Phase II. Dose Ranging Study of the Safety and Efficacy of Orally Administered Lyophilized Fecal Microbiota Product (PRIM-DJ2727) for the Treatment of Recurrent Clostridium difficile Infection (CDI)

NCT03298048

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Clinical Study Protocol

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Investigational Product

Lyophilized Healthy Donor Intestinal Bacteria

Sponsor
The University of Texas Health Science Center
1200 Pressler Room 743
Houston, TX 77030

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UTHealth The University of Texas Health Science Center at Houston
IRB NUMBER: HSC-SPH-17-0614
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SYNOPSIS

TITLE	Phase II. Dose Ranging Study of the Safety and Efficacy of Orally Administered Lyophilized Fecal Microbiota Product (PRIM-DJ2727) for the Treatment of Recurrent *Clostridium difficile* Infection (CDI)
PROTOCOL	HSC-SPH-17-0614
SETTINGS	Attending physicians will refer their patients with recurrent CDI (RCDI) for treatment with study drug (PRIM-DJ2727) and will continue to make clinical decisions related to overall health of their patients. Patients with RCDI can self-refer but must have attending physician who will provide non-transplant care. All subjects undergoing therapy will be handled as outpatients at the Kelsey-Seybold Clinic – Main Campus on W. Holcombe Blvd. or University of Texas School of Public Health, Center for Infectious Diseases.
STUDY PURPOSE	The purpose of the study is to determine the safety and efficacy of three different doses of lyophilized intestinal bacteria given orally in capsules for therapy in subjects with recurrent *C. difficile* Infection (RCDI).
STUDY DESIGN	Allocation: Randomized Endpoints: Safety, preliminary efficacy in preventing future bouts of CDI and improvement in intestinal flora diversity Intervention Model: Parallel assignment Masking: Double-blinded Primary Purpose: safe cure of CDI
STUDY SCHEDULE	Three hundred (360) eligible subjects (1:1:1:1 ratio) with recurrent CDI will be randomly assigned to receive PRIM-DJ2727 lyophilized intestinal bacteria capsules orally in one of 3 dosage schedules: microbiota from 50g stool microbiota/day for 2 consecutive days; 100g stool microbiota for the 1st day and 50g for the 2nd day; 100g stool/day for 2 consecutive days with bowel preparation and Imodium; and a 4th control group will receive 100g stool/day for 2 consecutive days without bowel preparation and Imodium. An aliquot (2mL) of stool sample collected before dosing and at follow-up days 1,7,14,30,60 and 90 after FMT will be stored at -80°C for future analysis (e.g. microbiome studies)
INCLUSION CRITERIA	All subjects 1. Male and female subjects 18 years of age or older. 2. Sexually active male and female subjects of childbearing potential agree to use an effective method of birth control during the study. 3. Female subjects of childbearing potential must have a negative pregnancy test on the day of the procedure prior to administration of study drug. 4. Subject/LAR willing and able to provide informed consent. 5. Able to follow study procedures and follow-ups. 6. Subject must have an attending physician who will provide non-transplant care. 7. Medical history of ≥ 3 bouts of CDI in outpatient or ≥ 2 bouts of CDI in inpatient with either group having ≥ 2 positive fecal tests for *C. difficile* toxin and at least one bout of CDI within 6 months of enrollment. 8. Received at least two courses of standard-of-care antibiotic therapy for CDI. 9. Have a refrigerator at home to store the second dose of FMT overnight.

EXCLUSION CRITERIA	1. Unable to take capsules orally. 2. Requiring systemic non-*C. difficile* antibiotic therapy within 14 days prior to FMT. 3. Unwilling to stop taking non-dietary probiotics 24-96 hours prior to FMT. 4. Unable to stop taking bile acid sequestrants (e.g. cholestyramine) 24-96 hours prior to FMT. 5. Unable to stop use of drugs with CDI activity: oral vancomycin, oral or IV metronidazole, fidaxomicin, rifaximin or nitazoxanide 24-96 hours prior to FMT and after FMT. 6. Receipt of CDI monoclonal antibodies as treatment for the most recent bout of CDI. 7. Life expectancy of < 6 months. 8. In the opinion of investigator, subject for any reason, should be excluded from the study.
DURATION OF FOLLOWUP	The duration of study participation for each subject is up to 216 days including screening (14 days), treatment (2 days) and follow-up (180-200 days).
STUDY POPULATION	The target population are adults (≥18 years old) with ≥3 bouts of CDI in outpatients or ≥2 bouts of CDI in an inpatient with either group having ≥ 2 positive fecal tests for *C. difficile* toxin and at least one bout of CDI within 6 months of enrollment.
STUDY BLINDING	This is a double-blinded study for the groups randomized to receive pre-treatment bowel prep and Imodium.
DEFINITION OF TREATMENT FAILURE	Treatment failure is defined as the presence of diarrhea (passage of ≥ 3 watery stools per 24-hour period for two consecutive days) and *C. difficile* toxin A/B positive stool within 60 days of FMT treatment plus receipt of anti-*C. difficile* antibiotics.
STUDY AGENT, DOSE OF ADMINISTRATION	Processed intestinal bacteria from a screened healthy donor will be administered lyophilized FMT to be given by the oral route. FMT will be given as follows: 1. Group 1: With bowel prep and Imodium 1st treatment day, lyophilized product generated from 50g of stool 2nd treatment day, lyophilized product generated from 50g of stool 2. Group 2: With bowel prep and Imodium 1st treatment day, lyophilized product generated from 100g of stool 2nd treatment day, lyophilized product generated from 50g of stool 3. Group 3: With bowel prep and Imodium 1st treatment day, lyophilized product generated from 100g of stool 2nd treatment day, lyophilized product generated from 100g of stool 4. Unblinded Group 4: (Without bowel prep and Imodium) 1st treatment day, lyophilized product generated from 100g of stool 2nd treatment day, lyophilized product generated from 100g of stool
EFFICACY EVALUATION	Primary Endpoint: • Safety of FMT (lyophilized capsules with various doses) • Prevention of subsequent bouts of CDI in 60 days post FMT. • Safety of FMT (lyophilized capsules with same doses) without using pre-FMT bowel preparation and Imodium • Prevention of subsequent bouts of CDI in 60 days post FMT without using pre-FMT bowel preparation and Imodium. Secondary Endpoint: • To characterize improvement in microbiota diversity from baseline in subjects treated with various doses of lyophilized capsules.

SAFETY EVALUATION	To ensure safe study conduct during the study, an independent, non-blinded, external Data and Safety Monitoring Board (DSMB) will be established to perform safety evaluations on an ongoing basis. A DSMB charter will be developed and will detail the review of the safety data. The DSMB will have the power to stop the trial.
STUDY STOPPING RULES	Study may be halted or discontinued if the DSMB identifies a trend of more illnesses or side effects in one or more of the treatment groups.

1. INTRODUCTION

1.1 Background

During the past several years, *C. difficile* Infection (CDI) has become more frequent and severe, more refractory to standard therapy, with predictable recurrence rates of 25% after standard therapy[1, 2]. It is now accepted that the disruption of the normal balance of colonic microbiota secondary to antibiotics facilitates the development of CDI and improvements in the diversity and quantity of microbiota is associated with recovery of infection and prevention of disease recurrence. Studies have shown that subjects with recurrent CDI (RCDI) have decreased diversity of colonic flora with absolute reductions in counts of anaerobic species of *Bacteroidetes* and *Firmicutes* in their stool compared to subjects recovering from single episodes of CDI [3]. Numerous case reports and retrospective case series have demonstrated the benefit of intestinal microbiota transplantation (FMT) in subjects with severe or RCDI with cure rates over 80%-90% [4-8], making FMT standard therapy when multiple recurrences are seen [9]. FMT involves administration of a suspension of intestinal bacteria obtained from a healthy individual into the GI tract of a patient with RCDI to promote normalization of flora [6].

1.2 Our Previous Study (HSC-SPH-13-0119 and HSC-SPH-14-0020)

Fecal Microbiota Transplantation Delivered by Colonoscopy

A recently published study by our group found a single infusion of 50 g fresh, frozen or lyophilized fecal microbiota product obtained from a small number of healthy donors in the treatment of 72 patients with recurrent CDI given by colonoscopy led to cure in 87% [10].

Our data from the recently published study suggested certain *Firmicutes* taxa might be negatively associated with CDI, which was consistent with the results of a previous study[11]. Noticeable changes in microbiota structures occurred by 7 days after FMT when the structures of the SUBJECTs closely resembled those of the donors. We observed multiple microbes at the family level consistently enriched in subjects with recurrent CDI after receipt of FMT: *Lachnospiraceae*, *Ruminococcaceae* and *Bacteroidaceae*. These bacteria are known to be dominant and are established early in life as key members of the human faecal microbiota. They are thought to play major ecologic roles in establishment and maintenance of colonic epithelial health [12,13]. *Lachnospiraceae* has also been

observed in an experimental animal model to suppress *C. difficile* [14]. It will be important to determine the microbial composition at a finer species or strain level to better understand more fundamentally what is happening with FMT.

The unique findings of our study were the comparative clinical efficacy and improvement of intestinal microbiome profiles resulting from the use of fresh, frozen and lyophilized donor microbiota products in the treatment of RCDI using a small number of non-related donors providing each product. Rates of cure were slightly less with the lyophilized product but well within the range of cures seen in the literature with fresh product [15]. We demonstrated equivalent efficacy in a small number of donors supporting other studies showing non-related donors with a healthy microbiome are suitable sources of microbiota for FMT in patients with dysbiosis.

