by completion of a screening diary demonstrating:
    a. Mean daily production of at least 3 soft or watery stools (BSFS scores 6 or 7); **or**
    b. More than 3 bowel movements per day on average with >25% of them being BSFS type 6 or 7

11. Stated willingness to comply with all study procedures and availability for the duration of the study
12. Clinical laboratory values within the laboratory's stated normal range; if not within this range, they must be without clinical significance, as determined by the investigator
13. Female subject must meet one of the following criteria:

    a) Participant is of childbearing potential and agrees to use one of the accepted contraceptive regimens from at least 30 days prior to the first study treatment administration through to at least 30 days after the last dose of the study treatment. An acceptable method of contraception includes one of the following:

        a) Abstinence from heterosexual intercourse

        b) Systemic contraceptives (combined birth control pills, injectable/implant/insertable hormonal birth control products, transdermal patch)

        c)Intrauterine device (with or without hormones)

        d) Condom with spermicide

    b) Participants of non-childbearing potential, defined as surgically sterile (i.e. has undergone complete hysterectomy or bilateral oophorectomy) or is in a menopausal state (i.e. at least 1 year without menses prior to the first study drug administration) are eligible

8.2 Exclusion Criteria

1. Patients with known or suspected intestinal strictures of clinical relevance as judged by the Investigator
2. Active inflammatory bowel disease (IBD) or fistula during the screening period as judged by the Investigator
3. Crohn's disease patients not being in clinical remission for the last 12 weeks prior to randomization
4. Diarrhea caused by other causes than SBS
5. Presence of clinically significant steatorrhea, requiring pancreatic enzymes supplementation
6. Presence of complete biliary obstruction
7. Presence of active cancer (except resected cutaneous basal or squamous cell carcinoma and except *in situ* cervical cancer) and/or the need to receive chemotherapy or radiotherapy during the study
8. History of allergic reaction to cholestyramine or any excipient of the investigational drug product or placebo such as lactose, or packaging components.
9. Females who are lactating at screening

10. Females who are pregnant according to the pregnancy test at screening or prior to the first study treatment administration
11. Significant history (at least 3 consecutive months in the year prior to Screening) of drug dependency or alcohol abuse (> 3 units of alcohol per day, intake of excessive alcohol, acute or chronic)
12. Subjects who took an Investigational Product (IP) in the 30 days prior to the first study drug administration
13. Any other clinically significant condition that is considered by the principal investigator as being susceptible to put the patient at greater safety risk, influence response to study product, or interfere with study assessments.

8.3 Informed Consent

Subjects are required to provide written informed consent prior to commencement of any protocol procedure. The consent form can be signed up to 28 days before screening visit. When a suitable subject presents with SBS, the Investigator or designee will explain the nature, purpose, risks and benefits associated with the study to the subject and ensure that the subject is given full and adequate written information. Subjects must be informed that they are free to discontinue from the study at any time. The subject should be given opportunity to ask questions and allowed time to consider the information provided. If the subject agrees to participate in the study, the Informed Consent Form (ICF) must be signed by the subject or his/her legal representative to indicate that the subject understands all aspects of the study. The subject's signed and dated informed consent must be obtained before any study procedures are performed. The Investigator will provide the subject with a copy of the ICF.

8.4 Study Withdrawal of Patients

A subject may withdraw from the study at any time at his/her own request without prejudice to further treatment or clinical care. In addition, subjects may be withdrawn at the discretion of the Investigator for safety, behavioral, compliance or administrative reasons. Patients should be withdrawn from the study in the event of any of the following:

1. If a patient does not continue to meet eligibility criteria at baseline, the patient will be withdrawn prior to randomization.
2. Safety issues as described in the Study/Treatment Arm or Individual Patient Stopping Rules.
3. If a patient withdraws consent during the treatment period, and more specifically consent to contribute additional outcome information, the patient shall be withdrawn from the study (although it is recommended that the investigator attempts to perform a Treatment Discontinuation Visit Evaluation). If the patient withdrawing consent to further treatment agrees to contribute additional outcome information, then the regular schedule of visits could be maintained, for safety and relevant outcome measures of efficacy.
4. If a patient is serially and persistently noncompliant with study procedures and/or visit schedules, the investigator or the Sponsor may withdraw the patient from the study.
5. A patient's treatment is unblinded by the Investigator.
6. If a female patient or a female partner of a male patient has a confirmed pregnancy (with the exception of a pregnancy resulting from *in-vitro* fertilization), this should be immediately reported to the Medical Monitor and the Sponsor (within 1 working day of discovery) and the female patient shall be withdrawn. The male patient would continue in the study.

The reason for patient withdrawal from the study will be noted on the e-CRF, reported to the Sponsor, and documented in the clinical study report. The investigator should attempt to follow patients withdrawn after first

dosing until resolution of any AEs, or at least 4 weeks after the last dose of study agent, or until completion of pregnancy. Upon withdrawal, no further dosing should be performed for these patients. Investigators shall make reasonable attempts to contact lost-to-follow-up patients for evaluation.

8.5 Treatment Discontinuation

Patients should discontinue treatment but remain in the study for adequate follow-up, for any of the following reasons:

- Patients may discontinue study drug treatment at any time if the patient, Investigator, or the Sponsor determines that it is not in the best interest of the subject to continue treatment.
- Unacceptable toxicity associated with treatment (see Study Treatment Arm Stopping Rule section for details).
- Clinically significant lab abnormalities or AEs that, in the Investigator's judgment, would preclude continued treatment.

The reason for treatment discontinuation will be noted on the e-CRF, reported to the Sponsor, and documented in the clinical study report.

If a patient discontinues treatment due to unacceptable toxicity or any other safety reason, that patient should continue to be assessed according to the planned visit schedule for safety, or at the minimum, attend a Treatment Discontinuation Visit four (4) weeks after having received the last dose of study drug.

A clear distinction should be made between study withdrawal and treatment discontinuation.

8.6 Replacement of Patients

A subject who withdraws during the pretrial evaluations but before receiving the treatment will not be considered as a drop-out and will not be included in the final statistical analysis.

Standbys should be recruited and available to replace any subject who withdraws prior to the first treatment administration.

Patients who are withdrawn or lost to follow-up during the study may be replaced in order to achieve the number of evaluable patients required, per the sample size calculations, to address the primary objective.

9. STUDY CONDUCT

9.1 Timing of Assessments

All scheduled clinic visits allow a +/- 1 day window. See Section 2 for the Schedule of Activities. The latest version of the consent form must be signed prior to subject's inclusion (prior to initiating any protocol procedure).

9.2 Study Visits and Procedures

Prior to any study specific screening activities, all potential patients will sign the Study Informed Consent Form (ICF). The consent forms will comply with all applicable regulations governing the protection of human subjects. An ICF, approved by the Sponsor and the site's institutional review board (IRB) or Ethics Committee (EC), must be used. The consent form can be signed up to 28 days before screening visit.

9.2.1 Screening and Eligibility Assessments (Visit 1, Day -15)

On Day -15 prior to randomization, interested SBS patients will undergo a screening session, Visit 1. Patients will arrive in the fasted state (i.e. fast for 4 hours before visit). This visit is comprised of the following elements:

1. Verification of identity and demographics (age, gender, race);
2. Confirmation of the SBS diagnosis will be obtained, with proper medical history data;
3. Description of the type and extent of intestinal surgical resection will be provided (if applicable);
4. Verification of all inclusion and exclusion criteria;
5. Collection of medical history including history of intestinal surgical resection or other possible cause of SBS, all current medications and therapies, non-prescription drug intake, and HPN if still administered. Particular attention will be paid to obtaining the history of diarrhea in the 3 months prior to screening, as well as prohibited drugs;
6. Measurement of body weight and height, and calculation of BMI;
7. Measurement of vital signs including heart rate, blood pressure and body temperature;
8. Complete physical examination;
9. An ECG will be performed;
10. All safety laboratory tests including hematology, serum chemistry, pregnancy test (if applicable) and urinalysis (dipstick). Screening laboratory results must be available and reviewed by the Investigator before randomization.
11. Patients will be given a bowel movement (BM) and BSFS diary, in which they will need to record daily the number of stools and their form, using the BSFS. Proper instructions will be provided for the use of the BSFS. Patients will be instructed to start recording in this Screening diary at Day -14 and to fill it out daily until Day -1 inclusive, in order to establish a baseline and confirm patients' eligibility.
12. All screening results must be available prior to Day 0 randomization so that the study Investigator can determine eligibility.
13. From screening to the first dose of the study, AEs will be recorded as screening events or as part of the medical history, as applicable.

