Statistical Analysis Plan

Sponsor Name:	Medically Associated Science and Technology (MAST) Program Cedars-Sinai Medical Center
Institution Study Number:	54792
Protocol 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)**
Protocol Version and Date:	Protocol Version 3.0, 9 January 2019 Investigator Sponsored Research (ISS) Protocol
Clinical Study Phase:	2
NCT03763175	
SAP Version:	2.0
SAP Version Date:	19 March 2019

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Version: **2.0** **Version Date:** **19 March 2019**

I confirm that I have reviewed this document and agree with the content.

APPROVALS	
Cedars-Sinai Medical Center	
	3/21/2019
Approved by: , MD Cedars-Sinai Medical Center	Date

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1. ABBREVIATIONS

Abbreviation	Description
AE	Adverse Event
ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BMI	Body Mass Index
BSFS	Bristol Stool Form Scale
CH_4	Methane
CO_2	Carbon dioxide
CSBM	Complete Spontaneous Bowel Movement
CRF	Case Report Form
EQ-5D-5L	A standardized measure of health status developed by the EuroQol group
ECG	Electrocardiogram
EOS	End Of Study
FAS	Full Analysis Set
H_2	Hydrogen
H_2S	Hydrogen sulfide
IBS	Irritable Bowel Syndrome
LOCF	Last Observation Carried Forward
MAST	Medically Associated Science and Technology
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
NRS	Numeric Rating Scale
PAC-SYM	Patient Assessment of Constipation – Symptom
PP	Per-Protocol
QTcF	QT Corrected with Fridericia's Formula
SAP	Statistical Analysis Plan
SD	Standard Deviation
SBM	Spontaneous Bowel Movement

Abbreviation	Description
TEAE	Treatment Emergent Adverse Event
WHO	World Health Organization

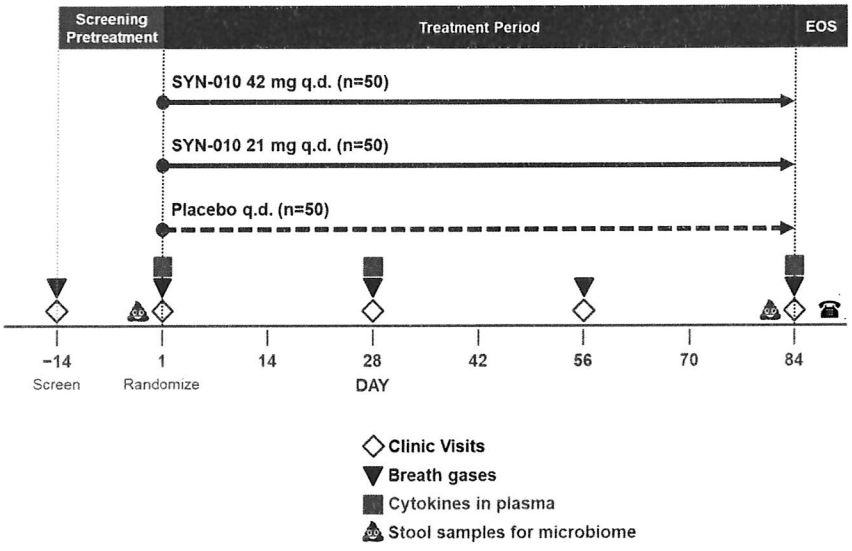
2. PURPOSE

This Statistical Analysis Plan (SAP) is based on Protocol Version 3.0 (9 January, 2019) and it describes in detail the statistical methodology and the statistical analyses to be conducted for the above-mentioned protocol.

Results obtained from the analyses outlined in this document will become the basis of the final clinical study report for this protocol. The purpose of this plan is to provide specific instructions as to how each analysis will be conducted.

2.1. STUDY DESIGN

Single-center, randomized, double-blind, placebo-controlled clinical trial comprising three periods:
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.



2.2. 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. Fifty subjects in each group will have 90% power for each SYN-010 group compared with the placebo by using the t-test with 2-sided alpha = 0.05.

2.3. RANDOMIZATION AND ADMINISTRATION OF STUDY DRUG

After successful completion of the Screening and Pre-treatment Period, 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). Subjects will take SYN-010 or matching placebo orally once daily at bedtime for 12 weeks.

3. ENDPOINTS

3.1. PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is the change from baseline in the weekly average number of complete spontaneous bowel movements [CSBMs] during the 12-week Treatment Period.