Lyophilized microbiota are of particular interest for FMT because of their potential for prolonging storage time, and more importantly, oral administration in non-frozen capsules with lower volume resulting in the need to ingest fewer capsules [16]. We hypothesize the slightly reduced efficacy of lyophilized material in this study related to loss of microbiota during the freezing process. We are currently exploring the use of lyophilized product given in capsules using a higher content of faecal microbiota and using a non-polyol cryoprotectant, which has been reported as possibly stabilizing culturable microbiota during the freezing process [17]. The frozen product was effective in the present study without the use of cryoprotectants, and we do not know the effect of low temperatures on the anaerobes of most importance in FMT. Damage from long-term freezing of faecal bacteria has been shown to be lessened by adding 10% glycerol to the samples [18]. We found that glycerol cannot be used in product to be lyophilized.

Lyophilized microbiota are of particular interest for FMT because of their potential for prolonging storage time, and more importantly, oral administration in non-frozen capsules with lower volume resulting in the need to ingest fewer capsules [16]. Lyophilized capsules were effective in the present study without the use of cryoprotectants, and we do not know the effect of low temperatures on the anaerobes of most importance in FMT. However, we are currently exploring the use of lyophilized product given in capsules using a higher content of fecal microbiota, using a cryoprotectant, to stabilize the microbiota during the freezing process [17]. Lyophilized donor fecal microbiota have been used successfully in a small number of people suffering from recurrent CDI in a case report and one small series [19,20] and need further study.

FMT by Oral Capsules of Lyophilized FMT Product versus Enema using Frozen FMT Product

In an ongoing study, 8 patients with RCDI were administered orally of 5g of lyophilized FMT product derived from 100g of fresh stool and 21 RCDI patients were given frozen product (via enema) obtained from 100g of fresh stool. Cure was seen in 5 of 8 (63%) for the oral product and 20 of 21 (95%) for the frozen product given by enema. We then increased the dose of lyophilized product to 10g given over 2 consecutive days (5g each day, derived from a total of 200g of fresh stool per treatment). We saw cure in 10 of 11 (91%) of treated subjects. This study demonstrated effectiveness of a single infusion via enema of frozen or lyophilized fecal microbiota capsules obtained from a small number of healthy donors in the treatment of recurrent CDI. Frozen FMT product has become widely used for microbial restoration in recurrent cases of CDI [21] and was shown to be equivalent to fresh donor product in a randomized double-blind comparison in 219 patients with recurrent CDI [22].

In vivo Study to Determine Storage Time for Frozen and Lyophilized FMT Product

Mice were divided into four groups for each storage time point assay, with each group containing 5 mice. The study design and time line are illustrated in Figure 1. All mice were treated with a mixture of antibiotics dissolved in drinking water. The mixture contained kanamycin (2 mg/day), gentamycin (0.175 mg/day), metronidazole (35 mg/kg/day) and vancomycin (40 mg/kg/day) (Thermo Fisher Scientific, Waltham, MA). The antibiotic treatment started at day -4 and continued for three days. Mice were then fed regular water with clindamycin (10 mg/kg) (Chem-Impex International Inc., Wood Dale, IL) until day zero when *C. difficile* by gavage was administered via intraperitoneal injection of 1.5x 10^5 colony-forming units (CFU) followed by FMT products. The time interval between *C. difficile* challenge and FMT administration was 30 to 60 minutes. The total volume for each gavage was less than 0.2 ml. The positive control of CDI disease group was fed with *C. difficile* alone in saline. The positive therapeutic control group was treated with fresh product. The test groups were treated with either frozen or lyophilized products stored for different time intervals (7, 9, 11 or 15 months). The mice were followed-up for clinical diarrhea occurrence (excretion of soft wet, unformed with moderate perianal a staining of the coat, or watery without solid pieces stool) [23]. Fecal samples were collected at various time points and immediately stored at -80°C for further analysis. The FMT products' efficacies were measured by time required to develop diarrhea after *C. difficile* challenge

starting at day zero.

We attempted to determine changes in major microbiota groups after freezing or lyophilization and storage for various lengths (7, 9, 11 or 15 months) by neutralization of CDI in mice experimentally infected. After antibiotic disruption of the intestinal microbiota, 27/27 (100%) of the mice treated only with *C. difficile* developed diarrhea within 2 days of challenge. Diarrhea occurred in the following number of challenged mice given fresh, frozen or lyophilized FMT product stored for 7 months, respectively: 0/22 (0%); 0/5 (0%); and 0/5 (0%) (p=1.00). The number of mice developing diarrhea with 95% confidence interval and statistical results comparing frozen with the fresh FMT product stored 9 months 3/5 (60%, 3, 95% CI: 1-6, p=0.0911), 11 months 5/5 (100%, 2, 95% CI: 2-3, p=0.0040), and 15 months 3/5 (60%, 2, 95% CI: 2-6, p=0.0566, frozen, 15 months). The values for lyophilized products stored at 9 months were 2/5 (40%, 6, 95% CI: 1-6, p=0.1756), lyophilized, for 11 months 3/5 (60%, 2, 95% CI: 1-6, p=0.1095) and for 15 months 2/5 (40%, 3, 95% CI: 2-3, p=0.0110). When comparing frozen and lyophilized products stored up to 7 months, there was no significant difference, with OR equal one (1.00) for both products. Both products showed a reduction in CDI neutralization after 7-month storage time.

2. OBJECTIVE

Primary endpoints of the study are to evaluate the safety of lyophilized product by capsules with various doses and the prevention of subsequent bouts of CDI in 60 days post FMT; and to evaluate the safety of lyophilized product by capsules with same doses without bowel preparation and Imodium, and the prevention of subsequent bouts of CDI in 60 days post FMT without bowel preparation and Imodium. The secondary endpoint is to characterize improvement in microbiota diversity from baseline in subjects treated with various doses of lyophilized capsules.

Lyophilized FMT intestinal bacteria inoculate from well-screened healthy volunteer donors providing ≥100 grams of stool/FMT will be generated. Donor intestinal bacteria suspensions will be diluted (1:5 dilution) ≥ 500mL for later lyophilization and encapsulation. Fecal samples from donors and recipients will be saved for future studies (e.g. microbiome analysis).

3. STUDY DESIGN

This is a single center, randomized, parallel assignment, double-blinded, safety and

efficacy study to be conducted in subjects with recurrent CDI. Approximately 360 subjects will be enrolled in the study and randomized at 1:1:1:1 ratio to receive lyophilized donor intestinal bacteria with various doses (group 1 receiving healthy microbiota collected from 50g stool for 2 consecutive days; group 2 receiving healthy microbiota collected from 100g stool on the 1st day of treatment and from 50g stool on the 2nd day of the treatment; group 3 will receive healthy microbiota collected from 100g stool for 2 consecutive days using bowel prep and Imodium. An unblinded group 4 will receive healthy microbiota collected from 100g stool for 2 consecutive days without using bowel preparation and Imodium. All subjects will be followed for approximately 180-200 days following FMT treatment for safety.

3.1 Study Structure

Recipient subjects must have a physician who agrees to accept care of the patient following FMT. Subjects consenting to treatment at Kelsey-Seybold Clinic (KSC) and the University of Texas School of Public Health must be willing to self-pay for the FMT with a money order or cashier's check, or debit/credit card in the amount of $1,500 (see Section 11). Insurance payment will not be accepted. All subjects will be treated at Kelsey-Seybold Clinic – Main Campus on Holcombe Blvd. Once the procedure is completed, the recipient's care will be returned to their attending physician. Letters will be sent to physicians regarding the study and FMT completion.

3.2 Definition of recurrent CDI for Study Entry

The definition of recurrent CDI for study entry is a medical history of ≥ 3 bouts of CDI in outpatients or ≥ 2 bouts of CDI in an inpatient with either group having ≥ 2 positive fecal tests for C. difficile toxin and at least one bout of CDI within 6 months of enrollment. Two of the three bouts must have a positive fecal C. difficile toxin test and one of the three bouts should be within 6 month of enrollment.

3.3 Outcome Measure

Fecal microbiota diversity and phyla and family will be measured at the Alkek Microbiome Center at Baylor College of Medicine or other qualified laboratory (to be clarified on form 1572). A number of clinical and safety endpoints will be examined during the trial related to worsening of underlying disease and events common in this population. Additionally we will look for symptoms of worsening diarrhea and test *C. difficile* toxins A/B.

3.4 Data Safety and Monitoring Board

A committee of 3 faculty level people will serve as the DSMB reviewing primary and

secondary parameters and data from the study. Safety will be the greatest concern. The DSMB has the capacity to stop the study. The Board will meet at least three times during the study: At the study beginning; after 8-10 patients in each group have been studied and at the completion of the study (all subjects reached 180-200 days follow-up after FMT).

Professional	Name
Pharmacist	Dr. Kevin Garey
Epidemiologist	Dr. Lu-Yu Hwang
Clinical Infectious Diseases Specialist	Dr. Javier Adachi

4. SUBJECTS SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

4.1.1 Male and female subjects18 years of age or older.

4.1.2 Sexually active male and female subjects of childbearing potential agree to use an effective method of birth control during the study.

4.1.3 Female subjects of childbearing potential must have a negative pregnancy test on the day of the procedure prior to administration of study drug.

4.1.4 Subject/LAR willing and able to provide informed consent.

4.1.5 Able to follow study procedures and follow-ups.

4.1.6 Subject must have an attending physician who will provide non-transplant care.

4.1.7 Medical history of ≥ 3 bouts of CDI in outpatient or ≥ 2 bouts of CDI in inpatient with either group having ≥ 2 positive fecal tests for C. difficile toxin and at least one bout of CDI within 6 months of enrollment.

