*U.S. Food and Drug Administration (FDA)*
*Investigational New Drug Application*

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**Study Title: S**afety of fecal microbiota **T**ransplantation: **O**penBiome **O**utcomes and **L**ongitudinal follow-up (STOOL) for recurrent *Clostridium difficile* infection

**Product:** Fecal Microbiota Preparations

**Proposed Indication:** Recurrent *Clostridium difficile* infection

**Date Submitted:** October 3, 2014

**Edition:** 1st

**ClinicalTrials.gov Identifier:** NCT02403622

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3. INTRODUCTORY STATEMENT AND GENERAL INVESTIGATIONAL PLAN

# I. Introductory Statement

Overarching Objective

There is emerging concern regarding suboptimal efficacy of standard antibiotic treatment for recurrent *Clostridium difficile* infection, leading to expanded interest in fecal microbiota transplantation, a highly promising therapy. However, little is known about the safety, particularly long-term, of fecal microbiota transplants as current retrospective data has had sporadic, inconsistent follow-up. The overarching objective of this study is to address this knowledge gap by collecting safety data from a multicenter cohort in a prospective, longitudinal manner.

The burden of *Clostridium difficile* infection

*Clostridium difficile* infection (CDI) has recently been reported as the most common healthcare-associated infection in the United States, and the emergence of community-acquired CDI is particularly concerning.[1 2] CDI is associated with a spectrum of symptoms ranging from diarrhea to abdominal pain, and severe infections may lead to toxic megacolon and death. The clinical burden of CDI is significant. In the United States, the incidence of CDI per 10,000 inpatient hospitalizations doubled between 2000 and 2005 (5.5 *versus* 11.2 cases).[3] Moreover, CDI presents substantial economic ramifications. Nosocomial CDI increases the cost of an otherwise matched hospitalization by four-fold, translating to a reported cost of up to $4.8 billion/year in the United States.[4–6]

The suboptimal efficacy of standard antibiotic treatment for CDI has prompted growing concerns. Since 2000, failure rates of metronidazole, the recommended first-line treatment for mild to moderate CDI, have increased from 2.5% to more than 18%.[7] Other reports from Canada, the United Kingdom, and Spain have also documented resistance to metronidazole and reduced susceptibility to vancomycin in CDI.[8–10] What is most concerning about the landscape of CDI is the incidence of recurrent CDI following antibiotic treatment with metronidazole, vancomycin or fidaxomicin – which continues to increase and has become a major challenge.[11–14] After 2 or more episodes of recurrence, the risk of subsequent recurrence has been reported to exceed 60% with antibiotic treatment.[12 15 16] Evidence-based treatment options are limited for patients experiencing multiple recurrent CDI episodes as highlighted in the most recent US clinical guidelines.[17]

An emerging treatment: Fecal Microbiota Transplantation

Given the shortcomings of standard antibiotic therapy, particularly in patients with recurrent CDI, alternative approaches have been explored. Although its mechanism of action is not fully elucidated, fecal microbiota transplantation (FMT) is a unique therapy that aims to address the underlying mechanism of recurrent CDI. Specifically, FMT aims to restore 'colonization resistance', re-establish diversity, and facilitate microbial homeostasis in order to protect against toxigenic CDI.[18–20] Systematic review/meta-analysis of observational data has described clinical cure in approximately 90% of patients, where a placebo response seems unlikely in a patient population with

prolonged and severe symptoms in the context of previous standard antibiotics.[21] Additionally, small randomized controlled trials have reported similarly impressive efficacy in a difficult to treat patient population.[22 23]

Although the efficacy of FMT appears convincing, there is a paucity of robust safety data for FMT. The current safety profile has been collected predominantly from retrospective reviews or systematic reviews of case series data, approaches that have methodological limitations in assessing the safety of FMT.[21 24 25] Broadly, the literature contains inconsistent long-term follow-up of adverse events, which have been spontaneously reported rather than actively sought, in turn increasing the risk of underreporting. [21] Randomized controlled trials have also had brief follow-up and one trial was stopped early for benefit at interim analysis, limiting the ability to meaningfully assess the long-term safety profile of FMT.[22 23] Although the current body of literature does not signal any significant safety risk for FMT, a high-quality prospective longitudinal study is needed to confirm the safety profile of FMT among recurrent CDI patients.

OpenBiome is a 501(c)(3) non-profit organization that is the first public FMT stool bank in the United States for CDI non-responsive to standard therapy. Under the FDA's current regulatory framework of enforcement discretion, OpenBiome has partnered with more than 100 health care institutions in over 30 states to expand safe, affordable access to FMT. Accordingly, our institute is uniquely positioned to meaningfully assess the safety profile of FMT, as our group and others have identified an absence of high-quality, prospective studies interrogating safety.[21]

## II. General Investigational Plan

The design will be a prospective, open-label, multi-center longitudinal cohort study to assess the short- and long-term safety of FMT as well as the clinical resolution of diarrhea among 150 patients with 3 or more episodes of CDI (defined as 3 unformed stools over 24 hours for 2 consecutive days and either a positive stool test for CDI or pseudomembranes on colonscopy/sigmoidoscopy).[26] Subjects will be adult outpatients referred to one of the study centers after at least three recurrent episodes of CDI and previous treatment with at least one 10-day course of oral vancomycin or fidaxomicin. After FMT by colonoscopy/sigmoidoscopy or enema, patients will be followed prospectively and monitored for clinical resolution and adverse events at:
3 days (telephone), 6 weeks (clinical assessment), 6 months (telephone or clinical assessment), and 12 months (telephone or clinical assessment) after FMT. Subjects who recur will be offered a second FMT by colonoscopy with a different donor. Microbiome analysis will be conducted from stool samples at baseline and each of the 4 follow-up intervals.

