A Randomized, Double-Blind, Placebo Controlled Trial of Aldafermin (NGM282) for Treatment of Chronic Diarrhea Due to Bile Acid Malabsorption (BAM)

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Compound Name:	Aldafermin (NGM282)
IND	[redacted] Investigator-held IND Letter of cross-reference for INDs [redacted] and [redacted] held by NGM
Development Phase:	II
Version Date of Protocol:	Version 6.0: September 29th, 2022

PROTOCOL HISTORY

Document	Amendment Type	Date
Original, Version 1.0	Not applicable	August 31, 2021
First protocol revision, Version 2.0	Not applicable	September 15, 2021
Second protocol revision, Version 3.0	Not applicable	October 7, 2021
Third protocol revision, Version 4.0	Amendment 1	February 9, 2022
Forth protocol revision, Version 5.0	Amendment 2	April 20, 2022
Fifth protocol revision, Version 6	Amendment 2	September 29th,2022

PROTOCOL SYNOPSIS

Protocol Title	A Randomized, Double-Blind, Placebo Controlled Trial of Aldafermin (NGM282) for Treatment of Chronic Diarrhea Due to Bile Acid Malabsorption ((BAM)
Study Number	21-009348
Development Phase	Phase II
Study Center	Mayo Clinic, Rochester, Minnesota, USA
Abstract	Bile acid malabsorption (BAM), also known as bile acid diarrhea (BAD), is a condition characterized by excessive biosynthesis of bile acids or inability to reabsorb sufficient bile acids in the terminal ileum (1,2). This results in unabsorbed bile acids in the colon leading to increased colonic secretion of fluid, thereby causing diarrhea. BAM/BAD contributes to symptoms in a subset of individuals with Irritable bowel syndrome (IBS), which is the most common lower gastrointestinal (GI) disorder that affects 11% of adults (3). Currently, IBS and subgroups diarrhea-predominant IBS (IBS-D), and IBS-constipation (IBS-C), are defined by symptoms alone. Apart from central hypervigilance and psychological disturbances in IBS (4),48% of IBS-D patients have accelerated colonic transit, 21% have increased rectal sensation (5), and 25-33% of IBS-D patients have abnormal bile acid (BA) synthesis or excretion (6). Aldafermin (NGM282) is an analog of fibroblast growth factor 19 (FGF19), a potent inhibitor of bile acid (BA) synthesis in animals and humans.
Hypothesis	Aldafermin reduces bile acid synthesis and fecal excretion of bile acids and normalizes symptoms of colonic dysfunction in BAM/BAD with increased fecal BA excretion.
Specific Aim	This single-center, randomized, double-blind, placebo-controlled study is designed to compare effects of aldafermin, 1 mg and placebo SQ daily, on bowel functions, hepatic synthesis and fecal excretion of bile acids in patients with diarrhea associated with bile acid malabsorption (BAM).
Number of Subjects	Approximately 36 patients with laboratory confirmed BAM will be enrolled.
Study Duration	The study includes a maximum 28-day prescreen period and a 28-day treatment period for a maximum duration of 56 days.
Study Population	**Inclusion criteria**: 1. Aged 18 to 75 years, inclusive at Visit 1 Screen. 2. Clinical diagnosis of functional diarrhea or IBS with diarrhea according to Rome III or IV criteria at Visit 1 Screen. 3. Clinical laboratory evidence of BAM (20-22), with at least one of the following results recorded in their past medical history: • Serum C4 ≥ 52 ng/mL • Fecal BA > 2337 µmoles / 48 hours • Total fecal BA > 1000 µmoles / 48 hours + 4 % primary BA • Fecal primary BA > 10% / 48 hours 4. Body mass index (BMI) 18.0 to 45.0 kg/m^2, inclusive at Visit 1 Screen 5. Understands the study procedures, is willing and able to comply with the study procedures, and is able to give informed consent 6. If treated with any of the following medications, dosing must be stable for 30 days prior to Visit 1 Screen. Patient must agree to maintain the same dose of medication throughout the study: • Tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs). • Bile acid sequestrants such as colestipol, cholestyramine and colesevelam. 7. Patients must use one highly effective method of contraception for 30 days before

	the study through 90 days after study completion for males and through 30 days after study completion for females. Highly effective methods of contraception include: • Oral, implantable, subdermal or injectable hormonal contraceptives • Standard intrauterine device or vaginal ring • Male or female condoms and diaphragms used with spermicide • Abstinence from heterosexual intercourse • Female partners exclusively sexually active with a surgically sterilized male partner • Females who are surgically sterile prior hysterectomy, bilateral salpingectomy, or bilateral oophorectomy) or postmenopausal (defined as: 12 consecutive months with no menses) are not considered to be of childbearing potential **Exclusion criteria:** 1. Pregnant or lactating 2. Structural or metabolic diseases/conditions that affect the gastrointestinal system 3. Use of the following medications at least 14 days prior to Visit 1 throughout the duration of the treatment period • Patients may elect to withdraw from bile acid sequestrants such as colestipol, cholestyramine and colesevelam or they may continue but they must continue at the same dose throughout the study. • GI medications including: • Anti-nausea agents including trimethobenzamide, promethazine, prochlorperazine, dimenhydrinate, hydroxyzine • Osmotic laxative agents including lactulose, sorbitol or PEG solutions as Miralax and Glycolax • Prokinetic agents including tegaserod, metoclopramide, prucalopride, domperidone, erythromycin, clarithromycin and azithromycin. • 5-HT3 antagonists including alosetron, ondansetron, tropisetron • Patients may use Gabapentin if they are on a stable dose, which is not greater than 1200 mg a day. • Drugs with a known pharmacological activity at 5-HT4, 5-HT2b or 5-HT3 receptors including tegaserod, ondansetron, granisetron and tropisetron • All narcotics including codeine, morphine, and propoxyphene, either alone or in combination • Anti-cholinergics including dicyclomine, hyoscyamine, propantheline. • Antimuscarinics • Tramadol • Peppermint oil • Systemic antibiotics and antibiotics directed at colonic flora including rifaximin and metronidazole

	5. Clinically relevant changes in dietary, lifestyle, or exercise regimen within 30 days prior to Visit 1 Screen and throughout the duration of the study 6. Any colonic or major abdominal surgery including bariatric surgery, gastric banding, stomach surgery and intestinal or colonic surgery. Procedures such as appendectomy, cholecystectomy, hysterectomy, caesarean section, or polypectomy are allowed as long as they have occurred at least 3 months prior to Visit 1 Screen. 7. .History of colorectal cancer, inflammatory bowel disease, diverticulitis, ischemic colitis, microscopic colitis or celiac disease 8. History of organic abnormalities of the GI tract, intestinal obstruction, stricture, toxic megacolon, GI perforation, or impaired intestinal circulation. 9. Other GI diseases such as GI bleeding or ulcerations 10. History of cerebrovascular disease including stroke, TIA, acute coronary syndrome, myocardial infarction or unstable angina 11. Clinically significant cardiac history or presence of electrocardiogram (ECG) findings at Visit 1 Screen: o Abnormal heart rate < 40 or > 100 beats per minute o QTc interval > 470 milliseconds (ms) o QRS interval ≥ 110 ms o PR interval ≥ 220 ms 12. Hepatic dysfunction including abnormal serum alanine aminotransferase [ALT] or aspartate transaminase [AST] > 3 × upper limit of normal [ULN]); total direct bilirubin > 2 × ULN, or alkaline phosphatase > 2 × ULN at Visit 1 Screen 13. Clinically significant renal insufficiency including serum creatinine > 2.5 mg/dL at Visit 1 Screen 14. History of severe head injury or history of seizures 15. History of suicide attempt or a hospitalization for a major psychiatric condition within 1 year prior to Visit 1 Screen. At Visit 1 Screen or during the optional remote consent and eligibility review, participants will complete the Hospital Anxiety and Depression questionnaire. If either score for anxiety or depression individually exceeds 8, the score will be discussed. The patient will be and advised whether to participate or whether to see their primary care physician. 16. History of alcohol use disorder or substance use disorder within 2 years of Visit 1 Screen. 17. Significant history or clinical manifestation of any endocrine, allergic, dermatological, hepatic, renal, hematological, pulmonary, GI, neurological or psychiatric disorder, malignancy (with the exception of treated basal cell carcinomas), or any other condition that would prevent the individual from participating in the study due to risk to the scientific validity of study assessments or to personal well-being of the patient. 18. Participated in another clinical study that includes an investigational drug or a biologic therapy within 30 days or 5 half-lives, whichever time period is longer, prior to Visit 1 Screen.

Criteria for Evaluation:	**Bowel pattern:** Assessed by patient-recorded daily bowel pattern diaries **Biomarkers:** Measurements of fasting serum C4 and FGF-19 will be undertaken at baseline, Day 14 and Day 28. **Pharmacokinetics (PK):** Trough concentrations of aldafermin will be determined by a validated PK laboratory assay performed at a bioanalytical CRO, Frontage Laboratories, at baseline, Day 14 and Day 28. **Safety**: Safety will be assessed through regular monitoring of adverse events, clinical laboratory results, safety 12-lead ECGs, physical examinations, and vital signs.