4.1.8 Received at least two courses of standard-of-care antibiotic therapy for CDI.

4.1.9 Have a refrigerator at home to store the second dose of FMT overnight.

4.2 Exclusion Criteria

4.2.1 Unable to take capsules orally.

4.2.2 Requiring systemic non-*C. difficile* antibiotic therapy within 14 days prior to FMT.

4.2.3 Unwilling to stop taking non-dietary probiotics 24-96 hours prior to FMT.

4.2.4 Unable to stop taking bile acid sequestrants (e.g. cholestyramine) 24-96 hours prior to FMT.

4.2.5 Unable to stop use of drugs with CDI activity: oral vancomycin, oral or IV metronidazole, fidaxomicin, rifaximin or nitazoxanide 24-96 hours prior to FMT and after FMT.

4.2.6 Receipt of CDI monoclonal antibodies as treatment for the most recent bout of CDI.

4.2.7 Life expectancy of < 6 months.

4.2.8 In the opinion of investigator, subject for any reason should be excluded from the study.

4.3 Vulnerable Population in Research:

The elderly population particularly is susceptible to recurrent *C. difficile* infection (CDI) and some elderly patients may be cognitively impaired. Informed consent can be obtained from legally authorized representatives (LAR) if potential subjects have condition or circumstances that could impact their ability to make decision or consent. These subjects may include but are not limited to the cognitively impaired, demented, incapacitated, or otherwise mentally or physically incapable of communication. Before their enrollment, the study coordinators must be certain that a caretaker can properly record data called for in the study.

The investigators or designee will assess subjects on their abilities to understand information and express a reasoned choice concerning the nature of the research, consequences of participations for the subject's own situation, and consequences of the alternatives to participation. The investigators or designee will ask questions about the main elements of the study and judge whether the potential participant has a clear understanding.

When an LAR agrees to the participation of the subject in the study, the consent would be obtained either from the patient's legal guardian with the authority to make decisions regarding medical treatment (with proper paperwork to establish this) or a person designated as a surrogate decision-maker by the patient in a medical power of attorney or Advance Directive. The investigator or designee will ask for a copy of the legal document from the authorized representative. The LAR and the person obtaining consent would personally sign and date the consent form. A copy of the signed consent document will be given to the LAR.

4.4 Subject Pre-screening, Screening and Re-screening:

Patients will be referred by a physician or self-referral to the study. Patient/LAR or physician will be provided a pre-screening form and requested to return the form with signed medical release and C. *difficile* related medical record (via email/fax/mail) for eligibility review. Pre-screening form and medical record received electronically will be kept securely in an access controlled network file service and will not be shared with anyone outside the study team. Any paper record will be kept securely in locked cabinet with access control. For potential subjects who are pre-screened but do not enroll subsequently, the identifying information may be kept for 180 days of release, after which all identifying information will be destroyed and not retained with study records. A non-

identifiable information log will be maintained to assess the number of patients pre-screened and the reasons for exclusion or not participating in the study.

If a potential subject seems eligible on pre-screening, an appointment will be set up for in person enrollment and screening. Study personnel will advise potential subject as to this study's purpose, requirements, and anticipated risks. Subject will be given the chance to review the informed consent form, will have the study explained in detail and any questions answered. Subjects who choose to enroll will sign the informed consent form, and the date of the consent form will serve as the first day of enrollment into the study. Subjects will be provided with a copy of the signed consent form.

Consented subjects who meet all inclusion and none of the exclusion criteria will be assigned a Subject ID (FMT-R-03-XXX) and randomized. Subjects who do not meet the inclusion criteria and/or meet exclusion criteria will be considered screen failures. Screen failures or subjects who exit the study before receiving Treatment Day 1 dose will not count towards total randomization and their assigned Subject IDs will be retired. Screen failures or subjects who exit the study before receiving treatment Day 1 dose are allowed to be re-screened once for eligibility. Re-screens will be re-consented and re-randomized to a new Subject ID to ensure maintenance of blinding.

Subject's attending physician will continue to treat the subject's primary disease(s) while the subject is a part of the study. The subject will continue all medications deemed as necessary and they will be recorded in the subject's study record. Physicians caring for these subjects will have no limitations in how the subject's primary disease(s) are managed. Antibiotics for *C. difficile*, non-dietary probiotics, and bile-acid sequestrants must be stopped 24-96 hours before the treatment. All other antibiotics must be stopped 14 days prior to treatment. Subjects will be recommended not to use non-dietary probiotics and bile-acid sequestrants within 60 days after FMT treatment; however, subjects who take probiotics and bile-acid sequestrants within 60 days after treatment will not be excluded.

4.5 Subject Withdrawal or Termination

Subject's participation is considered complete approximately 180-200 days after FMT procedure; however, subjects may withdraw from the study at any time for any reason, without any consequence. In addition, a subject may be withdrawn from the study by the investigator for any reasons including the following:

1. Lost to follow-up
2. Adverse Event (AE) or Serious Adverse Event (SAE)

3. Protocol violation/non-compliance
4. Subject decision to withdraw consent
5. Investigator decision to terminate the study

4.6 Handling of Withdrawals and Discontinuation of Treatment

Subjects are free to withdraw at any time. The primary reason for withdrawals from the study will be recorded in the study exit form. Subjects who withdraw or are withdrawn will be encouraged to complete an Early Termination Phone Visit to assess for AEs. Subjects who withdraw will be discontinued from all study procedures.

4.7 Lost to Follow-up

If subjects miss a treatment or follow-up, three attempts will be made by telephone or e-mail to contact and locate subjects. The contact attempts will be recorded on the study exit form.

4.8 Receipt of Systemic Antibiotics, Non-Dietary Probiotics and Bile-acid Sequestrants during the Trial

If a subject takes a systemic (non-topical) antibiotic within 60 days after FMT treatment, they will be excluded from the efficacy part of the study and only followed for safety. Exclusion from efficacy means subjects will no longer provide stool specimens. However, subjects will continue to have phone visits and submit diaries till 180-200 days (the end of the study) for safety assessment (AEs). If they take systemic antibiotics more than 60 days after FMT treatment, they will be included in all aspects of the study.

Subjects will be recommended not to use non-dietary probiotics and bile-acid sequestrants within 60 days after FMT treatment; however, subjects who take probiotics and bile-acid sequestrants within 60 days after treatment will not be excluded and continue to be followed for efficacy and safety.

4.9 Termination of Study

Although the Principal Investigator has every intention of completing the study, he may terminate the study at any time for clinical, safety or administrative reasons.

5. INVESTIGATIONAL PRODUCT PRIM-DJ2727 (MICROBIOTA SUSPENSION)

5.1 Manufacture and Storage

PRIM-DJ2727 is manufactured by using current Good Laboratory Practices in The University of Texas Health Science Center at Houston School of Public Health's Enteric Pathogen Diagnostic Laboratory (EPDL) which is a CLIA and CAP-certified laboratory. The FMT product for each treatment group contains bacteria from ≥100g filtered stool

from healthy donors. Product will be lyophilized at the University of Texas School of Public Health [redacted], and compounded and packaged into capsule formulation at the [redacted] Because of individual diet variation in the donors and the FMT product being lyophilized, the number of FMT capsules produced for each dose varies depending on the density of end product. Therefore, placebo capsules will be added to doses to maintain consistent number of capsules per treatment. Placebo capsules will be identical to the investigational product but will not contain intestinal bacteria. After packaging into capsules, the PRIM-DJ2727 will be stored in a sterilized refrigerator at 4°C at the EPDL for up to 6 months in a test tube rack clearly labelled with the lot number and expiration date.

5.2 Dose of FMT Product

Subjects will receive varying doses of PRIM-DJ2727 on two consecutive days of FMT. The clinical team will receive medications for each subject in labeled (see CMC protocol for label details) standard opaque pharmacy-type plastic containers for Day 1 or Day 2. Because of adding placebo capsules to maintain consistent number of capsules per treatment, the clinical trial personnel and subjects will be blinded as to the drug dose allocation.

Subjects in group 1 will receive healthy microbiota collected from 50g stool for 2 consecutive days with bowel prep and Imodium. Subjects in group 2 will receive healthy microbiota collected from 100g stool on the 1st day of treatment and from 50g stool on the 2nd day of the treatment with bowel prep and Imodium. Subjects in group 3 will receive healthy microbiota collected from 100g stool for two consecutive days with bowel prep and Imodium. Subjects in unblinded group 4 will receive healthy microbiota collected from 100g stool for two consecutive days without bowel prep and Imodium.

5.3 Method of Assigning Subjects to Study and Treatment

The four groups will be assigned randomly on 1:1:1:1 ratio by computer table randomization method at the University of Texas School of Public Health. After enrollment of subject in the study, the study coordinator will send an email to the authorized person for randomization at the University of Texas School of Public Health including the schedule (date and time) of study procedure. The study coordinator will receive an email from the authorized person providing the subject identification number and confirmation of date and time for study drug delivery. For subjects randomized to unblinded treatment group 4, it will be noted in the email not to use bowel prep and Imodium.

5.4 PRIM-DJ2727 Dispense and Handling

After randomization, PRIM-DJ2727 capsules will be delivered to the clinic site in two single dose standard opaque plastic containers for Day 1 and Day 2 treatment. Two single dose containers will be labeled with the study drug, the name of the PI, number of capsules, lot number, date, and instructions (see CMC protocol for label details). Study personnel delegated by the investigator will open the day 1 dose container and administer capsules to subject with water. Delegated study personnel will dispense day 2 dose container to subject with an ice-pack to take home. Subject will be instructed to keep the day 2 dose container in refrigerator at home.