9.2.2 Screening Telephone Contact (Phone 1, Day -8)

Each patient will be contacted by phone by the study coordinator, to inquire about the occurrence of AEs, any changes in medications, and use of the BM and BSFS screening diary.

9.2.3 Randomization (Visit 2, Day 0)

Eligible SBS patients will be invited to the study site for the selected baseline procedures.

1. Verification of completion of the BM and BSFS screening diary and review of eligibility criteria to decide if patient can continue to be randomized into the study. If yes:
2. Vital signs will be measured (heart rate, blood pressure and body temperature).
3. A urine pregnancy test will be performed if applicable. This test must be negative to dispense study medication.
4. Randomized patients will receive a 14-day supply of study treatment. Patients will be instructed to start taking their study medication the next day in the morning, i.e. on Day 1, and for 14 consecutive days, until Day 14 inclusive.
5. Patients will be given a new BM and BSFS diary for Period 1, in which they will need to record daily the number of stools and their form, using the BSFS, for Days 1 to 14 inclusive. Proper instructions will be provided for the use of the BSFS. They will also be provided the BM/BSFS diary for the Washout period, to be completed during Days 15 to 28.
6. Patients will be given a VAS questionnaire to complete on site. The four 11-point VAS scales (from 0 to 10), will be used by the patients to make an overall assessment of diarrhea, abdominal pain, bloating and urgency experienced during the last 24 hours. Zero will mean no symptom at all, and 10: very severe. The VAS assessments will be made at Days 0, 15, 28 and 43, but not during screening.

9.2.4 Period 1 Telephone Contact (Phone 2, Day 7)

Each patient will be contacted by phone by the study coordinator, to inquire about the occurrence of AEs, concomitant medications, compliance to study treatment and use of the BM and BSFS diary.

9.2.5 Washout (Visit 3, Day 15)

On the first day of the Washout period (**Day 15**), patients will return to the clinic with their BM and BSFS diaries, and the remaining study medication. Patients will arrive in the fasted state (i.e. fast for 4 hours before visit). The following assessments and procedures will be performed:

1. Complete physical examination;
2. Measurement of vital signs including heart rate, blood pressure and body temperature;
3. Measurement of body weight, and calculation of BMI;
4. All safety laboratory tests, including: urinalysis (dipstick), hematology, serum chemistry;
5. Recording of concomitant medications;
6. Study drug compliance check – patients will return all study medication from Period 1 to the study coordinator. Drug accountability will be performed.
7. Verification of completion of the BM and BSFS diary for Period 1, and start of diary for Washout. Patients will return the Period 1 diary and will be reminded to complete the Washout diary daily during Days 15 to 28.
8. The VAS symptoms questionnaire will be completed by the patient on site.
9. Recording of AEs and concomitant medications.

During the Washout period (**Days 15 – 28**), patients will not be taking any study medication, for 14 consecutive days. Other concomitant medications can be maintained as long as their dose and doing regimen remain unchanged (See

Permitted Medications section for details). Patients will be instructed to keep using their BM/BSFS diary during the washout period to report the daily number of bowel movements as well as stool form using the BSFS.

9.2.6 Washout Telephone Contact (Phone 3, Day 21)

Each patient will be contacted by phone by the study coordinator, to inquire about the occurrence of AEs, concomitant medications, compliance to study treatment and use of the BM and BSFS diary.

9.2.7 Washout (Visit 4, Day 28)

On the last day of the Washout period (**Day 28**), patients will return to the clinical with their BM/BSFS diary. Patients will arrive in the fasted state (i.e. fast for 4 hours before visit). The following assessments and procedures will be performed:

1. Complete physical examination;
2. Measurement of vital signs including heart rate, blood pressure and body temperature;
3. Measurement of body weight, and calculation of BMI;
4. All safety laboratory tests, including at a minimum: urinalysis (dipstick), hematology, serum chemistry;
5. A urine pregnancy test will be performed if applicable. This test must be negative to dispense study medication for Period 2.
6. Recording of concomitant medications;
7. Verification of the BM and BSFS Washout diary. The Washout diary will be given back to the patient and they will be instructed to continue using the diary for Day 28. The patient will also be provided a new diary for Period 2.
8. The VAS symptoms questionnaire will be completed by the patient on site.
9. Recording of AEs;
10. Study medication for Period 2 will be supplied at the end of the visit, with instructions to begin dosing on Day 29.

9.2.8 Period 2 Telephone Contact (Phone 4, Day 35)

Each patient will be contacted by phone by the study coordinator, to inquire about the occurrence of AEs, concomitant medications, compliance to study treatment and use of the BM/BSFS diary.

9.2.9 End-of-Study (Day 43, Visit 5)

At Day 43, the final clinic visit, all patients will return to the clinic with their BM and BSFS diaries, and the remaining study medication. Patients will arrive in the fasted state (i.e. fast for 4 hours before visit). The following assessments and procedures will be performed:

1. Complete physical examination;
2. Measurement of vital signs including heart rate, blood pressure and body temperature;
3. Measurement of body weight, and calculation of BMI;
4. A 12-lead ECG;
5. All safety laboratory tests, including at a minimum: urinalysis (dipstick), hematology, serum chemistry;
6. A urine pregnancy test will be performed if applicable;
7. Recording of concomitant medications;

8. Study drug compliance check - patients will return all study medication from Period 2 to the study coordinator. Drug accountability will be performed.
9. Verification of completion of the BM and BSFS diaries for Washout and for Period 2. The diaries will be given back to the study coordinator.
10. The VAS symptoms questionnaire will be completed by the patient on site.
11. Recording of AEs.

AEs still ongoing at the time of the visit will be assessed and evaluated for further follow-up as necessary. SAEs occurring during the 30-day period following the End-of-Study, Day 43 visit, should be reported to the medical monitor. (See Serious Adverse Event Reporting section for details)

10. INVESTIGATIONAL PRODUCT MANAGEMENT

10.1 Packaging, Labeling, and Shipping

The Sponsor will be responsible for ensuring the Test and Placebo investigational products are manufactured in accordance with Good Manufacturing Practice (GMP); the labeling should also comply with applicable regulatory requirement(s).

Study medication will be provided in patient kits containing enough medication for one period (14 days). Each patient kit consists of 2 labeled bottles packed inside a labeled carton. Patients randomized to “high” dose will receive 2 bottles of ECC, and patients randomized to “low” dose will receive 1 bottle of ECC and 1 bottle of matching placebo. Study medication will be specifically identified and will bear instructions to ensure proper order of intake at all times. Each bottle and carton label will bear a unique number linked to a randomized medication list to identify the kit contents (i.e. high dose or low dose treatment). Both bottles contained within one kit will have the same number as the outer carton. The randomized medication list will be linked to the patient randomization schedule at the IWRS level.

Each bottle and patient kit box will bear a label that conforms to the appropriate local regulations.

Shipments of patient kits to the clinical sites will be done using a Sponsor-defined courier. A temperature data logger will accompany the shipment to ensure an adequate chain of custody. Excursions outside of 15^{o}C to 30^{o}C will be promptly reported to the Sponsor.

10.2 Storage, Dispensing and Compliance Verification of Study Drug

All patient kits will be shipped from the Sponsor or Sponsor resources to the clinical sites.
Patient kits will be provided to study subjects only.

Upon receipt of the patient kit shipper container, the pharmacy site staff should record the receipt, date, time, and temperature of the product based on inspection or on the temperature logger reading. Any study drug that arrives in improper storage conditions or is damaged in any way must be reported to the Sponsor as soon as possible and shall not be administered until instructed otherwise. Each clinical site will maintain an inventory record of the patient kits received, stored (in a secure restricted area), and dispensed.

At the clinical sites, patient kits are to be stored under labeled storage conditions in a secure location with limited access. Any temperature excursions outside of 15°C to 30°C will be promptly reported to the Sponsor.