Study Endpoints	**Primary Endpoints:**
	• Change in fasting serum C4 levels from Baseline to Day 28
	• Change in stool consistency from Baseline to Day 28
	
	Secondary Endpoints:
	• Stool consistency based on Bristol Stool Form Scale, BSFS
	  o Absolute change in stool consistency from baseline to Day 14 and Day 28 of treatment
	  o Average daily stool consistency score at Day 14 and Day 28 of treatment
	• Maximum abdominal pain score rated by patient using an 11-point scale, 0 to 10 inclusive with 0 as none and 10 as worst imaginable pain
	• Absolute change in abdominal pain from baseline to Day 14 and Day 28 of treatment
	• Number of bowel movements per week
	• Chane in total fecal bile acid concentration between baseline and Day 28
	• Proportion of fecal secretory (CDCA+ DCA) bile acid as measured in a single, random stool sample by a validated laboratory assay.
	• Proportion of fecal primary (CDCA + CA) bile acid as measured in a single, random stool sample by a validated laboratory assay.
	• Total fecal bile acid concentration in a single, random stool sample as measured by a validated laboratory assay.
	• Total aldafermin concentration in serum as measured by a validated laboratory assay

Statistical Methods	**Sample size:** 30 patients, 15 in placebo group, 15 in aldafermin treatment group. **Statistical analyses**: Effects of treatment will be compared using Intent-to-Treat principles using ANCOVA with BMI, gender and baseline fasting C4 as covariates. Adverse events (AEs) will be listed and summarized by system organ class and preferred term, as well as according to severity and causality/relationship to study treatment. Observed values for clinical laboratory tests, vital signs and safety 12-lead ECGs will be summarized by treatment. Observed values will be classified as normal, abnormality that is not clinically significant, and clinically significant abnormality by treatment Results of other safety assessments will be listed and summarized as appropriate. **Statistical power:** Based on the prior open-label study, the proposed sample size of 15 per group has ~80% power (2-sided α=0.05) to detect clinically relevant effect sizes in serum C4 and stool consistency. **Anticipated results and significance:** Aldafermin will reduce serum C4 and percent

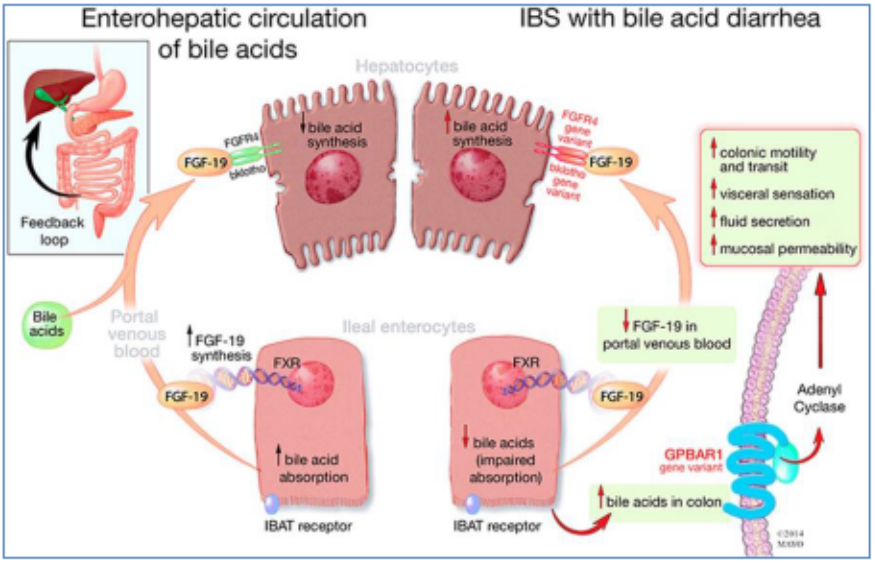
	fecal primary bile acids in a random stool sample; preliminary data will be obtained regarding efficacy on stool frequency and stool consistency in BAM/BAD with high BA excretion.

1. INTRODUCTION

1.1. Significance of the Problem

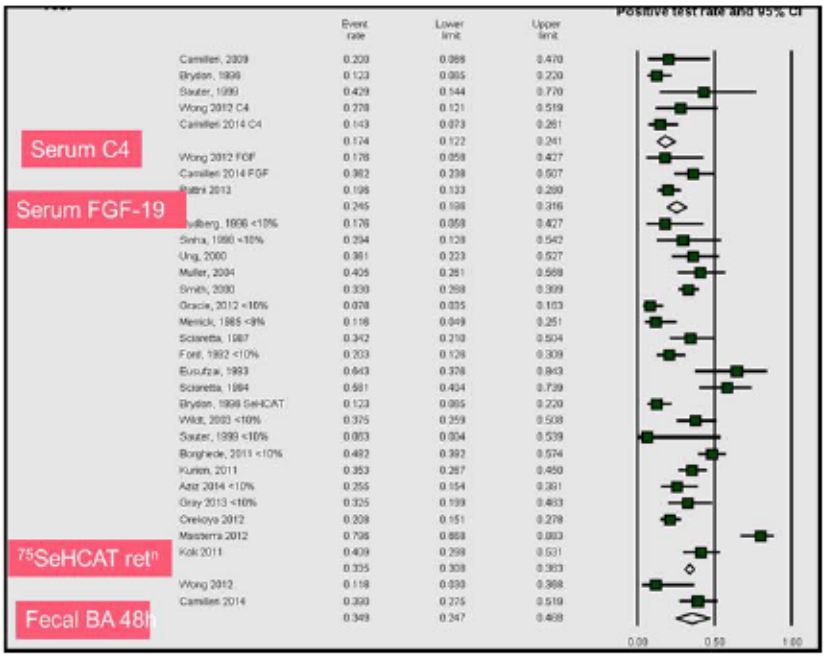
Bile acid malabsorption (BAM), also known as bile acid diarrhea (BAD), is a condition characterized by excessive biosynthesis of bile acids or inability to reabsorb sufficient bile acids in the terminal ileum (1, 2). This results in unabsorbed bile acids in the colon leading to increased colonic secretion of fluid, thereby causing diarrhea. BAM/BAD contributes to symptoms in a subset of individuals with irritable bowel syndrome (IBS), which is the most common lower gastrointestinal (GI) disorder.

IBS affects ~11% and IBS-D affects ~5% of the US population (3) and often impairs quality of life (5). BAM/BAD affects approximately one third of individuals with IBS-D. IBS is currently defined by symptoms (i.e., abdominal discomfort associated with bowel disturbances) in the absence of organic disease on routine testing. There is a large body of evidence that IBS-D symptoms are manifestations of colonic transit, mucosal immune function, visceral hypersensitivity, and central nervous system (CNS) hypervigilance (4,8,9).



Abbreviations: **FXR**: farnesoid X receptor; **FGF19**: fibroblast growth factor 19; **FGFR4**: FGF receptor 4; **GpBAR1**: G protein coupled bile acid receptor 1; **KLB**: klotho B;LRH1: liver receptor homolog-1; **TGR5 =GpBAR1**

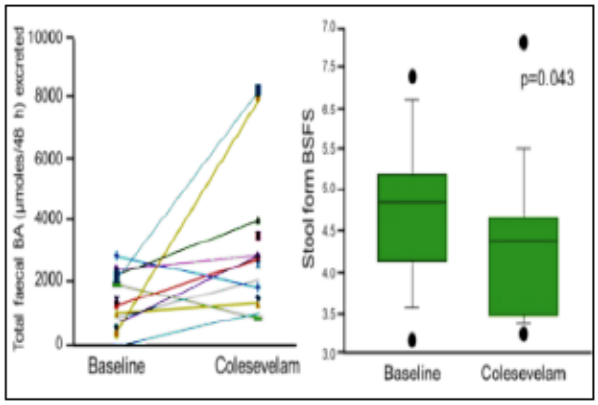
A graphical representation of the enterohepatic circulation of bile acids (10) summarizes the pivotal chemical steps in the control of bile acid synthesis that is critical to impact bile acid diarrhea. BAs are reabsorbed in the ileum, activate FXR, and increase FGF-19 synthesis. FGF-19 then binds to the FGFR-4 and klotho β receptors to decrease C4 and subsequent hepatic BA synthesis. In BAM, BAs are reabsorbed, but FGF-19 remains low, or there are mutations within the FGFR-4 or klotho β receptors that do not inhibit hepatic BA synthesis. BAs that enter the colon bind to the GPBAR1 receptor and cause increased colonic transit and secretion.



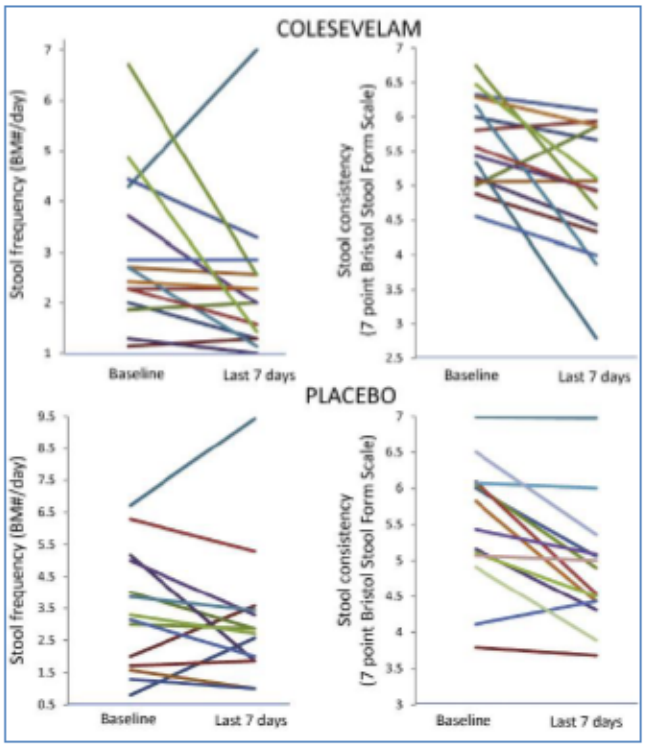
A systematic review and meta-analysis (11) showed an average 28% of IBS-D patients have evidence of increased BA synthesis or fecal *BA* excretion.