6. TREATMENT FAILURES AND RETREATMENT

Treatment failure is defined as the presence of diarrhea (passage of ≥ 3 watery stools per 24-hour period for two consecutive days) and C. difficile toxin A/B positive stool within 60 days of FMT treatment and receipt of anti-*C. difficile* antibiotics.

If diarrhea (passage of ≥ 3 watery stools per 24-hour period for two consecutive days) occurs and CDI recurrence is suspected, subjects should inform study team and be recommended to contact their physician to perform a stool test for C. difficile. EPDL will test scheduled stool specimens and any unscheduled illness stool submitted up to days 60-70 after treatment for C. difficile toxins A/B in weekly batches but this should not delay treatment. If the test is positive, they should be treated by their physician. The Principal Investigator will be available for consultation with the attending physician. Subjects who are determined treatment failures by the investigator will not be followed further for clinical parameters. They will be offered FMT retreatment with maximum dose (200g) from a different donor product if the subject and their physician wish to pursue this treatment. After at least 10-20 days of anti-CDI antibiotics (vancomycin or fidaxomicin) subjects will be eligible for second FMT after 24-96 hour washout. If there is a delay between completing treatment and second FMT, the subject will receive at least four days of vancomycin or fidaxomicin prior to FMT (PI can call in prescription if needed) to complete the treatment 24-96 hours before second FMT. Subjects will be recommended to follow a clear liquid diet the entire day before retreatment clinic visit. Bowel preparation and Imodium will not be required before retreatment. During retreatment clinic visit, vital signs and weight will not be assessed and subjects will be observed for 30 minutes after dosing.

Retreatment Dosage: Day 1 (Clinic Visit)

- Subjects are recommended to continue clear liquid diet until 1 hour after retreatment.

- Perform urine pregnancy test on all female subjects of childbearing potential prior to FMT administration.
- Administer Day 1 dose and dispense Day 2 dose.
- Observe the subject for 30 minutes after dosing.
- Remind subject that Day 2 dose should be taken the next morning on empty stomach with water and release the subject.

Retreatment Dosage: Day 2 (Phone Visit)

- Ask that subject has taken the Day 2 dose.
- Ask for any adverse events since Day 1 assessment.
- A form letter may be faxed or emailed to subject's physician about FMT completion.

Subjects will be withdrawn from the study after retreatment Day 2 phone visit. After any second FMT, the subjects' own physician will monitor and manage the patients' illnesses.

If a subject experiences diarrhea and C. difficile toxin A/B test is negative, then the subject is not considered a treatment failure as the presence of C. difficile was not established. The study investigator will evaluate the patient's clinical picture and help decide whether the subject remains on the study. The PI will be available to discuss with subject's physician about continuing illness to arrive at the best outcome for the subject.

7. STUDY PROCEDURES

7.1 Duration of Participation

The duration of study participation is up to 216 days. It includes the enrollment period up to 14 days, 2 days of treatment, and 180-200 days of follow up for treatment effect and safety.

7.2 Clinical Evaluations

- At the time of Enrollment, patients will be evaluated for inclusion and exclusion criteria. Demographics and current medications will be documented. Medical history will be obtained with particular attention to the recent CDI history (test result, treatment, hospitalization, weight and if any antibiotics used prior to first CDI episode). History will include obtaining information on solicited symptoms such as diarrhea, nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering and fever (> 99.9 F) for two-week period prior to enrollment to establish baseline patterns should an adverse event occur in the future.
- Subjects will collect fresh stool samples at home and bring to the EPDL in transportation provided containers with an ice pack within 6 hours of collection if possible or as quickly as

possible before FMT and at follow up days 1(+3), 7(+3), 14(+3), 30(+10), 60(+10) and 90(+10) and any unscheduled visit up to 60 days after FMT treatment (excluding weekends and holidays). Subject may collect pre-treatment stool on any day after enrollment and before bowel prep on day -1, and can bring stool sample on treatment Day 1 clinic visit. Stool samples collected from subjects until 60-70 days post FMT or at unscheduled visit up to 60-70 days will be tested for C. *difficile* toxins A/B at EPDL in weekly batches. All the stool samples will be stored at -80ºC for future microbiome analysis. If a subject is unable to bring stool sample for any reason (e.g. out of town), subject will be asked to ship the sample by Federal Express as expeditiously as possible. A FedEx shipping label, ice pack, Styrofoam container with shipping box and instructions will be provided to the subject to ship stool samples if they are not able to hand deliver the stools to the EPDL. The EPDL will be closed on weekends and holidays. If the follow-up day falls on Friday, Saturday or Sunday, subjects can ship the sample on Thursday or the following Monday. Stool samples that are received out of window are not a protocol deviation; however, missed stool samples are a protocol deviation.

- Subjects will be assessed for vital signs (blood pressure, heart rate, respiration rate, oral temperature, weight) at the day 1 of treatment clinic visit.
- Women of childbearing potential will undergo urine pregnancy testing (Qualitative HCG) to confirm absence of pregnancy at the day 1 of treatment clinic visit prior to administration of study drug.
- A diary completed by subject will be collected before FMT and on follow up days 7(+3), 14(+3) 30(+10), 60(+10), 90(+10) and 180(+20) days after FMT treatment. Subjects may submit diaries with stool samples at the University of Texas School of Public Health, or may fax, email, or mail to the study site. For diary copies received in email or fax, an attempt will be made to obtain the originals from subjects to be taken to the study site subsequently. Diaries serve as tools for subjects to record daily bowel movements and weekly weight. Subjects will be recommended to measure weight at home each Sunday morning before eating and record on follow up diaries providing they have a scale or balance. If they do not have the means to measure their weight this is not a protocol deviation. Additionally, the pre-treatment diary will include daily record of solicited symptoms from day of enrollment until a day before treatment Day 1 (prior to bowel prep) to establish baseline patterns should an adverse event occur in the future. Solicited symptoms include diarrhea, nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding,

fatigue/malaise, chills/shivering and fever (>99.9 F). The diaries on follow up days 7 and 180 will include a brief survey to assess subject-reported experience of FMT treatment. Diaries that are received out of the window or missed diaries are not a protocol deviation.

Study assessments and procedures will include the following:

- Enrollment visit will occur on day -14 to -1 during which the informed consent will be obtained; inclusion and exclusion criteria will be reviewed; demographics, current medications, the medical history, and the CDI history (including information on solicited symptoms for two-week period prior to enrollment) will be reviewed and recorded. Subjects may already be taking or just have been prescribed antibiotics per standard of care to control recurrent CDI symptoms at the time of enrollment. Subjects who have completed antibiotic therapy will receive four days of oral vancomycin or fidaxomicin daily (PI can call in prescription if needed) prior to the 24-96 hours washout period. A stool collection kit along with IRB approved pretreatment prep instructions, pre-treatment diary, stool collection instructions and directions to drop specimen will be provided to subject.
- A stool sample will be collected by subject during enrollment period (prior to bowel prep) and a pre-treatment diary card will be completed by subject. Study personnel can obtain the stool and diary from subject on treatment Day 1 clinic visit. This stool will go to EPDL at the University of Texas School of Public Health.
- Day -4 to -1 before treatment: remind the subject by email or phone call about clinic appointment time, stopping antibiotic/probiotic/ bile-acid sequestrant, pre-treatment preparation and bringing pre-treatment diary and stool. Day -4 to -1(96 to 24 hours) before treatment: the last day of *C. difficile* antibiotics, non-dietary probiotics and bile-acid sequestrants. Subjects continue taking routine prescription medications.
- Day -1 before treatment: subjects follow a clear liquid diet the entire day. In the evening around 6:00pm, subjects (treatment groups 1-3) who were instructed by the study team during the enrollment will ingest bowel prep.

Treatment Visit: Day 1 (Clinic Visit)

- Subjects are recommended to continue clear liquid diet until after treatment for 1 hour.
- Ensure subject in treatment groups 1-3 has taken bowel prep the evening before and 4mg (2 capsules) of loperamide (Imodium) the morning of treatment (If Imodium

is missed, can be taken within 12 hours of FMT treatment). If bowel prep is missed, subject can proceed with FMT and will be recorded as protocol deviation.

- Collect and review the pretreatment diary with subject to determine baseline pattern of solicited symptoms to assess for adverse events.
- Confirm that the C. difficile antibiotic was stopped 24-96 hours before the visit.
- Perform urine pregnancy test on all female subjects of childbearing potential prior to FMT administration.
- Weight and vital signs (blood pressure, heart rate, respiration rate, oral temperature) will be collected pre-treatment.
- Administer Day 1 dose and dispense Day 2 dose.
- Ask subject if experienced any objectionable taste or smell or difficulty swallowing the capsules and general reaction on taking PRIM-DJ2727 capsules.
- Observe the subject for 1 hour after dosing for any adverse events.
- Collect post-treatment vital signs (blood pressure, heart rate, respiration rate, oral temperature).
- Remind subject that Day 2 dose should be taken the next morning on empty stomach with water.
- Remind subject to continue taking routine prescription medications as needed, but antibiotics, non-dietary probiotics and bile-acid sequestrants may not be taken.
- Release subject with IRB approved aftercare instructions, follow-up paper diaries, visit scheduler, safe antibiotics list and specimen request form.

Second Treatment Dosage: Day 2 (Phone Visit)

- Ask that subject has taken the Day 2 dose.
- Ask for any adverse events since Day 1 assessment.
- Remind subject to follow the aftercare instructions provided on Day 1.
- Remind subject to continue taking routine prescription medications as needed, but antibiotics, probiotics and bile-acid sequestrants may not be taken.
- Remind subject to completely fill the diaries, and submit diaries and stools at follow-up visits according to schedule and instructions provided by study personnel.
- A form letter to subject's physician about FMT completion may be faxed or emailed.