Upon instructions from the IWRS, the pharmacy site staff will, at the time of dispensing, formally identify and remove the assigned Patient Kit box(es) from storage and will cross-check the expiry date on the labels on the Patient Kit box(es) with the target dates of use, at a minimum. The necessary Patient Kits will be sent to the site staff actually dispensing the study drug to the patients.

10.3 Study Drug Accountability

At Visit 2, the patient will receive one Patient Kit box which will contain sufficient study drug for Period 1. At Visit 3, the patient will return to the site the Patient Kit box containing all the remaining study drug from Period 1. The next dispensing visit is Visit 4 where the patient will receive another Patient Kit box which will contain sufficient study drug for Period 2. At the End-of-Study visit, the patient will return to the site the Patient Kit box containing all the remaining study drug from Period 2. If a patient withdraws or discontinues from the trial, they will be requested to return all study supplies promptly.

Complete and accurate records regarding the receipt, dispensing, and return or destruction of study drug for each patient in the study will be kept. Any used study drug containers, as well as any unused containers or unused portions of containers, or patient kits must be maintained until accounted for by the study monitor. Drug accountability will be performed at the end of each period to confirm that the required amount of study drug has been consumed. If the amount of study drug is not as expected, the patient will be contacted for clarification. After drug accountability by the monitor and approval from the Sponsor, the study drug and containers should be destroyed per the CRO's procedures.

11. TREATMENT AND PROTOCOL COMPLIANCE

Patients are expected to self-administer oral daily doses based on the protocol schedule and have all procedures done within the allowable time windows (detailed in the Schedule of Activities).

Regardless of allowable visit windows, each period or study drug treatment cycle should always be 14 calendar days. Investigational drug will then be supplied for this 14 consecutive days period. A 14-day treatment-free period (Washout) will be included between the 2 dosing periods.
In the event a patient misses a scheduled assessment visit, this should be rescheduled for the earliest possible date, no more than 2 days later. Patients who are persistently noncompliant may be withdrawn from the study at the investigator's or the Sponsor's discretion.

The following measures will be employed to ensure treatment and protocol compliance:

- Patients will be given clear instructions prior to study drug dispensing on how to self-administer the study drugs.
- Patients will be given clear instructions and reminders during each visit and telephone contact on how to use the BSFS and BM diary.
- Patients will be contacted by telephone on Days -8, 7, 21 and 35 to confirm continued compliance with the dosing regimen, use of concomitant medication, and use of BM/BSFS diaries.
- BM/BSFS diaries will be checked for compliance at each visit.

11.1 Permitted and Prohibited Concomitant Medications

The standard of care for patients with SBS includes several medications, nutritional recommendations and procedures destined to improve their quality of life, treat symptoms and prevent complications. This section outlines the general study-specific requirements in terms of concomitant medications, vitamin supplements and potential drug interactions.

11.2 Permitted Medications

All standard of care drugs will be permitted, unless they are listed in the Prohibited Medications List section. However, dose and dosing regimen of permitted drugs must remain constant during the whole study duration, until Day 42.

11.3 Prohibited Medications

After consulting the protocol, in case of doubt about a patient's concomitant medication, study personnel should consult with the Sponsor before enrolling/pursuing participation of a patient on study. If a medication other than those specified in the protocol (including OTC) is used after the first treatment administration or at any time before the end of the study, the Sponsor will decide whether the subject will be permitted to remain in the study, depending on the drug used, the time of drug intake, etc. The drug and dose taken will be noted.

Prohibited medications include:

- H2 receptors antagonists: cimetidine, famotidine, ranitidine and nizatidine (should be stopped at least 72 hours prior to Day 0 visit)
- Pancrelipase supplements
- Prostaglandin E drugs, such as misoprostol (should be stopped at least 24 hours prior to Day 0 visit)
- Over the counter antacid medications, containing: bismuth salts, aluminium and magnesium hydroxide, sodium alginate, sodium bicarbonate, etc. (should be stopped at least 24 hours prior to Day 0 visit)
- Drugs used to treat Diarrhea Predominant Irritable Bowel Syndrome (IBS-D), such as: alosetron (*Lotronex*), eluxadoline (*Viberzi*), rifaximin (*Xifaxan*), lubiprostone (Amitiza), linaclotide (*Linzess*) and trimebutine (Modulon) (should be stopped at least 72 hours prior to Day 0 visit)
- Antispasmodic drugs, such as dicyclomine (should be stopped at least 24 hours prior to Day 0 visit)
- Prokinetic drugs, such as: domperidone and metoclopramide (should be stopped at least 48 hours prior to Day 0 visit)
- Any kind of pre-, and probiotics (should be stopped at least 72 hours prior to Day 0 visit)
- Bile acids sequestrants, such as regular cholestyramine, colestipol and colesevelam (should be stopped at least 72 hours prior to Day 0 visit)
- Any kind of orally administered antibiotics, unless judged absolutely necessary by the investigators (should be stopped at least 72 hours prior to Day 0 visit)
- Gut trophic factors such as growth hormone and GLP-2 are also prohibited (should be stopped at least 48 hours prior to Day 0 visit)
- Products containing cannabinoids.

12. EFFICACY ASSESSMENTS

12.1 Weekly Number of Bowel Movements

The total number of bowel movements (defecations) occurring during the second week of ECC treatment (Days 8 to 14) compared those occurring during screening in the week prior to randomization, will be used to assess the primary endpoint. Use of loperamide will also be recorded. Bowel movements will be recorded by the patients on a daily basis, as follows:

- each day of the last 14 days of the screening period, prior to randomization, to establish the first baseline and confirm subject eligibility
- each day of the first 14-day treatment period
- each day of the 14-day washout period
- each day of the second 14-day treatment period

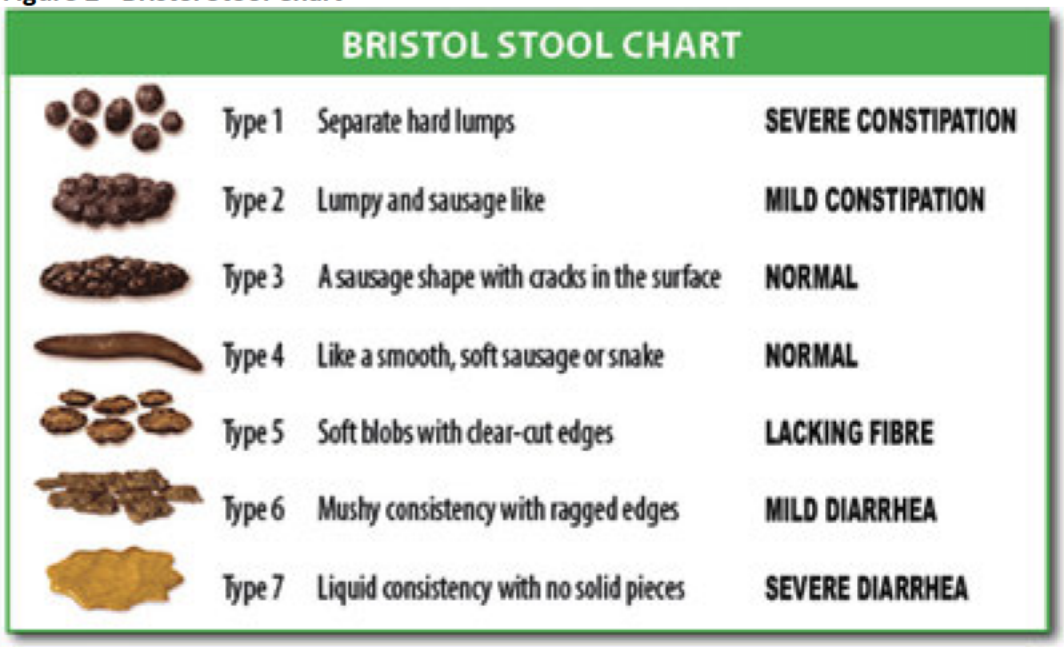
In summary, this implies that occurrences of bowel movements will be recorded daily by the patient, in the diary, from Day -14 (screening) up to Day 42, i.e. the last dosing day of the study.