1.2 Experimental Therapies for BAM/BAD

A prior open-label study of the BA sequestrant, Colesevelam, in 12 patients with IBS-D and high fecal bile acid excretion demonstrated biological relevance of increased fecal BA excretion (12).



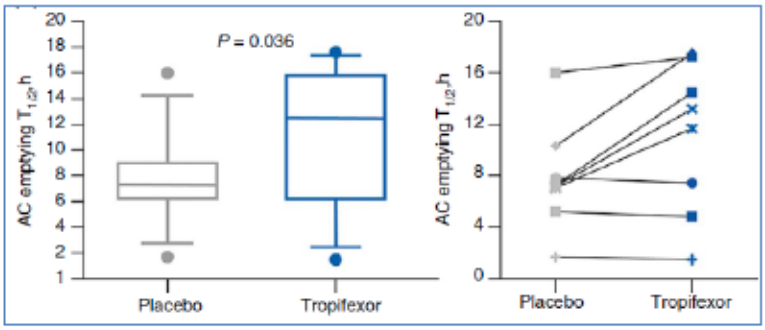
Effects of colesevelam on sequestration of bile acid *(methanol extraction of stool released the bile acids showing that increased amounts were bound)* and *reduced* stool form. Colesevelam improved stool consistency, sequestering and inactivating BA (shown by increased BA in stool after methanol extraction).



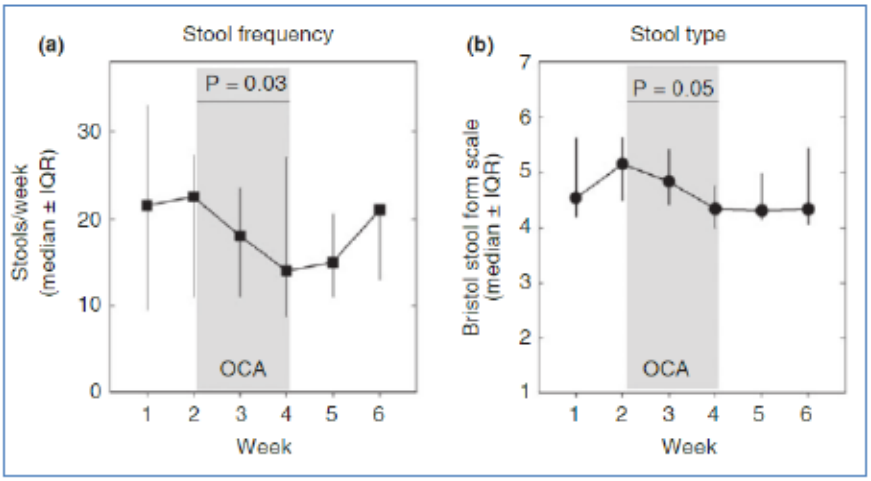
On the other hand, a randomized, controlled trial with colesevelam, 1.875g bid, of 4 weeks' duration (13) did not significantly impact symptoms (stool frequency and consistency) although there was evidence of biological efficacy, with significant changes in sequestered fecal total BA excretion ($P < .001$) and serum levels of C4 and FGF19 (both $P < .001$), and with a mean increase in fecal level of deoxycholic acid (10%; $P = 0.07$) compared to placebo.

Effects of colesevelam on stool frequency and consistency in patients with IBS-D and BAD in randomized controlled study

The **FXR agonist, tropifexor**, has been tested in a small multicenter study, randomized crossover study in patients with primary BAM/BAD (14). Tropifexor 60 µg once daily had acceptable safety and tolerability. Effects on changes in FGF19 and C4 showed effective target engagement; however, higher doses may be required to observe stool frequency changes. On the other hand, slowing of ascending colon emptying suggests therapeutic potential of tropifexor in patients with primary BAM/BAD.



Effect of tropifexor on gastrointestinal transit in the single center sub-study. A, Ascending colon emptying T1/2 (n = 8) on Day 12 of each phase. Left panel shows a statistically significant (p = 0.036) slowing of the mean ascending colon emptying with tropifexor vs. placebo on Day 12. Right panel shows the ascending colonic T1/2 in individual patients.



6-ethyl CDCA or **obeticholic acid** is another FXR agonist that has also been tested for idiopathic BAM/BAD (15). Obeticholic acid stimulates FGF-19, reduces bile acid synthesis and produces clinical benefits in bile acid diarrhea.

Summary: Prior clinical trials in small numbers of participants suggest potential efficacy of bile acid sequestration as well as biological but not clinical efficacy with experimental medications with potential for efficacy though increases in FGF-19, the feedback regulator of hepatic bile acid synthesis

1.3 Aldafermin

Aldafermin (NGM282) is a recombinant protein with 95.4% of its 190 amino acids identical to fibroblast growth factor 19 (FGF19) and with similar binding affinity to FGFR4 and KLB co-receptor on human hepatocytes. The molecule aldafermin mimics the actions of FGF19 on bile acid synthesis by decreasing the gene expression in human hepatocytes of CYP7A1 which is responsible for catalyzing the rate limiting step of cholesterol conversion to bile acids. This has been demonstrated by a rapid and dose-dependent reduction in levels of serum 7-alpha-hydroxy-4-cholesten-3-one (C4), a key intermediate metabolite of CYP7A1-mediated bile acid synthesis in mice and humans (16-18).

This investigator-initiated study will be conducted under an investigator-held IND [redacted] with a Letter of Authorization from NGM Biopharmaceuticals, Inc. based upon the existing aldafermin INDs [redacted] and [redacted] A prior study of aldafermin (NGM282) was conducted a few years ago in patients with functional constipation under IND [redacted]

In this prior study (19), we showed there was a dose related effect of aldafermin on colonic transit in patients with functional constipation, with significant effect on colonic transit (particularly ascending colon emptying) and bowel frequency with the 1mg dose of aldafermin in contrast to the stimulation with the 6 mg dose:

Data LSM (SEM)	Placebo (PLA) n=10	NGM282 1mg, n=10	NGM282 6mg, n=11	P ANOVA	NGM282 1mg vs PLA	NGM282 6mg vs PLA
GE $T_{1/2}$,min	132.4 (7.4)	97.8 (7.2)	94.5 (8.1)	0.002	0.003	0.001
CT GC24	2.03 (0.16)	2.35 (0.18)	3.30 (0.29)	< 0.001	0.070	< 0.001
CT GC48	3.36 (0.29)	4.29 (0.22)	4.66 (0.13)	< 0.001	0.001	< 0.001
AC $T_{1/2}$, h	20.0 (2.9)	16.7 (1.8)	12.2 (1.6)	0.049	**0.300**	0.015
# BM/week	5.1 (0.5)	8.0 (0.7)	15.1 (2.3)	< 0.001	**0.178**	< 0.001
Fasting C4 ng/mL	27.7 (7.9)	9.5 (4.3)	2.6 (1.9)	0.89	0.98	0.72
Δ Fasting C4 on Rx minus pre-Rx, ng/mL	5.9 (5.2)	-2.9 (4.0)	-8.3 (2.3)	0.056	0.12	0.018

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objective

To compare with a randomized trial (n=15 per treatment group), effects of Aldafermin, 1mg and placebo SQ treatment, on bowel functions, hepatic synthesis and fecal excretion of bile acids in patients with diarrhea associated with bile acid malabsorption.

2.2 Study Endpoints

Primary Endpoints

1. Change in fasting serum C4 levels from Baseline to Day 28

2. Change in stool consistency based on the Bristol Stool Form Scale, BSFS from Baseline to Day 28

Secondary Endpoints

1. Stool consistency based on Bristol Stool Form Scale, BSFS
   a. Absolute change in stool consistency from baseline to Day 14 and Day 28 of treatment.

   b. Average daily stool consistency score at Day 14 and Day 28 of treatment

2. Maximum abdominal pain score rated by patient using an 11-point scale, 0 to 10 inclusive with 0 as none and 10 as worst imaginable abdominal pain.

3. Absolute change in abdominal pain from baseline to Day 14 and Day 28 of treatment.

4. Number of bowel movements per week

5. Change in total fecal bile acid concentration between baseline and Day 28.

6. Proportion of fecal secretory (CDCA + DCA) bile acid as measured in a single, random stool sample by a validated laboratory assay.

7. Proportion of fecal primary (CDCA + CA) bile acid as measured in a single, random stool sample by a validated laboratory assay.

