Evaluation of the EfficAcy and Safety of SinglE, Daily Oral Doses of SYN-010 Compared to Placebo in Adult Patients with Irritable Bowel Syndrome with Constipation (EASE-DO)

Medically Associated Science and Technology (MAST) Program
Cedars-Sinai Medical Center
Los Angeles, CA

Investigator Sponsored Research (ISS) Protocol

Product Name: SYN-010

Chemical Name: Lovastatin Lactone Modified Release Capsules

USAN (United States Adopted Name): Lovastatin

Chemical formula: 1S-[1α(R*),3α,7α,8α(2S*,4S*),8aα]]-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl]-1-naphthalenyl 2-methylbutanoate ($C_{24}H_{36}O_5$)

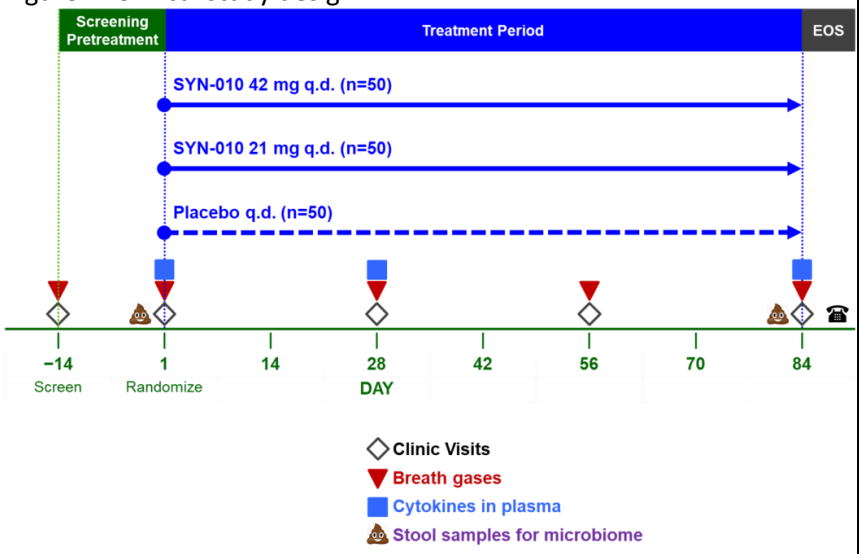
NCT03763175

Table of Contents

1. Project summary

Compound No.	
Name of Active Ingredient of Investigational Agent **Route of Administration**	SYN-010 lovastatin lactone modified release capsules 21mg given orally once daily 42mg given orally once daily
Title of Study	Evaluation of the EfficAcy and Safety of SinglE, Daily Oral Doses of SYN-010 Compared to Placebo in Adult Patients with Irritable Bowel Syndrome with Constipation (EASE-DO)
Study Center	Cedars-Sinai Medical Center 8700 Beverly Boulevard Los Angeles, CA 90048
Phase of Clinical Development	Phase II
Indication	Irritable Bowel Syndrome with Constipation (IBS-C)
Control Agent **Name of Active Ingredient of Control Agent** **Route of Administration**	Reference product is Placebo in matching presentation to be given orally once-daily.
Clinical Study Phase	Phase II
Study Objectives	Primary: Change from baseline in the weekly average number of CSBMs during the 12-week Treatment Period for SYN-010 relative to placebo. Secondary: Safety and tolerability of SYN-010 21 mg and 42 mg in IBS-C patients treated for 12 weeks. For SYN-010 relative to placebo: • Proportion of overall stool frequency responders. • Proportion of overall bloating responders. • Proportion of patients using rescue medication. • Proportion of overall responders in the SYN-010 21 mg and 42 mg treatment groups versus placebo. • Change from baseline for SYN-010, relative to placebo, in: o Weekly average score for worst abdominal pain at Weeks 1 through 12. o Weekly average number of CSBMs at Weeks 1 through 12. o Weekly average number of SBMs at Weeks 1 through 12. o Weekly average bloating score at Weeks 1 through 12. o Weekly average stool consistency (BSFS) at Weeks 1 through 12. Exploratory: For SYN-010 relative to placebo: • Proportion of patients with adequate relief.

	• Change from baseline for SYN-010 in patient reported outcomes (PRO) using validated questionnaires in the REDCap system.
	• Breath gas area-under-the-curve (AUC) (methane, hydrogen, and hydrogen sulfide) on lactulose breath test at EOS compared to Screening.
	• Spot breath tests at Randomization, Day 28, and Day 56.
	• Comparison of serum cytokine levels at baseline and EOS – measuring for potential immune or inflammatory signals to include the key cytokines of the Th1/Th2/Th17 pathways: IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17, GM-CSF and IFN-γ. Plus TNF-α, IL-12, IL-6, the two chemokines IL-8 and MCP-1, and the gut cytokine IL-25.
	• Microbiome measurements in stool:
	  ○ Bacteria (16S rRNA)
	  ○ Methanogens (qPCR)

Study Design	Single-center, randomized, double-blind, placebo-controlled clinical trial comprising three periods (Figure 1): 1. Screening and Pre-treatment Period (up to 17 days prior to the first dose of the study drug), 2. Treatment Period comprising Randomization (Day 1) and 12 weeks of dosing (with clinic visits at Weeks 4, 8, and 12), and 3. End-of-Study contact between 24 and 48 hours after Week 12 for patients who complete the Treatment Period. Figure 1: Clinical study design 
Main Inclusion Criteria	1. Male or female participants aged between 18 and 65 years inclusive.

	2. Patient must be willing and able to participate in the study for the required duration, understand and sign the informed consent form (ICF), and be willing to comply with all protocol-related visits and procedures. 3. Patient has had IBS-C symptoms (as defined by Rome IV diagnostic criteria) for at least 6 months prior to diagnosis. 4. Patient has an average score of ≥ 3.0 for daily abdominal pain at its worst (11-point numerical rating scale [NRS]) during and up to the 17 days immediately before randomization (i.e. Pre-treatment Period). 5. Patient has an average of ≤ 3 CSBMs per week or ≤ 5 SBMs per week during the 17 days immediately before randomization (i.e. Pre-treatment Period). 6. Patient has a breath methane level ≥ 10 ppm on a lactulose breath test administered at Screening. 7. Patient may be on a stable, continuous regimen of fiber or probiotics one month before the Screening Visit; however, they must maintain a stable dose regimen through Week 12. 8. Patient must agree to refrain from starting a new diet, changing stable dose of supplemental fiber, or changing exercise pattern that may affect IBS-C symptoms from the time of Screening through the end of the study. If the patient takes food products that are strong inhibitors of cytochrome P450 3A (CYP3A) (e.g. grapefruit juice, Seville orange juice, St. John's Wort), he/she must agree to refrain from taking these from the time of Screening through the end of the study. 9. Patient must agree to use an acceptable method of contraception from the time of signing the ICF to 30 days after the final dose of study drug if the patient is a sexually active female of child-bearing potential (defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal. Postmenopausal is defined as 12 consecutive months with no menses without an alternative medical cause). Adequate contraceptive measures include: oral contraceptives (stable use for two or more cycles before Screening); intrauterine device; Depo-Provera®; Norplant® System implants; partner with a vasectomy; double-barrier birth control (e.g. use of a condom plus diaphragm or condom plus either contraceptive sponge, foam, or jelly); or abstinence. According to drug research standards, male patient must agree to use an acceptable method of contraception and refrain from donating sperm from the time of signing the ICF to 90 days after the final dose of study drug.

	10. A female patient of child-bearing potential must be non-pregnant and non-lactating and have negative pregnancy tests at the Screening Visit and on Day 1 prior to dosing with study drug. 11. Patients must be able to understand and be willing to sign the written informed consent form. A signed informed consent form must be appropriately obtained prior to the conduct of any trial-specific procedure. 12. Willing and able to comply with the protocol, including follow-up visits and examinations.
Main Exclusion Criteria	1. Patient has loose (mushy) or watery stools for > 25% of their bowel movements (BMs) during the 12 weeks before Screening or during the Screening and Pre-treatment Periods. 2. Patient has a history of cathartic colon, laxative, or enema abuse. 3. Patient has a history of ischemic colitis. 4. Patient has a history of pelvic floor dysfunction. 5. Patient has a history of bariatric surgery for the treatment of obesity. 6. Patient has a history of surgery to remove a segment of the gastrointestinal (GI) tract at any time before the Screening Visit. 7. Patient has any history of myopathy, rhabdomyolysis, chronic myalgia, or familial history of hereditary muscular disorders. 8. Patient has been diagnosed with or has a family history of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or any other form of familial colorectal cancer. 9. Patient currently has any structural abnormality of the GI tract or a disease or condition that can affect GI motility, or any unexplained and clinically significant symptoms such as lower GI bleeding, rectal bleeding, heme-positive stool, iron-deficiency anemia, weight loss, or systemic signs of infection. 10. Patient has had any previous surgery involving the abdomen, pelvis, or retroperitoneal region during the last 12 months prior to Screening, with the exclusion of laparoscopic gallbladder surgery or appendix removal. 11. Patient has a history of diverticulitis or any chronic condition that could be associated with abdominal pain or discomfort and could confound the assessments in the study (e.g. inflammatory bowel disease, chronic pancreatitis, polycystic kidney disease, ovarian cysts, endometriosis, or lactose intolerance).

	12. Patient has history of severe renal insufficiency defined as an actual or estimated glomerular filtration rate of < 30 mL/min/1.73 m^2 within the 6 months prior to Screening Visit. 13. Patient has any history of a medical condition or a concomitant medical condition that, in the opinion of the investigator, would compromise the patient's ability to complete the study safely or could confound the assessments in this study (e.g. uncontrolled hypothyroidism). 14. Patient is known to have elevated liver enzyme levels (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) or creatine kinase levels that are ≥ 1.5 times the upper limit of normal (ULN) that have not been resolved within the 4 weeks prior to consent and/or these elevated levels are present at the Screening Visit laboratory assessment. 15. Patient has any abnormal laboratory results, electrocardiogram (ECG) findings, or physical examination findings deemed clinically significant by the investigator during the Screening Period. 16. Within 14 days prior to the Screening Visit, patient has used concomitant medications that are: (1) moderate-to-strong inhibitors of cytochrome P450 3A (CYP3A) and/or organ anion transporting polypeptide (OATP)1B1 (e.g. cyclosporine, verapamil, dronedarone, diltiazem, amiodarone, itraconazole, ketoconazole, posaconazole, voriconazole, clarithromycin, telithromycin, human immunodeficiency virus protease inhibitors, boceprevir, telaprevir, nefazodone, erythromycin, cobicistat-containing products); (2) other concomitant medications that are excluded from the lovastatin label (e.g. rifampin, colchicine, ranolazine); or (3) metformin or GLP-1 agonists. Patients should not take any of these concomitant medications during the treatment phase of the study without contacting the investigator. 17. Patient has hypersensitivity to statins; or has used any statins, fibrates, > 1 g/day of niacin, or gemfibrozil within the 3 months prior to the Screening Visit. 18. Patient reports current chronic or frequent use of drugs known to cause constipation (e.g. narcotics) for the 3 months prior to Screening. 19. Patient has taken over-the-counter IBS treatments (e.g. laxatives) or proton pump inhibitors within 3 days prior to the Screening Visit. 20. Certain drugs used for the treatment of IBS (e.g. low dose tricyclic antidepressants) may be allowed at the discretion of the Medical Monitor provided the patient remains on a stable dose for one month prior to the Screening Visit and throughout the study with the exception of tegaserod, lubiprostone,

	linaclotide, metoclopramide, prucalopride, domperidone, plecanatide, CandiBactin, Atrantil, Allimax/Allimed within 2 weeks prior to the Screening Visit or antibiotics within 2 months prior to the Screening Visit. 21. Patient has used an opioid chronically or frequently within the 3 months prior to the Screening Visit and for the duration of the study. 22. Patient is currently enrolled in, or plans to enroll in, another clinical study or has used any investigational drug or device within 1 month before signing the ICF through the completion of the study. 23. Patient has previously participated in a SYN-010 study. 24. Patient has a history of alcohol or drug abuse within the 12 months prior to the Screening Visit.
Additional Screening and Inclusion	During the Screening and Pre-treatment Period, patients will be provided an ID number and password-protected access to the REDCap website to record Baseline parameters (such as weekly average worst abdominal pain score and weekly number of CSBMs). During this period, patients will also (i) complete a lactulose breath test including analysis of methane, hydrogen, hydrogen sulfide and carbon dioxide; (ii) provide a stool sample for analysis of the microbiome; and (iii) provide a blood sample for analysis of cytokines. Patients will be eligible for randomization if they have a breath methane level of ≥ 10 ppm on the lactulose breath test and fully respond to the PRO questionnaires in the REDCap system on at least 11 of 14 days of the Screening and Pre-treatment Period. On Day 1, eligible patients will be randomized in a 1:1:1 ratio to receive study drug (SYN 010 21 mg, SYN-010 42 mg, or placebo). Study drug will be administered during the Treatment Period once daily at bedtime for up to 12 weeks. Rescue medication, bisacodyl, magnesium citrate, magnesium hydroxide, or lactulose, will be allowed for severe constipation (i.e. when symptoms become intolerable). Dosage and frequency should be followed according to package insert. For the Randomization Visit only, rescue medication will not be allowed on the day before, the day of, or the calendar day after the Randomization Visit. Patients will be asked to refrain from starting a new diet, changing stable dose of supplemental fiber and/or changing their exercise patterns that may affect IBS-C symptoms from the time of Screening throughout the duration of the study. Patients will also be asked to refrain from taking food products that are strong inhibitors of cytochrome P450 3A (CYP3A) (e.g. grapefruit juice, Seville orange juice, St. John's Wort), from the time of Screening throughout the duration of the study.

