NCT Number:	NCT04598295
Official Title:	SH-DS01 on Fecal Metagenomic Stability
Document Type:	Study Protocol with SAP
Document Date:	04/21/2020

PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	A randomized, double blind, placebo-controlled study to evaluate the impact of a multi-strain synbiotic on fecal metagenomic stability, gut barrier integrity, and metabolic output of the gut microbiota in premenopausal adult females.
Principal Investigator	Anthony Lembo

B1. PURPOSE OF PROTOCOL

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder that is commonly seen in clinical practice. Specifically, it is a functional bowel disorder that is thought to result from a disorder of gut–brain interaction. Though patients with IBS often have a heterogeneous symptom profile, the predominant theme is the presence of abdominal pain or discomfort that is usually relieved by defecation. Genetic background and environmental factors are important in the pathogenesis of IBS, but the precise cause of IBS is still unknown.

Probiotics are live microorganisms with a vast array of therapeutic potential for gastrointestinal disease. Several probiotics strains have shown beneficial outcomes in constipation-predominant IBS (IBS-C) patients, especially as an adjunct to conventional treatment. However, a number of controversial issues regarding the roles of probiotics in pathogenesis of IBS-C remain to be clarified, including precise mechanism of action.

This protocol aims to assess the impact of a mix of 24-beneficial strains on fecal metagenomic stability and individual gastrointestinal symptoms specifically in a cohort of subjects with IBS-C or IBS-M.

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

The study agent is a 24-strain cocktail of live microorganisms (Seed Health Inc.). Seed Health Inc. is providing the consortia of probiotics, whose individual ingredients carry the designation Generally Recognized as Safe (GRAS) by the US FDA, which is a designation applied to all food ingredients. The live mircroorganisms included are: *Bifidobacterium breve SD-BR3-IT, Lactobacillus rhamnosus SD-LR6-IT, Lactobacillus plantarum SD-LP1-IT, Bifidobacterium longum SD-BB536-JP, Lactobacillus reuteri SD-LRE2-IT, Bifidobacterium infantis SD-M63-JP, Lactobacillus rhamnosus HRVD113-US, Bifidobacterium lactis HRVD574-US, Bifidobacterium breve HRVD521-US, Lactobacillus casei HRVD300-US, Bifidobacterium longum HRVD90b-US, Bifidobacterium lactis SD-BS5-IT, Bifidobacterium lactis SD150-BE, Lactobacillus rhamnosus SD-GG-BE, Lactobacillus reuteri RD830-FR, Bifidobacterium adolescentis SD-BA5-IT, Lactobacillus crispatus SD-LCR01-IT, Lactobacilus salivarius SD-CRL1328-IT, Lactobacillus fermentum SD-LF8-IT, Bifidobacterium longum SD-CECT7347-SP, Lactobacillus casei SD-CECT9104-SP, Bifidobacterium lactis SD-CECT8145-SP, Lactobacillus plantarum SD-LDL400-UK, Bifidobacterium lactis SD-MB2409-IT*. Prebiotic extracts *Punica granatum*, *Inonotus obliquus and. Pinus sylvestris* are also included in the capsules.

Previous studies performed on individual live microorganisms in the Seed Health inc. synbiotic have shown significant improvement in multiple markers of digestive health intestinal transit time, ease of expulsion, bloating, discomfort and stool consistency. After 2 weeks of supplementation with *Bifidobacterium longum* BB536, healthy adults suffering from constipation experienced an increase in defecation frequency, alongside changes in fecal color, volume and shape. Lower concentrations of ammonia production were also reported during the period of *B. longum* BB536 administration. In addition to changes in fecal enzymes, which are important for controlling the amount of fecal putrefactive substances, a study in 110 healthy volunteers (50 males and 60 females) treated with *Lactobacillus plantarum* LP01 and *Bifidobacterium breve* BR03, reported that probiotic supplementation significantly improved the number of weekly bowel movements and symptoms associated with evacuations (consistency of feces and ease of expulsion); Abdominal bloating and anal itching-burning or pain- was also improved with probiotic use. The synbiotic also contains a number of strains that protect gut barrier integrity: *B. breve* BR3, *B. longum* BB-536, *Lactobacillus rhamnosus* GG*, Lactobacillus salivarius* LS1, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, *Bifidobacterium breve*, *Bifidobacterium longum*. *B. breve* BR3 and *L. salivarius* LS1 have been clinically shown to improve gut barrier function by reducing bacterial translocation as measured by fecal LPS in almost 100 men and women with

atopic dermatitis.