8. Total fecal bile acid concentration in a single, random stool sample as measured by a validated laboratory assay.

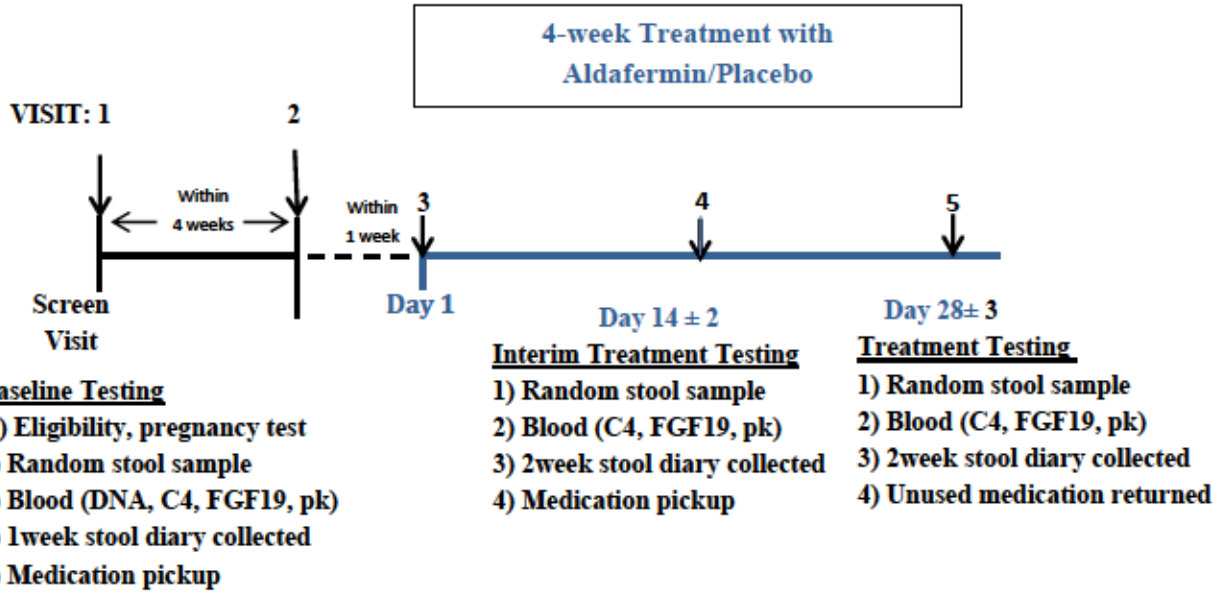
9. Total aldafermin concentration in serum as measured by a validated laboratory assay

3. STUDY DESIGN

3.1 Overview

This single-center, randomized, double-blind, placebo-controlled study is designed toevaluate the effects of aldafermin on daily bowel pattern, fasting serum C4 and FGF-19 and fecal bile acid excretion in a random stool sample in patients with BAM/BAD. The study includes a screening period (up to 28 days) and two 14-day treatment periods for a maximum duration of up to 56 days. Eligible patients will be randomized into 2 blocks, those staying of bile acid sequestrants and those not taking them, and stratified by gender and BMI, we anticipate 90% female and 75% BMI > 30 kg/m^2. A schematic diagram of the study design is shown below.

Aldafermin Study Design



3.1.1 Remote Consent and Eligibility Review (optional)

This is an **optional** prescheduled phone call or research e-consult for patients. The patients will receive a packet at their home containing the study consent form, study questionnaire, the 7-day bowel pattern diary, and a stool kit with instructions on how to collect a single, random stool sample. The patient and a study team member will review the informed consent. Adequate time for questions and discussion will be allowed. The patient can sign, date and time the consent form with either the Electronic Consent Technology-DocuSign or the paper form which needs to be placed in a prepaid addressed envelope to mailed back to the study team member when this review is over. Once the study team receives this signed copy, they will sign the consent form, make a hard copy to be returned to the patient and place a copy in the patient's medical record.

For the eligibility review, the patient will be asked their approximate height and weight and then complete one of the three standard questionnaires with the study team member over the phone call or e-consult. The questionnaire is the Hospital and Anxiety Depression questionnaire (HADs) and included in the protocol appendices. The patient will be asked to sign, date, and place them in the prepaid envelope. The study team member will review the bowel pattern diary with the patient to record in for at least 7 days prior to Visit 3. The stool collection instructions and timing will also be reviewed so the stool collection can be brought in at Visit 3. A study physician will then ask the patient general questions about their current health, past medical history, current medications/supplements and review the patient's questionnaire answers with them. The this point the study physician will discuss eligibility criteria with the patient. If the study physician believes the patient is eligible for the study, prohibited and rescue medications will be discussed with them. If the study physician believes the patient does not meet eligibility criteria, that will also be discussed with the patient. The patient will still be asked to send the paper consent form if you did not use DocuSign and questionnaires back to the study team. even if they do not qualify so the study team can reimburse them for their time.

For patients who have completed this remote consent and eligibility review, the above activities will not be repeated at Visit 1 Screen except their actual height and weight will be measured when they come in for Visit 1.

3.2 Visit 1 Screen

The patient will fast for 12 hours and avoidance of alcohol for 24 hours before the visit if they wish to have their screening safety bloods drawn. The patient and a study team member will review the informed consent. Adequate time for questions and discussion will be allowed. The patient will be given a signed hard copy of the informed consent and a copy will be placed in the patient's electronic medical history. A study physician will review the study's eligibility criteria, the patient's medical history and perform a physical examination. Height, weight and vital signs (pulse, blood pressure, respiration rate and temperature) will be obtained. If the patient does not have a prior laboratory assessment to confirm the presence of BAM/BAD (20-22) , a small volume blood sample will be drawn to test for serum C4. Blood and urine will be obtained for routine safety labs of hematology, chemistry, lipid panel and a dipstick urinalysis. A 12-lead electrocardiogram (ECG) will be performed. The patient will complete three standard questionnaires, the Short Bowel Disease questionnaire (short BDQ), , the Hospital and Anxiety Depression questionnaire and the Quality-of-Life questionnaire; these questionnaires are included in the protocol appendices. The patient will be given a bowel pattern diary to record in for at least 7 days prior to Visit 2; the diary page is included in the protocol appendices. The patient will be given a stool kit to collect a single, random stool sample to bring in at Visit 3.

If a patient elects to withdraw from taking some prohibited medications, a 14-day washout will be required after which the patient may proceed with laboratory tests, bowel pattern diary and stool collection.

Visit 1 activities may be combined with Visit 2 for greater convenience if the patient has not taken any of the prohibited medications within 2 weeks.

3.3 Visit 2 Pre-Randomization

The patient will return after a twelve-hour fast and avoidance of alcohol for 24 hours. Vital signs will be obtained. Blood will be obtained for DNA, C4, FGF-19 and pk analysis. Blood will be obtained for routine safety labs of hematology, chemistry, lipid panel if patient did not have them on Visit 1 Screen. Urine will be obtained for a pregnancy test for females of child-bearing potential.

Visit 2 may be combined with Visit 3 for greater convenience if requested by patient.

3.4 Visit 3 Randomization

Vital signs will be obtained. The patient will be asked about any changes in eligibility criteria, concomitant medications and adverse events. The patient will bring in a single, random stool sample and will be given a new stool collection kit to bring in a sample at the next visit. The patient will return the bowel pattern diary

and receive a new bowel pattern diary. Study medication, either aldafermin or placebo, will be dispensed. A nurse will demonstrate proper subcutaneous injection into the abdomen and observe the patient self-administer the first dose of the study medication. Dosing instructions and potential side effects will be reviewed. Patients will be instructed to self-administer aldafermin/matched placebo as a subcutaneous injection into the abdomen at home at a similar time each day, preferably in the evening, after dinner and before bed, for 4 weeks. Aldafermin/placebo syringes will be equilibrated to room temperature prior to use; this requires about 15 to 30 minutes at room temperature. The aldafermin/placebo syringe must be self-injected within an hour of being removed from the refrigerator. If the syringe is left at room temperature for longer than an hour, it must be discarded into a provided red sharps container and a replacement syringe used. The aldafermin/placebo syringes are provided as kits with four additional syringes. Additional or replacement study medication syringes are available. Patients must record time of dosing in the bowel pattern diary.

3.5 Visit 4 Interim Treatment Testing

About two weeks later, the patient will return after a twelve-hour fast and avoidance of alcohol for 24 hours. The patient will be asked about any changes in eligibility criteria, study medication dosing, concomitant medications and adverse events. Vital signs will be obtained. The patient will turn in any remaining study medication and receive a new supply. The patient will bring in a single, random stool sample and will be given a new stool collection kit to bring in a sample at the next visit. The patient will return the bowel pattern diary and receive a new bowel pattern diary. Blood will be obtained for C4, FGF19 and pk analysis. Urine will be obtained for a pregnancy test for females of child-bearing potential.

3.6 Visit 5 End of Study Testing

About two weeks later, the patient will return after a twelve-hour fast and avoidance of alcohol for 24 hours. The patient will be asked about any changes in eligibility criteria, study medication dosing, concomitant medications and adverse events. Weight and vital signs will be obtained. The patient will have an interview and a brief physical exam by a study physician. The patient will be asked to complete the Quality-of- Life (QoL) questionnaire once more. Blood and urine will be obtained for routine safety labs of hematology, chemistry, lipid panel and a dipstick urinalysis. If there is a clinically significant change in any lab test result, the test will be repeated in 30 days' time to ensure the result has returned to pre-study medication levels. A 12-lead electrocardiogram (ECG) will be performed. The patient will return any remaining study medication. The patient will turn in a single, random stool sample. The patient will return the bowel pattern diary. Blood will be obtained for C4, FGF19 and pk analysis.