## III. Product Name/Chemical Structure

Fecal Microbiota Preparations

## IV. Proposed Indication(s)

Recurrent *Clostridium difficile* infection

## V. Dosage, Route of Administration and Dosing Regimen

V-A Colonoscopic/sigmoidoscopic FMT (FMP250)

*Dosage Form*: Screened human donor stool, [redacted] homogenized, filtered [redacted] and aliquoted to sterile 250mL vessels.

*Route of Administration*: Infusion will occur in the most proximal region of the colon that is technically feasible. The material will be infused through the working channel of the endoscope.

*Dosing Regimen*: 250 mL x 1 dose. In the event of a clinical non-response (defined in 'Protocol' Section), a repeat single 250 mL dose will occur from a different donor.

V-B Retention Enema FMT (FMP250E)

*Dosage Form*: Screened human donor stool, [redacted] homogenized, filtered [redacted] and aliquoted to sterile 250mL vessels.

*Route of Administration*: Lubricated enema nozzle will be inserted into rectum and contents expelled into the distal colon with the subject requested to retain material for at minimum 30 minutes.

*Dosing Regimen*: 250mL x 1 dose. In the event of clinical non-response (defined in 'Protocol' Section), a repeat single 250 mL dose via colonoscopy will occur from a different donor.

4. [RESERVED, CFR 312.23]

5. INVESTIGATOR'S BROCHURE

[Follows]

6. PROTOCOL

# I. Study Overview and Specific Aims

The proposed multi-center, open-label, longitudinal cohort study aims to capture the safety profile of FMT in recurrent CDI, as well as to contribute additional efficacy data to the existing body of evidence. The proposed study includes collaboration with Investigators who are able to define the microbial landscape of patients pre- and post-FMT, and further elucidate if there is a microbial signature that predisposes patients to respond, fail treatment, or have adverse events from FMT.

Study Title
**S**afety of fecal microbiota **T**ransplantation: **O**penBiome **O**utcomes and **L**ongitudinal follow-up (STOOL) for recurrent *Clostridium difficile* infection

Specific aims (SA)

**SA1. Determine the short-term (<6 weeks) safety of FMT for the prevention of further CDI recurrence.**
Hypothesis: Careful donor screening for both infectious and non-infectious yet potentially microbiome-associated conditions minimize the risk of donor to recipient transmission of disease. Therefore, FMT will not be associated with meaningful number of *related* serious adverse events, recognizing that the recurrent CDI patient population is conventionally elderly with multiple comorbidities and may be at risk for FMT procedure-related complications compared to otherwise young and healthy individuals based on conventional literature.

**SA2. Determine the long-term (6-12 months) safety of FMT for the prevention of further CDI recurrence.**
Hypothesis: Careful donor screening for both infectious and non-infectious yet potentially microbiome-associated conditions minimize the risk of donor to recipient transmission of disease. Therefore, FMT will not be associated with meaningful number of *related* serious adverse events, recognizing that the recurrent CDI patient population is conventionally elderly with multiple comorbidities and may be at risk for developing new conditions, new medications or adverse events including death, but unrelated to FMT material.

**SA3. Determine the longitudinal robustness of FMT clinical cure rate for the prevention of further CDI recurrence.**
Hypothesis: We predict a clinical cure rate in keeping with the current literature (80-90%) at the12 month follow-up time interval. This predicted longitudinal response rate would exclude patients who require further antibiotics that put them at risk for CDI reinfection, not true recurrence.

**SA4. Collect pre- and post-FMT stool samples from patients with recurrent CDI as well as donor stools for microbiome analysis.**
Hypothesis: Prior to FMT, recurrent CDI patients will have deficiencies of certain normal colonic flora.[22] After FMT, these microbial populations will be restored, resulting in a durable transition to a diverse, stable community that resembles the composition of the healthy stool donor. We expect that this transformation will be stable in the absence of challenge with antimicrobial therapy.

## II. Subject Inclusion/Exclusion Criteria

Definitions

- ***Clostridium difficile* infection**: At least 3 unformed stools over 24 hours for 2 consecutive days and either positive stool test (ELISA or PCR) for *Clostridium difficile* toxins or pseudomembranes on colonoscopy (SHEA-IDSA guidelines).[26]
- **Clinical Cure**: Clinical resolution of diarrhea (<3 unformed stools for 2 consecutive days) with maintenance of resolution at 6-week follow-up period, at minimum, and no further need for antibiotic therapy for CDI. In keeping with SHEA-IDSA guidelines and an absence of 'test of cure,' subjects who meet aforementioned definition will be considered a clinical cure regardless of any incidental follow-up stool testing employed.[26]
- **Clinical Non-Response:** Ongoing or development of diarrhea and the requirement for antibiotic therapy for CDI.