Follow Up and Safety Assessment Period (Phone Visits): Post Treatment Days 1(+3), 7(+3), 14(+3), 30(+10), 60(+10), 90(+10) and 180(+20).

- Assess subject for any adverse events, new illness symptoms, worsening of baseline illnesses including adverse events of special interest (new or worsening autoimmune conditions and metabolic syndrome) and change in medication. Review and assess subject-reported weight data from diaries for AE (>5% change from baseline weight) at days 60 and 180 phone visits.
- Assess subject for solicited symptoms such as diarrhea (passage of ≥ 3 watery stools per 24-hour period for two consecutive days), nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering and fever (>99.9 F) for up to 60 days after treatment.
- If CDI recurrence is suspected, subjects should be recommended to contact their physician to perform a stool test for C. difficile. EPDL will test scheduled stool specimens and any unscheduled illness stool submitted up to days 60-70 after treatment for C. difficile toxins A/B in weekly batches but this should not delay treatment. If the test is positive, they should be treated by their physician. The Principal Investigator will be available for consultation with the attending physician. A form letter may be faxed or emailed to subject's physician about CDI recurrence.
- Subject will be reminded to submit completed diary (except on follow up visit Day 1) and stool sample (except on follow up visit Day 180) and to put stool collection date on provided container (if already submitted, stool collection date should be confirmed).
- Stool samples will be collected at home by the subject and dropped off at the EPDL in the University of Texas School of Public Health in transportation provided containers with ice pack within 6 hours of collection if possible or as quickly as possible (excluding weekends and holidays). Stool sample will not be collected for follow up visit Day 180. If the subject is unable to bring stool sample for any reason (e.g. out of town), subject will be asked to ship the sample by Federal Express as expeditiously as possible. A FedEx shipping label, ice pack, Styrofoam container with shipping box and instructions will be provided to the subject to ship stool sample if they are not able to hand deliver to the EPDL. The EPDL will be closed on weekends and holidays. If the follow-up day falls on Friday, Saturday or Sunday, subjects can ship the sample on Thursday or the following Monday. Stool samples that are received out of window are not a protocol deviation; however, missed stool samples are a protocol deviation.

- Diaries will be collected from subjects recording subject-reported daily bowel movements and weekly weight. The diaries on follow up days 7 and 180 will include a brief survey to assess subject-reported experience of FMT. Diary will not be collected on follow up day 1 after treatment. Subjects may submit diaries with stool samples to the University of Texas School of Public Health, or may fax, email, or mail to the study site. For diary copies received in email or fax, an attempt will be made to obtain the originals from subjects to be taken to the study site subsequently. Diaries that are received out of the window or missed diaries are not a protocol deviation.

Unscheduled Visit (Phone): Subjects who have a suspected episode of CDI within 60-70 days of FMT treatment may submit an unscheduled stool sample to EPDL.

Early Termination Visit (Phone): may occur any time before 180-200 days after FMT treatment.

7.3 Clinical Response Evaluation

The clinical evaluation will include an evaluation of CDI recurrence to assess clinical response on each follow-up contact for 60 days after FMT.

If subjects experience diarrhea (3 or more watery stools/day for two consecutive days) and suspect CDI recurrence, they should inform the study team and be recommended to contact their physician to perform a stool test for C. *difficile*. EPDL will test scheduled stool specimens and any unscheduled illness stool submitted up to days 60-70 after treatment for C. difficile toxins A/B in weekly batches but this should not delay treatment. If the test is positive, they should be treated by their physician. The Principal Investigator will be available for consultation with the attending physician. A form letter may be faxed or emailed to subject's physician about CDI recurrence.

Subjects requiring a systemic Non- C. *difficile* antibiotic within 60 days following FMT treatment will be excluded from the study for efficacy analysis, but will be followed for safety. Exclusion from efficacy means subjects will no longer provide stool specimens. However, subjects will continue to have phone visits and submit diaries till 180-200 days (the end of the study) for safety assessment (AEs). Subjects requiring a systemic Non- C. *difficile* antibiotic 60 days after FMT treatment will continue to be followed for efficacy and safety.

Subjects will be recommended not to use non-dietary probiotics and bile-acid sequestrants within 60 days after FMT treatment; however, subjects who take probiotics and bile-acid sequestrants within 60 days after treatment will not be excluded and continue

to be followed for efficacy and safety.

7.4 Patient Laboratory Evaluations – Fecal Sample

Stool samples will be collected by the subject and brought to the EPDL within 6 hours of collection if possible or as quickly as possible (if not feasible stool samples may be shipped via Federal Express) before FMT and at follow up days 1(+3), 7(+3), 14(+3), 30(+10), 60(+10), 90(+10) and any unscheduled visit up to 60-70 days after FMT treatment (excluding weekends and holidays). Stool samples collected from subjects until 60-70 days post FMT or unscheduled visit will be tested for C. difficile toxins A/B at EPDL in weekly batches. All the stool samples will be stored at -80ºC for future metagenomics/microbiome analysis.

The University of Texas Health Science Center at Houston Enteric Pathogens Diagnostic Laboratory may conduct future biochemical research on specimens routinely and specifically collected during this clinical study that may be used for future research and may be stored for up to 15 years.

7.5 Health Outcome Assessment

Information such as mortality from any cause and hospitalizations will be collected through AE assessments. Subjects will report their solicited and unsolicited adverse events to study personnel on follow-up phone visits.

7.6 Subjective FMT Experience Assessment

On Treatment Day 1 in clinic, study personnel will inquire from subject if experienced any objectionable taste or smell or difficulty swallowing the capsules and general reaction on taking PRIM-DJ2727 capsules. The diaries on follow up days 7 and 180 will include a brief survey to assess subject-reported experience of FMT treatment.

8. STUDY SCHEDULE

8.1 Enrollment (Day -14 to -1)

The following study activities/data will be collected at the enrollment visit:

- After full explanation of the study protocol, have each subject sign an Informed Consent Form (ICF) before performance of any study-related procedures (including screening procedures).
- Assess each subject to ensure all inclusion criteria are met and no exclusion criteria met.
- Obtain standard demographics (date of birth, gender, ethnicity, race)

- Obtain medical history to record baseline conditions, current medications and CDI history (test result, treatment, hospitalization, weight and if any antibiotics used prior to first CDI episode). History will include obtaining information on solicited symptoms such as diarrhea, nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering, and fever (>99.9 F) for two-week prior to enrollment to determine baseline patterns should an adverse event occur in the future.
- Instruct patients that should they enroll in the study, they will need to meet the following requirements:
    - Sexually active male and female subjects of childbearing potential must use an effective contraceptive method of birth control throughout the study which may include any of the following; abstinence, barrier methods (such as a condom or diaphragm) used with a spermicide, approved hormonal contraceptives (such as birth control pills, patches, implants or injections), intrauterine device (IUD), or surgical sterilization (such as tubal ligation, hysterectomy, or vasectomy) at any time in the past. Post-menopausal women ≥45 years of age must be amenorrheic for at least six months to be considered to not have childbearing potential.
    - Probiotics and bile-acid sequestrants should be stopped 24-96 hours before the FMT treatment.
    - On 24-96 hours before FMT, subjects take their last dose of antibiotic treatment for their CDI, non-dietary probiotics and bile-acid sequestrants. Any other antibiotics being taken must complete 14 days prior to FMT treatment.
    - On Day -1, all subjects must follow a clear liquid diet the entire day. In the evening around 6:00pm, subjects (treatment groups 1-3) must take bowel preparation i.e. 1 bottle (10 oz.) of magnesium citrate or patients with impaired kidney function take 250mL of GoLytely. GoLytely will be prescribed by the investigator or nurse practitioner. If bowel prep is missed, subject can still proceed with FMT (noted as protocol deviation).

- On Day 1, subjects (treatment groups 1-3) will take 4mg (2 capsules) of loperamide (Imodium) the morning of treatment (if Imodium is missed, can be taken within 12 hours after FMT treatment).
- On Day 1, subjects take first dose of oral capsules of PRIM-DJ2727 and recommended to continue their clear liquid diet for 1 hour after dosing.
- Stool samples will be collected by the subject before FMT and at follow up days 1, 7, 14, 30, 60, 90 and any unscheduled visit up to 60-70 days after FMT treatment and brought to the EPLD within 6 hours of collection if possible or as quickly as possible (excluding weekends and holidays). Subject can collect pre-treatment stool on any day after enrollment and before bowel prep on Day -1, and can bring stool sample on treatment day 1 clinic visit. If a subject is unable to bring the sample to the EPDL (e.g. out of town), it may be shipped by Federal Express as expeditiously as possible. A FedEx shipping label, ice pack, Styrofoam container with shipping box and instructions will be provided to subjects to ship stool sample if they are not able to hand deliver to the EPDL. The EPDL will be closed on weekends and holidays. If the follow-up day falls on Friday, Saturday or Sunday, subjects can ship the sample on Thursday or the following Monday.
- Subjects should inform the study team if they experience diarrhea (passage of ≥ 3 watery stools per 24-hour period for two consecutive days) suspecting CDI recurrence and contact their physician to perform a stool test for C. *difficile*. The EPDL will test scheduled stool specimens and any unscheduled illness stool submitted up to 60-70 days after treatment for C. difficile toxins A/B in weekly batches but this should not delay treatment. If the test is positive, they should be treated by their physician. The Principal Investigator will be available for consultation with the attending physician. A form letter may be faxed or emailed to subject's physician about CDI recurrence.