3.7 Study Design Rationale

BAM/BAD is increasingly recognized as a subtype of IBS-D (11,27). In an open-label study (12), we identified beneficial effects of colesevelam in the treatment of IBS symptoms in patients with high total fecal BA excretion (> 2000µmoles/48h), elevated primary bile acids (> 4%) with elevated total fecal bile acids (> 1,000 µmol/48h), or primary bile acids > 10% /48h, elevated C4 (> 40 ng/mL), and decreased FGF19 (<80 pg/mL).

4. STUDY POPULATION

4.1 Number of Patients

Approximately 36 patients with BAM/BAD irritable bowel syndrome-diarrhea will be enrolled.

4.2 Inclusion Criteria

Patients must meet ALL of the following inclusion criteria to be eligible for enrollment into thestudy:

1. Aged 18 to 75 years, inclusive at Visit 1 Screen.
2. Clinical diagnosis of functional diarrhea or IBS with diarrhea according to Rome III or IV criteria at Visit 1 Screen.
3. Clinical laboratory evidence of BAM (20-22), with at least one of the following results recorded in their past medical history:
   - Serum C4 ≥ 52 ng/mL
   - Fecal BA > 2337 µmoles / 48 hours
   - Total fecal BA > 1000 µmoles / 48 hours + 4 % primary BA
   - Fecal primary BA > 10% / 48 hours
4. Body mass index (BMI) 18.0 to 45.0 kg/m^2, inclusive at Visit 1 Screen
5. Understands the study procedures, is willing and able to comply with the study procedures, and is able to give informed consent
6. If treated with any of the following medications, dosing must be stable for 30 days prior to Visit 1 Screen. Patient must agree to maintain the same dose of medication throughout the study:
   - Tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs).
   - Bile Acid sequestrants such as colestipol, cholestyramine and colesevelam.
7. Patients must use one highly effective method of contraception for 30 days before the study through 90 days after study completion for males and through 30 days after study completion for females. Highly effective methods of contraception include
   - Oral, implantable, subdermal or injectable hormonal contraceptives
   - Standard intrauterine device or vaginal ring
   - Male or female condoms and diaphragms used with spermicide
   - Abstinence from heterosexual intercourse
   - Female partners exclusively sexually active with a surgically sterilized male partner
   - Females who are surgically sterile prior hysterectomy, bilateral salpingectomy, or bilateral oophorectomy) or postmenopausal (defined as: 12 consecutive months with no menses) are not considered to be of childbearing potential

4.3 Exclusion Criteria

Patients who meet ANY of the following exclusion criteria will not be eligible for enrollmentinto the study:

1. Pregnant or lactating

2. Structural or metabolic diseases/conditions that affect the gastrointestinal system

3. Use of the following medications at least 14 days prior to Visit 1 throughout the duration of the treatment period:

    - Patients may elect to withdraw from bile acid sequestrants such as colestipol, cholestyramine and colesevelam or they may continue but they must continue at the same dose throughout the study.

    - GI medications including:
        - Anti-nausea agents including trimethobenzamide, promethazine, prochlorperazine, dimenhydrinate, hydroxyzine
        - Osmotic laxative agents including lactulose, sorbitol or PEG solutions as Miralax and Glycolax
        - Prokinetic agents including tegaserod, metoclopramide, prucalopride, domperidone, erythromycin, clarithromycin and azithromycin.
    - 5-HT3 antagonists including alosetron, ondansetron, tropisetron.
    - Patients may use Gabapentin if they are on a stable dose, which is not greater than 1200 mg a day.

    - Drugs with a known pharmacological activity at 5-HT4, 5-HT2b or 5-HT3 receptors including tegaserod, ondansetron, granisetron and tropisetron

    - All narcotics including codeine, morphine, and propoxyphene, either alone or in combination
    - Anti-cholinergics including dicyclomine, hyoscyamine, propantheline.
    - Antimuscarinics
    - Tramadol
    - Peppermint oil
    - Systemic antibiotics and antibiotics directed at colonic flora including rifaximin and metronidazole

5. Clinically relevant changes in dietary, lifestyle, or exercise regimen within 30 days prior to Visit 1 Screen and throughout the duration of the study
6. Any colonic or major abdominal surgery including bariatric surgery, gastric banding, stomach

surgery and intestinal or colonic surgery. Procedures such as appendectomy, cholecystectomy, hysterectomy, caesarean section, or polypectomy are allowed as long as they have occurred at least 3 months prior to Visit 1 Screen.

7. History of colorectal cancer, inflammatory bowel disease, diverticulitis, ischemic colitis, microscopic colitis or celiac disease

8. History of organic abnormalities of the GI tract, intestinal obstruction, stricture, toxic megacolon, GI perforation, or impaired intestinal circulation.

9. Other GI diseases such as GI bleeding or ulcerations

10. History of cerebrovascular disease including stroke, TIA, acute coronary syndrome, myocardial infarction or unstable angina

11. Clinically significant cardiac history or presence of electrocardiogram (ECG) findings at Visit 1 Screen:
    1. Abnormal heart rate < 40 or > 100 beats per minute
    2. QTc interval > 470 milliseconds (ms)
    3. QRS interval ≥ 110 ms
    4. PR interval ≥ 220 ms

12. Hepatic dysfunction including abnormal serum alanine aminotransferase [ALT] or aspartate transaminase [AST] > 3 × upper limit of normal [ULN]); total direct bilirubin > 2 × ULN, or alkaline phosphatase > 2 × ULN at Visit 1 Screen

13. Clinically significant renal insufficiency including serum creatinine > 2.5 mg/dL at Visit 1 Screen

14. History of severe head injury or history of seizures

15. History of suicide attempt or a hospitalization for a major psychiatric condition within 1 year prior to Visit 1 Screen. At Visit 1 Screen or during the optional remote consent and eligibility review, participants will complete the Hospital Anxiety and Depression questionnaire. If either score for anxiety or depression individually exceeds 8, the score will be discussed. The patient will be and advised whether to participate or whether to see their primary care physician.

16. History of alcohol use disorder or substance use disorder within 2 years of Visit 1 Screen.

17. Significant history or clinical manifestation of any endocrine, allergic, dermatological, hepatic, renal, hematological, pulmonary, GI, neurological or psychiatric disorder, malignancy (with the exception of treated basal cell carcinomas), or any other condition that would prevent the individual from participating in the study due to risk to the scientific validity of study assessments or to personal well-being of the patient.

18. Participated in another clinical study that includes an investigational drug or a biologic therapy within

30 days or 5 half-lives, whichever time period is longer, prior to Visit 1 Screen

4.4 Patient Withdrawal and Replacement

If a patient is pregnant, the patient will be withdrawn from study treatment immediately.

A patient may be withdrawn for any treatment related adverse event or clinically significant findings in clinical labs or other vital signs that are deemed by the investigator to pose an unacceptable risk to the patient. If the study is interrupted due to any of the criteria listed above, the patient may undergo unscheduled assessments to evaluate his/her status. Additional subsequent visits may be required to assess event outcomes.

Study treatment may be discontinued, interrupted or dose reduced for any grade 3 adverse event that is possibly or probably related to study treatment. Study treatment will be discontinued for any grade 4 adverse event that is possibly or probably related to study treatment

Patients may also be discontinued from the study at any time for any of the following reasons:

- Adverse event
- Death
- Lost to follow-up
- Investigator decision
- Pregnancy
- Protocol deviation
- Study terminated by Sponsor
- Withdrawal by subject

A patient may be withdrawn from the study at any time due to the following adverse events based on the known safety profile and mechanism of the drug:

- Dehydration requiring hospitalization
- A laboratory result of total cholesterol and triglycerides > 2 x ULN
- Allergic reaction requiring hospitalization

A patient may be withdrawn from the study at any time due to the following adverse events:

- A laboratory result of ALT or AST > 3 x ULN, total direct bilirubin > 2 x ULN or serum creatine > 2.5 ULN
- Abnormal cardiac findings such as a clinically significant abnormal heart rate or rhythm, left bundle branch block with QRS interval > 110 msec or first-degree AV block with PR interval > 220 msec.

A patient may be withdrawn by the investigator or the sponsor if enrollment in the study is deemed inappropriate, the study plan is violated, or for administrative and/or safety reasons.

A patient may elect to discontinue study participation at any time for any reason without prejudice to his/her

future medical care by the physician or at the institution. The investigator will document the specific reason why consent was withdrawn.

4.5 Termination of the Clinical Study

The sponsor has the right to terminate this study at any time. Reasons for terminating the studymay include, but are not limited to, the following:

- The incidence or severity of adverse events (AEs) in this or other studies indicates a potential health hazard to subjects. Specifically, the study will be stopped if five or more patients report dehydration requiring hospitalization or allergic reaction requiring hospitalization
- Patient enrollment is unsatisfactory
- Upon request of health or regulatory authorities

The sponsor will notify the investigator if the study is placed on hold or if the sponsor decidesto discontinue the study. Health authorities, regulatory authorities, and Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs) will be informed about the terminationof the study in accordance with applicable regulations.

5. INVESTIGATIONAL MEDICINAL PRODUCT

5.1. Identity of the Investigational Medicinal Products

Study treatments include a pharmaceutical form of the active substance being tested, aldafermin, and a matched placebo being used as a reference.