Treatment Schedule and Dose Adjustment and Management	Oral administration of one (1) capsule each day at bedtime.
Criteria for Evaluation Study Endpoints	Three analysis populations are defined for this study. The Intent to Treat (ITT) patient population will consist of all patients who are randomized. The full analysis set will consist of all randomized patients who receive at least 1 dose of study drug. The full analysis set will be used for the efficacy and safety analyses. Efficacy analysis will be conducted according to the randomized treatment the patients receive, and safety analysis will be conducted according to the actual treatment the patients receive. The per-protocol set will consist of all patients in the full analysis set who do not have major protocol deviations as specified in the statistical analysis plan.
Plan for Statistical Analysis	Efficacy: The analysis for the change from baseline in weekly number of CSBMs will be performed using the analysis of covariance (ANCOVA) with treatment group and the Baseline value as covariates using the ITT analysis set. The primary analysis will be conducted using the observed values. For missing data, two sensitivity analyses will be conducted by using the multiple imputation and last observation carried forward (LOCF) methods. Details will be included in the statistical analysis plan (SAP). For secondary and exploratory analyses, the Chi-squared test will be used to determine differences between treatment groups for categorical variables. For continuous-type variables, analysis of covariance (ANCOVA) methods will be used with treatment group and the corresponding baseline value as covariates. For the responder endpoints, the analysis will be conducted by imputing the missing data as “non-responders”. A sensitivity analysis will be conducted by imputing the missing data as “responders”. All statistical tests will be conducted at a 2-sided significance level of 5% unless otherwise specified. Due to the COVID-19 pandemic, study enrollment was temporarily stopped, at which time 53 patients had been randomized to study drug treatment groups. A futility interim analysis will be conducted on these 53 patients (49 have completed the study and 4 have withdrawn/early terminated). Safety: All AEs will be analyzed in terms of descriptive statistics and qualitative analysis. Adverse events will be listed for each patient and summarized by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA). Safety laboratory data, ECGs, physical examination findings and vital signs measurements will be listed for each patient and presented descriptively by treatment group time points.

Sample Size Rationale	A total of 150 adult IBS-C patients: 50 in the placebo group and 50 in each of the SYN-010 21 mg and SYN-010 42 mg treatment groups. The sample size calculation is based on the following assumptions: the weekly CSBM change from baseline is 0.5 for the placebo group, and 1.5 for each active group, with common standard deviation of 1.5, the study will have 90% power for each SYN-010 group compared with the placebo by using the t-test with alpha=0.05.

2. General information

STUDY TITLE: Evaluation of the EfficAcy and Safety of SinglE, Daily Oral Doses of SYN-010 Compared to Placebo in Adult Patients with Irritable Bowel Syndrome with Constipation (EASE-DO)

SPONSOR INSTITUTION: Cedars-Sinai Medical Center – Medically Associated Science and Technology (MAST)

INDICATION: Irritable Bowel Syndrome with Constipation (IBS-C)

Investigational drug(s) and route of administration:	**SYN-010 21mg, 42mg, Placebo**		
Study purpose:	Proof-of-concept, dose finding, health economics		
Clinical study phase:	II		
Version no.:	3.0	Date:	9 Jan. 2019
Amendment no.:	23411	Date:	09 Apr 2019
Institution study no.:	**54792**		

The study will be conducted in compliance with the protocol, ICH-GCP and any applicable regulatory requirements.

Confidential

This protocol submitted to Synthetic Biologics is the property of the investigator named as Principal Investigator within this document. The information contained herein is confidential and is intended solely for the guidance of the clinical investigation and submission to Synthetic Biologics for an appeal for financial or clinical drug supply support. Reproduction or disclosure of this document whether in part or in full to parties not associated with the clinical investigation, or its use for any other purpose, without the prior written consent of the Principal Investigator is not permitted. Any changes or amendments to the conduct of this protocol must be resubmitted in writing to Synthetic Biologics.

Throughout this document, symbols indicating proprietary names (®, TM) are not displayed. Hence, the appearance of product names without these symbols does not imply that these names are not protected.

Principal Investigator:	**Ali Rezaie, MD, MSc** Director, GI Motility Director, Bioinformatics Medically Associated Science and Technology (MAST) Program Cedars-Sinai Medical Center Thalians Building 8730 Alden Drive Second Floor East Los Angeles, CA 90048 **Phone: 310-423-0617 (Clinical)** **Phone: 310-423-1955 (Laboratory)** **Email:** ali.rezaie@cshs.org
Data Management and Coordinating Center:	**Christine Chang, RN** Research Nurse Medically Associated Science and Technology (MAST) Program Cedars-Sinai Medical Center Thalians Building 8730 Alden Drive Second Floor West Los Angeles, CA 90048 **Phone: 310-423-0617 (Clinical)** **Phone: 310-423-1955 (Laboratory)** **Email:** christine.chang@cshs.org **Rashin Sedighi, RN, PhD** Clinical Research Coordinator Medically Associated Science and Technology (MAST) Program Cedars-Sinai Medical Center Thalians Building 8730 Alden Drive Second Floor West Los Angeles, CA 90048 **Phone: 310-423-0617 (Clinical)** **Phone: 310-423-1955 (Laboratory)** **Email:** rashin.sedighi@cshs.org

Signatures

The undersigned confirm that they are the authors of this protocol and agree to conduct this trial in accordance with all stipulations of the protocol and in accordance with International Conference on Harmonisation (ICH) and all applicable local Good Clinical Practice (GCP) guidelines, including the Declaration of Helsinki

_____ _____

Ali Rezaie, MD MSc Date
Principal Investigator

_____ _____

Christine Chang, RN Date
Study Coordinator

3. Introduction

3.1 Background

Irritable bowel syndrome (IBS) is a gastrointestinal (GI) syndrome characterized by chronic abdominal pain and altered bowel habits in the absence of any organic cause. The symptoms of IBS not only adversely affect a patient's health-related quality of life (QoL), but also place a significant financial burden on society due to reduced work productivity and increased use of healthcare-related resources[1,2]. Patients with IBS frequently complain of abdominal bloating and increased gas production in the form of flatulence or belching. The prevalence in North America and Europe is approximately 10-15%[3,4]. Irritable bowel syndrome affects all ages and genders however there is a 2:1 female predominance in North America[5]. Irritable bowel syndrome is classified into 4 subtypes based on stool pattern: IBS with constipation (IBS-C), IBS with diarrhea, mixed IBS, and un-subtyped IBS. Irritable bowel syndrome with constipation is defined as the presence of hard or lumpy stools with ≥ 25 percent of bowel movements and loose or watery stools with < 25% of bowel movements[6].

SYN-010 is a modified release, oral formulation of lovastatin being developed for the treatment of IBS-C. The SYN-010 program is based predominantly on research by Dr. Mark Pimentel (Director, Medically Associated Science and Technology Program, Cedars-Sinai Medical Center) and collaborators[7,8,9] hypothesizing that reduction in intestinal methane (methane) production can reverse constipation and improve global symptoms in IBS-C. Methane production in humans is due to methanogenic archaea in the intestine, predominantly *Methanobrevibacter smithii* (*M. smithii*). Methane, the key product of anaerobic respiration of methanogens, had been perceived to produce no ill effects in humans aside from gaseous distention. However, several research groups worldwide have shown that a significant percentage of patients with IBS-C excrete methane, and elevated methane production by methanogens correlates with constipation and related symptoms in both IBS-C and chronic idiopathic constipation[7,8,9,10,11,12,13]. A direct causative role for methane in IBS-C was demonstrated in a recent case report, wherein a woman undergoing fecal microbiota transplantation (FMT) for *C. difficile* infection unknowingly received stool containing a high concentration of methanogens. The FMT recipient rapidly developed severe symptoms of IBS-C that were subsequently reversed by ablation of methane production[14].

Further details can be found in the Investigator's Brochure, which contains comprehensive information on the investigational product.

3.2 Summary of findings from pre-clinical studies with potential clinical significance

A series of preclinical studies have sought to understand the mechanism by which methane exerts its effects on gastrointestinal motility. The precise mechanism remains under investigation; however, these studies indicate that methane may exert its actions in the intestine by modulation of the cholinergic pathway of the enteric nervous system[15]. *In vivo* studies in dogs and *ex vivo* studies using guinea pig ileal strips showed that methane stimulates non-propagating contractions, preventing propulsion of materials through the intestinal tract[16,17].

The active ingredient of SYN-010 is lovastatin lactone, which was first approved by the FDA in 1987; it is a cholesterol-lowering agent (Mevacor®, Merck & Co., Inc.)[18] and has a history of safe use at oral doses up to 80 mg/day. In its cholesterol-lowering indication, lovastatin lactone is a prodrug and must be converted to the β-hydroxyacid form to inhibit the enzyme HMG-CoA reductase (HMGR). The preclinical safety of lovastatin lactone and β-hydroxyacid was established in detailed preclinical and toxicology studies conducted by Merck[19].

Interest in lovastatin as a potential inhibitor of methanogenesis originated with recognition that the rate-limiting step in the synthesis of lipid membranes in the methanogen *M. smithii* is catalyzed by HMGR. As detailed below, it has since been determined that HMGR is not the target for lovastatin anti-methanogenic activity and the methane reducing effects of SYN-010 can be separated from potential systemic effects such as cholesterol lowering.

The ability of lovastatin to inhibit methane production was established in *ex vivo* studies investigating the effects of nine statins (including lactone and β-hydroxyacid forms) on methane production by stool samples from IBS-C patients. In these studies, lovastatin lactone was identified as the only effective statin inhibitor of methane production, while the cholesterol-lowering metabolite lovastatin β-hydroxyacid was ineffective. In fact, the β-hydroxyacid forms of pravastatin, simvastatin, rosuvastatin and atorvastatin were all ineffective inhibitors of methane production in his system. These experiments also demonstrated the dose-dependence of methane inhibition by lovastatin lactone and a head-to-head comparison of lovastatin lactone, lovastatin lactone-diol and lovastatin β-hydroxyacid affirmed that the lactone form of lovastatin is the anti-methanogenic species[20].

An additional important feature of lovastatin as a methane lowering agent is that neither lovastatin lactone nor the β-hydroxyacid appear to be microbicidal. Neither species eradicated *M. smithii* or bacteria in the intestine of rats administered daily oral doses of each compound for 10 days[21].

3.3 Rationale of the study

SYN-010 has previously been evaluated in consecutive Phase 2a clinical trials. Sixty-three (63) IBS-C patients with high breath methane (>10 ppm) at screening were enrolled in a multicenter, randomized, controlled, double-blinded clinical trial (RCT) in which they received SYN-010 21 mg, SYN-010 42 mg or Placebo once daily for 4 weeks. Fifty-four (54) subjects who completed the RCT continued into an open-label extension (EXT) in which all subjects received SYN-010 42 mg once daily for an additional 8 weeks.

The SYN-010 Phase 2a studies were intentionally designed as mechanistic proof-of-concept studies, wherein reductions in breath methane were employed as a rapid and cost-effective means by which to determine if SYN-010 could be effective in treating an underlying cause of symptoms in IBS-C. Breath methane was reduced relative to baseline in SYN-010 treatment groups, and lower breath methane levels correlated with an increased number of complete spontaneous bowel movements (CSBMs) at week 12, consistent with the proposed methane-inhibiting action of lovastatin lactone[22].

Since lovastatin has not previously been used to treat IBS-C patients, the SYN-010 Phase 2a studies were also focused on the safety of the SYN-010 dosage form. Daily doses of SYN-010 were well-tolerated by IBS-C patients over the 12-week treatment period (at least 8 weeks of SYN-010 42 mg). SYN-010 did not cause clinically meaningful or persistent changes in serum liver and muscle markers in IBS-C patients at daily doses of 21 mg and 42 mg. Modest decreases from baseline in lipid parameters observed after 7 days of SYN-010 21 mg or 42 mg had largely faded by 28 days and were not evident after 12 weeks of dosing[23]. Very few adverse events were reported over 12 weeks of SYN-010 treatment and all were of mild or moderate intensity. No serious adverse events were reported and there were no incidences of drug-related diarrhea, which is an important potential benefit of SYN-010 as an IBS-C therapy.

Although the Phase 2a studies were not prospectively powered for formal statistical evaluation of clinical endpoints, compelling improvements in CSBMs, abdominal pain, and bloating were observed in SYN-010 treatment groups. These clinical findings have been presented in multiple public forums

and a panel of clinical advisors affirmed that the Phase 2a data validate the need to evaluate optimal dosing of SYN-010 in a larger patient population over a longer dosing period.