- Subjects will need to complete and submit diaries before FMT and on follow up days 7, 14, 30, 60, 90 and 180 days after FMT treatment. Subject can deliver diaries with stool sample, or may be fax, email, or mail to the study site. For diary copies received in email or fax, an attempt will be made to obtain the originals from subjects to be taken to the study site subsequently. Diaries serve as a tool for the subject to record daily bowel movements and weekly weight. Subjects will be recommended to measure weight at home each Sunday morning before eating and record on follow up diaries, providing they have a scale or balance. If they do not have the means to measure their weight this is not a protocol deviation. Additionally, the pre-treatment diary will include daily record of solicited symptoms from enrollment until a day before treatment Day 1 to determine baseline patterns. Solicited symptoms include nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering and fever (>99.9 F) The diaries on follow up days 7 and 180 after treatment will include a brief survey to assess subject-reported experience of FMT treatment.
- Subjects will receive phone call from study personnel at follow up visit days 1, 7, 14, 30, 60, 90 and 180 days after FMT treatment (within windows specified in Table 1) Subjects will be assessed for any adverse events, new illness symptoms, worsening of baseline illnesses including adverse events of special interest (new or worsening autoimmune conditions, and metabolic syndrome) and change in medication. Subject-reported weight data from diaries will be assessed for AE at days 60 and 180 phone visits. Subjects will be assessed for CDI recurrence and solicited symptoms (diarrhea, nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering and fever (> 99.9 F) for up to 60 days after treatment. Subjects will be reminded to submit completed diaries and stool samples. Diary will not be

collected on follow up visit Day 1. Stool sample will not be collected on follow up visit day 180.

- Provide subject with stool collection kits at enrollment for collecting stool samples before treatment and at follow up days along with IRB approved pretreatment prep instructions, pre-treatment diary, stool collection instructions and directions to drop specimen.

8.2 Pretreatment Preparation (Day -4 to -1) Email or Phone Reminder

- Remind the subject of the clinic appointment time.
- Remind the subject not to take antibiotics or probiotics 24-96 hours before FMT treatment day 1.
- Remind the subject to maintain a clear liquid diet on Day -1.
- Remind all subject to collect a stool sample (if not submitted), for subject groups 1-3, this should be before beginning the Magnesium Citrate or GoLytely bowel preparation.
- Remind the subject (treatment groups 1-3) to consume a 10 oz. bottle of Magnesium Citrate or 250ml of GoLytely bowel preparation for subjects with impaired kidney function (investigator or nurse practitioner will call in a prescription if needed) on evening (around 6:00pm) before the FMT treatment Day 1 clinic visit.
- Remind the subject to take 4mg (2 capsules) of loperamide (Imodium) for study groups 1-3 on the morning of the first day (Day 1) of the FMT treatment (can be taken within 12 hour after FMT treatment if dose missed before).
- Remind the subject to continue taking routine prescription medications.
- Remind the subject to complete and bring the pretreatment diary card to the clinic visit.

8.3 FMT Treatment (Day 1)

Subjects will arrive at the clinic on Treatment Day 1.

8.3.1 Before Administering PRIM-DJ2727

- Medication containers (Day 1 and Day 2) of PRIM-DJ2727 will be taken from the EPDL to the clinic by study personnel.
- Obtain pretreatment stool sample from patient's at-home collection and deliver to EPDL if stool sample has not been delivered to EPDL prior to Day 1 Treatment clinic visit.
- Obtain and review the pretreatment diary card with subject to determine baseline pattern of solicited symptoms.

- Ensure subject (treatment groups 1-3) consumed a 10 oz. bottle of magnesium citrate or 250mL of GoLytely on Day -1. If bowel prep is missed, subject can still proceed with FMT and will be recorded as protocol deviation.
- Ensure subject (treatment groups 1-3) has taken 4mg (2 capsules) of loperamide (Imodium) that morning (can be taken within 12 hours after FMT if dose missed before).
- Confirm subject took their last doses of antibiotic treatment for the CDI 24-96 hours before, non-dietary probiotics and bile-acid sequestrants are not being taken, and any antibiotic for non-CDI treatment has not been taken in the last 14 days.
- If applicable, perform urine pregnancy test.
- Collect pre-treatment weight and vital signs (blood pressure, heart rate, respiration rate, oral temperature).

8.3.2 Administering PRIM-DJ2727

- Administer PRIM-DJ2727 capsules with water. Instruct subject that capsules are to be swallowed and not chewed. If subjects chew capsules, ask them to swallow and continue administering capsules.

8.3.3 After Administering PRIM-DJ2727

- Observe subject in the clinic for $\geq$ 60 minutes and assess for any adverse events.
- Ask subject if experienced any objectionable taste or smell or difficulty swallowing the capsules and general reaction on taking PRIM-DJ2727 capsules.
- Collect post-treatment vital signs (blood pressure, heart rate, respiration rate, oral temperature).
- Provide subject with container of PRIM-DJ2727 for Day 2 treatment
- Remind the subject to take Day 2 PRIM—DJ2727 capsules empty stomach next morning with water.
- Remind subject to continue taking routine prescription medications as needed, but antibiotics, non-dietary probiotics and bile-acid sequestrants may not be taken.
- Release subject with IRB approved aftercare instructions, paper diaries, visit scheduler, safe antibiotics list and specimen request form.

8.4 FMT Treatment (Day 2 Phone Visit)

- Ask subject to ensure PRIM-DJ2727 Day 2 capsules dose was taken.
- Ask for any adverse events since Day 1 assessment.
- Remind subject to follow the aftercare instructions provided on Day 1.
- Remind subject to take routine prescription medications as needed, but antibiotics, non-dietary probiotics and bile-acid sequestrants may not be taken.
- Remind subject to completely fill the diaries, and submit diaries and stools at follow-ups according to schedule and instructions provided by study team.
- A form letter may be sent to subject's physician about FMT completion.

8.5 Phone Visit Follow-up Days 1(+3), 7(+3), 14(+3), 30(+10), 60(+10), 90(+10) and 180 (+20) after treatment

- Contact the subject by phone and/or email at follow up days 1, 7, 14, 30, 60, and 90 after treatment, within windows specified in Table 1 to:
- Remind subject to collect stool sample that will be placed in a transportation container with ice pack and delivered to the EPDL within 6 hours of collection if possible or as quickly as possible (excluding weekends and holidays) to be stored in the EPDL at -80^{o} degrees for possible future testing. Remind subject to put stool collection date on provided container (if already submitted, stool collection date should be confirmed). Stool sample will not be collected for follow up visit Day 180. If hand delivery is not possible, stool sample may be shipped by Federal Express as expeditiously as possible. A FedEx shipping label, ice pack, Styrofoam container with shipping box and instructions will be provided to the subject to ship stool samples if they are not able to hand deliver to the EPDL. The EPDL will be closed on weekends and holidays. If follow-up day falls on Friday, Saturday or Sunday, subjects can collect and ship the sample on Thursday or the following Monday.
- Remind subject to bring completed diaries with the stool sample, or fax, or email, or mail to the study site. For diary copies received in email or fax, an attempt will be made to obtain the originals from subjects to be taken to the study site subsequently. Diary will not be collected on follow up visit day 1 after treatment.
- Assess subject for any adverse events, new illness symptoms, worsening of baseline illnesses including adverse events of special interest (new or worsening autoimmune conditions, and metabolic syndrome) and change in medication. Assess and review subject-reported weight data from diaries for AE (>5% change from baseline weight) at days 60 and 180 phone visits.

- Assess subject for solicited symptoms such as diarrhea (passage of ≥ 3 watery stools per 24-hour period for two consecutive days), nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering and fever (> 99.9 F) up to 60 days after treatment.
- If CDI recurrence is suspected, subjects should be recommended to contact their physician to perform a stool test for C. *difficile*. EPDL will test scheduled stool specimens and any unscheduled illness stool submitted up to 60-70 days after treatment for C. difficile toxins A/B in weekly batches but this should not delay treatment. If the test is positive, they should be treated by their physician. The Principal Investigator will be available for consultation with the attending physician. Subjects who are determined treatment failures by the investigator will not be followed further for clinical parameters. A form letter may be sent to subject's physician about CDI recurrence.

9. ASSESSMENT OF SAFETY AND EFFICACY

9.1 Assessment of Safety (Definition)

9.1.1 **Adverse event:**

Any untoward medical occurrence associated with the use of PRIM-DJ2727 whether or not considered drug related, and can therefore be

- Any symptom not previously reported by the subjects (medical history/pre-treatment diary). Wherever possible, a specific disease rather than individual associated signs and symptoms should be identified by the investigator. However, if an observed or reported sign or symptom is not considered a component of a specific disease by the investigator, it should be recorded as a separate adverse event.
- Clinical worsening of baseline parameters according to subject.
- A condition felt to be possibly related to treatment first detected or diagnosed after study drug administration

All adverse events (AEs) that occur within 180-200 days after treatment will be recorded as indicated above. Recurrence of CDI is not to be recorded as an adverse event unless hospitalization ≥ 24 hours is required for management, then recorded as serious adverse event.

9.1.2 Suspected adverse reaction:

Any adverse event for which there is a reasonable possibility that the study drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means

there is evidence to suggest a causal relationship between the drug and the adverse event.

9.1.3 **Serious adverse event:**

Any adverse event or suspected adverse reaction is considered serious if in the view of the investigators it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Hospitalization ≥ 24 hours
- Prolongation of existing hospitalization
- Substantial disruption of the ability to conduct normal life functions
- Congenital abnormally/birth defect
- Important medical events that jeopardize the patient, such as allergic bronchospasm requiring intensive treatment
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

An emergency room visit without hospital admission will not be recorded as a serious adverse event (SAE) under this criterion, nor will hospitalization for a procedure scheduled or planned before signing of informed consent.