5.2. Drug Accountability, Dispensing, and Destruction

The study pharmacist will maintain accurate records of receipt of all study treatment materials. In addition, accurate records will be kept regarding when and how much study treatment is dispensed and used by each subject. Reasons for deviation from the expected dispensing regimen also must be recorded. Study treatment will be reconciled. The study pharmacist will provide access to study treatment and study treatment records as required for reconciliation to be timely completed.

At the conclusion of the study, any used and unused study treatment containers will be returned to the sponsor or designee, or destroyed, after data review is complete, as per sponsor instructions. If no supplies remain, this fact will be documented in pharmacy product accountability records.

5.3. Administration of Investigational Medicinal Products

Unblinded pharmacy personnel will be responsible for providing aldafermin or placebo to blinded study personnel for dispensing as per the randomization scheme. Study treatment will be dispensed to subjects in a

blinded manner.

Patients will self-administered study treatment by subcutaneous injection into the abdomen once daily at approximately the same clock time each day during the treatment period. Patients will be counseled on the importance of administering study treatment as instructed and of recording the time of each dose accurately in the patient's bowel pattern diary throughout the treatment period.

5.4. Compliance

As this is a multiple-dose study, treatment compliance will be assessed for each administration of study treatment. Comments will be recorded if there are any deviations from planned study treatment administration procedures.

5.5. Randomization

Patients who complete screening assessments and meet all eligibility criteria will be assigned a unique randomization identification number at the time of study treatment administration. Once a randomization number has been allocated to a subject, it may not be assigned to another patient.

DESIGN: Randomized placebo-control trial based using **BLOCK RANDOMIZATION** so that equal numbers of patients are randomized to placebo and aldafermin 1 mg in **two blocks:**

a. **Patients OFF bile acid sequestrants**
b. **Patients ON bile acid sequestrants.**

Randomized treatment assignment will be considered blinded as per the following: The randomization assignments will be available to the study pharmacy staff that is preparing the study treatment, who will not be involved in any other aspect of the study including administration of the study treatment. Randomization assignments will not be made available to the patients or study staff responsible for the monitoring and evaluation of safety assessments.

In the absence of a medical emergency, the blinded randomization for this study will not be revealed to the Principal Investigator and study team until all data are entered in the database, edit checks are performed, queries closed, and the database is officially locked.

Breaking the blind is expressly forbidden except in the event of a medical emergency where the identity of the study treatment must be known in order to properly treat the patient. In the event of a medical emergency, it is requested that the investigator contact the sponsor prior to breaking the blind. If breaking the blind is required because of a medical emergency, the treatment identity would be revealed by the investigator or designee, for that patient only. Reasons for treatment unblinding must be clearly explained and documented.

5.6. Prior and Concomitant Medications

Any medication (including over-the-counter or prescription medicines, vitamins, and/or herbalsupplements) or vaccines that a patient uses from 30 days prior to Visit 1 Screen throughout the study must be recorded along with reason for use, dose and frequency and dates of use.

5.7. Rescue Medication

Because the study includes placebo treatment and is approximately 56 days long, loperamide may be used as a rescue medication **except** within 48 hours of a stool collection. The maximum dose of loperamide allowed is 4 mg in a 24-hour period. The use of loperamide as a rescue medication must be recorded in the bowel pattern diary.

6. STUDY ASSESSMENTS AND PROCEDURES

6.1. Screening and Eligibility

Patient eligibility will be assessed based on protocol inclusion and exclusion criteria. All screening evaluations must be completed and reviewed to confirm potential patients meet all eligibility criteria.

Screening procedures must be completed within 28 days prior to initiation of study medication.

At the Visit 1 Screen or during the optional remote consent and eligibility review, potential patients will have a detailed verbal presentation of the nature, purpose, risks, and requirements of the study in addition to receiving detailed written information. Patients will have adequate opportunity to ask the appropriate person of the study team (ie, Principal Investigator or designee) about any aspect of the study. If a patient decides that he/she is willing to participate in the study, he/she will be asked to sign the informed consent form. The clinical personnel obtaining written consent from the patient will also sign the form to confirm consent has been obtained. Once signed, the original consent will be maintained for the study records and a signed copy given to the patient. A copy will be scanned into the patient's electronic medical record. The investigator will verify that informed consent has been obtained from each patient prior to the patient undergoing any study-related procedures.

Visit 1 Screen consists of:

- Complete medical history
- Physical examination, including height, weight, and vital signs (heart rate, blood pressure, temperature and respiration rate)
- Safety 12-lead ECG
- Clinical laboratory tests: The standard Comprehensive Metabolic Panel which includes calcium, creatinine, sodium, potassium, albumin, alkaline phosphatase, total bilirubin, carbon dioxide, chloride, glucose, total protein, BUN, AST, and ALT. Hematology includes a CBC with differential. A lipid panel including total cholesterol, HDL, LDL and triglycerides will be performed. Analysis of the urine will be completed by a standard urine dipstick method with microscopy.

- Urine human chorionic gonadotropin (hCG) pregnancy test (for females of childbearing potential)

6.2. Adverse Event

An AE is any untoward medical occurrence that does not necessarily have a causal relationship with the study treatment. An AE can therefore be any unfavorable and unintended sign, symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Adverse events can include, but are not limited to, any of the following:

- Unfavorable changes in general condition
- Subjective or objective signs/symptoms
- Concomitant disease or accidents
- Clinically relevant adverse changes in laboratory parameters over the course of thestudy
- Pre-existing conditions that worsen in severity, increase in frequency, or have new signs/symptoms

6.3 Serious Adverse Event

An AE should be classified as an SAE if it meets one of the following criteria:

Fatal:	The AE resulted in death.
Life-threatening:	The AE placed the subject at immediate risk of death. This classification does not apply to an AE that hypothetically might cause death if it were more severe.
Hospitalization:	The AE required or prolonged an existing inpatient hospitalization. Hospitalizations for elective medical or surgical procedures or treatments planned before the signing of informed consent in the study or routine check-ups are not SAEs by this definition.
Disabling/ incapacitating:	The AE resulted in a persistent or significant incapacity or substantial disruption of the subject's ability to conduct normal life functions.
Congenital anomaly or birth defect:	An adverse outcome in a child or fetus of a subject exposed to the study treatment before conception or during pregnancy.

Medically significant:	The AE did not meet any of the above criteria but could have jeopardized the patient and might have required medical or surgical intervention to prevent one of the outcomes listed above or involves suspected transmission via a medicinal product of an infectious agent.

The Principal Investigator will promptly review all information relevant to the safety of the investigational drug and notify the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but no later than 7 calendar days after receipt of the information. The Principal Investigator will also notify the FDA in an IND safety report of potential serious risks, as soon as possible, but in no case later than 15 calendar days after the Principal Investigator determines that the information qualifies for reporting.

Elective hospitalization and/or surgery for clearly preexisting conditions (i.e., a surgery that has been scheduled prior to the patient's entry into the study) will not be reported as an SAE. All other hospitalizations, including elective hospitalizations for any condition that was not preexisting, will be reported as an SAE.

Any SAE that is ongoing when the patient completes the study or discontinues the study will befollowed by the Investigator until the event resolves, stabilizes, or returns to baseline status.

6.4 Adverse Event Severity

The severity of each AE will be assessed at the onset by a physician. When recording the outcome of the AE the maximum severity of the AE experienced will also be recorded. The severity of each AE will be graded according to the Common Terminology Criteria for AdverseEvents (CTCAE) version 5.0:

Grade 1:	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2:	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling, limiting self-care activities of daily living (i.e., preparing meals, shopping for groceriesor clothes, using the telephone, managing money).
Grade 4:	Life-threatening consequences, urgent intervention indicated.
Grade 5:	Death related to AE

6.5 Adverse Event Relationship

The investigator is obligated to assess the causal relationship between the study treatment and each occurrence of an AE. The AE causal relationship to study treatment must be characterized as one of the following categories:

Not Related:	The AE does not follow a reasonable temporal sequence from administration of the study treatment, does not abate upon discontinuation of the study treatment, does not follow a known or hypothesized cause-effect relationship, and (if applicable) does not reappear when the study treatment is reintroduced, furthermore, there may exist a clear alternative medical explanation (e.g., underlying disease state) or association with study procedure or conduct.
Unlikely Related:	The temporal association between the AE and the study treatment is such that the study treatment is not likely to have any reasonable association with the AE.
Possibly Related:	The AE follows a reasonable temporal sequence from administration of the study treatment and follows a known or expected response to the study treatment but could have also been produced by a number of other factors.
Probably Related:	The AE follows a reasonable temporal sequence from administration of the study treatment and cannot be reasonably explained by the known characteristics of the patient's clinical state, environmental, or toxic factors, orother modes of therapy administered to the patient.
Related:	The AE follows a reasonable temporal sequence from administration of the study treatment, abates upon discontinuation of the study treatment, follows a known or hypothesized cause-effect relationship, and (if appropriate) reappears when the study treatment is reintroduced.