A CSBM is a spontaneous bowel movement [SBM] that is associated with a sense of complete evacuation. An SBM is a bowel movement that occurs in the absence of rescue medicine use or other laxative use (e.g. suppository or enema) on the calendar day of bowel movement or the calendar day before the bowel movement.

3.2. SECONDARY EFFICACY ENDPOINTS

The secondary efficacy 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 subject 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 subject'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 subject 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 subject with a weekly abdominal pain intensity response in at least 50% of the weeks of treatment (6 of 12 weeks). A weekly abdominal pain intensity response is defined as a decrease in the subject'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 changes 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 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 overall bloating responders with different cutoff points for the % improvement from baseline in weekly average bloating score, i.e. ≥ 10%, 20%, 40%, 50%, 60%, and 70%.

3.3. EXPLORATORY EFFICACY ENDPOINT

Exploratory endpoints are listed as the following parameters:

- Proportion of subjects who have adequate relief of Irritable Bowel Syndrome (IBS) symptoms.
- Change from baseline for SYN-010 in patient reported outcomes (PRO) using validated questionnaires in the REDCap system.
    - Change from baseline in weekly PAC-SYM scores.
    - Change from baseline in weekly straining score.
    - Assessment of Quality of Life measures using the EQ-5D-5L questionnaire.
- 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.

3.4. SAFETY ASSESSMENTS

Safety assessments will include the collection of AEs, ECG recordings, laboratory tests (clinical chemistry, hematology, lipid, cytokine, and creatine phosphokinase), physical examinations, urine and serum pregnancy tests (when applicable), and rescue medication use.

4. ANALYSIS POPULATIONS

4.1. RANDOMIZED SUBJECT POPULATION

The randomized subject population will consist of all subjects who are randomized.

4.2. FULL ANALYSIS SET

The Full Analysis Set (FAS) will consist of all randomized subjects 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 subjects receive, and safety analysis will be conducted according to the actual treatment the subjects receive.

4.3. PER PROTOCOL ANALYSIS SET

The Per Protocol (PP) analysis set will consist of all subjects in the full analysis set who do not have any major protocol deviations. The major protocol deviations will include the following, but not limited to:

- Did not meet important eligibility criteria
- Missed more than 20% of planned medication

The Per Protocol (PP) analysis set will be identified before unblinding the study, and will be used in a secondary analysis of the primary endpoint and selected secondary endpoints.

4.4. PROTOCOL DEVIATIONS

Protocol deviations identified by site staff and study monitors will be documented before database lock.

5. EFFICACY ASSESSMENTS

5.1. DAILY DIARY, BREATH TESTS, AND OTHER QUESTIONNAIRES

Subjects will be provided an ID number and password-protected access to the REDCap website to 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 using the Numeric Rating Scale (0-10), straining, and bloating assessment using a Likert scale (0-4). Questionnaires will also be completed weekly for PAC-SYM and Adequate Relief question. 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.

Lactulose breath tests will be performed during the Screening Visit and 12-Week Visit. 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 hours. 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.

Methane breath tests will be performed during the Randomization Visit and the 4- and 8-Week Visits. 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.

6. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

6.1. GENERAL METHODS

Unless otherwise specified, for numeric data, descriptive statistics will include the number of subjects with data to be summarized (n), mean, standard deviation (SD), median, minimum (min), and maximum (max). The same number of decimal places as in the raw data will be presented when reporting min and max, 1 more decimal place than in the raw data will be presented when reporting mean and median, and 2 more decimal places than in the raw data will be presented when reporting SD. If the raw data have 3 decimals or more, 3 decimals will be presented for mean, median, min and max, and SD.

All categorical/qualitative data will be presented using absolute or relative frequency counts and percentages. All percentages will be presented with 1 decimal point. Percentages equal to 100 will be presented as 100% and percentages will not be presented for zero frequencies but the categories whose counts are zero will be displayed for the sake of completeness.

P-value > 0.9999 will be present as '>0.9999' and p-value < 0.0001 will be presented as '< 0.0001'.

All analyses and summary outputs will be generated by treatment group (Placebo, SYN-010 21 mg, SYN-010 42 mg) using SAS® version 9.3 (or higher).

Data collected in this study will be presented in by-subject listings.

6.2. BASELINE AND STUDY DAY DEFINITIONS

Baseline definition will vary upon the data/variables for summaries/analyses.