Inclusion criteria

- Adult (age 18-75 years old)
- Outpatient
- Third or further documented CDI episode **and**
- Unable to maintain CDI cure after standard therapy with oral vancomycin or fidaxomicin
    - Previous treatment with at least one course of tapered/pulse vancomycin **or**
    - Inability to taper or stop vancomycin or fidaxomicin without developing diarrhea requiring antibiotic therapy.
- Improvement of CDI symptoms on vancomycin or fidaxomicin

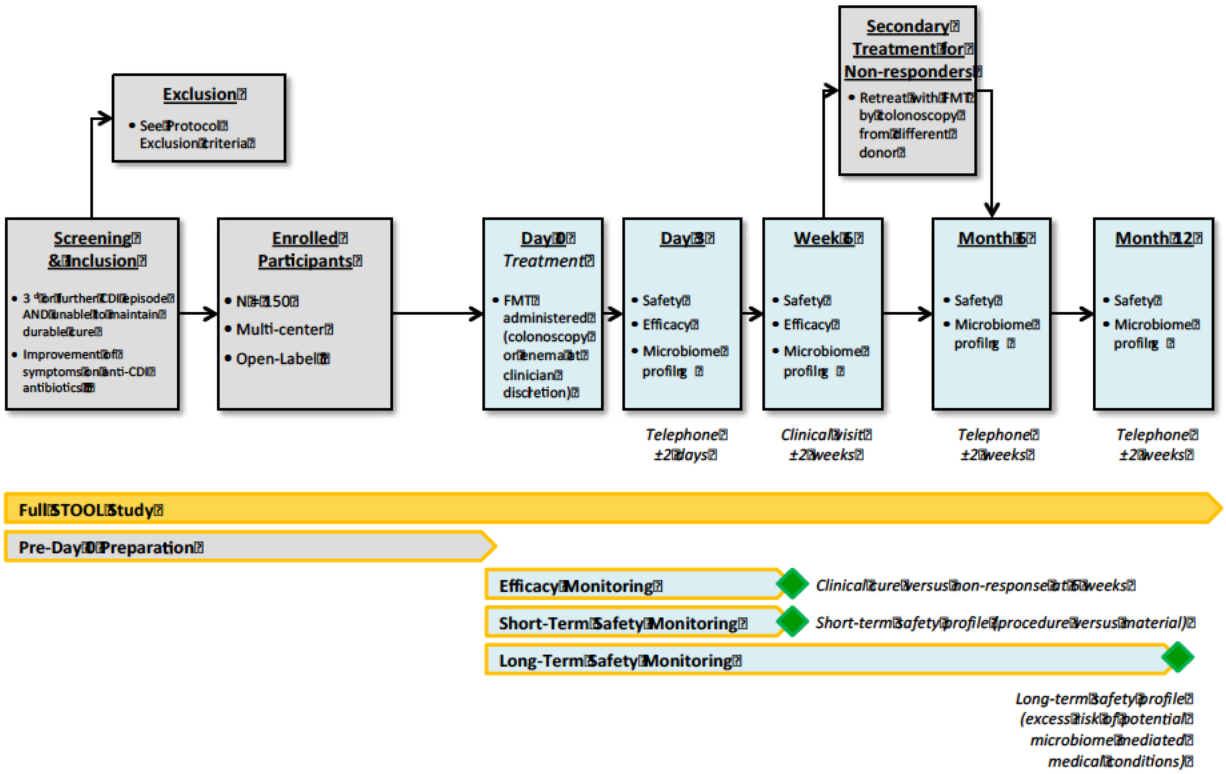
Exclusion criteria

- Unable to comply with study follow-up procedures at discretion of MD
- Unable to provide informed consent at discretion of MD
- Participating in another clinical trial
- Pregnant or nursing currently or planned pregnancy in next 1 year
- Evidence of toxic megacolon or gastrointestinal perforation
- Peripheral white blood cell count >30 x $10^9$/L and/or temperature >38 degrees Celsius
- Admission to an intensive care unit within prior 7 days for any reason
- Previously undergone FMT
- Severely immunocompromised patients

    - HIV infection (any CD4 count)
    - AIDS-defining diagnoses
    - Inherited/primary immune disorder
    - Immunosuppressant medications
        - Current or recent (<3 months) treatment with anti-neoplastic agents
        - Current or recent (<3 months) treatment with calcineurin inhibitors (tacrolimus, cyclosporine)
        - Current or recent (<3 months) treatment with mycophenolate mofetil
        - Current or recent (<3 months) treatment with monoclonal antibodies to B and T-Cells, anti-TNF, glucocorticoids, antimetabolities (azathioprine, 6-mercaptopurine)
- Neutropenia with absolute neutrophil count (ANC) $<0.5 \times 10^9/L$
- Active gastroenteritis due to infectious cause other than CDI
- Short gut syndrome
- Colostomy
- Ascites
- End-stage liver disease
- Untreated, in-situ colorectal cancer
- Irritable bowel syndrome
- Inflammatory bowel disease including Crohn's disease and ulcerative colitis
- Microscopic colitis including collagenous colitis and lymphocytic colitis
- Severe food allergy (anaphylaxis) that cannot be confirmed as having been excluded from a donor's diet within the five days prior to donation
- Anorectal disorder/severe rectal sphincter tone abnormality or inability to retain enema material
- Unable or unwilling to tolerate colonoscopy/sigmoidoscopy, colonoscopy prep, or enema for any reason at discretion of MD
- Severe underlying disease that the patient is not expected to survive for the subsequent 12 months at the discretion of the MD.
- Any conditions for which, in opinion of MD, the treatment may pose a health risk