Based on the potential clinical benefit observed in Phase 2a, this Phase 2b clinical study will evaluate in more detail the clinical effects of two dose strengths of SYN-010 administered over a longer treatment period (12 weeks per FDA guidelines) to a larger number of IBS-C patients. The study will seek more definitive evidence regarding potential symptom improvements and safety in IBS-C patients. The study also seeks to provide new information of relevance to both efficacy and safety by measuring changes to the microbiome in patient stool samples; serum level of cytokine markers of inflammation; and expanded breath gas measurements.

3.4 SYN-010 (lovastatin lactone modified release capsules)

3.4.1 Description

SYN-010 is a hydroxypropyl methylcellulose (HPMC) capsule filled with enteric-coated tablets from which lovastatin is released at different intestinal pH values. The tablets are designed to pass through the stomach unchanged then release a small amount of lovastatin into the duodenum and the majority of the lovastatin dose into the ileocecal junction and colon. The amount of lovastatin to be released into the small and large intestine is anticipated to be consistent with the relative levels of methane-producing archaea in each location of the intestine[24].

Each tablet in the SYN-010 dosage form contains 7 mg of lovastatin combined with USP excipients and coated with two different EUDRAGIT® enteric polymers that dissolve at either pH 5.5 (duodenal release; DR) or pH 7.0 (ileocecal release; ICR).

- SYN-010, 21 mg (D1-I2) comprises an opaque, green, size 1 HPMC capsule containing 1 x pH 5.5-coated tablet (DR) and 2 x pH 7.0-coated tablets (ICR).
- SYN-010, 42 mg (D1-I5) comprises an opaque, green, size 1 HPMC capsule containing 1 x pH 5.5-coated tablet (DR) and 5 x pH 7.0-coated tablets (ICR).

3.4.2 Mechanism of action

Methane production (methanogenesis) is a ubiquitous process in the human intestine, disposing of hydrogen and other by-products formed during bacterial fermentation. Methane production in humans is almost entirely due to the archeon *M. smithii*. Elevated intestinal methane production reduces intestinal motility and is a cause of constipation, pain and bloating in IBS-C.

Interest in lovastatin as a potential inhibitor of methanogenesis originated with recognition that the rate-limiting step in the synthesis of archaeal lipid membranes is catalyzed by HMG-CoA reductase (HMGR) which is the target enzyme for cholesterol-lowering statins. Subsequent *in vitro* studies (described above) and computational studies have since determined that HMGR is not the target for lovastatin anti-methanogenic activity; rather, lovastatin lactone appears to exert a direct effect on methanogenesis enzymes. The mechanism by which lovastatin lactone inhibits methane production by *M. smithii* has not been conclusively determined; however, detailed computational studies showed that lovastatin lactone may competitively inhibit F420-dependent methylenetetrahydromethanopterin dehydrogenase (mtd), an enzyme that is integral to the *M. smithii* methanogenesis pathway[25].

Methanogenic archaea reside predominantly in the human colon, with lower methanogen levels measured in the small intestine of some patients. Methane production at both sites contributes to reduced gastrointestinal motility and rat studies suggest that the ileocecal region may be of particular significance[21]. SYN-010 utilizes a dual-pulse release profile to deliver a portion of the lovastatin dose to the small intestine and the majority of the dose to the ileocecal junction and colon where the most methane-producing organisms are found; the relative amounts of lovastatin released into the small and large intestine are consistent with the anticipated relative levels of methanogens in each location.

Lovastatin lactone exerts its methane-reducing effect in the intestinal lumen and systemic absorption of lovastatin is not required for its therapeutic effect in IBS-C. The pharmacokinetic profile of SYN-010 has been evaluated in a repeated-dose study in heathy volunteers. Two of the most therapeutically relevant findings from the PK study were:

- Conversion of the anti-methanogenic lovastatin lactone to the cholesterol-lowering β-hydroxyacid metabolite was significantly reduced in the SYN-010 formulation, and this was reflected in reduced systemic levels of the β-hydroxyacid and an absence of clinically meaningful or persistent SYN-010 effects on serum liver, lipid and muscle markers;
- Conversion of the anti-methanogenic lovastatin lactone to the cholesterol-lowering β-hydroxyacid metabolite was significantly reduced in the SYN-010 formulation, and this was reflected in reduced systemic levels of the β-hydroxyacid and an absence of clinically meaningful or persistent SYN-010 effects on serum liver, lipid and muscle markers.

In addition to the pharmacokinetic study, sparse PK sampling was conducted in IBS-C patients during Phase 2a clinical testing. After daily administration of SYN-010 21 mg or SYN-010 42 mg, plasma trough levels of both lovastatin lactone and β-hydroxyacid were low and variable, such that ≥ 50% of patients had undetectable plasma levels of each lovastatin species after both 7 and 28 days of treatment[23]. Reduced systemic levels of lovastatin species and high concentrations of lovastatin lactone in the stool are compelling findings regarding the use of SYN-010 as an intestine-acting anti-methanogenic therapy.

4. Study objectives

4.1 Primary

Change from baseline in the weekly average number of CSBMs during the 12-week Treatment Period for SYN-010 relative to placebo.

4.2 Secondary

Safety and tolerability of SYN-010 21 mg and 42 mg in IBS-C patients treated for 12 weeks.

For SYN-010 relative to placebo:

- Proportion of overall stool frequency responders.
- Proportion of overall bloating responders.
- Proportion of patients using rescue medication.

- Proportion of overall responders in the SYN-010 21 mg and 42 mg treatment groups versus placebo.
- Change from baseline for SYN-010, relative to placebo, in:
  - Weekly average score for worst abdominal pain at Weeks 1 through 12.
  - Weekly average number of CSBMs at Weeks 1 through 12.
  - Weekly average number of SBMs at Weeks 1 through 12.
  - Weekly average bloating score at Weeks 1 through 12.
  - Weekly average stool consistency (BSFS) at Weeks 1 through 12.

4.3 Exploratory

For SYN-010 relative to placebo:

- Proportion of patients with adequate relief[27].
- Change from baseline for SYN-010 in patient reported outcomes (PRO) using validated questionnaires in the REDCap system.
- Breath gas area-under-the-curve (AUC) (methane, hydrogen, and hydrogen sulfide) on lactulose breath test at EOS compared to Screening.
- Spot breath tests at Randomization, Day 28, and Day 56.
- Comparison of serum cytokine levels at baseline and EOS – measuring for potential immune or inflammatory signals to include the key cytokines of the Th1/Th2/Th17 pathways: IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17, GM-CSF and IFN-γ. Plus TNF-α, IL-12, IL-6, the two chemokines IL-8 and MCP-1, and the gut cytokine IL-25.
- Microbiome measurements in stool of bacteria (16S rRNA) and methanogens (qPCR).

5. Study design and investigational plan

5.1 Pre-treatment period

Screening and Pre-treatment Period (up to 17 days prior to the first dose of study drug):

During the Screening and Pre-treatment Period, patients will be provided an ID number and password-protected access to the REDCap website to record baseline parameters (such as weekly average worst abdominal pain score and weekly number of CSBMs). During this period, patients will also (i) complete a lactulose breath test including analysis of methane, hydrogen, hydrogen sulfide, and carbon dioxide; (ii) provide a stool sample for analysis of the microbiome; and (iii) provide a blood sample for analysis of cytokines and baseline tests. Patients will be eligible for randomization if they have a breath methane level of ≥ 10 ppm on the lactulose breath test and fully respond to the PRO questionnaires in the REDCap system on at least 11 of 14 days of the Screening and Pre-treatment Period.

5.2 Treatment phase

Treatment Period comprising Randomization (Day 1) and 12 weeks of dosing (with clinic visits at Weeks 4, 8, and 12).

On Day 1, eligible patients will be randomized in a 1:1:1 ratio to receive study drug (SYN-010 21 mg, SYN-010 42 mg, or placebo). Study drug will be administered during the Treatment Period and taken once daily at bedtime for up to 12 weeks.

5.3 Post-treatment period or follow-up period

End-of-Study contact between 24 and 48 hours after Week 12 for patients who complete the Treatment Period.

6. Statistical methods and determination of sample size

6.1 General considerations

A statistical analysis plan (SAP) describing all the statistical analyses to be completed will be finalized prior to database lock. Unless otherwise specified, for numeric data, descriptive statistics will include the number of patients with data to be summarized (n), mean, standard deviation (SD), median, minimum (min), and maximum (max). Categorical data will be presented using frequency counts and percentages. All statistical tests will be conducted at a 2-sided significance level of 5% unless otherwise specified.

6.2 Disposition of patients

Patient disposition will be summarized for the study, along with reasons for early termination. Numbers (%) of patients in each analysis population will be tabulated.

6.3 Protocol deviations

All protocol deviations will be reviewed and documented before database lock. Protocol deviations will be recorded by the site staff, and study monitor. Major protocol deviations will be defined in the SAP before unblinding the study.

6.4 Analysis populations

Three analysis populations are defined for this study:

- The randomized patient population will consist of all patients who are randomized;
- The full analysis set will consist of all randomized patient who receive at least one dose of study drug. The full analysis set will be used for the efficacy and safety analyses. Efficacy analysis will be conducted according to the randomized treatment the patients receive, and safety analysis will be conducted according to the actual treatment the patients receive;
- The per-protocol set will consist of all patients in the full analysis set who do not have major protocol deviations as defined in the SAP.

6.5 Demographics and baseline characteristics

Demographics and baseline characteristics will be summarized by treatment for the full analysis set. Demographics and baseline characteristics of the patient sample will be described using summary statistics.

6.6 Efficacy analyses

The primary endpoint is the change from baseline in the weekly average number of CSBMs during the 12-week Treatment Period.

The secondary endpoints are defined as below:

- Proportion of overall responders during the 12-week Treatment Period (overall 12-week responders). An overall 12-week responder is defined as a patient with a weekly response in at least 50% of the weeks of treatment (6 of 12 weeks). A weekly response is defined as a decrease in the patient's weekly average score for worst abdominal pain in the past 24 hours of at least 30% compared to baseline and a stool frequency increase of 1 or more CSBMs per week compared with baseline.
- Proportion of overall stool frequency responders during the 12-week Treatment Period. An overall stool frequency responder is defined as a patient with a weekly stool frequency response in at least 50% of the weeks of treatment (6 of 12 weeks). A weekly stool frequency response is defined as a stool frequency increase of 1 or more CSBMs per week compared with baseline, with abdominal pain unchanged or improved compared with baseline.
- Proportion of overall abdominal pain intensity responders during the 12-week Treatment Period. An overall abdominal pain intensity responder is defined as a patient with a weekly abdominal pain intensity response in at least 50% of the weeks of treatment (6 of 12 weeks). A weekly response abdominal pain intensity response is defined as a decrease in the patient's weekly average score for worst abdominal pain in the past 24 hours of at least 30% compared to baseline, with stool frequency unchanged or improved compared with baseline.
- Proportion of overall bloating responders during the 12-week Treatment Period. An overall bloating responder is defined as a patient with a weekly bloating response in at least 50% of the weeks of treatment (6 of 12 weeks). A weekly bloating response is defined as a weekly average bloating score of at least 30% improvement compared to baseline, with stool frequency unchanged or improved compared with baseline.
- Proportion of patients using rescue medication.

Additional secondary endpoints are change from baseline. The change from baseline at Visit X is defined as Value at Visit X – Value at Baseline. The percent change from baseline is defined as (change from baseline)/baseline value. If the baseline value = 0 or missing, then the percent change from baseline will be missing.

- Change from baseline in weekly average score for worst abdominal pain at Weeks 1 through 12.
- Percent change from baseline in weekly average score for worst abdominal pain at Weeks 1 through 12.
- Change from baseline in weekly average number of CSBMs at Weeks 1 through 12.
- Change from baseline in weekly average number of SBMs at Weeks 1 through 12.
- Change from baseline in the weekly average bloating score at Weeks 1 through 12.
- Change from baseline in weekly stool consistency score (BSFS) at Weeks 1 through 12.
- Proportions of overall abdominal pain intensity responders with different cutoff points for the % improvement from baseline in weekly average score for worst abdominal pain, i.e., ≥ 10%, 20%, 40%, 50%, 60% and 70%.
- Proportions of overall stool frequency responders with different cutoff points for the improvements from baseline in the weekly number of CSBMs ≥ 2, 3, 4, 5, and 6.
- Proportions of patients with improvements of ≥ 10%, 20%, 30%, 40%, 50%, 60%, and 70% from baseline in the weekly average bloating score in at least 50% of the weeks of treatment (6 of 12 weeks).

The exploratory endpoints are defined as below:

- Proportion of patients with adequate relief[26].
- Change from baseline for SYN-010 in patient reported outcomes (PRO) using validated questionnaires in the REDCap system.
- Change from baseline in the area-under-the-curve (AUC) of breath CH_4 production, based on the 120-minute lactulose breath test.
- Change from baseline in breath CH_4 production based on a single-point breath CH_4 test.

The efficacy analyses will be conducted based on the full analysis set and per-protocol set. The analysis for the primary endpoint will be performed using the analysis of covariance (ANCOVA) with treatment group and the baseline value as covariates. The primary analysis will be conducted using the observed values. For missing data, two sensitivity analyses will be conducted by using the multiple imputation and last observation carried forward (LOCF) methods. Details will be included in the SAP.

For missing data imputation, the primary analysis will be conducted based on the following imputation method: If a subject drops out of the study or otherwise does not report efficacy data for a particular treatment period week, the subject will not be considered a responder for that week. The weekly response will not be considered a responder if there are more than three days with missing assessments during a week.