Table 1: Baseline Definitions

Data/Variables	Baseline Definition
Laboratory, vital signs, and ECG	The last non-missing value prior to the first dose of the study drug
Breath CH_4 AUC measurement	AUC of breath CH_4 measurements collected during lactulose breath test performed at Screening after an overnight fast
Singe-point breath CH_4 measurement	A single-point breath CH_4 measurement collected at Randomization after an overnight fast
Weekly average score for worst abdominal pain in the past 24 hours	(Sum of daily pain scores) / (number of days with pain score assessment during the 7 days prior to the first dose of study drug)
Stool frequency of CSBMs per week/Weekly average number of CSBMs	7 x (sum of daily number of CSBMs) / (number of days with CSBM assessment

	during the 7 days prior to the first dose of study drug)
Stool frequency of SBMs per week/Weekly average number of SBMs	7 x (sum of daily number of SBMs) / (number of days with SBM assessment during the 7 days prior to the first dose of study drug.
Weekly average of bloating score	Sum of daily bloating scores / (number of days with bloating score assessments during 7 days prior to the first dose of study drug)
Weekly average of straining score	Sum of daily straining scores / (number of days with straining score assessments during 7 days prior to the first dose of study drug)
Adequate Relief	The last non-missing value prior to the first dose of the study drug
Weekly stool consistency score (BSFS)	Sum of (i × number of type i stools) / total number of stools during 7 days prior to the first dose of study drug, where stool consistency i = 1, 2,…7
EQ-5D-5L score	The last non-missing value prior to the first dose of the study drug

Study day will be defined as follows:

- The first dose of study drug is designated as Day 1.
- For visit days after Day 1, study day = visit date - Day 1 date + 1.
- For visit days prior to Day 1, study day = visit date - Day 1 date. Thus, study days for screening visit are negative numbers. There is no "Day 0".

6.3. VISIT WINDOWS

The unscheduled or early termination (ET) visit will be mapped to a scheduled visit for analysis using the date of collection/assessment as a basis to determine study day and then study day will be mapped to the intended visit. The table below contains the analysis visit windows.

Once analysis visit windows get assigned, all visits, including scheduled visits, unscheduled visits, and early termination visits will be eligible for being flagged as the "analyzed record" within the analysis visit window, a subject's individual analysis visit window could potentially contain more than one visit. In the event of multiple visits falling within an analysis visit window or in case of a tie, the following rules will be used in sequence to determine the "analyzed record" for the analysis visit window:

- If there is a scheduled visit/week for the analysis visit window, then the scheduled visit/week data will be used.

- If there is no scheduled visit/week for the analysis visit window, the data closest to the scheduled day will be used.
- If there is no scheduled visit/week for the analysis visit window and there is a tie between the data in the number of days before and after the scheduled day, the later data will be used.

The data not flagged as the "analysed record" will also be listed in subject listings.

Table 2: Definitions of Analysis Windows

Study Day Window	Scheduled day	Scheduled Visit/Week
Days (-17) to (-1)	Day -1	Visit 1/Screening
Day 1	Day 1	Visit 2/Randomization
Day 2 - 42	Day 28	Visit 3/Day 28
Day 43 – 70	Day 56	Visit 4/Day 56
Day 71 - 84	Day 84	Visit 5/Day 84
Day 85 - 86	Day 85	End-of-Study Contact

6.4. MISSING DATA

For the analyses of various weekly responses, the week with missing assessments for more than 3 days will be treated as "non-responder" for the week. 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.

For the analyses of various weekly average scores for IBS symptoms, the weekly average score will be set to "missing" if there are more than 3 days with missing assessments during a week, and the data will be considered as "missing" for the week. An observed-cases approach to missing data will be applied to the continuous type variables; this means that if the data is considered as "missing" for the week, the average of the non-missing data over the 12 weeks of the treatment period will be the subject's value for that week.

Subjects will be assumed to have not had bowel movement nor taken rescue medication if the corresponding daily question is not answered.

7. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

7.1. SUBJECT DISPOSITION AND WITHDRAWALS

The number of all randomized subjects and the number and percentage of subjects in each analysis population will be presented by treatment group. The number of subjects who completed the study and the number who discontinued prematurely and the reason for discontinuation will be presented by treatment group for randomized subjects.

For the screen failure subjects, reasons for screen failure will be summarized separately.

7.2. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Demographics and baseline characteristics will be summarized by treatment group for the full analysis set.