<u>Target enrollment and rationale</u>
The longitudinal cohort sample size (n=150) was chosen to allow recruitment in a suitable time frame. Strong consideration will be given to resubmitting documentation including IND amendment and IRB ammendment for a larger sample size and/or prolonged follow-up at the completion of the study.

# III. Design Methodology



## IV. Therapeutic Administration: Fecal Microbiota Transplantation Protocol

Donor selection, laboratory screening, and monitoring

A. Donor Clinical Assessment: OpenBiome utilizes adult, healthy, universal donors who have given informed consent with oversight from Massachusetts Institute of Technology (MIT) IRB/COUHES. Refer to the OpenBiome Master File (BB-MF 15543) for donor selection details. An accompanying authorization letter from OpenBiome can be found in *Appendix 1*. The OpenBiome Donor Risk Factor Questionnaire (*Appendix 2*) is detailed elsewhere. Briefly, the Donor Risk Factor Questionnaire is modified from a similar questionnaire developed by the American Association of Blood Banks for blood donors. The stool donor questionnaire is used to exclude potential donors with infectious disease risk factors (eg. high risk sexual behavior, risk factors for variant Creutzfeldt-Jakob disease, high risk travel), medications that may mediate the microbiome, and a wide array of medical conditions that may have a theoretical link the microbiome.

B. Donor Laboratory Screening: Refer to the OpenBiome Master File (BB-MF 15543) for donor laboratory screening details. An accompanying authorization letter from OpenBiome can be found in *Appendix 1*. Briefly, both serologic and stool test are completed on prospective donors. All tests are outsourced to third-party Clinical Laboratory Improvement Amendments (CLIA)-certified testing facilities. As a condition for participation in this program, donors are required to submit written authorization for the disclosure of these test results to the OpenBiome, in compliance with the Health Insurance Portability and Accountability Act (HIPAA).

C. Donor Monitoring: Refer to the OpenBiome Master File (BB-MF 15543) for donor monitoring details. An accompanying authorization letter from OpenBiome can be found in *Appendix 1.* Briefly, the OpenBiome Donor Prospective Health Status Questionnaire (*Appendix 3*) details the routine questions asked before processing of a specimen, with initiation of clinical assessment by the Chief Medical Officer if a positive answer disclosed by a donor.

Subject preparation: determining eligibility, pre-treatment, and special considerations

A. Subject Clinical Assessment

1. Potential subjects will undergo a medical interview to determine eligibility for the study and a physical exam will be performed at the screening visit.

2. Baseline status, at MD discretion, will be conducted: HIV (type 1 and 2), hepatitis A (IgM, G), hepatitis B (HBsAg, anti-HBcIgM and anti-HBcIgG), hepatitis C (HCV antibody), and Treponema pallidum screening (EIA with reflex to RPR).
3. Baseline complete blood count, liver function panel, creatinine and random blood glucose, and stool *C. difficile* PCR will be documented prior to FMT.

B. Pre-Treatment Medications

1. Subjects will complete at least a 10 day course of vancomycin or fidaxomicin for the most recently diagnosed acute CDI prior to undergoing FMT.
2. To minimize symptoms, antibiotic therapy will be continued by subjects until 3 days prior to the FMT procedure then subsequently discontinued.
3. Any probiotics will be continued by subject until 3 days prior to the FMT procedure then subsequently discontinued.
4. The day before the procedure, the subject will be prepped with standard bowel prep if undergoing colonoscopic/sigmoidoscopy delivery at MD discretion. Patients receiving FMT via enema administration need not undergo a bowel prep at MD discretion.

C. Specimen Collection

Patients must be willing and able to provide stool samples for microbial characterization via high-throughput 16S rRNA sequencing. Patients will be asked to provide a baseline stool sample prior to the FMT procedure (Day 0), as well as follow-up stool samples collected at Day 3, Week 6, Month 6 and Month 12 after FMT.

Samples will be collected with a standard stool collection hat. Immediately after passage, approximately 1 gram of stool will be suspended in 5 ml of RNALater (Life Technologies) using a para-pak collection vial with embedded scoop (Meridian Biosciences). Samples will then be shipped at room temperature to the OpenBiome study site where they will be frozen within 7 days of passage, consistent with the technical specifications for stabilization of nucleic acids in RNAlater. To avoid contamination of the OpenBiome production facility, sample processing and sequencing will take place at the Massachusetts Institute of Technology in a BL-2 laboratory with appropriate containment.

D. Female Subjects:

1. Females of childbearing potential will have a urine pregnancy test on the day of enrollment and be excluded if pregnant.