The following studies were completed with individual strains:

- *Lactobacillus plantarum* LP01 (LMG P-21021), *Bifidobacterium breve* BR03 (DSM 16604) or *Bifidobacterium animalis* subspecies *lactis* BS01 (LMG P-21384):

Over a period of 5 years (2003 to 2008), the study involved 300 healthy volunteers (151 males and 149 females; age 24 to 71 y) with evacuation disorders and hard stools. Subjects were divided into 3 groups: 80 subjects in the group A received placebo, 110 subjects in the group B received mixed *L. plantarum* LP01 and *B. breve* BR03 (2.5 billion colony-forming units/day of each strain), and 110 subjects in the group C received *B. animalis* subspecies *lactis* BS01 (2.5 billion colony-forming units/day) daily for 30 days (T30). Subjects were assessed at 15 (T15) and 30 days (T30) of supplementation. A statistically significant difference was recorded at T15 and T30 versus T0 both in group B (T15 vs. T0 P<0.001; T30 vs. T0 P<0.001) and in group C (T15 vs. T0 P<0.001; T30 vs. T0 P<0.001). No differences were observed in the placebo-control group (T15 vs. T0 P=0.148; T30 vs. T0 P=0.507). These differences were reflected in the number of weekly evacuations but similar statistically significant improvements were observed with consistency of feces, ease of expulsion, sensation of complete emptying, anal itching, burning or pain and also abdominal bloating.

- *Lactobacillus plantarum* LP01 and *Bifidobacterium breve* BR0:

Study involved 50 patients (24 males,26 females) (mean age range = 26–64 years) with IBS according to Rome II criteria. Patients were randomly assigned to receive either the active preparation containing *Lactobacillus Plantarum* LP01 and *Bifidocterium Breve* BR0 (5 billion colony-forming units) or placebo powder containing starch identical to the study product once daily for 4 weeks. Pain score in different abdominal locations after treatment decreased in probiotics group of 38% versus 18% (P <0.05) of placebo group after 14 days and of 52% versus 11% (P <0.001) after 28 days. The severity score of characteristic IBS symptoms significantly decreased in probiotic group versus placebo group after 14 days 49.6% versus 9.9% (P < 0.001) and these data were confirmed after 28 days (44.4% versus 8.5%, P < 0.001).

- *Bifidobacterium longum* BB536:

Bifidobacterium longum BB536 was administered to 40 healthy adult female volunteers and the effects on defecation frequency and fecal characteristics were assessed. They were administered BB536 (2 billion colony-forming units) daily in 200 ml of milk for three weeks. (Milk was used as the control diet). The defecation frequency of volunteers in the interval period was 0.54 ± 0.18 times per day. They suffered constipation slightly. The frequency increased to 0.65 ± 0.21 and 0.72 ± 0.22 after ingesting milk and BB536 milk, respectively. These increases were statistically significant when compared with the interval period (p < 0.001).

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Study Design

This is a 1:1 randomized, double blind 12-week comparison of effects of SH-DS01 by Seed Health Inc. versus placebo, in a cohort of 100 women with IBS with constipation or IBS with mixed bowel habits. 50 IBS-C or IBS-M patients will receive the daily synbiotic for 12 weeks, while 50 IBS-C or IBS-M patients will receive the placebo.

- ***Initial Screening Session (1 hour)*** – The purpose of this screening is to determine if the subject meets the qualifications to participate in the research study. After the screening is complete they will be notified whether they meet qualification or not. If the subjects qualify they will be provided with the supplementation and instructions for future study appointments. Informed Consent: This will be explained by a member of the study staff in an individual setting. After it has been explained and all questions have been answered, the subject will be asked to sign the consent form.

- Brief Medical History Form: After completing this short survey, the medical history will be reviewed and either approval will be issued to continue with the study or exclusion from further participation.
- Physical Exam:
    - Measurement of Height, Weight: the subject will be asked to remove shoes and any items in pockets (i.e. loose change, wallet, watch, cell phone, etc.) prior to having height (stadiometer) and weight (digital scale) measured.
- Medical history and interval history to include current medications, recording of adverse events (including intercurrent illness and any new diagnoses).
- Other Vital Signs: Temperature, Heart Rate, Blood Pressure
- ***Study Procedures for Qualified Participants:*** If the subject meets the study requirements and is cleared for study enrollment they will be asked to complete the following tasks:
    - **Pre-supplement wash-out (1 week):** during which the participant is to stop taking probiotics or antibiotics, in order to eliminate any residual effects of the synbiotic intervention. During this period, subjects will be provided with a single blinded placebo. Participants who are judged to be "responders" will be excluded from further participation in the trial.