Demographics and baseline characteristics include: age, gender, race, ethnicity, baseline breath methane values, height, weight, and body mass index (BMI).

- Height (in cm) = height (in inches) * 2.54
- Weight (in kg) = weight (in lbs) * 0.4536

7.3. MEDICAL/SURGICAL HISTORY

Medical/surgical history collected at Screening will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

These data will be summarized by system organ class (SOC) and preferred term (PT) and treatment group for the full analysis set.

7.4. MEDICATION

Medications will be coded with generic names using IBM Micromedex, which is a collection of drug databases.

7.4.1. Prior Medication

Prior medications will be defined as medications with a start date prior to the first dose of study drug. The stop date of the medication may be before or after the first dose of study drug or the medication may be ongoing. If a start date is completely missing, the medication will be considered a prior medication.

7.4.2. Concomitant Medications

Concomitant medications will be defined as medications with a stop date on or after the first dose of study drug or any medication that is ongoing. The start date of the medication may be before or after the first dose of study drug. A medication with completely missing use dates or partially

missing use dates without evidence that the medication was stopped prior to the first dose of study drug will be considered a concomitant medication.

Concomitant medications will be summarized by ATC level 2 term, PT, and treatment group for the full analysis set.

7.5. PROTOCOL DEVIATIONS

Major protocol deviations will be summarized by treatment group for the full analysis set. The categorization (e.g., informed consent, inclusion/exclusion, missing assessment) and classification (major/minor) of protocol deviations will be performed prior to database lock.

8. EFFICACY ANALYSIS

Observed data at each time point and change from baseline to each post-baseline visit/time point will be summarized by treatment group. For categorical data, frequency counts and percentages will be presented over time by treatment group.

All efficacy analyses will be performed on the full analysis set, and efficacy analyses will be conducted according to the randomized treatment the subjects receive. Sensitivity analyses of the primary efficacy endpoint and selected secondary efficacy endpoints will be performed. In addition, an analysis of the primary efficacy endpoint and selected secondary efficacy endpoints will be performed for the per protocol population.

8.1. PRIMARY EFFICACY ENDPOINT AND ANALYSIS

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

The analysis for the primary endpoint will be performed using the analysis of covariance (ANCOVA) with treatment group and the baseline value as covariates based on the full analysis set. The primary analysis will be conducted using the observed values. To control the overall false positive rate at 0.05, the Hochberg testing procedure will be used. Specifically, let P_1 and P_2 be the P-values from the ANCOVA comparing the 42 and 21 mg dose group with the placebo, respectively, if $\text{Max}(P_1,P_2) \leq 0.05$, then the primary endpoint will achieve statistical significant for both doses. Otherwise, if $\text{Min}(P_1,P_2) \leq 0.025$, then the primary endpoint will achieve statistical significance for the dose that has smaller P-value. If $\text{Min}(P_1,P_2) > 0.025$, then the primary endpoint will not achieve statistical significance for either dose group. The nominal P-values and 95% confidence intervals (CI) will be provided from the ANCOVA model.

Two sensitivity analyses will be conducted by using the multiple imputation and last observation carried forward (LOCF) methods to impute the missing data for the primary endpoint. The same ANCOVA model will be used for the sensitivity analyses.

In addition, the analyses of the primary endpoint will be performed based on the PP population using the same statistical model.

8.2. SECONDARY AND EXPLORATORY EFFICACY ENDPOINTS AND ANALYSIS

8.2.1. Secondary Efficacy Endpoints

The secondary efficacy endpoints are listed below:

- The proportion of overall responders during the 12-week Treatment Period (overall 12-week responders).
- Proportion of overall stool frequency responders during the 12-week Treatment Period.
- Proportion of overall abdominal pain intensity responders during the 12-week Treatment Period.
- Proportion of overall bloating responders during the 12-week Treatment Period.

- Proportion of patients using rescue medication.

The comparisons of the proportions will be performed using the Chi-squared test for equal proportions based on the full analysis set. The Chi-squared test from SAS Proc Freq will be used for the comparison of the treatment differences (each SYN-010 dose group versus placebo). Nominal P-values and 95% Newcombe-Wilson CIs for treatment differences will be presented.