12.2 Bristol Stool Form Scale

The Bristol Stool Form Scale (BSFS) is a diagnostic medical tool designed to categorize and score the form of human stools into seven categories (30,31). It is used mostly in clinical research. It is sometimes also referred to as the Bristol stool chart (BSC), Bristol stool form scale, or BSF scale.

It was developed at the Bristol Royal Infirmary as a clinical assessment tool in 1997, and is widely used as a research tool to evaluate the effectiveness of treatments for various diseases of the bowel, especially Irritable Bowel Syndrome and chronic constipation. BSFS scores of 6 or 7 indicate diarrhea.

Figure 2 - Bristol Stool Chart



Reference: Lewis and Heaton (1997)[30]

All patients will be asked to examine their stools at every bowel movement and to provide a general estimation of their form, according to the Bristol Stool Chart. This will be done at every bowel movement, every day as per the Schedule of Activities. This information will also be recorded in the patient diary.

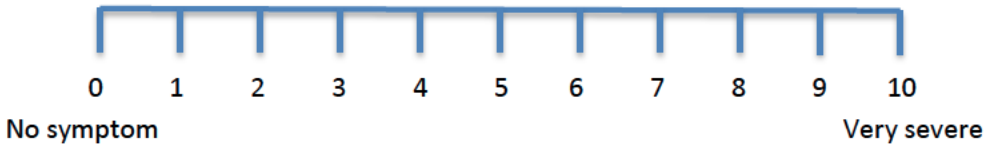
The Mean Diarrhea Composite Index ([weekly bowel movement frequency X mean daily BSFS score] + loperamide use [weekly mg X 3]) at baseline and during the second week of treatment (i.e. Days 8-14, and Days 36-42) will also be estimated.

12.3 Patient Visual Analog Scale (VAS)

An 11-point Visual Analog Scale will be used to rate the patient's experience on the following 4 clinical symptoms:

a. severity of diarrhea
b. abdominal pain
c. bloating
d. urgency

The Analog Scale will look as follows:



All patients will be asked to put an X on the scale, where they grade the severity of their symptoms, as experienced over the last 24 hours. The VAS will be used by the patients on Days 0, 15, 28 and 43.

13. SAFETY ASSESSMENT

Safety evaluations will include reporting of Adverse Events (AEs), clinical laboratory assessments (clinical chemistry, hematology and urinalysis), ECGs, and clinical evaluation of vital signs and physical examinations performed at regular intervals during the study.

All AEs will be assessed, documented, and reported in accordance with ICH GCP guidelines.

13.1 Documentation of Adverse Events

AEs will be collected from the time of informed consent signing until the last scheduled study follow-up. From screening to the first dose of the study, AEs will be recorded as screening events or as part of the medical history, as applicable. An AE is any untoward medical occurrence (which does not necessarily have a causal relationship with the treatment). An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not related to the study drug. This includes any occurrence that was new in onset or aggravated in severity or frequency from the baseline condition. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was screened in the study are **not** to be considered AEs unless the condition deteriorated in an unexpected manner during the study (e.g. surgery was performed earlier than planned).

Abnormal results of diagnostic procedures, including laboratory test abnormalities, are considered AEs if they result in any one of the following:

- Discontinuation of study treatment;
- Require treatment or any other therapeutic intervention;
- The necessity for further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality);
- Association with clinical signs or symptoms that may have a significant clinical impact, as determined by the Investigator.

Patients are encouraged to report AEs spontaneously or in response to general, non-directed questioning. All AEs are to be followed until resolution or until a stable clinical endpoint is reached. The investigator should question patients about AEs and changes in pre-existing illnesses since their last visit and must record the information in the patients' medical records. The onset and end dates, severity, relationship to study agent, action taken, and outcome must be recorded for each AE. All AEs are to be recorded on the appropriate e-CRF and in detail on the source documents.

SAEs must be reported immediately to the medical monitor, i.e. no later than 24 hours after they are known. AEs that are ongoing at the end of the study should be marked as ongoing. However, it is the responsibility of the Investigator to follow up on these events until resolution, according to standard medical care. Any SAE that the investigator becomes aware of outside of the study period should be reported to the medical monitor, for a period up to 30 days post-last investigational drug dose.

Patients who experience AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the Investigator. All AEs and laboratory abnormalities encountered during the study should be followed until resolution or stabilization of the event(s). Any action taken and follow-up results must be recorded in the patient's

medical record. Follow-up laboratory results should be filed with the patient's source documentation and e-CRF. For all AEs that require the patient to discontinue treatment, relevant clinical assessments and laboratory tests should be repeated on at least a monthly basis until final resolution or stabilization of the event(s). These assessments should be captured in the source data and SAE forms but will not be entered in the e-CRF.

All AEs for randomized patients will be recorded in the e-CRF and the patient's source documents. AEs for patients who are screened but not subsequently randomized in the study will be recorded only in the patient's source documents. The following data should be documented for each AE:

- Description of the event;
- Classification of "serious" or "not serious";
- Date of first occurrence and date of resolution (if applicable);
- Severity;
- Causal relationship to study drug(s);
- Action taken;
- Outcome;
- Concomitant medication or other treatment given.

13.2 Adverse Event Severity

The severity of an AE should be determined according to the definitions below. Clinically significant laboratory tests should be recorded as AEs in the patient's source documents and e-CRF.

Severity will be assessed according to the following criteria:

Classification	**Definition**
Mild (Grade 1)	Mild level of discomfort and does not interfere with regular activities
Moderate (Grade 2)	Moderate level of discomfort and significantly interferes with regular activities
Severe (Grade 3)	Significant level of discomfort and prevents regular activities
Life-threatening (Grade 4)	Any adverse drug experience that places the patient, in the view of the investigator, at immediate risk of death

13.3 Adverse Event Causality

Causality will be assessed according to the following criteria:

Classification	Definition
Definitely Related	Reasonable temporal relationship to study drug administration Follows a known response pattern (i.e., drug is known to cause this AE) There is no alternative etiology
Probably Related	Reasonable temporal relationship Follows a suspected response pattern (i.e., based on similar drugs) Little or no evidence for a more likely alternative etiology
Possibly Related	Reasonable temporal relationship Some evidence for a more likely alternative etiology
Unrelated	Does not have a temporal relationship. Or, Definitely due to alternative etiology

ICH guidelines (March, 1995) clarify “reasonable causal relationship” to mean “that there are facts [evidence] or arguments to suggest a causal relationship.”

The causality assessment must be made by the Investigator based on information available at the time that the AE/SAE worksheet is completed. The initial causality assessment may be revised as new information becomes available.

13.4 Study Drug Action Taken

The investigator will classify the study drug action taken with regard to the AE. The action taken should be classified according to the categories shown below:

Classification	Definition
Dose Not Changed	Study drug dose not changed in response to an AE;
Drug Withdrawn	Study drug administration permanently discontinued in response to an AE;
Not Applicable	Action taken regarding study drug administration does not apply. "Not applicable" should be used in circumstances such as when the investigational treatment had been completed before the AE began and no opportunity to decide whether to continue, or withdraw treatment is possible.

13.5 Adverse Event Outcome

An AE should be followed until the investigator has determined and provided the final outcome. The outcome should be classified according to the categories shown below:

Classification	Definition
Recovered/Resolved	Resolution of an AE with no residual signs or symptoms
Recovered/Resolved with Sequelae	Resolution of an AE with residual signs or symptoms
Not Recovered/Resolved (Continuing)	Either incomplete improvement or no improvement of an AE, such that it remains ongoing
Fatal	Outcome of an AE is death. "Fatal" should be used when death is at least possibly related to the AE
Unknown	Outcome of an AE is not known (e.g., a patient lost to follow-up)

13.6 Treatment Administered

The Investigator will ensure adequate medical care is provided to patients for any AEs, including clinically significant laboratory values related to study drug. In addition, the Investigator will describe whether any treatment was administered for the AE. "Yes" is used if any treatment was administered in response to an AE and may include treatments such as other medications, hospitalization, surgery, or physical therapy. "No" indicates the absence of any kind of treatment for an AE.