The Principal Investigator will use clinical judgment to determine the causal relationship. Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, aswell as the temporal relationship of the event to the study treatment administration should be considered and investigated. The Principal Investigator should consult the IB and the Product Information of marketed products within the study treatment class, when applicable. For each AE, the Principal Investigator must document in the medical notes that he/she has reviewed the AE and has provided an assessment of causality. There may be situations in which an SAE has occurred, and the Principal Investigator has minimal information to include in the initial report; however,the Principal Investigator should always make an initial assessment of causality for every event. The Principal Investigator may change his/her opinion of causality based on subsequent receipt of information and send an SAE follow-up report with theupdated causality assessment. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Any SAE that is ongoing when the subject completes the study or discontinues the study will be followed by the Principal Investigator until the event resolves, stabilizes, or returns to baseline status.

6.6 Clinical Laboratory Tests

Clinical safety laboratory tests will be performed at the study site at Visit 1 Screen or Visit 2 and Visit 5 End of Study. These tests include the Comprehensive Metabolic Panel, calcium, creatinine, sodium, potassium,

albumin, alkaline phosphatase, total bilirubin, carbon dioxide, chloride, glucose, total protein, BUN, AST, and ALT. Hematology includes a CBC with differential. A lipid panel including total cholesterol, HDL, LDL and triglycerides will be performed. Analysis of the urine will be completed by a standard urine dipstick method with microscopy. The investigator will the laboratory report, document the review, and record any clinically relevant changes as AEs.

6.7 Electrocardiograms

Single 12-lead ECGs will be recorded with subjects in a supine position at Visit 1 Screen and Visit 5 End of Study. Additional ECGs may be taken at any other times, if deemed necessary by the investigator. All ECG tracings will be reviewed by the Principal Investigator or designee.

6.8 Assessment of Abdominal Pain, Stool Frequency and Consistency

Patients will complete a daily bowel pattern diary throughout the study to record and assess their most severe abdominal pain on an 11-point scale and timing of each bowel movement, consistency based on Bristol stool form scale, ease of passage, and sense of completeness of evacuation. Ease of stool passage was also assessed based on a 7-point scale (1: manual disimpaction; 2: enema needed; 3: straining needed; 4: normal; 5: urgent w/o pain; 6: urgent w/pain; 7: incontinent). The Bristol stool scale and a sample diary page are included in the protocol appendices.

6.9 Assessment of Bile Acid Kinetics

Assessment of BA synthesis and fecal excretion (uniquely available in USA at Mayo Clinic):

Serum 7α-hydroxy-4-cholesten-3-one (7α-HCO or C4) measures hepatic cholesterol synthesis and is a validated method for BAM/BAD (18,19). Serum C4 (ref. 20, assay based on ref. 21) is positively correlated with fecal BA excretion [$r_s = 0.61$, $p<0.001$ (4)].

Serum fibroblast growth factor 19 (FGF19), a measure of feedback regulation of BA synthesis (22), will be measured by ELISA (R&D Systems, Minneapolis, MN).

Fecal BA excretion: Using HPLC/tandem mass spectrometry, we adapted a method (23) to measure fecal total and individual BAs in a RANDOM stool sample. We have previously shown that fecal total BA (4), and secretory primary and secondary BAs [chenodeoxycholic (CDCA) and deoxycholic acids (DCA)] are higher in IBS-D, whereas CDCA is decreased and non-secretory lithocholic acid increased in IBS-C (24)]. Total fecal bile acids and primary CDCA will be measured.

6.10 Assessment of Aldafermin Pharmacokinetics

Trough concentrations of aldafermin will be determined by a validated PK laboratory assay performed at a bioanalytical CRO, Frontier Laboratories, using serum samples obtained at baseline, Day 14 and Day 28.

Anonymized serum samples will be provided for pk analysis. The samples will be labelled with a de-identified study number that cannot be linked to the patient's identity without use of a key which will be kept in a password-protected secure server at Mayo accessible only to the study team.

Samples will be stored at Frontier Laboratories only until the pk analysis is performed which must be done in a reasonable amount of time. After performing the pk analysis, Frontier Laboratories will discard the samples.

7. STATISTICAL CONSIDERATIONS

7.1 Sample Size and Statistical Power

Based on the endpoints of interest in patients with bile acid diarrhea [12], the proposed sample sizes have ~80% power (2-sided α=0.05) to detect clinically relevant effect sizes in serum C4 and stool form (expressed as detectable difference [Δ] in 2 groups:

Table 1: Detectable differences based on 80% power in patients with BAD ***not*** receiving BA sequestrant

Response	Mean (SD)	Detectable Δabsolute with n=9 per group
Serum C4 ng/mL	72.3 (45.6)	64.1 (1.34*SD or 89 % of baseline mean)
% fecal primary BA	15.7 (20)	28.1 (1.79*SD or 179 % of baseline mean)
# BMs per week	15.1 (7.1)	10.0 (1.41*SD or 66 % of baseline mean)
Stool form (BSFS 1-7 scale)	4.4 (1.12)	1.58 (1.41*SD or 35.9 % of baseline mean)

Based on our previous open-label study demonstrating effects of a bile acid sequestrant (colesevelam) as a typical baseline results for patients on bile acid sequestrants:

Table 2: Detectable differences based on 80% power in patients with BAD receiving BA sequestrant

Response	Mean (SD)	Detectable Δabsolute with n=6 per group
Serum C4 ng/mL	112.5 (50.6)	90.8 (1.79*SD or 80.7 % of baseline mean)
% fecal primary BA	2.7 (7.4)	13.3 (1.80*SD or 492% of baseline mean)
# BMs per week	21.7 (7.1)	12.75 (1.80*SD or 58.8 % of baseline mean)
Stool form (BSFS 1-7 scale)	4.6 (0.6)	1.08 (1.80*SD or 23.5% of baseline mean)

7.2 Statistical Analysis

Effects of treatment will be compared using Intent-to-Treat principles using ANCOVA with BMI, gender and baseline fasting C4 as covariates. In order to reach 30 subjects, accrual will be set at 36 to replace any volunteer(s) who withdraw prior to taking less than 1 day of dosing. Any Subject who receives more than 1

day of dosing will be consider completed using the Intent-to-Treat analysis.

Primary endpoints are change in fasting serum C4 levels from baseline to Day 28 and change in stool consistency based on Bristol Stool Form Scale, BSFS from baseline to Day 28.

Secondary endpoints are

- Absolute change in stool consistency from baseline to Day 14 and Day 28 of treatment
- Average daily stool consistency score at Day 14 and Day 28 of treatment
- Maximum abdominal pain score rated by patient using an 11-point scale, 0 to 10 inclusive with 0 as none and 10 as worst imaginable pain
- Absolute change in abdominal pain from baseline to Day 14 and Day 28 of treatment
- Number of bowel movements per week
- Change in total fecal bile acid concentration between baseline and Day 28
- Proportion of fecal secretory (CDCA+ DCA) bile acid as measured in a single, random stool sample by a validated laboratory assay.
- Proportion of fecal primary (CDCA + CA) bile acid as measured in a single, random stool sample by a validated laboratory assay.
- Total fecal bile acid concentration in a single, random stool sample as measured by a validated laboratory assay.
- Total aldafermin concentration in serum as measured by a validated laboratory assay

Adverse events (AEs) will be listed and summarized by system organ class and preferred term, as well as according to severity and causality/relationship to study treatment.

Observed values for clinical laboratory tests, vital signs and safety 12-lead ECGs will be summarized by treatment. Observed values will be classified as normal, abnormality that is not clinically significant, and clinically significant abnormality by treatment.

8. ETHICAL CONSIDERATIONS

8.1. Ethical Conduct of the Study

This study will be conducted in accordance with the ethical principles that have their origin inthe Declaration of Helsinki and that are consistent with GCP, International Council for Harmonisation (ICH) guidelines, and other applicable regulatory requirements (i.e., local requirements).

8.2. Institutional Review Board Approval

Before initiating a study, the Principal Investigator must have written and dated approval from the IRB/IEC for the study protocol, written ICF, subject recruitment materials and procedures (i.e., advertisements or websites), and any other written information to be provided to subjects.

Approval from the committee must be documented in a letter to the Principal Investigator specifying the

protocol number, protocol version, documents reviewed, and the date on which the committee met and granted the approval.

All documents subject to review during the study, including any modifications made to the protocol after receipt of IRB approval, must also be submitted to the committee for approval prior to implementation. The Principal Investigator must also provide periodic reports as required and promptly report important safety information (ie, SAEs) and protocol violations, as appropriate, to the IRB.

As part of the Principal Investigator's written application to the IRB, he should provide the committee with a current copy of the IB. If the IB is updated during the study, the Investigator should supply an updated copy to the committee.

8.3 Designation of an Independent Monitor

Dr. [redacted] PhD (Mayo Clinic) will serve as independent monitor to perform an independent review of ongoing safety. *In accordance with IRB review, we shall ask Dr. [redacted] to constitute an independent DSMB if required.*

As a sponsor-investigator study, the study will be monitored annually by the Mayo Office of Research Regulatory Support.

8.4 Safety Review Plan

Study progress and safety will be reviewed quarterly (and more frequently, if needed). Progress reports including patient recruitment, retention/attrition, and AEs will be provided to the Independent Monitor every year. An annual report will be compiled and will include a list and summarization of AEs. In addition, the annual report will address:

1. Whether AE rates are consistent with pre-study assumptions;
2. Reason for dropouts from the study;
3. Whether all participants met entry criteria;
4. Whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and
5. Conditions whereby the study might be terminated prematurely (i.e.,, voluntary dropout of > 40% of cohort).

The annual report will be reviewed by the independent monitor and will be forwarded to the IRB on an annual basis.