Death and life-threatening AEs possibly related to the study are reported by study coordinator or investigator within 24 hours to the IRB. Other SAEs potentially related to the study will be reported to the IRB within 7 days.

9.1.4 Unexpected adverse event:

An adverse event or suspected adverse reaction is considered 'unexpected' if it is not described in the general investigational plan or is not listed at the specificity or severity that has been observed.

9.1.5 Adverse events of special interest (weight gain, new or worsening autoimmune conditions, and metabolic syndrome):

Adverse events of special interest such as new or worsening autoimmune conditions and metabolic syndrome will be asked from subjects during follow up phone visits. Subjects will be asked at the enrollment visit that how much they weighed before the first CDI episode to take into consideration any weight loss resulting from CDI bouts. To obtain baseline data, subjects will be weighed on treatment day 1 clinic visit prior to administration of FMT treatment. After FMT treatment, subjects will be recommended to measure weight at home each Sunday morning before eating and record on follow up diaries, providing they have a scale or balance.

To account for normal weight fluctuations, subject-reported weekly weight data from diaries will be assessed for AE (>5% change from baseline weight) at days 60 and 180 phone visits.

9.2 Medical Conditions

1. During the follow up phone visits, the subject will be asked for any adverse event or medication change since the medical history/diary at baseline.
2. Investigators will determine if any adverse event should be further studied. All actions will be recorded with medical condition and dates of adverse event and medication taken. The donor will be contacted and tested, if necessary.
3. Subjects should be instructed on treatment days to contact the study personnel if they have any questions regarding an adverse event or the appropriateness of a medication after FMT.

9.3 Monitoring for Safety

The following definitions of terms are guided by the International Conference of Harmonization and US Code of Federal Regulations (21 CFR 312.32).

9.3.1 Severity

The adverse event will be documented according to the following descriptors:

- *Mild*: associated with no limitation of usual activities or only slight discomfort
- *Moderate*: associated with limitation of usual activities or significant discomfort
- *Severe*: associated with inability to carry out usual activities or very marked discomfort

9.3.2 **Relationship**

The relationship of an adverse event to FMT will be assigned by the Investigator according to the following definitions:

- *Probable*: a reaction that follows a reasonable temporal sequence from the procedure that follows a known or expected response pattern to the suspected procedure and that could not be reasonably explained by the known characteristics of that subject's clinical state
- *Possible*: a reaction that follows a reasonable temporal sequence from the procedure that follows a known or expected response pattern to the procedure but could readily have been produced by a number of other factors

- *Unlikely*: a reaction that does not follow a reasonable temporal sequence from the procedure but for which causality from FMT cannot be ruled out.
- *Not related*: a reaction for which sufficient data exist to indicate that the etiology is unrelated to the procedure

9.4 Pre-existing Signs and Symptoms and Medical Conditions

Medical conditions that are present at or before the procedure that manifest with the same severity or frequency will not be recorded as an adverse event. Similarly, signs or symptoms related to a pre-existing disease will not be recorded as adverse event unless there is an increase in the severity or frequency of the signs or symptoms. The pre-existing conditions will be recorded in the Medical History.

9.5 Progression of Underlying Conditions and Anticipated events as an Adverse Event

If the symptoms of the primary CDI continue on the study, this is not an AE. If new symptoms develop or worsen after treatment, this is considered an AE.

Anticipated events include diarrhea, nausea, vomiting, gas/flatulence, abdominal distension, abdominal cramps/pain, constipation, rectal bleeding, fatigue/malaise, chills/shivering and fever (> 99.9 F), and may or may not be causally related to the FMT treatment or CDI. These anticipated events will be solicited from subjects at enrollment visit, via pre-treatment diary and at follow-up phone visits. For blinded treatment groups (1-3), mild to moderate diarrhea resulting from taking bowel prep starting a day before treatment and/or mild constipation from taking Imodium on treatment day up to one day after treatment are expected side effects, and should not be recorded as adverse events.

9.6 Recording and Documenting Adverse Event

The study team must completely and promptly record each new adverse event and serious adverse event that occurs after the procedure, even if the relationship of adverse event to the procedure is assessed by the Investigator to be “unlikely” or “not related”. In addition, the investigator must document and follow serious adverse events and adverse events of special interest that occur from the procedure through 180-200 days after the FMT. The Investigator should attempt, if possible, to establish a diagnosis based on the presenting signs and symptoms. If an adverse event meets the definition of a serious, unexpected and suspected (related to study drug/procedure) adverse reaction (SUSAR) then the Investigator must also complete the serious adverse event form/IND safety report, and send any supporting source documents to the University of

Texas Health Science Center IRB and FDA as soon as the event is discovered within the defined timeline.

At each visit, after the subject has had an opportunity to mention any problems spontaneously, the Investigator (or designee) will inquire about adverse event by asking the standard questions listed, such as:

- Have you had any new medical problems since your last visit?
- Have any medical problems present at your last visit changed, i.e., stopped, worsened, or improved?
- Have you taken any new medicines, other than study drug, since your last visit?

Any spontaneous adverse event information provided by the subject will be recorded. If an adverse event has not resolved at the time of the final visit, the Investigator should evaluate the status of the adverse event at the follow-up phone contact and reflect the status of the adverse event (e.g. ongoing or resolved).

The DSMB will issue a final report identifying adverse events related to study drug.

9.7 Investigator Reporting of Suspected Unexpected Serious Adverse event (SUSAR)

Adverse events that meet all three definitions described above of a Suspected (related or possibly related to study drug or participation in the research), Unexpected and Serious Adverse Reaction (SUSAR) must be reported to the University of Texas Health Science IRB and FDA expeditiously. The investigator must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event.

The investigator must report all suspected unexpected serious adverse reaction events to the University of Texas Health Science IRB/CPHS via iRIS within 7 calendar days after investigator recognizes or classifies the event as SUSAR unless the report involves the death of a participant, in which case the event needs to be reported to IRB within 24 hours after first knowledge by the investigator via facsimile or email or by telephone.

The investigator must also report all suspected unexpected serious adverse reactions to the FDA as soon as possible but within a maximum of 15 calendar days of first knowledge by the investigator, unless the event is *unexpected fatal or life-threatening suspected adverse reaction,* in which case the event needs to be reported to the FDA as soon as possible but in no case later than 7 calendar days after the investigator's initial receipt of the information.

The reports should identify the subject by their unique subject number instead of names. The completed serious adverse event report forms will be used by the investigators in regulatory filings. The investigator is responsible for continuing to report to the University of Texas Health Science IRB and FDA within 15 calendar days of any new or relevant follow-up information obtained concerning the SUSAR event. The results of any additional assessments conducted must also be reported to the University of Texas Health Science IRB and FDA.

Serious and unexpected adverse events can be anticipated to occur during the course of the study regardless of drug exposure. Examples of such anticipated events may include but not limited to known consequences of any underlying disease or RCDI, events anticipated from any background regimen, events common in study population or recurrence or worsening of a condition relative to pretreatment baseline. Such events will not be reported individually as they are not informative as single cases to conclude that the event is a suspected adverse reaction.

If an investigator is unsure about whether to report a finding as an adverse event, he will record the finding as an AE in study data. All adverse events will be reported in the annual and final clinical study reports as required by the applicable regulations.

9.8 Notification of Post-Study Serious Adverse event

Investigators are not obligated to actively seek follow-up information for subjects with an adverse event after the conclusion of the study (i.e.>180-200 days after the FMT procedure). However, if the investigator becomes aware of an adverse event that occurs after the subject completes and the adverse event is recognized by the Investigator to be at least possibly related to study procedure, the investigator must notify the University of Texas Health Science Center IRB and FDA within 15 calendar days of making the determination about the event.

9.9 Grading Adverse Event Severity

Adverse Events are to be graded by severity by the site investigator following FDA guidelines (http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf). For the classification of adverse events, severity is not the same as seriousness, which is defined in section 9.6. Severity is an indication of the *intensity* or a specific event (e.g., mild, moderate or severe). Classification of an event as serious relates to an event's outcome or intervention criteria and is usually associated with events that pose a threat to a

subject's life or functioning (Severity Grading Table). An event can be severe but not serious, such as a migraine. Using the definitions in Severity Grading Table at the end of the protocol, the site investigator will categorize the severity of an adverse event on the AE reports.

9.10 Halting Criteria

Halting will take place if a serious adverse event is seen that is at least possibly related to FMT and if there is any suspected or confirmed transmission of a pathogen from the product to a study subject. The study will be halted temporarily until the DSMB makes a decision. Halting will also take place if the DSMB is concerned enough to stop the study to collect and analyze more clinical data.

If a subject develops infection after FMT caused by a bacterial pathogen (e.g. urinary tract infection, sepsis, pneumonia), any potential pathogen isolated by a laboratory (e.g. *E. coli* from urinary tract) will be obtained if at all possible and compared with the same species identified from the donor using either culture or ribotyping techniques to determine if the FMT was responsible for transmission of a bacterium from the product to a subject. A form will be given to subjects to provide to their doctors if an infection is diagnosed after FMT, requesting to work with their laboratory to send the isolate to the University of Texas School of Public Health at 1200 Pressler Street, Room 740, Houston TX 77030 for further study. The research program will pay for any reasonable costs for obtaining and shipping of the bacteria.

9.11 Efficacy Endpoint

The primary endpoints of the study are to evaluate the safety of lyophilized product by capsules with various doses and the prevention of subsequent bouts of CDI in 60 days post FMT; and to evaluate the safety of lyophilized product by capsules with same doses without bowel preparation and Imodium, and the prevention of subsequent bouts of CDI in 60 days post FMT without bowel preparation and Imodium. The secondary endpoint is to characterize improvement in microbiota diversity from baseline in subjects treated with various doses of lyophilized capsules.