To control the overall false positive rate, a fixed sequence testing procedure will be used for the testing of the secondary endpoints listed above. Specifically, the secondary endpoints will be tested formally if statistical significance is achieved for the primary endpoint for both dose groups, otherwise, the secondary endpoints will not be tested formally. If statistical significance is achieved for the primary endpoint for both dose groups, then the secondary endpoints will be tested formally in the order of the list for the 42 mg dose. The first listed secondary endpoint will be tested at the 2-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 2-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 for the 42 mg dose group. If statistical significance is achieved for all listed secondary endpoints of the 42 mg dose group, then the testing procedure will be repeated for the 21 mg dose group.

The 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 3 days with missing assessments during a week.

Two sensitivity analyses for the overall responders will be conducted: (1) missing data for the responders will be imputed as "responders"; (2) the overall responders are defined based on the LOCF for missing values of the weekly average score for worst abdominal pain and/or weekly average number of CSBMs.

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. Weekly average score for worst abdominal pain = (sum of daily worst abdominal pain scores) / (number of days with worst abdominal pain score assessment for the specific week).

- Percent 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 = (the weekly average score for worst abdominal pain for the specific week - the weekly average score for worst abdominal pain at baseline) / (the weekly average score for worst abdominal pain at baseline).

- Change from baseline in weekly average number of CSBMs at Weeks 1 through 12. Weekly average number of CSBMs = 7 x (sum of daily number of CSBMs) / (number of days with CSBM assessment) for the specific week.

- Change from baseline in weekly average number of SBMs at Weeks 1 through 12. Weekly average number of SBMs = 7 x (sum of daily number of SBMs) / (number of days with SBM assessment) for the specific week.

- Change from baseline in weekly average bloating score at Weeks 1 through 12. Weekly average bloating score = (sum of daily bloating scores / number of days with bloating score assessment) for a specific week.

- Change from baseline in weekly stool consistency score (BSFS) at Weeks 1 through 12. Weekly stool consistency score = sum of (i × number of type i stools in a week) / total number of stools in the specific week, where i = 1, 2,…7.

- 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 overall bloating responders with different cutoff points for the % improvement from baseline in weekly average bloating score, i.e. ≥ 10%, 20%, 40%, 50%, 60%, and 70%.

The same analysis of covariance (ANCOVA) model will be used for the analysis of the continuous secondary endpoints. The model will include treatment group and the corresponding baseline value as covariates. Nominal P-values and least squares treatment means and their differences will be presented, accompanied by 95% CIs for each time point. The comparisons of the proportions will be performed using the Chi-squared test for equal proportions.

All analyses will be based on the full analysis set.

8.2.2. Exploratory Efficacy Endpoints

Exploratory endpoints are listed as the following parameters:

- Proportion of subjects who have adequate relief of IBS symptoms.
- Change from baseline for SYN-010 in PROs using validated questionnaires in the REDCap system.
    - Change from baseline in weekly PAC-SYM scores, the averages of the PAC-SYM scores, abdominal, rectal, and stool symptom scores.
        - The average of the PAC-SYM scores is based on the 12 items from PAC-SYM, and is the sum of non-missing item scores within the instrument / the total number of non-missing items (score range, 0–4). The averages of the abdominal, rectal, and stool symptom scores are based on items 1-4, 5-7, and 8-12, respectively. Each average score is the sum of non-missing item scores within the subscale / the total number of non-missing items for that subscale (score range, 0–4).
    - Change from baseline in weekly straining score at Weeks 1 through 12. Weekly average of straining score = (sum of daily straining scores / number of days with straining score assessment) for a specific week.
    - Assessment of Quality of Life measures using the EQ-5D-5L questionnaire.

- 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 same analysis of covariance (ANCOVA) model will be used for the analysis of the continuous exploratory endpoints except for the serum cytokine levels.

For the EQ-5D-5L scores, the summary statistics will be provided for the 5 dimensions as stated in the 'EQ-5D-5L User Guide' by Mandy van Reenen and Bas Janssen. In addition, summary statistics will be provided for the EQ VAS scores and change from baseline in EQ VAS scores by treatment group.

8.2.3. Additional Exploratory Efficacy Endpoints

Additional exploratory efficacy endpoints will be evaluated as follows:

- Change from baseline in the area-under-the-curve (AUC) of breath H_2 production, based on the 120-minute lactulose breath test.

- 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).

The serum cytokine levels (IL-2, IL-4, IL-5, IL-9, IL-10, IL-13, IL-17, GM-CSF, IFN-γ, TNF-α, IL-12, IL-6, IL-8, MCP-1, IL-25) and the microbiome measurements in stool of bacteria (16S rRNA) and methanogens (qPCR) will be summarized by treatment group with mean and standard deviation, median, and range at each time point. The nominal P-values from the Wilcoxon rank sum test for the change from baseline will be provided.