8.5 Informed Consent

The investigator will fully inform the patient of all pertinent aspects of the study, including the approval of the

study by the IRB. Before informed consent may be obtained, the investigator or a designee should provide the patient ample time and opportunity to inquire about details of the study and to decide whether or not to participate.

Prior to a patient's participation in the study, the IRB-approved ICF must be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

The written ICF and any other written information to be provided to patients should be revised whenever important new information becomes available that may be relevant to the patient's consent. Any revised written ICF or study materials to be available and/or supplied to patients should receive the IRB's approval in advance of use. The patient will be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the study. The communication of this information shouldbe documented.

8.6 Confidentiality

All information generated in this study is considered highly confidential and must not be disclosed to any person or entity not directly involved with the study.

Prior to study participation, the investigator shall inform the patient that the monitor(s), sponsorrepresentatives, auditor(s), IRB, and the regulatory authorities will be granted direct access to the patient's original medical records for verification of clinical study procedures and/or data,and that, by signing a written ICF, the patient is authorizing such access.

In addition, prior to study participation, the patient must be informed that the records identifyingthe patient will not be made publicly available; if the results of the study are published, the patient's identity will remain confidential.

8.7 Protocol Compliance

The Principal Investigator will conduct the study in compliance with the protocol agreed to by the regulatory authorities (if applicable) and that was approved by the IRB.

The Principal Investigator should not implement any deviation from, or changes to, the protocol without prior review and documented approval from the IRB of an amendment, except where necessary to eliminate immediate hazard(s) to study patients or when the change involves only logistical or administrative aspects of the study (i.e., change in monitor, change of telephone number).

When an important deviation from the protocol is deemed necessary for an individual patient, the Principal Investigator must determine the impact of the deviation on the patient's participation and/or the assessment of safety or efficacy in the study. Any significant protocol deviations affecting patient eligibility and/or safety must be reported by the Principal Investigator or site delegate to the IRB and regulatory authorities, as applicable, prior to implementation.

The Principal Investigator should document and explain any deviation from the approved protocol.

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APPENDIX I : BOWEL PATTERN DIARY

Daily Diary: Day 1

Date ☐☐☐☐☐☐

If you had no bowel movements today, please check this box: ☐

Please rate your most severe abdominal pain over the last 24 hours, on a ten-point scale with 0 = no pain, 10 = worst imaginable pain _____

Please record the time the study medication was injected _____:_____ *pm*

	Describe the consistency of bowel movement	Describe the ease of passage of bowel movement	Did you feel like you completely emptied your bowels?
	1 Hard lumps 2 Lumpy sausage 3 Cracked sausage 4 Smooth sausage 5 Soft lumps 6 Mushy 7 watery	1 Manual Disimpaction 2 Enema needed 3 Straining needed 4 Normal 5 Urgent w o/pain 6 Urgent w /pain 7 Incontinent	1 No 2 Yes
1 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐
2 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐
3 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐
4 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐
5 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐
6 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐
7 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐

Any unusual negative events today? **No** ☐ **Yes** ☐ *(complete below)*

Event _____ Mild / Moderate / Severe Resolved / Ongoing
Event _____ Mild / Moderate / Severe Resolved / Ongoing
Event _____ Mild / Moderate / Severe Resolved / Ongoing

Taken any medications other than those you routinely use? **No** ☐ **Yes** ☐ *(complete below)*

Medication _____ Medication _____
Dose _____ Dose _____
Time(s) _____ Time(s) _____

APPENDIX II : SHORT BOWEL DISEASE QUESTIONNAIRE

In the past 12 months, have you experienced the following?

QUESTION	YES	NO
1. 2 or less than 2 bowel movements/week		
2. Excessive straining or sensation of incomplete evacuation of stool on more than 25% of occasions		
3. Lumpy stools on more than 25% of occasions		
At least 3 months of continuous or recurrent symptoms of:		
4. Abdominal pain or discomfort relieved by defecation		
5. Abdominal pain or discomfort associated with a change in stool frequency		
6. Abdominal pain or discomfort associated with a change in stool consistency		
7. More than 3 bowel movements per day		
8. Loose watery stools		
9. Bloating		
10. Swallowing difficulties		
11. Upper abdominal pain after meals more than once a month		
12. Abdominal bloating after meals		
13. Nausea regularly more than once a month		
14. Vomiting regularly more than once a month		
15. Heartburn regularly more than once a week		
16. Acid reflux regularly more than once a week		

Sign_____
Date _____

APPENDIX III : HOSPITAL and ANXIETY DEPRESSION QUESTIONNAIRE

Please read each item and **circle** the reply which best describes how you have been feeling during the past week. Don't devote too much time to your responses; your immediate reaction will probably be more accurate than a long thought-out response.

1. I feel tense or 'wound up' :

   Most of the time
   A lot of the time
   Occasionally
   Not at all

2. I still enjoy the things I used to enjoy :

   Definitely as much
   Not quite so much
   Only a little
   Hardly at all

3. I get a frightened feeling, as if something awful is about to happen :

   Very definitely and quite badly
   Yes, but not too badly
   A little, but it doesn't worry me
   Not at all

4. I can laugh and see the funny side of things :

   As much as I always could
   Not quite so much now
   Definitely not so much now
   Not at all

5. Worrying thoughts go through my mind :

   A great deal of the time
   A lot of the time
   From time to time
   Only occasionally

6. I feel cheerful :

   Not at all
   Not often
   Sometimes
   Most of the time

7. I can sit at ease and feel relaxed :

   Definitely
   Usually
   Not often
   Not at all

8. I feel as if I am slowed down :

   Nearly all the time
   Very often
   Sometimes
   Not at all

9. I get a frightened feeling, like 'butterflies in the stomach' :

   Not at all
   Occasionally
   Quite often
   Very often

10. I have lost interest in my appearance :

    Definitely
    I don't take as much care as I should
    I may not take quite as much care
    I take just as much care as ever

11. I feel restless as if I have to be on the move :

    Very much indeed
    Quite a lot
    Not very much
    Not at all

12. I look forward with enjoyment to things :

    As much as I ever did
    Rather less than I used to
    Definitely less than I used to
    Hardly at all

13. I get sudden feelings of panic :

    Very often indeed
    Quite often
    Not very often
    Not at all

14. I can enjoy a good book or TV program :

    Often
    Sometimes
    Not often
    Very seldom

APPENDIX IV : QUALITY of LIFE QUESTIONNAIRE

Quality of Life Questionnaire

PLEASE READ THIS CAREFULLY

ON THE FOLLOWING PAGES YOU WILL FIND STATEMENTS CONCERNING BOWEL PROBLEMS (IRRITABLE BOWEL SYNDROME) AND HOW THEY AFFECT YOU.

FOR EACH STATEMENT, PLEASE CHOOSE THE RESPONSE THAT APPLIES BEST TO YOU AND **CIRCLE** THE NUMBER OF YOUR RESPONSE.

IF YOU ARE UNSURE ABOUT HOW TO RESPOND TO A STATEMENT, PLEASE GIVE THE BEST RESPONSE YOU CAN. **THERE ARE NO RIGHT OR WRONG RESPONSES.**

About how you feel

Please think about your life over the **past month (last 30 days)** and look at the statements below. Each statement has five different responses. For each statement, please circle the response that best describes your feelings.

Q1. I feel helpless because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q2. I am embarrassed by the smell caused by my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q3. I am bothered by how much time I spend on the toilet. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q4. I feel vulnerable to other illnesses because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q5. I feel fat/bloated because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q6. I feel like I'm losing control of my life because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q7. I feel my life is less enjoyable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY

4 QUITE A BIT
5 A GREAT DEAL

Q8. I feel uncomfortable when I talk about my bowel problems. *(Please circle one number)*
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q9. I feel depressed about my bowel problems. *(Please circle one number)*
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q10. I feel isolated from others because of my bowel problems. *(Please circle one number)*
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q11. I have to watch the amount of food I eat because of my bowel problems. *(Please circle one number)*
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q12. Because of my bowel problems, sexual activity is difficult for me. *(Please circle one number)*
(If not applicable, please circle "NOT AT ALL")
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q13. I feel angry that I have bowel problems. *(Please circle one number)*
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q14. I feel like I irritate others because of my bowel problems. *(Please circle one number)*
1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q15. I worry that my bowel problems will get worse. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q16. I feel irritable because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q17. I worry that people think I exaggerate my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q18. I feel I get less done because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q19. I have to avoid stressful situations because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q20. My bowel problems reduce my sexual desire. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q21. My bowel problems limit what I can wear. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q22. I have to avoid strenuous activity because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY

4 QUITE A BIT
5 A GREAT DEAL

Q23. I have to watch the kind of food I eat because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q24. Because of my bowel problems, I have difficulty being around people I do not know well. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q25. I feel sluggish because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q26. I feel unclean because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q27. Long trips are difficult for me because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q28. I feel frustrated that I cannot eat when I want because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q29. It is important to be near a toilet because of my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY

Q30. My life revolves around my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q31. I worry about losing control of my bowels. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q32. I fear that I won't be able to have a bowel movement. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q33. My bowel problems are affecting my closest relationships. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 A GREAT DEAL

Q34. I feel that no one understands my bowel problems. *(Please circle one number)*

1 NOT AT ALL
2 SLIGHTLY
3 MODERATELY
4 QUITE A BIT
5 EXTREMELY