9.12 Stool Analysis

Stool samples for future metagenomics/microbiome analysis will be collected before FMT and at follow up days 1, 7, 14, 30, 60 and 90 and unscheduled visit (if any up to 60 days) after FMT treatment. Stool samples collected up to 60 days after FMT treatment including unscheduled specimen (if any) will be tested for C. difficile toxins A/B at EPDL in weekly batches. All collected stool samples will be stored at -80°C. Procedures for

stool sample collection and handling storage will be recorded.

10. DATA COLLECTION AND ANALYSIS

10.1 Recording of Data

The Investigator and/or designee must maintain adequate and accurate study related documents including source documents and regulatory documents. The study documents will be reviewed and signed by the investigators periodically. Study records will remain on file for a minimum of two years after the completion/termination of this study.

10.2 Statistical Methods

10.2.1 General Considerations

All analyses will be performed, and all tables, figures, and data listings will be prepared using SAS (Cary, NC). Summary statistics for continuous variables will include the mean, standard deviation, median, minimum, and maximum value; categorical variables will be presented as counts and percentages. For all analyses, baseline is defined as the date of fecal transplantation.

10.2.2 Power Calculations

Assuming recipients receiving 100g for both treatment days, 100g on the first treatment day and 50g on the second treatment, or 50g for both treatment days, or 100g for both treatment days without bowel prep and Imodium has 88%, 80%, 70% and 80% efficacy in preventing post RCDI respectively, it is determined that 348 recipients (1:1:1:1 ratio) would provide 85% power to detect a statistically significant difference in the distributions of efficacy between the 4 treatment groups at the two-sided $\alpha = 0.05$ level of significance. Assuming 10% of patients would be lost to follow-up, 360 (90 in each group) recipients are planned.

10.2.3 Analysis Populations

Two primary analyses will be performed:

1) Analysis for safety and efficacy between three groups (1-3) receiving varied doses of lyophilized product generated from stool (50g for both treatment days; 100g on the first treatment day and 50g on the second treatment; 100g for both treatment days)
2) Analysis for safety and efficacy between two groups (3 and 4) with and without bowel preparation and Imodium receiving same high doses of lyophilized product (100g for both treatment days).

- All analyses will be conducted using the intent-to-treat population. Intent-to-treat is defined as all subjects who have started the procedure.
- Listings and summary tables with descriptive statistics will be prepared for demographic data (gender, date of birth, race/ethnicity), and medical history data.

10.2.4 Randomization Procedures

An independent statistician will develop a randomization list using SAS. Randomization in permuted blocks will be used to achieve balance across treatment groups. The randomization scheme consists of a sequence of blocks such that each block contains a pre-specified number of treatment assignments in random order. The purpose of this is to balance the randomization scheme at the completion of each block. The target sample size is 360 evaluable subjects.

10.2.5 Analyses of Safety

All adverse events and serious adverse events will be coded using the Medical Dictionary for Regulatory Activities Version 12.0, which is a registered trademark of the International Federation of Pharmaceutical Manufacturers and Associations. The number and percentage of subjects reporting at least one occurrence of an adverse event and/or serious adverse event for each system organ class and preferred term will be tabulated. Term will also be tabulated by severity and by the relationship to the procedure. For multiple occurrences of the same adverse event with different severities, the adverse event with the highest severity will be tabulated. For multiple occurrences of the same adverse event with different relationships to the procedure (related and not related); the adverse event will be tabulated as related. All adverse events and or serious adverse events for all subjects will be presented in data listings. Narratives will also be prepared for each serious adverse event.

10.2.6 Efficacy Analyses

While we will perform standard statistical analyses as outlined above, we do not expect to achieve statistical significance only trends.

11. SUBJECT PAYMENT PROCESS

Subjects will self-pay $1,500 by money order, cashier's check or debit/credit card. Debit/credit card payments will be entered at the URL **https://go.uth.edu/sph-cid** by personnel at the University of Texas School of Public Health. A copy of transaction will be placed in the subject file.

12. PROTECTING PRIVACY

This study protocol, documentation data, and all other information generated, will be held in strict confidence by the Investigators. The representatives of the Food and Drug Administration (FDA), the University of Texas Health Science Center at Houston, Kelsey Research Foundation, Kelsey-Seybold Clinics, and/or companies engaged with UTHealth for the commercialization of the results of the study may review research for the purposes of verifying research data.

13. CONTACTS ABOUT STUDY

For study details, the study coordinators at Kelsey Research Foundation [redacted]

For product related issues - Dr. Herbert L. DuPont [redacted]

For subjects at Kelsey-Seybold Clinic - Dr. Ned Snyder [redacted]

For University of Texas/Memorial Hermann Hospital - Dr. Andrew DuPont [redacted] The University of Texas Health Science Center Committee for the Protection of Human Subjects [redacted].

Kelsey Seybold Clinic Review Committee at [redacted]

TABLE 1: STUDY SCHEDULE:

Assessment and Procedures	Enrollment (day -14 to -1)	Treatment Days		Follow Up and Safety Assessment Period via Telephone							Un scheduled Visit	Early Termination Visit
		Day 1	Day 2	Day 1 (+3)	Day 7 (+3)	Day 14 (+3)	Day 30 (+10)	Day 60 (+10)	Day 90 (+10)	Day 180 (+20)		
	Clinic	Clinic	Phone	Phone	Phone	Phone	Phone	Phone	Phone	Phone	Phone	Phone
Informed Consent	X											
Inclusion/Exclusion Criteria	X											
Demographics	X											
Medical/CDI History	X											
Concomitant medications	X			X	X	X	X	X	X	X		
Randomization at University of Texas School of Public Health	X											
Pre-treatment preparation reminder (day -4 to -1)[1]	X											
Urine Pregnancy Test[2]		X										
Confirmation of bowel prep and Imodium (groups 1-3)[3]		X										
Vital signs[4] and Weight[5]		X										
Stool Collection	X[6]			X	X	X	X	X	X		X	
Study Drug Dose		X	X									
Diaries Distribution	X	X										
Diaries Receipt [7]		X			X	X	X	X	X	X		X
AE Assessment/Monitoring for Safety		X	X	X	X	X	X	X	X	X	X	X

[1] Reminder can be via email or phone.
[2] Perform urine pregnancy test only on female subjects' of childbearing potential
[3] If bowel prep is missed, subject can still proceed with FMT (noted as protocol deviation).
[4] Vital signs before treatment, observe, and repeat vitals after at least 60 minutes
[5] Weight measured before treatment only. Subjects will measure weight at home each Sunday morning before eating and record on diaries if they have a scale or balance.
[6] Subject can collect stool any day after enrollment and before bowel prep on Day -1, and can bring stool sample on treatment day 1 clinic visit
[7] Diaries can be submitted with stool samples or emailed, faxed or mailed (original can be provided later)

TABLE 2: SEVERITY GRADING

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Flatulence	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	NA	**NA**
Belching (burping)	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	NA	**NA**
Abdominal distension or bloating	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	**NA**

Increased diarrhea	Increase of ≤ 3 stools over baseline per 24- hour period	Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea if not present at baseline OR increase of ≥ 7 stools over baseline per 24- hour period OR IV fluid replacement indicated if not indicated at baseline	Life-threatening consequences, e.g., hypotensive shock
Abdominal cramping/pain	Discomfort/pain causing no or minimal interference with usual social and functional activities	Discomfort/pain causing greater than minimal interference with usual social and functional activities	Discomfort/pain causing inability to perform usual social and functional activities	Disabling pain causing inability to perform basic self-care OR inpatient hospitalization ≥ 24 hours
Constipation	Occasional or intermittent symptoms, occasional use of stool softeners, laxatives, dietary modifications or enema	Persistent symptoms with regular use of laxatives or enemas indicated	Symptoms causing inability to perform usual social and/or functional activities	Life-threatening consequences, e.g., obstruction, toxic megacolon
Colitis	No symptoms, regardless of pathologic or radiographic evidence of inflammation	Abdominal pain, mucus or blood in the stool	Abdominal pain, fever, change in bowel habits with ileus; peritoneal signs	Life-threatening consequences, e.g., perforation, bleeding, ischemia, necrosis, toxic megacolon

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Fever	37.7 – 38.6°C (99.9 – 101.5° F)	38.7 – 39.3°C (101.6 – 102.8° F)	39.4 – 40.5°C (102.9 – 104.9° F)	> 40.5°C (104.9° F)
Fatigue/malaise	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions
Chills	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	NA
Rectal discomfort or irritation	No symptoms or symptoms not requiring medical intervention	Symptomatic with medical intervention (topical medications / treatments) indicated	Symptoms causing inability to perform usual social and functional activities or requiring medical intervention other than topical medications / treatments	NA

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life- threatening
Rectal bleeding	Mild or intermittent without transfusion	Persistent without transfusion	Requires transfusion	Life-threatening consequences
Nausea	Transient (≤ 24 hours) or intermittent nausea with nor or minimal interference with oral intake	Persistent nausea resulting in decreased intake for 24-48 hours	Persistent nausea resulting in decreased intake > 48 hours OR aggressive rehydration indicated, e.g., IV fluids	Life-threatening consequence, e.g., hypotensive shock
Vomiting	Transient (≤ 24 hours) or intermittent vomiting with nor or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated, e.g., IV fluids	Life-threatening consequence, e.g., hypotensive shock

Parameter	Grade 1: Mild	Grade 2: Moderate	Grade 3: Severe	Grade 4: Potentially Life-threatening
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Adverse event not identified elsewhere in this table	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Symptoms causing inability to perform basic self-care functions OR medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death

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