2. Female subjects must not be and should not become pregnant nor breast-feed an infant while in this study. In order to reduce the risk of pregnancy, the subject or her partner should use one or more of the acceptable methods of birth control listed below, regularly and consistently, while enrolled in this study. Acceptable methods of birth control, which will continue throughout the study and for one month after the study, include:

- Approved oral contraceptive/birth control pill
- Intra-uterine device (IUD)
- Hormone implants
- Contraceptive injections (Depo-Provera)
- Barrier method (diaphragm with spermicidal gel or condoms)
- Transdermal contraceptives (birth control patch)
- Vaginal contraceptive ring (birth control rings)
- Sterilization (tubal ligation, hysterectomy or vasectomy)
- Abstinence

3. If subjects become pregnant or suspect that they are pregnant during this study, they will be instructed to immediately inform the study personnel. If subjects become pregnant or suspect that they are pregnant while in this study, a pregnancy test will be performed. If pregnancy is confirmed, they will be withdrawn from the study if FMT therapy has not yet occurred. If FMT has occurred and conception occurs within the study period, the study physician will assist the patient in getting obstetrical care and will follow the progress of the pregnancy. The study physician will request access to subject and/or infant's medical records for up to 8 weeks after delivery.

<u>Preparation and handling of stool for FMT infusion</u>

1. Frozen material will thaw for 4 hours at room temperature. After thawing, material may remain at room temperature for up to 4 hours, or be kept refrigerated/on ice for up to 8 hours.
2. Standard protocol for handling biohazardous material will be employed in order to avoid contamination and risk to healthcare handlers. Sterile microbiological technique will be employed during material transfer peri-procedure.

<u>FMT procedure guidelines</u>

1. Colonoscopy/sigmoidoscopy
    - The procedure will be completed by a trained gastroenterologist or general surgeon in a standard endoscopy unit.
    - Colonoscopy/sigmoidoscopy will be performed under direct visualization, aiming to reach the most proximal part of the colon using safe endoscopic maneuvers and minimal insufflation.
    - Once at the most proximal location, 250 mL of the fecal material will be passed through the endoscope channel using a sterile syringe.

- Standard normal saline flush(s) will be utilized after all the fecal material has been infused.

2. Enema
   - The procedure will be completed by a trained Registered Nurse, Physician Assistant, or physician in an outpatient clinic, endoscopy recovery area, or standard endoscopy unit.
   - Fecal material will be transferred into conventional enema bottle/bag.
   - Patient will be placed in left lateral decubitus position with foot of bed elevated to facilitate retention.
   - Lubricated enema nozzle will be inserted into rectum and contents expelled into the distal colon with the subject requested to retain material for at minimum 30 minutes but targeting 1 hour.
   - The patient will be asked to transfer to right lateral decubitus position after 15 minutes. If unable to retain material for 1 hour, total retention time will be recorded.

<u>Baseline assessment and follow-up</u>

1. Subjects will be instructed to contact the clinical team if any symptoms of diarrhea occur or any adverse events occur. Follow-up assessments will focus on capturing any serious adverse events, new medical conditions, or changes in patients' pre-FMT medical conditions since last study contact.
2. After passing eligibility assessment, subjects will be assessed at baseline (Day 0 = day of procedure) to capture and document clinical and FMT characteristics (*Appendix 4*).
3. A study representative will contact subjects at Day 3 (+/- 2 days) post-FMT (*Appendix 4*).
4. An in-person clinical assessment between Week 6 (+/- 2 weeks) post-FMT will be performed by FMT provider (*Appendix 4*). If this visit cannot be arranged, the patient's primary care provider may complete the assessment under the guidance and instruction of the FMT provider.
5. A study representative will contact subjects at Month 6 (+/- 2 weeks) and Month 12 (+/- 2 weeks) post-FMT (*Appendix 4*).
6. A study representative will contact subjects to remind them to collect and mail stool samples.
7. A full Subject Contact Schedule (*Appendix 5*) and Informed Consent (*Appendix 6*).

<u>In the event of clinical non-response</u>

1. Colonoscopy/sigmoidoscopy: If patients experience clinical non-response and require vancomycin/fidaxomicin within 6 weeks post-FMT, after a 10 day course subject may opt to undergo a second FMT via colonoscopy with an alternate donor at the physician's discretion. Subjects will be followed per protocol for duration of study.
2. Enema: If patients experience clinical non-response and require vancomycin/fidaxomicin within 6 weeks post-FMT, after a 10 day course subject may opt to undergo a second FMT via colonoscopy with an alternate donor at the physician's. Subjects will be followed per protocol for duration of study.

Withdrawal

1. All reasons for study withdrawal will be reported.
2. Classification of withdrawal:
    - Post-enrollment but pre-FMT
    - Post-FMT but lost to follow-up at:
        - Day 3
        - Week 6
        - Month 6
        - Month 12
            - All reasonable efforts will be exhausted to contact subjects, and data analysis will be based on the last available assessment.
    - Adverse event or serious adverse event mediating withdrawal
    - Rescind informed consent
    - Procedure-related ineligible patients
        - Patients undergoing colonoscopy/sigmoidoscopy and discovered to be ineligible due to newly discovered exclusionary criteria (eg. in-situ colorectal cancer). FMT will not be performed as part of this study and no data will be collected.