13.7 Clinically Significant Assessments

Study assessments including safety laboratory tests, ECGs, and vital signs should be assessed and those deemed a clinically significant worsening from baseline documented as an AE. When possible, a clinical diagnosis for the study assessment should be provided rather than the abnormal test result alone (e.g. urinary tract infection, anemia). In the absence of a diagnosis, the abnormal study assessment itself should be listed as the AE (e.g. bacteria in urine or decreased hemoglobin).

An abnormal study assessment is considered clinically significant if the patient has one or more of the following:

- Concomitant signs or symptoms related to the abnormal study assessment;
- Further diagnostic testing or medical/surgical intervention;
- Discontinuation from study drug treatment.

Repeat testing to determine whether the result is abnormal, in the absence of any of the above criteria, does not necessarily meet clinically significant criteria. The determination of whether the study assessment results are clinically significant must be made by the Investigator. A laboratory abnormality judged to be Grade 4, in itself, may not constitute a SAE unless the clinical status of the patient indicates a life-threatening AE.

13.8 Serious Adverse Events

SAEs are generally any AEs that result in one or more of the following:

- Fatal (death, regardless of cause, that occurs during participation in the study or occurs after participation in the study but within 30 days post-study, and is suspected of being a delayed toxicity due to administration of the study drug);
- Is immediately life-threatening (i.e., presents an immediate risk of death at the time of the AE, not an AE that hypothetically might have caused death if it were more severe);
- Requires or prolongs inpatient hospitalization;
- Causes persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect;
- Other important medical events that may jeopardize the patient and/or require medical or surgical intervention to prevent one of the outcomes listed above.

Definition of Inpatient Hospitalization:

An inpatient hospitalization is defined as an admission for any length of time. A hospitalization for administration of study drug, for routine or planned clinical procedures, or for "social" reasons (not the result of any adverse change in the patient's condition) should not be considered an AE and should not be reported as a SAE. If the patient experiences any adverse change in condition during hospitalization, the condition must be reported as an AE or SAE according to the above definitions.

Definition of Life-Threatening Adverse Experience:

An adverse experience is life-threatening if the patient was at immediate risk of death from the event as it occurred (i.e., it does not include a reaction that, had it occurred in a more serious form, might have caused death). For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though drug-induced hepatitis can be fatal.

Definition of Disabling/Incapacitating Experience:

An adverse experience is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the patient's baseline ability to carry out normal life functions.

13.9 Serious Adverse Event Reporting

Any SAE that occurs during this study, including death from any cause, must be reported by the Investigator (or designee) to the medical monitor **within 24 hours** of awareness of occurrence, regardless of causality or expectedness. The initial notification must contain a description of the observed symptoms and an assessment of causality. Initial reports must be followed up by a written detailed report and submitted within 24 hours of the occurrence of the SAE. The designated contact for SAE reporting is the medical monitor:

PI
Neox Clinical Research
V Jámě 1, 110 00 Praha 1, Czech Republic
Tel: PI
Fax: PI
Email: PI

It is imperative that the medical monitor be informed (by email) within 24 hours of a SAE so that SAE reporting to the applicable health authorities can be met within the required time frame. The medical monitor will be responsible for evaluating the reported events for completeness and accuracy and will follow-up with the clinical site for any information missing. The medical monitor or designee will also be responsible for expedited reporting to the applicable health authorities, regulatory agencies and independent ethics committees (IECs)/institutional review boards (IRBs). The medical monitor or designee will be responsible for notifying the Sponsor of SAEs as specified in the safety and communications plans.

Because of the need to report to health authorities all serious adverse reactions in a timely manner, it is vitally important that an Investigator report immediately any adverse experiences which would be considered serious, even if the Investigator does not consider the adverse experience to be clinically significant or drug related. Should the Investigator become aware of a SAE (regardless of relationship to study drug) that occurs within 30 days after the last dose of the study drug, the SAE must be reported in accordance with the procedures specified in this protocol.

All SAEs that are not resolved by the end of the study, or that were not resolved upon discontinuation of the patient's participation in the study, are to be followed until the SAE resolves, the SAE stabilizes, the SAE returns to baseline values (if a baseline value is available), or it is shown that the SAE is not attributable to the study drug or study conduct.

13.10 Suspected Unexpected Serious Adverse Reactions (SUSAR)

SUSARs are SAEs that are possibly related or related to the study drug and are unexpected (i.e., not listed in the Investigator's Brochure). SUSARs will be collected and reported expeditiously, by the Investigator (or designee) to the medical monitor (i.e. within 24 hours of occurrence) who will then report to the competent authorities and independent ethics committees (IECs)/institutional review boards (IRBs) according to regulations, and to the Sponsor as per the safety and communications plans. Medical and scientific judgment of the Investigator is to be exercised in deciding whether expedited reporting is appropriate in other situations, such as for important medical events that are not immediately life-threatening or do not result in death or hospitalization, but jeopardize the patient or the patient population.

13.11 Pregnancy

Females of childbearing potential who have a negative pregnancy test and are enrolled in the study are to continue contraception until 30 days after the last administration of study drug. If the subject becomes pregnant during the study, the study drug should be immediately discontinued, and the Investigator should report the pregnancy to the medical monitor immediately. The Investigator should counsel the subject with regard to the possible untoward effects on the fetus. The subject will be withdrawn from the study and end of study visit performed as soon as possible. The subject should be monitored until the conclusion of the pregnancy. The outcome of all pregnancies must be followed up and documented even if the subject was discontinued from the study.

Pregnancies are considered immediately reportable AEs (**within 24 hours**). All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs.

13.12 Clinical Laboratory Assessments

Blood and urine samples will be collected according to the Schedule of Activities (Section 2) and analyzed at each individual clinical site. Specific instructions for the collection, processing, and shipment of samples are presented in the Study Laboratory Manual. Laboratory test results that are abnormal and considered clinically significant by the Investigator or designee must be reported as AEs. Screening laboratory results must be available and reviewed by the Investigator before randomization. The safety laboratory test panels are shown in Table 2 below.

Table 2 - Laboratory Tests Panels

Serum Chemistry	**Hematology**	**Urinalysis (dipstick)***
Albumin	Hematocrit	Nitrite
Creatinine	Hemoglobin	Urobilinogen
Total protein	Red blood cells	Protein
Potassium	Platelet count	pH
Sodium	Leukocytes	Blood
Calcium	**WBC Differential (absolute and %)**	Leukocytes
Bicarbonate	Eosinophils	Density
Phosphate	Basophils	Ketones
Alkaline phosphatase	Neutrophils	Bilirubin
ALT	Lymphocytes	Glucose
AST	Monocytes	
Total bilirubin		**Other (Urine)**
Uric acid		Appearance (clarity and color)
Glucose		
Total cholesterol		
Triglycerides		
Lipase		

* If dipstick urine is positive for leukocytes, nitrite, urobilinogen, protein, or blood, microscopic examination of urine will be performed for further evaluation.

In addition, according to the Schedule of Activities, the following tests are also performed:

- Pregnancy (β-human chorionic gonadotropin) tests for females of childbearing potential: Serum or urine samples will be obtained as specified in the Schedule of Activities (Section 2) and analyzed at the local labs (serum and urine). The urine pregnancy tests on Day 0 and Day 28 must be negative before dispensing of study drug. A positive result during the study will result in immediate interruption of study drug.

13.13 Medical History, Vital Signs and Physical Examinations

The medical history at screening will include all queries by the medical and clinical staff related to the subject's well-being and history of relevant past medical events/experiences. Medical history will include all demographic data (age, gender, race, body weight adjusted for indoor clothing, height, and BMI) and baseline characteristics. Alcohol and smoking habits will also be recorded. History of intestinal surgical resection or other possible cause of SBS, all recent and current medications and therapies, non-prescription drug intake, and home parenteral nutrition if still administered, will be recorded. Particular attention will be paid to obtaining the history of diarrhea in the 3 months prior to screening, including estimated weekly bowel movement frequency and stool morphology (using the BSFS). This will help in establishing a background diarrhea pattern and confirming patient eligibility for the study.

Vital signs and complete physical examinations will be performed by a medically qualified and licensed individual according to the Schedule of Activities (Section 2).