Visit 2 (Single-Blind Placebo Run-In)

- **Symptom assessment; questionnaires.**
- **Vital Signs:** Weight, Heart Rate, Blood Pressure.
- **Single-Blind Placebo**: Subjects will be provided with a single-blind placebo for a period of 28 days pre-randomization
- **Urine Pregnancy Test**

Visit 3 (Randomization)

- **Vital Signs:** Weight, Heart Rate, Blood Pressure.
- **Symptom assessment; questionnaires.**
- **Physical Examination**
- **Blood Collection:** General laboratory tests such as complete blood count (CBC) will be performed for all study subjects prior to randomization to ensure the safe and appropriate treatment of subjects. Clinical laboratory tests will be overseen by the PI or a physician sub-investigator Functional analysis of serum and cells will be measured to look at LPS-binding protein, C-reactive protein (CRP) and a cytokine panel. Serum and cells will be used or banked for translational/mechanistic studies.
- **Stool Sample Collection:** Subject will be provided with a take-home stool collection kit to collect stool samples.
- **Assess pill count**: If pill count from run-in indicates <80% adherence/compliance, subject will not be randomized
- **Randomization**: Subject will be randomized into double-blind portion of the study. Subjects will be given enough study medication to last until next visit.

Visit 4 (Day 42, Midpoint)

- **Symptom assessment; questionnaires.**

- **Physical Examination**
- **Blood Collection:** Serum and cells will be used or banked for translational/mechanistic studies.
- **Stool Sample Collection:** Subject will be provided with a take-home stool collection kit to collect stool samples.
- **Review of concomitant medication use.**
- Subjects will be given enough study medication to last until next visit.

Visit 5 (Day 84)

- **Symptom assessment; questionnaires.**
- **Physical Examination**
- **Blood Collection:** Serum and cells will be used or banked for translational/mechanistic studies.
- **Stool Sample Collection:** Subject will be provided with a take-home stool collection kit to collect stool samples.
- **Review of concomitant medication use.**

Visit 6 (Day 92, at home follow-up)

- **Symptom assessment; questionnaires.**

Cara App

The app used for the study is called Cara. It is available to the general public via the standard Apple app store. It uses encrypted transmission via HTTPS to transmit data from individuals to a cloud. Their cloud is provided by Microsoft Azur. The app as well as the sponsor will have access to the data.

Questionnaires

Irritable Bowel Symptom Severity Scale (IBS-SSS)

IBS Severity Scoring System (IBS-SSS) is a widely used questionnaire to assess the symptom severity. It measures abdominal pain intensity, abdominal pain frequency, abdominal distension, dissatisfaction with bowel habits, and influence of IBS on life in general ("life interference") during the preceding week on a 0-100 scale, with the total IBS-SSS score ranging between 0 and 500, with higher scores indicating more severe symptoms.

IBS-Global Improvement Scale

Study patients completed an IBS global improvement scale (GIS) at visits 1, 2, 3 and 4. A GIS responder was defined as a patient who recorded either moderately or substantially improved to the question: "Compared with the way you usually felt during the 3 months before you entered the study, are your IBS symptoms over the past 4 weeks substantially worse, moderately worse, slightly worse, no change, slightly improved, moderately improved, or substantially improved?" Improvement in bowel urgency and global symptoms were analyzed for all IBS-C or IBS-M patients.

IBS-Adequate Relief Question

The IBS-Adequate Relief (IBS-AR) is a dichotomous single item that asks participants "Over the past week have you had adequate relief of your IBS symptoms?". It serves as an additional way to assess GI symptoms in the IBS-C or IBS-M patient cohorts.

Visceral Sensitivity Index

The Visceral Sensitivity Index (VSI) is a self-reported survey measure that will be given to the IBS-C or IBS-M cohort for completion. It will assess gastrointestinal-specific anxiety, the cognitive, affective, and behavioral response to fear of gastrointestinal sensations, symptoms, and the context in which these visceral sensations and symptoms occur.