## V. Study Observations and Measurements

Study Endpoints

*Safety Endpoints*

Safety will be assessed by short and long-term safety summaries classified into:

1. Serious Adverse Events (SAE)
2. Adverse Events (AE)
3. New medical conditions
4. Changes in pre-existing medical conditions

*Efficacy Endpoints*

Efficacy will be captured through:

1. Clinical cure rate of first FMT at 6-week follow-up
    - Colonoscopy
    - Sigmoidoscopy
    - Enema
2. Clinical cure rate of second FMT at 6-week follow-up, in patients with clinical non-response
    - Colonoscopy/sigmoidoscopy non-response: Alternate donor FMT by colonoscopy
    - Enema non-response: Alternate donor FMT by colonoscopy

*Microbiome Endpoints*

Sequencing data targeting the composition of the gut microbiome will be used to assess whether there are consistent microbial signatures associated with clinical cure, clinical non-response, or safety endpoints.

Data Collection

Case Report Forms will be utilized to capture and collect data (*Appendix 4*):

1. Baseline Assessment (Day 0 – Clinical Assessment)

*Section 1: Clinical Evaluation*

- Study patient identifier
- Date
- Demographics: Age, sex, race/ethnicity
- Pre-existing medical conditions
- Concomitant medications
- Vital signs including height, weight, calculated body mass index (BMI)
- CDI History, including:
    - Date of initial CDI diagnosis
    - Number of CDI recurrences since initial diagnosis
    - Baseline clinical symptoms and severity related to CDI
    - CDI treatments received to date

*Section 2: FMT Procedure*

- Study patient identifier
- Date
- Description of FMT procedures, including:
    - Route of administration (colonoscopy/sigmoidoscopy or enema)
    - Location in colon where FMT delivered
    - Sedation (if applicable)
    - Prep quality (if applicable)
    - Findings of colonoscopy (if applicable)
- Dose
- Unit ID/Lot# of each treatment
- Expiration Date
- Storage Condition
- FMT retention time (if less than 1 hour)
- Immediate adverse events

2. Short-term assessment I (Day 3 – Telephone Assessment)

- Study patient identifier
- Date
- Any change in baseline clinical symptoms related to CDI
- Assessment of SAEs, including:
    - Any complications related to FMT administration
    - Any potential infectious complications such as transmissible enteric infections, sepsis, peritonitis, or bacteremia
    - Any non-serious newly diagnosed infections (specific organism and body site)

    - Any new medical conditions / new medications with associated clinical course
    - Any changes in pre-existing medical conditions with associated clinical course

3. Short-term assessment II (Week 6 – Clinical Assessment)
- Study patient identifier
- Date
- Vital signs including height, weight, calculated body mass index (BMI)
- Any change in baseline clinical symptoms related to CDI including documentation of clinical cure or clinical non-response
- Assessment of SAEs, including:
    - Any complications related to FMT administration
    - Any potential infectious complications such as transmissible enteric infections, sepsis, peritonitis, or bacteremia.
- Any new non-serious diagnosed infections (specific organism and body site)
- Any new medical conditions / new medications with associated clinical course
- Any changes in pre-existing medical conditions with associated clinical course

4. Long-term assessment I (Month 6 – Telephone Assessment)
- Study patient identifier
- Date
- Self-reported height and weight, resulting calculated BMI
- Any recurrence of CDI with description of clinical course, including additional treatments received
- Assessment of any new SAEs
- Any new medical conditions / new medications with associated clinical course
- Any changes in pre-existing medical conditions with associated clinical course

5. Long-term assessment II (Month 12 – Telephone Assessment)
- Study patient identifier
- Date
- Self-reported height and weight, resulting calculated BMI
- Any recurrence of CDI with description of clinical course, including additional treatments received
- Assessment of any new SAEs
- Any new medical conditions / new medications with associated clinical course
- Any changes in pre-existing medical conditions with associated clinical course

Data Analysis

*Primary Study: Safety and Efficacy (Aims 1-3)*

Safety endpoints will be collected with raw events and crude rates reported for overall cohort. Clinical cure rates will be calculated at each site. Both unweighted pooled clinical cure rates and weighted pooled clinical cure rates will be calculated with corresponding 95% confidence interval (CI) for overall study, as well as pre-defined

subgroups including: age, gender, modified Horn index, delivery modality, donor, retention time, site. Weighted pooled rates will be analyzed using the random effects model (DerSimonian-Laird method). Heterogeneity across sites will be assessed using the $I^2$ statistics and the Cochran Q test, where $I^2 > 50\%$ or Cochrane $Q < 0.10$ will be considered to indicate significant heterogeneity. Statistics will be performed using Stata SE 12.0 (StataCorp, College Station, TX).