A complete physical examination (review of all body systems) will be performed at screening and Days 15, 28 and 43 visits.
A complete physical examination includes a review of the following systems: head/neck/thyroid, eyes/ears/nose/throat, respiratory, cardiovascular, lymph nodes, abdomen, skin, musculoskeletal, and neurological. Breast, anorectal, and genital examinations will be performed only when medically indicated. Clinically significant physical examination findings prior to the first dose of study drug will be recorded as medical history; after first dose, any clinically significant abnormal findings in physical examinations should be reported as AEs.

Vital signs include blood pressure (systolic and diastolic), heart rate and body temperature. These will be assessed following a 5-minute rest (seated) and before any scheduled blood sample collection.

13.14 Electrocardiograms

Standard, digital 12-lead ECGs will be performed twice, i.e. during screening and at the End-of Study, Day 43 visit. Sites will use their own ECG equipment.

Performance of all ECGs must adhere to the following guidelines in order to minimize variability and consistent assessments of each patient. ECG recording will be read and interpreted by an internal medicine specialist, at each participating clinical site.

- ECGs will be performed after the patient has been supine for at least 5 minutes;
- The ECG will be performed before any other procedures that may affect heart rate;
- The ECG will be performed during the screening period and on Day 43.

A hard copy of the ECG will be printed and signed by the Investigator at the site.

14. STATISTICAL ANALYSIS

A statistical analysis plan (SAP) will provide details of the methods of analysis to address all study objectives. The SAP may be amended during the course of the study but will be finalized before the database hard-lock for any analysis.

Data summaries by treatment group will be presented. For continuous variables, data will be summarized with the number of patients, mean, standard deviation, median, and minimum and maximum values by treatment group. For categorical variables, data will be tabulated in frequency tables to display the number and proportion of patients for each category by treatment group. Baseline assessments for each outcome variable will be defined as the last measurement obtained before the first dose of study drug.

14.1 Sample Size Determination

This is a cross-over trial in which patients will be randomized to two different sequences of treatment.
The primary outcome measure will be the change in the weekly frequency of bowel movements between baseline and Week 2 of treatment (ie. Days 8 to 14, and Days 36 to 42). Efficacy will be assessed as the difference vs. baseline as well as the difference between the two treatments.

Given that this is a cross-over study, patient will serve as their own control and the statistical analysis will be based on matched comparisons. Depending on the distribution of the difference between the two treatments the parametric Paired Student's t-test, or non – parametric Wilcoxon's sign rank test will be used. The current sample size computation will be based on the parametric test, since this is more conservative and hence demanding of a higher sample size.

The following assumptions have been employed:

i. The mean change in the lesser efficacious treatment will be 4 bowel movements per week.
ii. The SD for the change in bowel movements per week will be 2.0 based on extrapolation of results from the literature (32).
iii. The correlation of the changes between the two treatments will be 0.80 given that there is a strong patient effect on bowel movements. Hence the SD of the difference between treatment groups is 1.265 assuming an SD of 2.00 for each treatment group.
iv. The significance level will be 0.05.
v. The power of the study will be 80%.
vi. The effect size to be detected will be between 10% and 25%.
vii. Adjustment of 20% has been considered to compensate for attrition.

The following table describes the sample size requirements (number of patients) for the study by different effect size.

Table 3 - Sample Size Requirements by Size Effect

ECC - A	ECC - B	Percent Difference (%)	Absolute Difference	Sample Size
4.00	4.40	10	0.40	81
4.00	4.44	11	0.44	67
4.00	4.48	12	0.48	57
4.00	4.52	13	0.52	49
4.00	4.56	14	0.56	43
4.00	4.60	15	0.60	37
4.00	4.64	16	0.64	33
4.00	4.68	17	0.68	30
4.00	4.72	18	0.72	27
4.00	4.76	19	0.76	24
4.00	4.80	20	0.80	22
4.00	4.84	21	0.84	20
4.00	4.88	22	0.88	19
4.00	4.92	23	0.92	17
4.00	4.96	24	0.96	16
4.00	5.00	25	1.00	15

Values are the mean change in bowel movement frequency (number of bowel movements per week) between baseline and Week 2. Higher values indicate a greater reduction in stool frequency.

Based on the above assumptions and assuming a 25% difference between treatments, a total sample size of 18 patients is recommended. It is expected that about 6 patients will be randomized at each of the 3 participating clinical sites.

With 18 patients, we will have more than 80% power to detect a minimal reduction in the weekly number of bowel movements by 4.

14.2 Analysis Populations

- **The intent-to-treat (ITT) population** will include all randomized patients who complete the baseline evaluations, including those who are not exposed to study drug. The ITT population will be used for the primary analyses of all efficacy endpoints. Analysis will be done by randomized treatment.
- **The per-protocol (PP) population** will include all randomized patients who (i) are administered all study medication, in both study periods, in accordance with the protocol, including receipt of the two study drug doses in the randomized sequence, receipt of all dispensed study drug as described in the protocol, and non-use of medications prohibited by the protocol; (ii) have available information for the assessment of the primary endpoint, i.e. available weekly number of bowel movements at baseline and Week 2. The PP population will serve as the basis for secondary analyses of all efficacy endpoints.

For the purposes of identifying the PP analysis population, the protocol deviations/violations compiled during the study will be reviewed prior to database lock and, based on the PP definition above, will be classified as major vs. minor. Review of the relevant data available in the database will also be conducted to identify the patients to be included in the PP population.

- **The safety population** will include all randomized patients who received at least one dose of study drug treatment. This population will serve as the basis for analyses of all safety endpoints. Analysis will be done by actual treatment received.

14.3 Baseline and Demographic Characteristics

Demographic and baseline data will be summarized by treatment group using descriptive statistics. Demographics will include, among others, age, gender, ethnicity, weight, height, body mass index. Childbearing status and pregnancy test results will be summarized descriptively for each treatment group.

The demographics and baseline characteristics summary will be presented for the ITT and the PP populations to allow review of the characteristics of those included in the efficacy analyses.

Prior and Concomitant Medications

Medications used in this study will be coded by using the World Health Organization Drug Dictionary and categorized as follows:

- Prior medication: Any medication that started before the first dose of study drug, independently of when it ended.
- Concomitant medication: Medication continued or newly received at or after first dose of study drug to the last follow-up.

A given medication can be classified using the above, in one or more categories. If a medication has a missing or partial missing start/end date or time and it cannot be determined whether the medication was taken before the first dose of study drug or concomitantly, it will be considered as concomitant medication. Prior medications and concomitant medications will be summarized descriptively based on the ITT population.

14.4 Study Drug Exposure

The duration of study drug exposure is defined as follows: Last dose date minus first dose date plus 1 day, regardless of any interruptions in dosing. If the last dose date of study drug is missing, the patient's discontinuation or completion date will be used for analysis purpose. Duration of study drug exposure will be summarized descriptively as a continuous variable (number, mean, SD, median, minimum, and maximum), by study group, using the safety population.

14.5 Study Drug Compliance

Study drug compliance will be assessed at the end of each treatment period. Treatment compliance percentages will be summarized descriptively as continuous variables (number, mean, SD, median, minimum, and maximum) using the ITT and PP populations. The percentage of patients whose compliance is <80% or ≥80% will also be summarized, by study group.

14.6 Efficacy Analysis

14.6.1 Analysis of Primary Endpoint

For the primary objective of the study, descriptive statistics will be produced for the change in weekly frequency of bowel movements between baseline and the second week of treatment. A sequential analysis approach will be used where:

a. First, the efficacy of the High ECC dose (4.25 g daily) will be tested using a paired Student's t-test and a null hypothesis of 0. If this comparison shows a statistically significant improvement, then the efficacy of the Low ECC dose will be tested.
b. To assess the efficacy of Low ECC dose (1.7 g daily) a paired Student's t-test and a null hypothesis of 0 will be used, similarly to above. If this comparison shows a statistically significant improvement, then the two doses will be compared.
c. To compare the efficacy of the two doses, the absolute change in weekly frequency of bowel movements will be compared using a paired Student's t-test and a null hypothesis of 0 ($\delta_{High} - \delta_{Low} = 0$).
d. Given the sequential design of the analysis correction for multiplicity will not be required.

If the change in weekly frequency of bowel movements between baseline and the second week of treatment does not follow the normal distribution the non-parametric Wilcoxon signed rank test will be used.