SH-DS01 Capsules

Probiotics for Digestive Health/Oxidative Stress/GI Immunity:

Bifidobacterium breve SD-BR3-IT

Lactobacillus rhamnosus SD-LR6-IT

Lactobacillus plantarum SD-LP1-IT

Bifidobacterium longum SD-BB536-JP

Lactobacillus reuteri SD-LRE2-IT

Bifidobacterium infantis SD-M63-JP

Lactobacillus rhamnosus HRVD113-US

Bifidobacterium lactis HRVD574-US

Bifidobacterium breve HRVD521-US

Lactobacillus casei HRVD300-US

Bifidobacterium longum HRVD90b-US

Bifidobacterium lactis SD-BS5-IT

Bifidobacterium lactis SD150-BE

Lactobacillus rhamnosus SD-GG-BE

Lactobacillus reuteri RD830-FR

Probiotics for Women's Health:

Bifidobacterium adolescentis SD-BA5-IT

Lactobacillus crispatus SD-LCR01-IT

Lactobacilus salivarius SD-CRL1328-IT

Lactobacillus fermentum SD-LF8-IT

Probiotics for Dermatological Health:

Bifidobacterium longum SD-CECT7347-SP

Lactobacillus casei SD-CECT9104-SP

Bifidobacterium lactis SD-CECT8145-SP

Probiotics for Cardiovascular Health:

Lactobacillus plantarum SD-LDL400-UK

Bifidobacterium lactis SD-MB2409-IT

Prebiotics:

Indian Pomegranate [whole fruit and skin] (*Punica granatum*)

Prohibited Therapy During Study Period

- Patient is currently using or has in the past used recreational or medicinal cannabis, or synthetic cannabinoid based medications (including Sativex®) or supplements (including hemp oil/extracts) within one month prior to study entry and is unwilling to abstain for the duration for the study.
- Patient has consumed any probiotic product three days prior to screening and/or is unwilling to abstain from consuming these during the study.
- Intake of antibiotics in the past 2 weeks (i.e. penicillin, amoxicillin, cephalexin (Keflex), erythromycin (E-Mycin), clarithromycin (Biaxin), azithromycin (Zithromax), ciprofloxacin (Cipro), levofloxacin (Levaquin), ofloxacin (Floxin), co-trimoxazole (Bactrim), trimethoprim (Proloprim), tetracycline (Sumycin or Panmycin), doxycycline (Vibramycin), gentamicin (Garamycin), or tobramycin (Tobrex). The supplement in the present study may have a minor interaction with these medications.

B. Statistical Considerations

Statistical analysis

This study is a randomized, double-blind, placebo-controlled trial comparing study subjects treated with a daily synbiotic vs. placebo. We will perform all statistical tests all under the null hypothesis that there is no difference between the two groups (synbiotic and placebo) against the two-sided alternative that they are not equivalent. Analyses will be conducted using an Intention to Treat (ITT) analysis and will include all study subjects who are randomized into this trial. Study subjects who drop out of the study will be considered failures for subsequent outcomes.

C. Subject Selection

Inclusion criteria

1. Patient must be willing and able to give informed assent/ consent for participation in the study
2. Patient must be willing and able (in the PI's opinion) to comply with all study requirements.
3. Patient must be a premenopausal female over the age of 18.
4. Patient must have a documented history of IBS that is not completely controlled by current IBS drugs.
5. Patient must have a score of ≥150 on the IBS-SSS at screening.
6. Patient must have no clinically relevant (in the judgment of the PI) abnormal blood laboratory levels at screening or randomization.
7. The clinician will assess eligibility as per the Rome IV criteria (Recurrent abdominal pain or discomfort at least 1 day/week in the last 3 months associated with two or more of the following: Improvement with defecation. Onset associated with a change in frequency of stool).

Exclusion criteria

1. Patient has clinically significant unstable medical conditions other than IBS.
2. Patient has had clinically relevant symptoms or a clinically significant illness in the four weeks prior to screening or randomization.
3. Patient has clinically significant laboratory values (in the PI's opinion).
4. Patient has a history or presence of alcohol or substance abuse within the last two years prior to the study or daily consumption of five or more alcohol-containing beverages.
5. Patient is currently using or has in the past used recreational or medicinal cannabis, or synthetic cannabinoid based medications (including Sativex®) or supplements (including hemp oil/extracts) within one month prior to study entry and is unwilling to abstain for the duration for the study.