8.3. SUBGROUP ANALYSES

Subgroup analyses by race, gender, and BMI at Screening (<30, ≥ 30) will be conducted for the following endpoints, using their corresponding statistical analysis method as mentioned before:

- Change from baseline in weekly average number of CSBMs at Weeks 1 through 12
- Proportion of overall responders during the 12-week Treatment Period
- Proportion of overall abdominal pain intensity responders during the 12-week Treatment Period.
- Proportion of overall stool frequency responders during the 12-week Treatment Period.
- 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 bloating score at Weeks 1 through 12

All the subgroup analyses will be based on the full analysis set.

9. SAFETY

Safety analyses will be based on the full analysis set, and the analysis will be conducted according to the actual treatment the subjects receive. No inferential statistics will be performed, and only summary statistics will be provided. Missing safety data will not be imputed.

9.1. EXTENT OF EXPOSURE

Exposure to study drug (in days) is calculated as last dose date of study drug – first dose date of study drug + 1. Study drug exposure will be summarized by treatment group.

9.2. TREATMENT COMPLIANCE

Study drug compliance (%) is calculated as 100 x (total number of capsules dispensed – total number of capsules returned)/exposure of study drug (in days) x 1 capsule per day.

Compliance will be summarized by treatment group. In addition, the number and percentage of subjects in pre-specified compliance categories (< 80%, 80-100%, > 100%) will be summarized by treatment group.

9.3. ADVERSE EVENTS

Adverse events will be coded using MedDRA to classify events under primary SOC and PT.

A TEAE is defined as an AE that either begins or first recognized after the first dose of study drug or worsens after the first dose of study drug.

The TEAEs below will be summarized by SOC, PT and treatment group. Multiple occurrences of an AE are counted only once per subject per SOC and PT for summary tables.

- Incidence of all TEAEs
- Incidence of all TEAEs by maximum severity (in the following order: severe, moderate and mild) specified by investigators
- Incidence of TEAE by strongest relationship to study drug specified by investigators (in the following order: very likely/certain, probable, possible, unlikely, and unrelated).
- Incidence of serious TEAEs
- Incidence of Serious TEAEs related to study drug specified by Investigators
- Incidence of TEAEs leading to study drug withdrawn

All data collected in the AE case report form (CRF) will be listed in by-subject listings.

9.4. LABORATORY EVALUATIONS

If a continuous laboratory value is reported as either below or above the limits of quantification, the qualifiers should be dropped and the numeric value used in the analysis (e.g., "< 3" should be "3" and "> 200" should be "200").

The observed data at each time point and change from baseline at each post-baseline time point in hematology, serum chemistry and quantitative urinalysis test results will be summarized by treatment group.

For hematology, serum chemistry, lipid, cytokine, and creatine phosphokinase, normal ranges for each parameter will be used to categorize the test result as low (value lower than the lower limit), normal (value within the normal range), or high (value higher than the upper limit). Frequency counts and percentages will be presented over time by treatment groups for these categorical data. In addition, shifts from baseline to each post-baseline time point for each parameter will be summarized by treatment group.

9.5. VITAL SIGNS AND 24-HOUR AMBULATORY BLOOD PRESSURE

Observed data at each time point and the change from baseline at each post-baseline time point for vital signs will be summarized by treatment group.

9.6. ECG

Observed data at each time point and the change from baseline at Week 12 in ECG parameters (HR, RR, PR, QRS, and QTcF) will be summarized by treatment group.

The number and percentage of subjects with QT and QT corrected with Fridericia's formula (QTcF) values falling into the following categories at Week 12 will be summarized by treatment group:

- Change from baseline of 30 – 60 msec in QT and QTcF.
- Change from baseline of > 60 msec in QT and QTcF
- Week 12 value > 450 and baseline <= 450 msec in QT and QTcF
- Week 12 value > 500 and baseline <= 500 msec in QT and QTcF

QTcF (msec) is calculated as: QT (msec)/ $RR^{1/3}$, where RR = 60 / heart rate (bpm).

The number and percentage of subjects with other clinically significant ECG findings and rhythm findings will also be summarized at Week 12 by treatment group.

9.7. RESCUE MEDICATION

The number and percentage of subjects using protocol-specified rescue medication at any time post-baseline will be summarized by treatment.