*Secondary Study: Microbiome (Aim 4)*

DNA will be extracted from each frozen specimen collected from both donors and recipients. The V4 region of the 16S rRNA gene will be amplified using multiplexed bar-coded primers as previously described.[27] Up to 192 samples will be pooled onto a single lane of an Illumina MiSeq for high-throughput sequencing. Quality trimmed and filtered sequence data will be clustered into Operational Taxonomic Units (OTUs) for computational analysis. Each recipient will receive an engraftment score based on their similarity (as quantified by the Jensen-Shannon Divergence) to the donor used in their FMT. Recipients will also be assessed for the long-term stability of their community and it's similarity to samples collected and previously published from both healthy and dysbiotic patients.

## VI. Adverse Event Monitoring

We will capture both short and long-term safety profiles, including:
1. FMT procedure-related adverse events from colonoscopy/sigmoidoscopy or enema (e.g. perforation, sedation related)
2. FMT material-related adverse events (e.g. transmissible infection, allergic reaction)
3. Short-term safety: Both solicited and unsolicited adverse events will be recorded by, telephone follow-up at Day 3 (+/- 2 days) and clinical assessment at Week 6 (+/- 2 weeks).
4. Long-term safety evaluations will focus on any new medical conditions/medications, which may be related or unrelated to FMT. This information will be elicited at the 6 month and 12 month follow-up telephone call and documented.

<u>VI-A. Adverse Events Reporting</u>

A. Definitions

*1. Adverse Event (AE)*
An AE is any untoward medical occurrence in a patient or study subject administered FMT that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a FMT, whether or not related to the FMT.

• AEs may be new events or may be pre-existing conditions that have become aggravated or have worsened in severity or frequency.
• AEs may be clinically significant changes from baseline in physical examination, laboratory tests, or other diagnostic investigation (e.g. laboratory results, radiographic findings).
Pregnancy is not an AE; however, if a female subject becomes pregnant during the conduct of the study, the Investigator will notify Sponsor who is responsible for notifying the FDA.

*2. Serious Adverse Event (SAE)*
A serious adverse event is any adverse experience occurring during or after FMT that results in any of the following outcomes:

• Death

• Life-threatening experience
*Note:* An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

• Requires inpatient hospitalization or prolongation of existing hospitalization
*Note*: Adverse events requiring hospital admissions that are less than 24 hours in duration do not meet this criterion. A scheduled hospitalization for a pre-existing condition that has not worsened during participation in the study does not meet this criterion. Pre-planned hospitalizations for an elective medical/surgical procedure or routine check-ups do not meet this criterion. Admission for CDI relapse will not be included in this definition.

• Results in persistent or significant disability or incapacity

• Results in a congenital anomaly or birth defect

• Results in an important medical event
*Note*: Important medical events are those that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

*3. Planned Hospitalization*
A hospitalization planned prior to FMT is to be considered a therapeutic intervention and not the result of a new SAE. If the hospitalization or procedure is executed as planned, it will be recorded in the subject's medical history or procedures. However, if the event/condition worsens during the study, it must be reported as an AE.

*4. Adverse reaction*

An adverse reaction means any adverse event caused by FMT. Adverse reactions are a subset of all suspected adverse events for which there is reason to conclude that FMT caused the event.

*5. Suspected Adverse Reaction*
Suspected adverse reaction means any adverse event for which there is a reasonable possibility that FMT caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between FMT and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a therapy.

*6. Unexpected*
An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator brochure or is not listed at the specificity or severity that has been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

VI-B. Solicited Adverse Events

**Short-term**
The following short-term AEs and their associated intensities will be solicited:

- Fever
  - Mild 37.7-38.6°C
  - Moderate 38.7-39.3°C
  - Severe 39.4-40.5°C
  - Potentially Life Threatening >40.5°C

- Chills
  - Mild: No or minimal interference with usual social and functional activities
  - Moderate: Greater than minimal interference with usual social and functional activities
  - Severe: Inability to perform usual social and functional activities

- Fatigue/Malaise
  - Mild: No or minimal interference with usual social and functional activities
  - Moderate: Greater than minimal interference with usual social and functional activities
  - Severe: Inability to perform usual social and functional activities
  - Potentially Life Threatening: Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions

- Anorexia (loss of appetite)
  - Mild: Loss of appetite without decreased oral intake

- Moderate: Loss of appetite with decreased oral intake but without significant weight loss
- Severe: Loss of appetite with decreased oral intake associated with significant weight loss
- Potentially Life Threatening: Life-threatening consequences or aggressive intervention indicated (TPN or tube feeding)

- Abdominal pain
  - Mild: Pain causing no or minimal interference with usual social and functional activities
  - Moderate: Pain causing greater than minimal interference with usual social and functional activities
  - Severe: Pain causing inability to perform usual social and functional activities
  - Potentially Life Threatening: Disabling pain causing inability to perform basic self-care functions OR hospitalization (other than an emergency room visit) indicated

- Bloating
  - Mild: No or minimal interference with usual social and functional activities
  - Moderate: Greater than minimal interference with usual social and functional activities
  - Severe: Inability to perform usual social and functional activities

- Gas/Flatulance
  - Mild: No or minimal interference with usual social and functional activities
  - Moderate: Greater than minimal interference with usual social and functional activities
  - Severe: Inability to perform usual social and functional activities

- Constipation
  - Mild: Irregularity of BMs not requiring dietary modification, laxative, or enema
  - Moderate: Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas
  - Severe: Obstipation with manual evacuation indicated
  - Potentially Life Threatening: Life-threatening consequences (e.g. obstruction)