6. Patient has consumed any probiotic product three days prior to screening and/or is unwilling to abstain from consuming these during the study.
7. Intake of antibiotics in the past 1-month (i.e. penicillin, amoxicillin, cephalexin (Keflex), erythromycin (E-Mycin), clarithromycin (Biaxin), azithromycin (Zithromax), ciprofloxacin (Cipro), levofloxacin (Levaquin), ofloxacin (Floxin), co-trimoxazole (Bactrim), trimethoprim (Proloprim), tetracycline (Sumycin or Panmycin), doxycycline (Vibramycin), gentamicin (Garamycin), or tobramycin (Tobrex). The supplement in the present study may have a minor interaction with these medications.
8. Patient has any known or suspected hypersensitivity to pomegranate, pine, or mushrooms, or any of the excipients of the Supplement Synbiotic Product (SSP).
9. Patients of child bearing potential unless willing to ensure that they use effective contraception, for example, oral contraception, double barrier, intra-uterine device, during the study and for three months thereafter.
10. Patients who are pregnant, lactating, or planning pregnancy during the course of the study and for three months thereafter.
11. Patients who have been part of a clinical trial involving any investigational product in the previous six months.
12. Any other significant disease or disorder which, in the opinion of the PI, may either put the patient at risk because of may either put the patient at risk because of participation in the study, may influence the result of the study, or affect the patient's ability to participate in the study.
13. Following a physical examination, the patient has any abnormalities that, in the opinion of the investigator would prevent the patient from safe participation in the study.
14. Patients unwilling to abstain from donation of blood during the study.
15. There are plans for the patient to travel outside the USA during the study.
16. Obesity (BMI > 30)
17. Implantable device such as heart pacemaker.
18. Patients unwilling to abstain from donation of blood during the study.
19. History of inflammatory bowel disease.
20. History of diverticulosis.
21. History of cardiovascular disease.
22. History of kidney/liver/serious infection.
23. History of diabetes or other hormone diseases.
24. History of abdominal surgery.
25. Currently suffering from high blood pressure.
26. Following a physical examination, the patient has any abnormalities that, in the opinion of the investigator would prevent the patient from safe participation in the study.
27. There are plans for the patient to travel outside the USA during the study.

B4. POSSIBLE BENEFITS

No direct benefit to subjects is expected.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

The possible risks for the study include risks of the symbiotic, typical blood drawing risks and risks to taking questionnaires.

Synbiotic
Side effects are transient bloating, loose stools, gas and diarrhea. Also, a rare allergic reaction to pomegranate.

Blood Drawing
The risks and discomforts of blood drawing from a vein include the possibility of pain or bruising at the site of the blood draw; occasional feeling of lightheadedness; and rarely, infection at the site of the blood draw.

Surveys/Questionnaires
Some of the questions asked as part of this study may make the subject feel uncomfortable.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment
Patients will be identified in the following ways:

- Clinical practice of the Center for Functional Bowel Disorders and GI Motility at BIDMC
- GI referrals from BIDMC
- Review of medical records, data repositories, and appointment logs
- Clinical Query 2
- Advertisements

Potential study participants (obtained from the means listed above) will be contacted by phone and a brief phone screening will be initiated to assess subject's eligibility before scheduling the office visit.

Potential study participants identified through Clinical Query 2 or appointment logs will be mailed a letter or postcard with opt-in information.

Consent
During the screening visit the Investigator will fully explain the purpose of the study to the patient and all questions and concerns regarding the study will be addressed as well (informed consent process) in a private area in the Division of Gastroenterology.

Subject Protection
None of the subjects in this study will be vulnerable to coercion or undue influence

B7. STUDY LOCATION

Privacy
All data will be kept in a password protected folder on the BIDMC shared drive. All blood and stool samples will be coded without patient identifiers and stored in keycard protected floors in research areas at BIDMC. When the mucosal samples will be sent to outside collaborators/labs, they will not have any PHI labeled.

Physical Setting
All samples will be stored in freezers on keycard protected floor 6 of Dana at BIDMC. All data generated from this study will also be stored in password protected folder of BIDMC shared drive.

B8. DATA SECURITY

All data will be kept in a password protected folder on the BIDMC shared drive. Serum, urine and mucosal samples will be labeled without patient identifiers.

B9 Multi-Site Studies

Is the BIDMC the coordinating site? ☐ Yes ☒ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☒ No

B10 Dissemination of Research Results

Patients will not be informed about the results of the study. Results might be presented/published.