For the responder endpoints, the analysis will be conducted by imputing the missing data as “non-responders”. Two sensitivity analyses for the primary endpoint will be conducted: (1) missing data for the responders will be imputed as “responders”; (2) the overall responders are defined based on the last observation carried forward (LOCF) for missing values of the weekly average score for worst abdominal pain and/or weekly average number of CSBMs.

For the analyses of the secondary and exploratory endpoints, the Chi-squared test will be used to determine differences between each active treatment group versus placebo group for categorical variables. P-values and 95% Newcombe-Wilson confidence intervals (CIs) for treatment differences will be presented. For continuous-type variables, analysis of covariance (ANCOVA) methods will be used with treatment group and the corresponding baseline value as covariates. P-values and least squares (LS) treatment means and their differences will be presented, accompanied by 95% CIs for each time point.

A fixed sequence testing procedure will be used for the statistical testing of the primary and secondary endpoints. More specifically, the primary endpoint will be tested at the two-sided alpha = 0.05 level first. If statistical significance is not achieved for the primary endpoint, then the testing procedure will stop, and the secondary endpoints will not be tested formally. If statistical significance is achieved for the primary endpoint, then the secondary endpoints will be tested formally in the order of the list in section 4.2. The first listed secondary endpoint will be tested at the two-sided alpha = 0.05 level. If statistical significance is not achieved, then the testing procedure will stop. If statistical significance is achieved for the first listed secondary endpoint, then the following secondary endpoint will be tested at the two-sided alpha = 0.05 level. The testing

procedure will continue for the testing of the secondary endpoints until statistical significance is not achieved for a secondary endpoint.

Due to the COVID-19 pandemic, study enrollment was temporarily stopped, at which time a total of 53 patients had been randomized to study drug treatment groups. 49 of these patients have completed the study and 4 have withdrawn or early terminated. A futility interim analysis will be conducted based on the data from these 53 patients. A recommendation to stop enrollment of the study will be made by the unblinded statistician(s) if, for both dose groups, the conditional power is less than 20% based on the primary endpoint AND the difference of the overall 12-week responder rates is less than 2%. The interim analysis will be conducted by a group of people that are not involved in the daily activities of the study. Details will be included in the SAP.

6.7 Safety analyses

All AEs, whether considered drug-related or not, will be reported with a diagnosis, start/stop dates, action taken, whether treatment was discontinued, any corrective measures taken, outcome, and other possible causes. For all events, the relationship to treatment and the intensity of the event will be determined by the investigator.

All AEs will be analyzed in terms of descriptive statistics and qualitative analysis. Treatment emergent adverse events will be listed for each patient and summarized by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA).

Safety laboratory data, ECGs, physical examination findings, and vital signs measurements will be listed for each patient and presented descriptively by treatment group and time point.

Safety variables may include but not limited to the following: laboratory changes (complete blood counts, electrolytes, chemistry, and coagulation), changes in vital signs (blood pressure, heart rate, respiratory rate, and temperature), and ECG and, in some instances, as produced at the investigator's discretion.

6.8 Determination of sample size

The sample size calculation is based on the following assumptions: the weekly CSBM change from baseline is 0.5 for the placebo group, and 1.5 for each active group, with common standard deviation of 1.5; 50 patients in each group will have 90% power for each SYN-010 group compared with the placebo by using the t-test with alpha=0.05.

7. Study population

7.1 Study population and statistical assumptions

A total of 150 adult IBS-C patients will be enrolled in a single site: 50 in the Placebo group and 50 in each of the SYN-010 21 mg and SYN-010 42 mg treatment groups. The sample size calculation is based on the t-test to achieve 90% power for each SYN-010 group when compared with the placebo group.

7.2 Eligibility criteria

Patients must meet all of the following inclusion and exclusion criteria to be included in the study.

7.2.1 Inclusion criteria

1. Male or female participants aged between 18 and 65 years inclusive.
2. Patient must be willing and able to participate in the study for the required duration, understand and sign the informed consent form (ICF), and be willing to comply with all protocol-related visits and procedures.
3. Patient has had IBS-C symptoms (as defined by Rome IV diagnostic criteria) for at least 6 months prior to diagnosis.
4. Patient has an average score of ≥ 3.0 for daily abdominal pain at its worst (11-point numerical rating scale [NRS]) during and up to the 17 days immediately before randomization (i.e. Pre-treatment Period).
5. Patient has an average of ≤ 3 CSBMs per week or ≤ 5 SBMs per week during the 17 days immediately before randomization (i.e. Pre-treatment Period).
6. Patient has a breath methane level ≥ 10 ppm on a lactulose breath test administered at Screening.
7. Patient may be on a stable, continuous regimen of fiber or probiotics one month before the Screening Visit; however, they must maintain a stable dose regimen through Week 12.
8. Patient must agree to refrain from starting a new diet, changing stable dose of supplemental fiber, or changing exercise pattern that may affect IBS-C symptoms from the time of Screening through the end of the study. If the patient takes food products that are strong inhibitors of cytochrome P450 3A (CYP3A) (e.g. grapefruit juice, Seville orange juice, St. John's Wort), he/she must agree to refrain from taking these from the time of Screening through the end of the study.
9. Patient must agree to use an acceptable method of contraception from the time of signing the ICF to 30 days after the final dose of study drug if the patient is a sexually active female of child-bearing potential (defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal. Postmenopausal is defined as 12 consecutive months with no menses without an alternative medical cause). Adequate contraceptive measures include: oral contraceptives (stable use for two or more cycles before Screening); intrauterine device; Depo-Provera®; Norplant® System implants; partner with a vasectomy; double-barrier birth control (e.g. use of a condom plus diaphragm or condom plus either contraceptive sponge, foam, or jelly); or abstinence. According to drug research standards, male patient must agree to use an acceptable method of contraception and refrain from donating sperm from the time of signing the ICF to 90 days after the final dose of study drug.
10. A female patient of child-bearing potential must be non-pregnant and non-lactating and have negative pregnancy tests at the Screening Visit and on Day 1 prior to dosing with study drug.
11. Patients must be able to understand and be willing to sign the written informed consent form. A signed informed consent form must be appropriately obtained prior to the conduct of any trial-specific procedure.
12. Willing and able to comply with the protocol, including follow-up visits and examinations.

7.2.2 Exclusion criteria

Patients who meet any of the following criteria will be excluded from this study:

1. Patient has loose (mushy) or watery stools for > 25% of their bowel movements (BMs) during the 12 weeks before Screening or during the Screening and Pre-treatment Periods.
2. Patient has a history of cathartic colon, laxative, or enema abuse.
3. Patient has a history of ischemic colitis.
4. Patient has a history of pelvic floor dysfunction.
5. Patient has a history of bariatric surgery for the treatment of obesity.
6. Patient has a history of surgery to remove a segment of the gastrointestinal (GI) tract at any time before the Screening Visit.
7. Patient has any history of myopathy, rhabdomyolysis, chronic myalgia, or familial history of hereditary muscular disorders.
8. Patient has been diagnosed with or has a family history of familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, or any other form of familial colorectal cancer.
9. Patient currently has any structural abnormality of the GI tract or a disease or condition that can affect GI motility, or any unexplained and clinically significant symptoms such as lower GI bleeding, rectal bleeding, heme-positive stool, iron-deficiency anemia, weight loss, or systemic signs of infection.
10. Patient has had any previous surgery involving the abdomen, pelvis, or retroperitoneal region during the last 12 months prior to Screening, with the exclusion of laparoscopic gallbladder surgery or appendix removal.
11. Patient has a history of diverticulitis or any chronic condition that could be associated with abdominal pain or discomfort and could confound the assessments in the study (e.g. inflammatory bowel disease, chronic pancreatitis, polycystic kidney disease, ovarian cysts, endometriosis, or lactose intolerance).
12. Patient has history of severe renal insufficiency defined as an actual or estimated glomerular filtration rate of < 30 mL/min/1.73 m^2 within the 6 months prior to Screening Visit.
13. Patient has any history of a medical condition or a concomitant medical condition that, in the opinion of the investigator, would compromise the patient's ability to complete the study safely or could confound the assessments in this study (e.g. uncontrolled hypothyroidism).
14. Patient is known to have elevated liver enzyme levels (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]) or creatine kinase levels that are ≥ 1.5 times the upper limit of normal (ULN) that have not been resolved within the 4 weeks prior to consent and/or these elevated levels are present at the Screening Visit laboratory assessment.
15. Patient has any abnormal laboratory results, electrocardiogram (ECG) findings, or physical examination findings deemed clinically significant by the investigator during the Screening Period.
16. Within 14 days prior to the Screening Visit, patient has used concomitant medications that are: (1) moderate-to-strong inhibitors of cytochrome P450 3A (CYP3A) and/or organ anion transporting polypeptide (OATP)1B1 (e.g. cyclosporine, verapamil, dronedarone, diltiazem, amiodarone, itraconazole, ketoconazole, posaconazole, voriconazole, clarithromycin, telithromycin, human immunodeficiency virus protease inhibitors, boceprevir, telaprevir, nefazodone, erythromycin, cobicistat-containing products); (2) other concomitant medications that are excluded from the lovastatin label (e.g. rifampin, colchicine, ranolazine); or (3) metformin or GLP-1 agonists. Patients should not take any of these concomitant

medications during the treatment phase of the study without contacting the investigator.

17. Patient has hypersensitivity to statins; or has used any statins, fibrates, > 1 g/day of niacin, or gemfibrozil within the 3 months prior to the Screening Visit.
18. Patient reports current chronic or frequent use of drugs known to cause constipation (e.g. narcotics) for the 3 months prior to Screening.
19. Patient has taken over-the-counter IBS treatments (e.g. laxatives) or proton pump inhibitors within 3 days prior to the Screening Visit.
20. Certain drugs used for the treatment of IBS (e.g. low dose tricyclic antidepressants) may be allowed at the discretion of the Medical Monitor provided the patient remains on a stable dose for one month prior to the Screening Visit and throughout the study with the exception of tegaserod, lubiprostone, linaclotide, metoclopramide, prucalopride, domperidone, plecanatide, CandiBactin, Atrantil, Allimax/Allimed within 2 weeks prior to the Screening Visit or antibiotics within 2 months prior to the Screening Visit.
21. Patient has used an opioid chronically or frequently within the 3 months prior to the Screening Visit and for the duration of the study.
22. Patient is currently enrolled in, or plans to enroll in, another clinical study or has used any investigational drug or device within 1 month before signing the ICF through the completion of the study.
23. Patient has previously participated in a SYN-010 study.
24. Patient has a history of alcohol or drug abuse within the 12 months prior to the Screening Visit.

7.3 Excluded therapies and medications, previous and concomitant

- Prior use of SYN-010.
- Concurrent use of another investigational drug or device therapy (i.e. outside of study treatment) during or within 4 weeks of trial entry (signing of the informed consent form).
- Major surgery within 30 days prior to start of study drug.

7.4 Removal of patients from therapy or assessment

The investigator may discontinue treating a patient with a study drug or withdraw the patient from the study at any time for safety or administrative reasons. The patient may decide to discontinue study drug or withdraw from the study at any time for any reason. The reason for discontinuation must be documented.

7.5 Withdrawal of patients from the study

Patients **must be withdrawn from the trial** (treatment and procedures) for the following reasons:

- Patient withdraws consent from study treatment and study procedures. A patient must be removed from the trial at his/her own request or at the request of his/her legally acceptable representative. At any time during the trial and without giving reasons, a patient may decline to participate further. The patient will not suffer any disadvantage as a result.
- Patient is lost to follow-up.
- Death.
- Pregnancy. Lovastatin is a pregnancy Category X drug and should not be taken during pregnancy. Female subjects who become pregnancy must immediately discontinue taking the study drug. Subjects will be instructed that known or suspected pregnancy occurring

during the study should be confirmed and reported to the investigator, who will then withdraw the female subjects from the study without delay. Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy. The investigator should also be notified of any pregnancy occurring during the study and for 30 days after completion of the study. **Within 24 hours** of awareness of any pregnancy associated with study drug exposure during the study and for 30 days after the last dose of study drug, the investigator must record the event on the supplied pregnancy form and send it to the safety vendor. Any pregnancy will be followed to term or termination, and the status of mother and child will be reported on the pregnancy form after delivery. If delivery occurs after study closure, the outcome should be reported directly to the Sponsor.

Patients **may be** withdrawn from the study for the following reasons:

- The patient is non-compliant with study drug, trial procedures, or both, including the use of concomitant medications not prescribed by the study protocol.
- If, in the investigator's opinion, continuation of the trial would be harmful to the patient's well-being.
- The development of a secondary infection or other condition.
- Development of an intercurrent illness or situation which would, in the judgment of the investigator, significantly affect assessments of clinical status and trial endpoints.
- Use of illicit drugs or other substances that may, in the opinion of the investigator, have a reasonable chance of contributing to toxicity or otherwise skewing trial result.

Any patient removed from the trial will remain under medical supervision until discharge or transfer is medically acceptable.

In all cases, the reason for withdrawal must be recorded in the CRF and in the patient's medical records.