The primary analysis will be conducted on the ITT population. The PP population will be used in a secondary analysis.

14.6.2 Analysis of Secondary Efficacy Endpoints

For all secondary endpoints, no official statistical hypothesis will be tested, and statistical comparisons may be conducted for exploratory purposes only.

- For the total number of bowel movements per week at week 1 and for the whole week 2 period, similar methods to those described for the primary endpoint will be used.

- The mean daily stool form score according to BSFS will be summarized over time by treatment dose using descriptive statistics. The mean daily score during the first week, second week and during the whole 2-week period of treatment will be compared to the baseline score using the paired Student's t-test or the Wilcoxon signed rank test, as appropriate based on the data distribution.

- For the total number of bowel movements with a BSFS ≥6 after 1 and 2 weeks of treatment and for the Mean Diarrhea Composite Index, descriptive statistics will be used by treatment dose. Within-group comparisons with the baseline values and between-group comparisons will be conducted as described in the primary endpoint.

- The proportion of patients treated with loperamide will be described by treatment dose and among treated patients the mean daily dose of loperamide in mg will be summarized.

- Overall evaluation of diarrhea, abdominal pain, urgency and bloating will be summarized over time by treatment dose using descriptive statistics. Within-group comparisons with the baseline score will be conducted using the paired Student's t-test or the Wilcoxon signed rank test, as appropriate.

14.7 Safety Analyses

All safety data will be included in subject data listings. Summary tables will be based on the safety population. No statistical comparisons will be conducted.

14.7.1 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 21.1 or later).

For the purpose of analyses and tabulations, AEs will be classified as pretreatment AEs, TEAEs, or post-treatment AEs. More specifically: Pretreatment AE are those that started before the first dose of study drug. TEAE are those that increased in severity (compared to pre-treatment) or that appeared at or after the first dose of study drug but before the last follow-up of each period. Post-treatment AEs are those that increased in severity or that appeared after the last follow-up, planned for 4 weeks after the last dose of study drug. For AEs with missing or incomplete start dates, if there is no clear evidence that the AEs started before or after the first dose of study drug, then the AEs will be classified as TEAEs.

AE summary tables, including the number and percentage of patients with at least one AE overall and by MedDRA system organ class (SOC) and preferred term (PT), will be presented by treatment group for TEAE only and will include the following: All TEAEs, TEAEs by relationship, TEAEs by maximal severity, TEAEs leading to treatment discontinuation, serious TEAEs and fatal TEAEs. All AEs, including pre-and post-treatment AEs, will be presented in individual patient data listings. For these summaries, patients with multiple events will be counted only once per PT. For the TEAEs by relationship and TEAEs by maximal severity, at each level of summarization, the event with the strongest drug relationship and highest level of severity, respectively, will be presented.

All AEs must be listed. In addition, separate listings will be provided for patients who die, experience a SAE, or discontinue the study because of an AE. These listings will include treatment, patient's age and gender, duration of follow-up, amount of ECC received, and time since last intake.

14.7.2 Clinical Laboratory Assessments

Safety laboratory variables (e.g., hematology, biochemistry, and urinalysis results) will be presented in the International System of units at each time point using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change from baseline values for each variable will also be presented by treatment group. The number and percentage of patients with shift changes from baseline (normal, high, and low according to the reference range as provided by the laboratories) to post-baseline will be tabulated by treatment group.

14.7.3 Vital Signs

Vital signs (blood pressure, temperature and heart rate) will be presented at each time point using descriptive statistics (mean, standard deviation, median, minimum, and maximum). Change from baseline values for each variable will also be presented by treatment group.

The number and percentage of patients with shift changes from baseline to post-baseline based on abnormal criteria will be presented by treatment group.

14.7.4 ECG

For ECG measurements, a summary of raw values at baseline and end of study and change from baseline values will be provided for the following standard digital ECG measurements: PR, QT, and QT corrected for heart rate (QTc) intervals, QRS duration, and HR. The number and percentage of patients with shift changes from baseline (normal, not clinically significant, and potentially clinically significant according to overall ECG evaluation as provided by the laboratories) to end of study will be tabulated.

In addition, the number and percentage of patients with QT/QTc intervals values of ≤500msec and >500msec, as well with changes from baseline in the value of QT/QTc intervals by >30msec will also be tabulated.

15. DATA HANDLING AND RECORD KEEPING

15.1 Case Report Forms

All patient data generated by the study will be recorded in each patient's e-CRFs. Data reported on the e-CRFs that are derived from source documents should be consistent with the source documents or the discrepancies should be explained. e-CRFs will be considered complete when all missing and/or incorrect data have been resolved and all safety data have been recorded.

The Investigator, or designated representative, should complete the e-CRF as soon as possible after information is collected. e-CRFs must be completed only by persons designated by the Investigator. The e-CRF system should enable an audit trail that will provide the user's identification information and the date and time of any entry/correction. The completed e-CRF will be reviewed by the Sponsor, study monitor or Sponsor's designees on a routine basis.

The Investigator must approve formally (via signature) all the information in the e-CRFs for the patients for whom he/she is responsible.

A copy of the e-CRFs will be provided to the Sponsor with the final report.

Subjects who do not meet protocol criteria will be entered on a screening/enrolment log and no e-CRF will be completed.

15.2 Source Documentation

Source documents are considered to be all information in original records and certified copies of original records of clinical findings, observations, data, or other activities in a clinical study necessary for the reconstruction and evaluation of the study. The Investigator and designees agree to maintain accurate e-CRFs and source documentation as part of the case histories. Source documents are the originals of any documents used by the Investigator, sub-Investigator, or hospital/institution that will allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial. All data entered into the e-CRF also must be available in the source documents. The Investigator will allow designated representatives of the Sponsor and regulatory bodies to have direct access to the source documents to verify the data reported in the e-CRFs in the course of trial-related monitoring, audits, IRB/IEC review and regulatory inspections.

15.3 Record Retention

Study records and source documents must be retained for at least 25 years after the completion or discontinuation of/withdrawal from the study, or 2 years after the last approval of a marketing application in an International Conference on Harmonization (ICH) region, whichever is the longest time period. If it becomes necessary for the Sponsor to review any documentation relating to the study, the Investigator must permit access to such records.

The Investigator should contact the Sponsor before destroying any records pertaining to the study to ensure they no longer need to be kept.

15.4 Data Management and Processing

Data management activities will be performed using the Omnicomm Trial Master electronic data capture (EDC) software in accordance with the data management facility's SOPs.
Data entered will be checked for accuracy through QC checks. When the database is declared to be complete and accurate, it will be locked.

16. MONITORING

In accordance with current applicable regulations, Good Clinical Practice (GCP), and the Sponsor's Standard Operational Procedures, monitors from the CRO will contact the site before the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory, ethical, and Sponsor requirements. When reviewing procedures for data collection, the discussion will include identification, agreement, and documentation of data items which will be recorded in each patient's e-CRF.

The study will be monitored to ensure the following:

- Data are authentic, accurate, and complete;
- The safety and rights of patients are being protected;
- The study is being conducted in accordance with the currently approved protocol, any other study agreements, GCP, and all applicable regulatory requirements.

The Investigator and the head of the medical institution (where applicable) agree to allow the monitor direct access to all relevant documents.

17. QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor's designee will perform the quality assurance and quality control activities of this study. Designated personnel from the quality assurance unit(s) of the clinical facility will be responsible for maintaining QA systems to ensure that the trial is conducted and that clinical data is generated, documented and reported in compliance with the protocol and ICH Guideline E6 for GCP.

Designated personnel from the corresponding operation unit (usually of the clinical and statistical facilities) will be responsible to maintain and assure the quality control (QC) of all data generated and documented in compliance with the protocol. However, responsibility for the accuracy, completeness, and reliability of the study data presented to the Sponsor lies with the principal or qualified Investigator generating the data.

The Sponsor or its designated representative will conduct a study site visit to verify the qualifications of the principal Investigator and sub-Investigators, inspect clinical site facilities as needed, and inform the Investigator of responsibilities and procedures for ensuring adequate and correct study documentation.

Study data for randomized patients will be entered into an e-CRF by clinical site staff using a secure, validated web-based electronic data capture (EDC) application. Select data for patients not randomized will also be collected in e-CRFs.