7.6 Screen failures

Patients withdrawn during the Screening Period due to abnormal ECG, liver function tests, creatine kinase level, opiate use, or pregnancy will not be allowed to repeat the Screening Period. All other patients withdrawn during the Screening Period will be reviewed and evaluated on an individual basis and may be allowed to re-enter the Screening Period at the discretion of the investigator and the study team.

8. Treatments

8.1 Randomization

Randomization will occur after a questionnaire collection period of at least 14 days, with compliance in fully responding to questions on at least 11 of the 14 days before the start of the Treatment Period.

After the successful completion of the Screening and Pre-treatment Period, eligible subjects will be randomly assigned in a 1:1:1 ratio to the following treatment groups: SYN-010 21 mg, SYN-010 42 mg, or placebo.

Patients will be assigned to the protocol specified treatments using a unique identification number which will be assigned when a patient is evaluated for inclusion into the study.

Randomization will be conducted through the research pharmacist using an online generator (www.sealedenvelope.com). Randomization will be in blocks of 6.

8.2 Investigational product

8.2.1 SYN-010

SYN-010 will be supplied by Synthetic Biologics, Inc. as a 21 mg or 42 mg capsule of lovastatin in a modified-release formulation.

SYN-010 is a capsule containing enteric-coated tablets of lovastatin designed to release drug in both the duodenal (pH 5.5) and ileocecal (pH 7.0) regions of the GI tract.

8.3 Comparator treatment to be administered

8.3.1 Placebo

The placebo is a capsule containing enteric-coated tablets without drug product that will be identical in appearance to SYN-010 and packaged in identical containers. The placebo will be supplied by Synthetic Biologics, Inc.

8.4 Full description and handling of treatments

8.4.1 SYN-010

SYN-010 will be supplied as a 21 mg or 42 mg capsule of lovastatin in a modified release formulation. SYN-010 is a green, size 1 HPMC capsule containing either 3 (21 mg) or 6 (42 mg) enteric-coated tablets of lovastatin (each 7 mg) designed to release drug in both the duodenal (pH 5.5) and ileocecal (pH 7.0) regions of the GI tract. The placebo is an HPMC capsule containing enteric coated tablets without drug product that will be identical in appearance to SYN-010 and packaged in identical containers.

8.4.1.1 General warning

SYN-010 capsules should not be chewed or opened.

The active ingredient of SYN-010 (lovastatin) is currently listed as a pregnancy category X drug and should not be taken during pregnancy. Female subjects who become pregnant must immediately discontinue taking study drug. Subjects will be instructed that known or suspected pregnancy occurring during the study should be confirmed and reported to the investigator, who will then withdraw the female subjects from the study without delay. The investigator should also be notified of any pregnancy occurring during the study but confirmed after completion of the study.

Clinical studies with the commercially-available cholesterol-lowering lovastatin formulation Mevacor® (Merck) showed that lovastatin, like other inhibitors of HMG-CoA reductase, occasionally causes myopathy manifested as muscle pain, tenderness or weakness with creatine kinase (CK) above ten times the upper limit of normal. Myopathy sometimes takes the form of

rhabdomyolysis with or without acute renal failure secondary to myoglobinuria, and rare fatalities have occurred. The risk of myopathy is increased by high levels of HMG-CoA reductase inhibitory activity in plasma. SYN-010 is designed to limit systemic exposure of HMG-CoA reductase inhibiting lovastatin species and no myopathy or rhabdomyolysis were observed in Phase 1 or Phase 2a clinical trials with up to 12 weeks of SYN-010 42 mg dosing. Transient and reversible increases in serum CK were observed in two subjects treated with SYN-010 42 mg for 8 weeks in the Phase 2a clinical trial; however, these were deemed unrelated to study drug by the investigator and returned to normal without discontinuation of SYN-010.

Clinical studies with the commercially-available cholesterol-lowering lovastatin formulation Mevacor® (Merck) also found persistent increases (to more than 3 times the upper limit of normal) in serum transaminases occurred in 1.9% of adult patients who received lovastatin for at least one year in early clinical trials. When the drug was interrupted or discontinued in these patients, the transaminase levels usually fell slowly to pretreatment levels. The increases usually appeared 3 to 12 months after the start of therapy with lovastatin and were not associated with jaundice or other clinical signs or symptoms. SYN-010 is designed to limit systemic exposure to lovastatin species and SYN-010 21 mg and 42 mg doses did not cause clinically meaningful or persistent alterations to serum transaminase levels in Phase 1 or Phase 2a clinical trials.

8.4.1.2 Dose handling

All study drug has been manufactured according to Good Manufacturing Practice for Medicinal Products and the relevant regulatory requirements. The study drug will be distributed by PCI Pharma Services. All packaging and labeling operations have been performed according to Good Manufacturing Practice for Medicinal Products and the relevant regulatory requirements.

Study drug at the clinical study sites should be stored at controlled room temperature in a locked area accessible only to appropriate study personnel. Study drug bottles, and any unused study drug, will be retained by the study site until final reconciliation is completed, then destroyed by the study site according to local procedures. The investigator must provide an explanation for any missing bottles or missing capsules. Any missing bottles or capsules must be documented in the applicable source documentation and Accountability Log.

8.4.1.3 Dose calculation

SYN-010 doses were selected based on previous clinical experience in Phase 1 and Phase 2a clinical studies. These studies showed that SYN-010 provided continuous delivery of lovastatin lactone throughout the colon, and stool concentrations for the SYN-010 21 mg and 42 mg doses were equivalent to concentrations that inhibited methane production by ~60% and ~90% respectively in stool samples from an IBS-C patient *in vitro*. Moreover, these SYN-010 doses did not cause significant or persistent systemic effects on blood chemistry, hematology or urinalysis parameters over 12 weeks of dosing in Phase 2a clinical trials.

8.4.1.4 Dose administration

The study drugs, SYN-010 21 mg or 42 mg (active) or placebo (control), will be administered orally once daily at bedtime for 12 weeks.

8.4.1.5 Dose modification

No dose adjustments will be allowed.

8.5 Blinding

In compliance with applicable regulations, in the event of a SUSAR completed for patients on investigational drug SYN-010, the patient's treatment code will be unblinded and reported to the health authorities. SUSARs involving placebo would not be required to be submitted to the health authorities, however local and institutional regulations should be followed. Any safety reports completed, do not need to be unblinded before submitting to other investigators. Submission of safety reports to the ethics committee should follow local reporting regulations.

8.5.1 Emergency unblinding by the investigator

Unblinding may occur for emergency purposes only. The investigator should note that the occurrence of a serious adverse event or progressive disease should not routinely precipitate the immediate unblinding of the label. If unblinding is necessary for the treatment of a patient for a serious adverse event, every attempt should be made to contact the investigator prior to unblinding. If this is not feasible, then the investigator must be contacted within 24 hours of unblinding.

8.6 Drug logistics and accountability

All study drugs will be stored at the investigational site in accordance with Good Clinical Practice (GCP) and Good Manufacturing Practices (GMP) requirements and the instructions given by the clinical supplies department of the Institution and will be inaccessible to unauthorized personnel.

8.6.1 Storage

All investigational product will be kept in a locked area with access limited to study staff only. The locked area will be maintained under controlled temperature conditions at 20°C-25°C (68°F-77°F), and away from light and moisture.

8.6.2 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of the agent(s) (investigational or free of charge) using the Drug Accountability Record or another comparable drug accountability form.

8.6.3 Destruction and return

At the end of the study, unused supplies of SYN-010 and other investigational agents should be destroyed appropriately and according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.7 Treatment compliance

Upon receipt of the study drug, the investigator, or a responsible party designated by the investigator, will conduct an inventory of the supplies and verify that study drug supplies are received intact and in the correct amounts. A full drug accountability log will be maintained at the study site at all times. All discrepancies must be accounted for and documented.

Subjects will be expected to return all unused study drug and empty containers to the site at each clinic visit, and the number of capsules dispensed and returned will be documented by study staff.

The study personnel will account for all drugs dispensed and returned. All unused supplies of SYN-010 and other investigational agents will be destroyed appropriately and according to institutional policies. Destruction will be documented in the Drug Accountability Record form.

Subjects who are not compliant with treatment protocol, including laboratory evaluations, will be subject to withdrawal from the study at the discretion of the investigator.

8.8 Prior and concomitant therapy

Any prior or concomitant therapy that the patient may be taking and may be necessary for the patient's welfare taken within two weeks prior to the start of the study and during the study must be recorded in the patient's source documentation and in the CRF (including start/stop dates, dose frequency, route of administration, and indication).

The patient may be on a stable, continuous regimen of fiber, or probiotics one month before the Screening Visit; however, he/she must maintain a stable dose regimen through Week 12. Additionally, certain drugs used for the treatment of IBS (e.g. low dose tricyclic antidepressants) may be allowed at the discretion of the Medical Monitor provided the patient remains on a stable dose for one month prior to the Screening Visit and throughout the study with the exception of tegaserod, lubiprostone, linaclotide, metoclopramide, prucalopride, domperidone, plecanatide, CandiBactin, Atrantil, Allimax/Allimed within 2 weeks prior to the Screening Visit or antibiotics within 2 months prior to the Screening Visit.

Rescue medication will be allowed for severe constipation (i.e. when symptoms become intolerable). For guidance purposes for this protocol, intolerable symptoms may be when a subject has not had a complete spontaneous bowel movement (CSBM) in the preceding three days with an abdominal pain score of ≥ 6 on an 11-point (0-10) scale each day during that time period. However, intolerability is ultimately a subjective judgment, and principal investigators and their patients make the final decision regarding what is intolerable and requires use of the rescue medication. For the Randomization Visit only, rescue medication will not be allowed the day before, the day of, or the calendar day after the Randomization Visit. If rescue medication is taken during this time, it must be recorded as a protocol deviation and the subject should not be randomized. The use of protocol-specified rescue medication and non-protocol-specified laxatives will be recorded in the subject record.

Permitted rescue medications include bisacodyl, magnesium citrate, magnesium hydroxide, and lactulose. Dosage and frequency should be followed according to package insert on rescue medication and should not exceed recommended doses as approved by package insert.

9. Procedures and variables

9.1 Schedule of assessments

Table 1: Schedule of assessments

X = required; O = only if directed by symptoms or reported AE; ET = early termination

Visit No.	**1**	**--**	**2**	**3**	**4**	**5**	**EOS**
Type	Screen	Pre-treatment	Randomize				Call/Visit
Days	-17 to -14	-17 to -1	1	28	56	84 or ET	85
Window (Days)	--	--	--	±5	±5	±5	+1
Participant Information							
Informed Consent[1]	X	--	--	--	--	--	--
Inclusion/Exclusion[1]	X	--	--	--	--	--	--
IBS-C Criteria (ROME IV)[1]	X	--	--	--	--	--	--
Demographics[2]	X	--	--	--	--	--	--
Medical History[3]	X	--	X	--	--	--	--
Prior/Concomitant Medications	X	--	X	X	X	X	X
Diary compliance/ eligibility to randomize[4]	--	X	X	--	--	--	--
Physical							
Physical Exam[5]	X	--	X	O	O	X	O
Vital Signs[6]	X	--	X	X	X	X	O
12-Lead ECG[7]	X	--		--	--	X	--
Drug Dispensing							
Drug Dispensing	--	--	X	X	X	--	--
Accountability/Returns	--	--	--	X	X	X	--
Adverse Events							
AEs	--	--	X	X	X	X	X
Blood Samples							
Clinical Chemistry (CMP)	X	--	X	X	--	X	--
Lipid Panel			X			X	
Cytokine Analysis	--	--	X	X	--	X	--
Hematology	X	--			--	X	--
Serum Pregnancy[8]	X	--	--	--	--	--	--
Creatine Kinase (CK)	X	--	--	O	O	O	O
Urine Samples							

Urine Drug Screen	X	--	--	--	--	--	--
Urine Pregnancy[8]	--	--	X	X	X	X	--
Patient Reported Outcomes (REDCap)							
Diary Dispensing or Return[9]	X	--	--	--	--	X	--
Diary Compliance[10]	--	X	X	X	X	X	--
EQ-5D-5L QoL Questionnaire[11]	--	--	X	--	--	X	--
Stool Samples[12]							
Bacteria (16S rRNA)	--		X	--	--	X	--
Methanogens (qPCR)	--		X	--	--	X	--
Breath Gases							
Lactulose breath test[13]	X	--	--	--	--	X	--
Single-point breath test	--	--	X	X	X	--	--

[1]Informed consent, inclusion/exclusion criteria, and ROME IV may be reviewed and signed prior to Visit 1.

[2]Date of birth, gender, race/ethnicity.

[3]Patients will complete the information in the REDCap system during the Screening Period.

[4]Patients will be eligible for randomization if they fully respond to the PRO questions on at least 11 of 14 days.

[5]General examination, including head, ears, eyes, nose, throat, endocrine, cardiovascular, respiratory, abdomen, skin, neurological, extremities, and musculoskeletal features.

[6]Body weight, blood pressure (requires the patient to be semi-supine for at least 5 minutes prior to the test) height (Screening Visit only), radial pulse rate, respiratory rate, and temperature.

[7]12-lead ECG requires the participant to be supine for at least 5 minutes prior to the test.

[8]All female participants of child-bearing potential.

[9]Provide and train the patient to use the REDCap system with questionnaire.

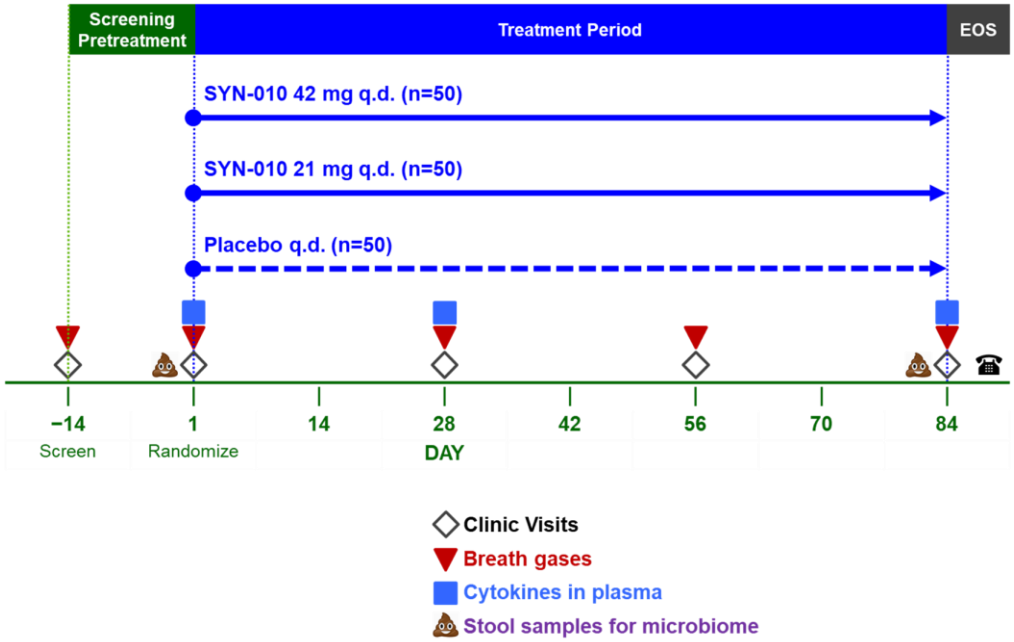
[10]Ongoing review and confirmation of patient PRO questionnaire compliance. Patients will complete the information contained in the REDCap system each day, assessing stool frequency, SBMs, CSBMs, and use of rescue medication or other laxative use; stool consistency using the Bristol Stool Chart; abdominal pain assessment using visual NRS scale (0-10) and visual Likert scale (0-4) for straining and bloating assessment. Questionnaires will also be completed weekly for the PAC-SYM questionnaire and Adequate Relief question.

[11]EQ-5D-5L questionnaire will be completed in the REDCap system on Day 1 prior to study drug administration and at Week 12 or upon early termination.

[12]Pre-treatment stool sample can be collected any time during the week preceding and including Day 1 and will only be analyzed if the patient is randomized into the study; Day 84 stool sample can be collected any time during the week preceding and including Day 84.

[13]Patients are fasted overnight prior to lactulose breath testing. Lactulose breath test measures hydrogen, methane, hydrogen sulfide, and carbon dioxide levels.

9.2 Timing of assessments



9.3 Assessment parameters

Subjects who provide written consent to participate will be screened for inclusion in the study. Consent to participate must be obtained prior to completing any study procedures and may be obtained prior to the Screening Visit.

9.3.1 ROME IV criteria

The investigator or designee will assess whether the subject meets the following ROME IV IBS criteria[28]:

Subject has had IBS symptoms at least six months prior to diagnosis and is experiencing abdominal pain, on average, at least one day per week for the three months preceding the Screening Visit and associated with two or more of the following:

- Abdominal pain or discomfort related to defecation;
- Abdominal pain or discomfort associated with a change in frequency of stool; and/or
- Abdominal pain or discomfort associated with a change in form (appearance) of stool.

On symptomatic days, subject reports more than 25% of stools are Type 1 or 2 and less than 25% of stools are Type 6 or 7 on the Bristol Stool Form Scale.

In addition to the ROME IV criteria, subjects must meet the inclusion and exclusion criteria listed above.

9.3.2 Demographics and medical history

The study staff will record the subject's sex, date of birth, and ethnic origin.

Medical history findings (i.e. previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected:

- Not pertaining to the study indication;
- Start before signing of the informed consent;
- Considered relevant to the study;
- Differentiated from adverse events.

Findings will be recorded in REDCap via the medical history questionnaire (Appendix 13.6).

9.3.3 Physical exam

Complete physical exams will be performed during the Screening Visit and Visits 2 and 5 by the investigator or a physician co-investigator. The general examination will include evaluation of the head, ears, eyes, nose, throat, endocrine, cardiovascular, respiratory, abdomen, skin, neurological, extremities, and musculoskeletal systems. Abnormalities noted at the Screening Visit will be recorded as medical history. Any clinically significant change from the Screening Visit will be recorded as an adverse event.

9.3.4 Vital signs

Vital sign measurements, including body weight, blood pressure, radial pulse rate, respiratory rate, and temperature will be recorded at all clinic visits except the End-of-Study Call/Visit. Vital signs will be collected after the subject has been semi-supine for at least five minutes prior to the test. Height will be recorded at the Screening Visit only.

9.3.5 12-lead ECG

A standard 12-lead ECG will be performed by a trained research nurse during the Screening Visit and Visit 5. ECGs will be interpreted by a trained cardiologist.

9.3.6 Blood draw

Blood will be drawn by a trained research nurse and collected for hematology, clinical chemistry, lipid, cytokine, and creatine phosphokinase. Laboratory analyses will be performed in the central clinical laboratory and MAST research laboratory at Cedars-Sinai Medical Center. Reference ranges will be supplied by the clinical laboratory and used by the investigator to assess the laboratory results for clinical significance and pathological changes. Blood samples will be used for serum pregnancy tests in women of childbearing potential.

Clinical chemistry (CMP) will be assessed on Visits 1 (Screening Visit), 2, 3, and 5. Creatine kinase (CK) will be assessed during Visit 1, however may be assessed as needed during the remaining study visits as indicated by the development of symptoms suggestive of myopathy and/or rhabdomyolysis.

Blood will be drawn during all visits except Visit 4 and the End-of-Study Call/Visit; however, blood may be drawn on Visit 4 and/or the End-of-Study Call/Visit as dictated by occurrence of subject symptom or adverse events.

9.3.7 Urine samples

Subjects will be asked to provide up to one cup of urine for drug and toxicology analysis during the Screening Visit. Urine samples will also be collected for pregnancy tests from women of childbearing potential during Visits 2, 3, 4 and 5.

9.3.8 Daily diary and questionnaires

A study diary will be completed each day assessing stool frequency, SBMs, CSBMs, and the use of rescue medication (Appendix 13.3). Stool consistency will be assessed using the Bristol Stool Chart (Appendix 13.2). Severity of abdominal pain Will be assessed via visual NRS scale (0-10), while severity of bloating and straining will be assessed via Likert scale (0-4)

The PAC-SYM questionnaire and adequate relief question (Appendix 13.4) will be completed weekly. The EQ-5D-5L questionnaire (Appendix 13.5) will be completed on Day 1 prior to study drug administration and at Week 12 or upon early termination.

Subjects will be instructed on how to use REDCap to complete the assessments. During the Screening and Pre-treatment Period, subjects must complete the daily assessment for at least 14 days and have full responses to the questions on 11 or more of the 14 days before the start of the Treatment Period.

9.3.9 Stool samples

Subjects will be asked to provide up to one cup of stool for analysis in the MAST research laboratory. Stool samples will be analyzed for total bacterial and methanogen levels.

9.3.10 Breath gases

9.3.10.1 Lactulose breath test

Lactulose breath tests will be performed during the Screening Visit and Visit 5. Subjects will be asked to undergo a 24-hour preparatory period just prior to the test. Subjects will maintain a bland diet during the first 12 hours and fast during the remaining 12. A baseline breath test will be collected by exhaling into disposable collection bag with a volume of 750 mL. Following the baseline breath sample, subjects will consume 10 g lactulose dissolved in water. Subsequent breath samples will be collected every 15 minutes over the remainder of the 120-minute test. Breath samples will be analyzed for hydrogen, methane, carbon dioxide, and hydrogen sulfide levels immediately after collection via gas chromatograph. The gas chromatograph will be used as approved.

9.3.10.2 Methane breath test

Methane breath tests will be performed during Visits 2, 3, and 4. Subjects will exhale into a disposable collection bag with a volume of 750 mL. Breath samples will be analyzed immediately after collection via gas chromatograph. The gas chromatograph will be used as approved.

9.3.11 Adverse events

Investigators should refer to the Safety Information section of the current IB for SYN-010, including the DCSI (development core safety information), for the expected side effects of SYN-010. As with any agent, there is always the potential for unexpected AEs, including hypersensitivity reactions. The IB will be updated if any new relevant safety data are obtained.

Therapeutic monitoring should be performed following dose selection of SYN-010 in a manner consistent with the local clinical standard of care. In general, patients should be closely monitored for side effects of all concomitant medications regardless of the path of drug elimination.

All concomitant medications must be recorded in the patient's source documentation.

Patients must be carefully monitored for AEs. This monitoring also includes clinical laboratory tests. Adverse events should be assessed in terms of their seriousness, intensity, and relationship to the study drug treatment.

The institutional sponsor and investigator are responsible to comply with the local regulation and legislation for adverse events reporting.

9.3.11.1 Definitions

Adverse event (AE)

In a clinical study, an AE is any untoward medical occurrence (i.e. any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a patient or clinical investigation patient after providing written informed consent for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of an investigational product.

A surgical procedure that was planned prior to the start of the study by any physician treating the patient should not be recorded as AE. The clinical manifestation of any failure of expected pharmacological action (lack of efficacy) is not recorded as an AE if it is already reflected as a data point captured in the CRF. If, however, the event fulfills any of the criteria of an SAE, it must be recorded as and AE and reported as an SAE.

- Conditions that started before signing of informed consent and for which no symptoms or treatments are present until signing of informed consent are recorded as medical history (e.g. seasonal allergy without acute complaints).
- Conditions that started before signing of informed consent and for which symptoms or treatment are present after signing of informed consent, at unchanged intensity, are recorded as medical history (e.g. allergic pollinosis).
- Conditions that started or deteriorated after signing of informed consent will be documented as adverse events.

Serious adverse event (AE)

An SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria:

- Results in death.
- Is life-threatening.
    - The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
    - A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:
        - The admission results in a hospital stay of less than 12 hours.
        - The admission is pre-planned.
        - The admission is not associated with an AE.
    - Results in persistent or significant disability / incapacity.
    - Disability means a substantial disruption of a person's ability to conduct normal life's functions.
        - Is a congenital anomaly/birth defect.
        - Is another medically important serious event as judged by the investigator.

9.3.11.2 Reporting of serious adverse events

The definition of serious adverse events (SAEs) is given above in section 7.3.1.1.

Each serious adverse event must be followed up until resolution or stabilization, by submission of updated reports to the designated person. The investigator must promptly report to the Institutional Review Board all events, incidents, information, and outcomes that represent a possible unanticipated problem involving risks to subjects or others. An isolated laboratory abnormality that is assigned as serious, according to the investigator is not reportable as an SAE, unless the investigator assesses that the event meets standard ICH criteria for an SAE. Baseline laboratory abnormalities that are part of the disease profile should not be reported as an SAE, specifically when they are allowed or not excluded by the protocol inclusion/exclusion criteria.

When required, and according to local law and regulations, serious adverse events that are determined by the Investigator to be related to the investigational drug must be reported by the investigator or the institution safety officer to the Ethics Committee and Regulatory Authorities.

Per the emergency unblinding section above (section 6.4.1), if SAE is considered to be related to the treatment, the Cedars-Sinai safety group will unblind the subject and determine if the subject was administered investigational drug and if so, will proceed with expedited reporting to the FDA.

If the SUSAR requires expedited safety reporting, MedWatch Form should be submitted within the timelines specified in 21 CFR 312.32 using:

- A MedWatch form available at http://www.fda.gov/medwatch/

Copies of all MedWatch reports shall be sent electronically within 15 days to:

Electronic Mailbox:
Michael Kaleko, MD, PhD (Research & Development): mkaleko@syntheticbiologics.com
Amy Sloan (Regulatory Affairs): asloan@syntheticbiologics.com

Address: 9605 Medical Center Drive, Suite 270
Rockville, MD 20850

Phone: (240) 238-3862

At the conclusion of the study, all safety reports and associated data should be forwarded to Dr. Kaleko at Synthetic Biologics.

9.3.12 Pregnancies

The investigator must report any pregnancy occurring in a study patient, or in his partner, during the patient's participation in this study. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE.

For a study patient, the outcome of the pregnancy should be followed up carefully, and any abnormal outcome of the mother or the child should be reported.

For the pregnancy of a study patient's partner, all efforts should be made to obtain similar information on course and outcome, patient to the partner's consent.

9.3.13 Further safety

Individual subject stopping criteria: If any subject demonstrates (i) clinical signs of potential serious liver injury, hepatotoxicity, and/or hyperbilirubinemia jaundice, or (ii) myopathy/rhabdomyolysis with or without acute renal failure secondary to myoglobinuria, or (iii) a hypersensitivity reaction determined by the investigator to be associated with treatment, that subject should be withdrawn from therapy.