Instances of missing, discrepant, or uninterpretable data will be queried with the Investigator for resolution. Any changes to study data will be enacted in the e-CRF and documented in an audit trail, which will be maintained within the clinical database.

18. COMPLIANCE, PROTOCOL AMENDMENT AND DEVIATION

18.1 Compliance

An application dossier for the commencement of a clinical trial, must be submitted to the national competent authorities of Poland and the Ethics Committees, prior to the start of the study. The authorization to conduct the clinical trial must be obtained prior to initiation of any screening activities, including ICF signature.

It is very important that no modifications to the protocol be made without prior approval from the Sponsor. Changes that significantly affect the safety of the patients, the nature, the scope and the scientific integrity of the study will require IRB/IEC notification/approval before their implementation. Exceptions are cases where the modification is necessary to abrogate an apparent immediate risk to the patients. The Sponsor's designee will submit all protocol modifications to IRB/IEC and the required regulatory authorities. When there is a need for immediate deviation from procedures enunciated in the protocol, the Investigator will contact the Sponsor to discuss the course of action and possible alternatives, if at all possible, before any implementation of changes. Any deviation from protocol must be fully documented in the source documentation and in the study documentation on protocol deviations.

18.2 Protocol Amendment

Administrative amendments to the protocol will be classified as amendments of typographical errors, clarifications of confusing wording, name changes, and minor modifications that have no impact on the safety of the patients or the science of the study. Administrative amendments will be submitted to the IRB/IEC for information only. JSS Medical Research will ensure that acknowledgement is received and filed. Any other amendment will be classified as a substantial amendment and will be submitted by the Sponsor's designee to the appropriate regulatory authorities and the IRBs/IECs for approval.

18.3 Protocol Deviation

Should a protocol deviation occur, the Sponsor must be informed as soon as possible. Important protocol deviations and their reasons will be summarized in the clinical study report. In accordance with applicable regulatory authority mandates, the Investigator is responsible for reporting protocol deviations to the IRB/IEC.

19. STUDY TERMINATION

At any time, the Sponsor may terminate this study in its entirety or at a specific clinical site. In addition, for reasonable cause, the IRB/IEC and/or the Investigator at a clinical site may terminate the study at their center. In such cases, the Sponsor should be informed immediately and if at all possible, before implementation.

Conditions that may lead to reasonable cause and warrant termination include, but are not limited to:

- Investigator noncompliance and/or lack of adherence to protocol procedures;
- Unsatisfactory patient enrollment;
- Lack of evaluable and/or complete data;
- Potentially unacceptable risk to patients;
- Changes in the Sponsor's drug development plans;
- Decision by regulatory authorities.

The reason(s) for clinical study termination must be properly documented. If the trial is prematurely terminated or suspended for any reason, the clinical site or the qualified investigator (or delegate) should promptly inform the trial subjects, should assure appropriate therapy and follow-up for the subjects and should inform the regulatory authority(ies) when required.

20. ETHICAL CONSIDERATIONS

This study will be conducted in compliance with the study protocol, the ethical principles that have their origins in the Declaration of Helsinki, ICH Guideline E6 for Good Clinical Practice (GCP), the FDA GCP Code of Federal Regulations (CFR) Title 21 (part 56), the European regulation EU 536/2014, the Tri-Council Policy Statement (Canada) and Division 5 part C section of Health Canada.

IRB/EC committees will review and approve this protocol and informed consent forms. Substantial amendments to the protocol and ICF will be submitted to the IRB/EC and written approval will be obtained prior to implementation. All patients must provide signed informed consent where applicable, before participation in the study. Before screening activities commence, each subject will be given a copy of the ICF to read, as well as a full explanation of the purpose of the study, the procedures to be carried out, and the potential AE(s). Once this essential information is provided to the subject and the physician in charge or delegate has the conviction the subject understands the implications of participating in the study, and if the subject chooses to continue the screening process, they will be requested to sign and date a properly executed ICF. Subjects will be assured they may withdraw from the study at any time without jeopardizing their medical care or future study participation (for which they qualify). Subjects will be given a signed copy of the ICF. If an amended or revised ICF is introduced during the study, each subject's further consent must be obtained.

This study will be performed by qualified clinical investigators and in accordance with GCP. The study specifically incorporates all of the following features:

- Multicenter, randomized study design;
- Prospectively stated objectives and analytical plan;
- Accepted, pre-specified outcome measures for safety and efficacy;
- A detailed protocol to promote consistency across sites;

- Compliance with current GCP, with assessment via regular monitoring;
- Quality assurance procedures performed at study sites and during data management to ensure that safety and efficacy data are adequate and well documented.

This protocol and the ICF will be submitted to an Institutional Review Board (IRB) (or Independent Ethics Committee (IEC)) prior to initiation of the study and the study will not start until the Board has approved the documents. Notification of the Board's approval will be appended to the final report. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, sample ICF, advertisements (if applicable), written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the Sponsor's designee, as allowable by local applicable laws and regulations.

The investigators and the Sponsor will preserve the confidentiality of all subjects taking part in the study, in accordance with GCP and local regulations. Subjects should be identified by a unique subject identifier on all study documents provided to the sponsor. In compliance with local regulations/ICH GCP Guidelines, it is required the investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and IRB access to review the subject's original medical records for verification of study-related procedures and data. The investigator is obligated to inform the subject that his/her study-related records will be reviewed by the above-named representatives without violating the subject's confidentiality.

21. FINANCE AND INSURANCE

Financial aspects of the study are addressed in a separate clinical study agreement.

The Investigator/institution is required to have adequate current insurance to cover claims for negligence and/or malpractice according to national regulations. The Sponsor's designee will provide insurance coverage for the clinical study as required by national regulations. This should include sufficient insurance coverage should any serious events or deaths result, either directly or indirectly, from the execution of the present protocol.

The present article is not to be interpreted as engaging the Sponsor's responsibility in the event of fault or negligence of the subjects, investigators, or any persons or employees under the control of the clinical site.

22. PUBLICATION POLICY AND CLINICAL STUDY REPORT

22.1 Confidentiality and Publication Policy

Both the use of data and the publication policy are detailed within the clinical study agreement.
Any and all scientific, commercial, and technical information disclosed by the Sponsor in this protocol or any other documents and communications should be considered the confidential and proprietary property of the Sponsor. The Investigator shall hold such information in confidence and shall not disclose the information to any third party except to the Investigator's staff on a "need to know" basis, as long as the said staff has been made aware that the information is confidential and who are bound to treat it as such.

The Investigator shall not use any and all information for any purpose other than determining interest in performing the study and, if the parties decide to proceed with the study, for the purpose of conducting the study. The

Investigator understands that the information developed from this clinical study will be used by the Sponsor for the development of the study drug and therefore may be disclosed as required to other clinical Investigators, potential and current business partners and associates, Health Canada, the FDA, and possibly other regulatory agencies. The Investigator also understands that, to allow for the use of the information derived from the clinical study, he/she has the obligation to provide the Sponsor with complete results and accompanying data developed in the study.

No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between the Sponsor and the Investigator and/or the Investigator's institution.

22.2 Clinical Study Report

A full clinical study report, written in accordance with the ICH E3 Guideline, will be submitted in accordance with local regulations.

23. REFERENCES

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APPENDIX 1: Sample Bowel Movement and BSFS Diary

Daily Symptom and Stool Diary – Period 1

DAY	DATE (e.g. Monday 05/Jun/18)	Loperamide taken today (e.g. 2 pills)	Time Study Drug Taken (24 hr clock)		STOOL FORM AND TIME (Stool Form = 1 to 7 from chart below; Time = time of stool, 24 hour clock (e.g. 15:30 for 3:30 pm)											
					1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
1				Form												
				Time												
2				Form												
				Time												
3				Form												
				Time												
4				Form												
				Time												
5				Form												
				Time												
6				Form												
				Time												
7				Form												
				Time												

1		Separate hard lumps, like nuts	4		Like a sausage or snake, smooth and soft	7	 Watery, no solid pieces
2		Sausage shaped but lumpy	5		Soft blobs with clear cut edges		
3		Like a sausage or snake, but with cracks on its surface	6		Fluffy pieces with ragged edges, a mushy stool		