An increase in serum aminotransferases to > 3x ULN will be followed by repeat testing within 48 to 72 hours to confirm the abnormality and determine whether they are increasing or decreasing. If an abnormality develops during the course of the study that meets any of the following criteria, the subject should be withdrawn:

- ALT or AST > 8x ULN;
- ALT or AST > 5x ULN for more than 2 weeks;
- ALT or AST > 3x ULN and (total bilirubin > 2x ULN or INR > 1.5); or
- ALT or AST > 3x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

If an ECG abnormality develops during the course of the study that meets the following criteria, the subject should be withdrawn:

- Any QTc interval > 500 milliseconds, correction by Fridericia's formula;
- Any QTc interval increase from baseline > 60 milliseconds, correction by Fridericia's formula;
- New onset of arrhythmias such as ventricular tachycardia, multifocal ectopic tachycardia, ventricular triplets, supraventricular tachycardia, atrial fibrillation, second- or third-degree heart block, or bradyarrhythmias; or
- New onset of ECG signs of cardiac ischemia such as, but not limited to, ST segment abnormalities (elevation or depression).

Study stopping criteria: A blinded safety review of liver function tests will be conducted when 25%, 50%, and 75% of the subjects have been enrolled. If 5% of the enrolled subjects in the study have persistent (e.g. confirmed on a repeated test and not resolved after 1 week) elevated liver function tests (AST, ALT) > 3xULN or experience myalgia or myositis with elevated CPK of grade 3 (severe), the investigator or designee will convene an independent drug safety group to assess the safety profile of the investigational drug for this study. In addition, any hepatocellular injury meeting the definition of Hy's Law will require study enrollment to be halted until an independent drug safety group is convened and their recommendation is received. Any potential Hy's Law case will be handled as a suspected unexpected serious adverse reaction.

9.3.13.1 Progressive disease

If progressive disease leads to signs and symptoms that meet the criteria for an SAE (i.e., hospitalization, disability, death, or important medical event), the signs and symptoms should be reported as an SAE and not the underlying progressive disease.

9.3.13.2 Death

If any patient dies during the trial or within 30 days of the End-of-Treatment Visit, the investigator or the institution's safety officer will document and record the cause of death in detail using the institutions SAE reporting procedure. The investigator or institution safety officer will complete the appropriate MedWatch report criteria and provide notification to Synthetic Biologics immediately.

10. Data handling and quality assurance

10.1 Data recording

The investigator will be responsible for assuring that all the required data will be collected and properly documented. It is the expectation that all data has source documentation available at the site. The site must implement processes to ensure compliance with Good Clinical Practice (GCP).

10.2 Monitoring

The investigator will appoint two monitors outside of the study team. The monitors are responsible for checking the quality of data and ensuring that all activities adhere to the study protocol. Additionally, the monitors ensure that legal and ethical requirements as stated in local laws and the

principles of GCP are being followed. Further information about the monitoring plan is included on a separate document titled “Data Safety Monitoring Plan”.

10.3 Data processing

10.3.1 Data collection

The study staff will enter the data required by the protocol into an electronic database from the source documents (e.g. medical records and study-specific data capture tools as needed). Data entered will be reviewed for completeness and accuracy.

10.3.2 Data handling and quality assurance

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each study participant. All information recorded in the database for this study must be consistent with the subjects’ source documentation (i.e. medical records).

The final data will be analyzed in accordance with the SAP.

10.4 Audit and inspection

Inspections by regulatory health authority representatives (i.e. FDA and IEC[s]/IRB[s]) are possible. The investigator should notify Synthetic Biologics immediately if a notification of inspection is received from the FDA regarding this study.

10.5 Archiving

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities’ request.

Patient (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

11. Premature termination of the study

- If risk-benefit ratio becomes unacceptable owing to:
    - Safety findings from this study (e.g. SAEs);
    - Results of parallel clinical studies;
    - Results of parallel animal studies (on e.g. toxicity, teratogenicity, carcinogenicity, or reproduction toxicity).
- If the study conduct (e.g. recruitment rate, drop-out rate, data quality, protocol compliance) does not suggest a proper completion of the trial within a reasonable timeframe.

The investigator has the right to close his/her center at any time.

For any of the above closures, the following applies:

- Closures should occur only after consultation between involved parties.
- All affected institutions (e.g. IEC[s]/IRB[s], competent authority[ies], study center, head of study center) must be informed as applicable according to local law.
- In case of a partial study closure, ongoing patients, including those in post study follow-up, must be taken care of in an ethical manner.

12. Ethical and legal aspects

12.1 Ethical and legal conduct of the study

The procedures set out in this protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abide by Good Clinical Practice (GCP) guidelines and under the guiding principles detailed in the Declaration of Helsinki. The study will also be carried out in keeping with applicable local law(s) and regulation(s).

Documented approval from appropriate IEC(s)/IRBs will be obtained for all participating centers before start of the study, according to GCP, local laws, regulations and organizations. When necessary, an extension, amendment or renewal of the EC/IRB approval must be obtained and also forwarded to Synthetic Biologics.

Strict adherence to all specifications laid down in this protocol is required for all aspects of study conduct; the investigator may not modify or alter the procedures described in this protocol.

Modifications to the study protocol will not be implemented by the investigator without discussion and agreement by Synthetic Biologics. However, the investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to the trial patients without prior IEC/IRB/Synthetic Biologics approval/favorable opinion. As soon as possible, the implemented deviation or change, the reasons for it and if appropriate the proposed protocol amendment should be submitted to the IEC/IRB/head of medical institution. Any deviations from the protocol must be explained and documented by the investigator.

The investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The investigator is responsible for personally overseeing the treatment of all study patients. The investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The investigator will be responsible for assuring that all the required data will be collected and properly documented.

12.2 Patient information and consent

This section must adhere to the following regulations as applicable:

- Section 4.8 of the ICH E6 Guideline for Good Clinical Practice.
- Health Insurance Portability and Accountability Act (HIPAA).

The following standard text should be adapted as needed:

Each patient/legal representative or proxy consenter will have ample time and opportunity to ask questions and will be informed about the right to withdraw from the study at any time without any disadvantage and without having to provide reasons for this decision.

Only if the patient/legal representative or proxy consenter voluntarily agrees to sign the informed consent form and has done so, may he/she enter the study. Additionally, the investigator and other information provider (if any) will personally sign and date the form. The patient/legal representative or proxy consenter will receive a copy of the signed and dated form.

The signed informed consent statement is to remain in the investigator site file or, if locally required, in the patient's note/file of the medical institution.

In the event that informed consent is obtained on the date that baseline study procedures are performed, the study record or patient's clinical record must clearly show that informed consent was obtained prior to these procedures.

If the patient is not capable of providing a signature, a verbal statement of consent can also be given in the presence of an impartial witness (independent of Synthetic Biologics and the investigator). This is to be documented by a signature from the informing physician as well as by a signature from the witness.

For minors or adults under legal protection, consent shall be given by the legal guardian(s). The consent of a minor or adult under legal protection shall also be requested where such a person is able to express his/her own will. His/her refusal or the withdrawal of his/her consent may not be disregarded.

In emergency situations, when prior consent of the patient is not possible, the consent of the patient's legal representative(s) or proxy consenter, if present, should be requested. The patient should be informed about the study as soon as possible and his/her consent to continue the study should be requested.

The informed consent form and any other written information provided to patients/legal representatives or proxy consenters will be revised whenever important new information becomes available that may be relevant to the patient's consent, or there is an amendment to the protocol that necessitates a change to the content of the patient information and/or the written informed consent form. The investigator will inform the patient/legal representative or proxy consenter of changes in a timely manner and will ask the patient to confirm his/her participation in the study by signing the revised informed consent form. Any revised written informed consent form and written information must receive the IEC/IRB's approval/favorable opinion in advance of use.

12.3 Publication policy

Synthetic Biologics recognizes the right of the investigator to publish results upon completion of the study. All investigator-initiated studies are the property of the investigator and publications generated from such studies are at the discretion of the investigator. Synthetic Biologics encourages investigators to publish the results of all studies supported through the Investigator Sponsored Study (ISS) Program.

Investigators are encouraged to submit a draft manuscript of supporting publication(s) or abstract(s) for a courtesy review prior to submission of the final version for publication or congress presentation. This will be reviewed promptly and will not be withheld unreasonably.

The investigator should ensure that the information regarding the study be publicly available on the internet at www.clinicaltrials.gov.

12.4 Confidentiality

All records identifying the patient will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available.

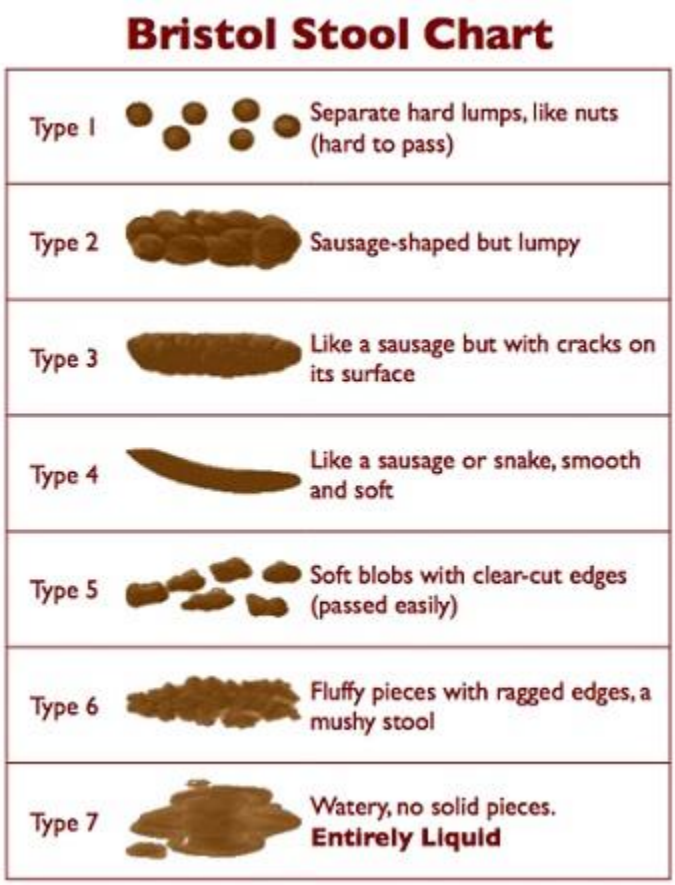
Should direct access to medical records require a waiver or authorization separate from the patient's statement of informed consent, the Investigator will obtain such permission in writing from the appropriate individual.

13. Appendices

13.1 Laboratory tests

CLINICAL CHEMISTRY	HEMATOLOGY	URINE SAMPLE
Electrolytes Bicarbonate Calcium, Total Chloride Potassium Sodium **Lipids** Cholesterol, Total Low Density Lipoprotein (LDL) Triglycerides (TG) **Liver Function Tests** Albumin Alanine Aminotransferase (ALT) Alkaline Phosphatase (ALP) Aspartate Aminotransferase (AST) Bilirubin, Total Protein **Metabolic Parameters** Glucose **Renal Function Tests** Creatinine Urea Nitrogen (BUN) **Other Tests** Creatine kinase (CK) Serum Pregnancy (bHCG; Screening)[1]	**Full Blood Exam** Hematocrit Hemoglobin Packed Cell Volume Platelet Count Mean Cell Volume Mean Cell Hemoglobin Mean Cell Hemoglobin Conc. Red Blood Cell count White Blood Cell Counts (total and differential)	**Drug Screen (Screening)** Amphetamine Barbiturates Benzodiazepines Cocaine Narcotics **Other Tests** Urine Pregnancy[2]
[1]Only women of child-bearing potential. [2]Urine pregnancy test can utilize a dipstick.		

13.2 Bristol stool chart



13.3 Daily questionnaire

Did you have a bowel movement today?

If yes, did it feel complete?

What did it look like? Please use the Bristol Stool Chart as a reference.

Did you have more than one bowel movement today?

If yes, did it feel complete?

What did it look like?

Did you have more than two bowel movements today?

If yes, did it feel complete?

What did it look like?

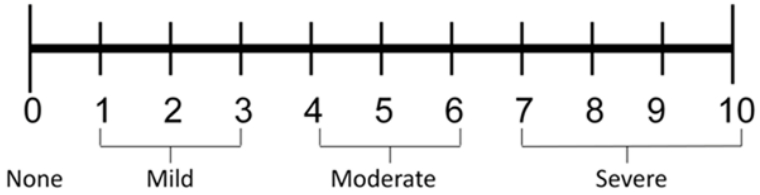
Did you take a laxative today?

If yes, please specify: Protocol-recommended rescue medication (bisacodyl, magnesium citrate, magnesium hydroxide, or lactulose) or Other laxative (oral, suppository, or enema)

If other, please specify the name of the laxative taken.

How severe has straining been during your bowel movements over the last 24 hours.

0 None (absent)
1 Mild (not very severe)
2 Moderate (somewhat severe)
3 Severe
4 Very Severe



Using the scale above, please rate the worst level of abdominal pain you have felt over the past 24 hours.

Bloating is a condition in which the belly (abdomen) feels full and tight or may look or feel swollen/distended. Patients may experience abdominal fullness, pressure, or a sensation of trapped gas.

Please rate the worst level of bloating you have felt over the last 24 hours.

0 None (absent)
1 Mild (not very severe)
2 Moderate (somewhat severe)
3 Severe
4 Very Severe

13.4 PAC-SYM

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

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

How severe have each of these symptoms been in the past week?					
	Absent (0)	Mild (1)	Moderate (2)	Severe (3)	Very Severe (4)

Discomfort in your stomach					
Pain in your stomach					
Bloating in your stomach					
Stomach cramps					
Painful bowel movements					
Rectal burning during or after a bowel movement					
Rectal bleeding or tearing during or after a bowel movement					
Incomplete bowel movement, as though you didn't "finish"					
Stools that were too hard					
Stools that were too small					
Straining or squeezing to try to pass stools					
Feeling like you had to pass a stool but you couldn't (false alarm)					

Overall, have you had adequate relief of your IBS symptoms over the last 7 days?

13.5 Quality of life questionnaire

Under each heading, please check the ONE box that best describes your health TODAY.

MOBILITY

I have no problems in walking about
I have slight problems in walking about
I have moderate problems in walking about
I have severe problems in walking about
I am unable to walk about

SELF-CARE

I have no problems washing or dressing myself
I have slight problems washing or dressing myself
I have moderate problems washing or dressing myself

I have severe problems washing or dressing myself
I am unable to wash or dress myself

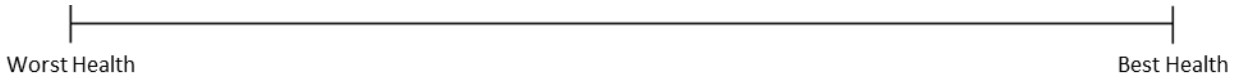
USUAL ACTIVITIES (e.g. work, study, housework, family, or leisure activities)
I have no problems doing my usual activities
I have slight problems doing my usual activities
I have moderate problems doing my usual activities
I have severe problems doing my usual activities
I am unable to do my usual activities

PAIN/DISCOMFORT
I have no pain or discomfort
I have slight pain or discomfort
I have moderate pain or discomfort
I have severe pain or discomfort
I have extreme pain or discomfort

ANXIETY/DEPRESSION
I am not anxious or depressed
I am slightly anxious or depressed
I am moderately anxious or depressed
I am severely anxious or depressed
I am extremely anxious or depressed

PLEASE RATE YOUR OVERALL HEALTH TODAY

The scale is from 0 to 100, where 0 is the WORST health you can imagine and 100 is the BEST health you can imagine.



13.6 Medical history

SECTION 1: DEMOGRAPHICS
Age
Gender
Height (inches)
Weight (pounds)

DEFINITIONS OF RACIAL CATEGORIES:

White:
A person having origins in any of the original peoples of Europe, North Africa, or the Middle East.

Black or African American:
A person having origins in any of the Black racial groups of Africa.

Asian:
A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

American Indian or Alaska Native:
A person having origins in any of the original peoples of North and South America, including Central America, and who maintains tribal affiliation or community attachment.

Native Hawaiian or Other Pacific Islander:
A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

Please choose all that apply.

Please indicate whether you are of Hispanic ethnicity.

DEFINITION:

Hispanic or Latino:
A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin regardless of race.

SECTION 2: MEDICAL HISTORY

What year was your constipation diagnosed?
Have you ever had an obstruction (blockage) of your bowel?
Have you ever had a fistula (abnormal tube-like connection between two organs)?
Have you had a perforation (cut/hole) in your bowel?
Have you had a fissure (tear in the skin) or abscess (pocket of inflamed tissue filled with pus) around the anus?

SECTION 3: SURGICAL HISTORY

Have you had an appendectomy (removal of the appendix)?
If yes, date of your appendectomy:

Have you had a cholecystectomy (removal of the gallbladder)?
If yes, date of your cholecystectomy:

Have you had a bowel resection (removal of part of the bowel)?
If yes, date of your bowel resection:

SECTION 4: SOCIAL HISTORY

Do you exercise?
Type of exercise? Please choose all that apply.
Aerobic
Strength training
Yoga/stretching

How would you describe your level of physical activity?

Inactive: Rare physical work or exercise
Active: Moderate physical work or leisure activities, such as running, cycling, swimming, or aerobics, for less than 30 minutes per day and less than 3 days per week
Very Active: Strenuous physical work or activities, such as running, cycling, swimming, or aerobics, for more than 30 minutes per day and at least 3 days per week

Do you, or did you, drink alcohol?
If yes, what type of alcohol do you drink?
How many standard drinks do you have per week?
1 glass of wine = 1.5 standard drinks
1 bottle of beer = 1 standard drink
1 oz. hard liquor = 1 standard drink

Do you, or did you ever, smoke?
If yes, what year did you start?
Did you quit?
If yes, what year did you quit?
On average, how many cigarettes do you, or did you, smoke per week?

Do you "vape" (use e-cigarettes)?

Are you exposed to secondhand smoke?

Do you, or did you ever, smoke marijuana?
If yes, what year did you start?
Did you quit?
If yes, what year did you quit?

Do you use recreational drugs (not including marijuana)?
If yes, what type of recreational drugs do you use?

Have you ever used recreational drugs in the past?
If yes, what type of recreational drugs did you use in the past?

SECTION 5: ADDITIONAL MEDICAL HISTORY
(For female subjects only.)
Have you ever been pregnant?
If yes, how many times have you been pregnant?

Have you ever taken hormone replacement therapy?
If yes, how many years have you been taking/did you use hormone replacement therapy?

Do you take birth control pills?

Are you menopausal?
If yes, natural or surgical menopause?

Are you pre-menopausal? Please choose all that apply.
No

Yes, hot flashes
Yes, night sweats
Yes, vaginal dryness

Have you had a hysterectomy?
If yes, what type of operation did you have?
Uterus
Uterus + one ovary
Uterus + both ovaries

14. References

1. Cash B, Sullivan S, Barghout V. Total costs of IBS: employer and managed care perspective. Am J Manag Care. Apr 2005; 11(1 Suppl):S7-16.
2. Drossman DA, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. Gastroenterol. Dec 2002;123(6):2108-2131.
3. Hungin AP, Whorwell PJ, Tack J, Mearin F. The prevalence, patterns and impact of irritable bowel syndrome: an international survey of 40,000 subjects. Aliment Pharmacol Ther. Mar 2003;17(5):643-650.
4. Drossman DA, Dumitrascu DL. Rome III: New standard for functional gastrointestinal disorders. J Gastrointestin Liver Dis. Sep 2006;15(3):237-241.
5. Brandt LJ, Chey WD, Foxx-Orenstein AE, et al. An evidence-based position statement on the management of irritable bowel syndrome. Am J Gastroenterol. Jan 2009;104(Suppl 1):S1-35.
6. Talley NJ, Zinsmeister AR, Van Dyke C, Melton LJ 3rd. Epidemiology of colonic symptoms and the irritable bowel syndrome. Gastroenterol. Oct 1991;101(4):927-934.
7. Pimentel M, Gunsalus RP, Rao SSC, Zhang H. Methanogens in human health and disease. Am J Gastroenterol. Jul 2012;1(1) Suppl:28-33.
8. Pimentel M, Mayer AG, Park S, Chow EJ, Hasan A, Kong Y. Methane production during lactulose breath test is associated with gastrointestinal disease presentation. Dig Dis Sci. Jan 2003;48(1):86–92.
9. Kunkel D, Basseri RJ, Makhani MD, Chong K, Chang C, Pimentel M. Methane on breath testing is associated with constipation: a systematic review and meta-analysis. Dig Dis Sci. 2011 Jun;56(6):1612-8.
10. Furnari M, Savarino E, Bruzzone L, Moscatelli A, Gemignani L, Giannini EG, Zentilin P, Dulbecco P, Savarino V. Reassessment of the role of methane production between irritable bowel syndrome and functional constipation. J Gastrointestin Liver Dis. 2012 Jun;21(2):157-63.
11. Ghoshal U, Shukla R, Srivastava D, Ghoshal UC. Irritable Bowel Syndrome, particularly the constipation-predominant form, involves an increase in Methanobrevibacter smithii, which is associated with higher methane production. Gut Liver. 2016 Nov 15;10(6):932-938.
12. Lee KM, Paik CN, Chung WC, Yang JM, Choi MG. Breath methane positivity is more common and higher in patients with objectively proven delayed transit constipation. Eur J Gastroenterol Hepatol. 2013 Jun;25(6):726-32.
13. Suri J, Kataria R, Malik Z, Parkman HP, Schey R. Elevated methane levels in small intestinal bacterial overgrowth suggests delayed small bowel and colonic transit. Medicine (Baltimore). 2018 May;97(21):e10554.

14. Chang BW, Rezaie A. Irritable Bowel Syndrome-Like Symptoms Following Fecal Microbiota Transplantation: A Possible Donor-Dependent Complication. Am J Gastroenterol. 2017 Jan;112(1):186-187.
15. Park YM, Lee YJ, Hussain Z, Lee YH, Park H. The effects and mechanism of action of methane on ileal motor function. Neurogastroenterol Motil. 2017 Sep;29(9). doi: 10.1111/nmo.13077.
16. Pimentel M, Lin HC, Enayati P, van den Burg B, Lee HR, Chen JH, Park S, Kong Y, Conklin J. Methane, a gas produced by enteric bacteria, slows intestinal transit and augments small intestinal contractile activity. Am J Physiol Gastrointest Liver Physiol. 2006 Jun;290(6):G1089-95.
17. Jahng J, Jung IS, Choi EJ, Conklin JL, Park H. The effects of methane and hydrogen gases produced by enteric bacteria on ileal motility and colonic transit time. Neurogastroenterol Motil. 2012 Feb;24(2):185-90, e92.
18. Mevacor® (lovastatin) tablets. Prescribing information, 2014. Merck & Co., Inc., Whitehouse Station, NJ 08889, USA.
19. Duggan DE, Chen IW, Bayne WF, Halpin RA, Duncan CA, Schwartz MS, Stubbs RJ, Vickers S. The physiological disposition of lovastatin. Drug Metab Dispos. 1989 Mar-Apr;17(2):166-73.
20. Marsh E, Morales W, Chua KS, Marsh Z, Weitsman S, Wacher V, Kim JH, Pimentel, M. (2015) [Abstract 1771] Lovastatin lactone inhibits methane production in human stool homogenates. Am J Gastroenterol. 110 (Suppl. 1): S753.
21. Morales W, Marsh E, Yu A, Marsh Z, Weitsman S, Barlow GM, Rezaie A, Chang C, Wacher V, Pimentel, M. (2015) [Abstract Mo 2051] Lovastatin improves stool form in Methanobrevibacter smithii colonized rats with constipation. Gastroenterology 148 (Suppl. 1): S-779-80.
22. Gottlieb K, Wacher V, Sliman J, Coughlin O, McFall H, Rezaie A, Pimentel M. (2016) [Abstract Su1210] SYN-010, a proprietary modified-release formulation of lovastatin lactone, lowered breath methane and improved stool frequency in patients with IBS-C: results of a multi-center randomized double-blind, placebo-controlled Phase 2a trial. Gastroenterology 150 (Suppl. 1): S496-7.
23. Wacher V, Gottlieb K, Sliman J, Coughlin O, Kokai-Kun, J, McFall H, Pimentel M (2016) [Abstract 562] SYN-010 modified-release lovastatin does not significantly alter lipid parameters at doses that reduce methane and alleviate symptoms in patients suffering irritable bowel syndrome with constipation (IBS-C). Am J Gastroenterol. 111 (Suppl. 1): S256.
24. Hubert S, Chadwick A, Wacher V, Coughlin O, Kokai-Kun J, Bristol A. Development of a modified-release formulation of lovastatin targeted to intestinal methanogens implicated in Irritable Bowel Syndrome with constipation. J Pharm Sci. 2018 Feb;107(2):662-671.
25. Muskal SM, Sliman J, Kokai-Kun J, Pimentel M, Wacher V, Gottlieb K. Lovastatin lactone may improve irritable bowel syndrome with constipation (IBS-C) by inhibiting enzymes in the archaeal methanogenesis pathway. Version 3. F1000Res. 2016 Apr 8 [revised 2016 Jan 1];5:606.
26. Camilleri M, Lembo AJ, Lavins BJ, MacDougall JE, Carson RT, Williams VS, Nelson LM, Shiff SJ, Currie MG, Kurtz CB, Johnston JM. Comparison of adequate relief with symptom, global, and responder endpoints in linaclotide phase 3 trials in IBS-C. United European gastroenterology journal. 2015 Feb 1;3(1):53-62.
27. Passos MC, Lembo AJ, Conboy LA, Kaptchuk TJ, Kelly JM, Quilty MT, Kerr CE, Jacobson EE, Hu R, Friedlander E, Drossman DA. Adequate relief in a treatment trial with IBS patients: a prospective assessment. Am J Gastroenterol. 2009 Apr 1;104(4):912-9.
28. Lacy BE, Mearin F, Chang L, Chey WD, Lembo AJ, Simren M, Spiller R. Bowel disorders. Gastroenterology. 2016 May;150(6):1393-1407.e5.