- Diarrhea
  - Mild: Transient or intermittent episodes of unformed stools OR increase of ≤3 stools over baseline per 24-hour period
  - Moderate: persistent episodes of unformed to watery stools OR increase of 4-6 stools over baseline per 24-hour period
  - Severe: Bloody diarrhea OR increase of ≥7 stools per 24-hour period OR IV fluid replacement indicated

- Potentially Life Threatening: Life-threatening consequences (e.g. hypotensive shock)

- Nausea
  - Mild: Transient (<24 hours) or intermittent nausea with no or minimal interference with oral intake
  - Moderate: Persistent nausea resulting in decreased oral intake for 24-48 hours
  - Severe: Persistent nausea resulting in minimal oral intake for >48 hours OR aggressive rehydration indicated (intravenous fluids)
  - Potentially Life Threatening: Life-threatening consequences (e.g. hypotensive shock)

- Vomiting
  - Mild: Transient or intermittent vomiting with no or minimal interference with oral intake
  - Moderate: Frequent episodes of vomiting with no or mild dehydration
  - Severe: Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated (intravenous fluids)
  - Potentially Life Threatening: Life-threatening consequences (e.g. hypotensive shock)

**Long-term**

The following long-term AEs and relevant details will be solicited:

- Gastrointestinal disorders
  - Inflammatory bowel disease
  - Irritable bowel syndrome
  - Celiac disease
  - Colon cancer
- Chronic infectious diseases (no therapy, outpatient, inpatient, ICU care)
- Autoimmune diseases
  - Inflammatory arthritis
  - Thyroid disease
  - Connective tissue diseases
  - Idiopathic thromobocytopenic purpura
- Metabolic disease
  - Diabetes Type 2 (diet controlled, oral hypoglycemic, insulin, uncontrolled/complication associated)
  - Metabolic syndrome (including obesity independently)
  - Non-alcoholic fatty liver disease
- Neuro-psychiatric disease
  - Multiple sclerosis
  - Depression/Anxiety
- Cardiovascular disease
  - Myocardial infarction (NSTEMI/STEMI)

- Stroke
- Cardiovascular related death

VI-C. Monitoring of Adverse Events

Each subject will be monitored for the occurrence of AEs, including SAEs. Each subject will be followed for safety monitoring at each follow-up interval.

- Subjects will be questioned at each follow-up interval regarding stool patterns, abdominal pain, fevers, and subjective well-being. First, subjects will be questioned about the possible occurrence of adverse events in a *generalized* way, such as, "How have you been feeling since your last visit?" Subsequent solicited questions and relevant details will be asked if there is a positive response. Site Investigators will be contacted by study representative if there is a suspected or reported AE
- Subjects having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the site Investigator
- AEs, actions taken as a result of AEs, and follow-up results will be recorded as well as in the subject's source documentation. Follow-up laboratory results should be filed with the subject's source documentation
- Subjects will receive a follow-up phone call 6 and 12 months post-FMT to record any SAEs, new medical conditions/new medications, or changes in pre-existing medical conditions

VI-D. Assessment of Adverse Events

*1. Assessment of Severity*

The severity of AEs will be assessed according to the following definitions:
•**Mild**: the AE is noticeable to the patient and/or the Investigator, but does not interfere with routine activity
•**Moderate**: the AE interferes with routine activity, but responds to symptomatic therapy or rest
•**Severe**: the AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy

*2. Assessment of Causality*

The Investigator must assess the relationship of any AE (including SAEs) to FMT, as *related* or *not related*, based on clinical judgment and using all available information, and may include consideration of the following factors:
• Possible alternative causes of the AE, including the disease under treatment, pre-existing conditions, concomitant use of other drugs, and presence of environmental or genetic factors
• The temporal association between FMT exposure and onset of the AE
• Whether the manifestations of the AE are consistent with known actions or theoretical toxicity of FMT

The causal relationship between FMT and the AE will be assessed using one of the following categories:

**Not Related:** An AE is not associated with FMT if:
• Temporal relationship is lacking (e.g., the event did not occur within a reasonable time frame following administration of FMT); or
• Other causative factors more likely explain the event (e.g. pre-existing condition, other concomitant treatments);
**Related:** An AE is attributed to FMT if:
• There is a positive temporal relationship (e.g. the event occurred within a reasonable time frame following FMT); and
• The AE is more likely explained by FMT than by another cause

VI-E. Reporting Safety Observations by the Investigator to the Sponsor

*1. Reporting of Non-serious AEs*

All AEs, regardless of seriousness, severity, or causal relationship to FMT, will be recorded in the AE section of the subject case report form.

*2. Reporting of FMT Exposure during Pregnancy*

If a female subject or the female partner of a male subject becomes pregnant during the course of study, the Investigator must report this occurrence to the Sponsor within **72 hours** of becoming aware of the pregnancy. The Investigator is required to follow up on the pregnancy until it has completed. The outcome of the pregnancy and the status of the newborn (if applicable) will be reported within 24 hours of becoming aware. If the female partner of a male subject becomes pregnant, the Investigator must obtain consent to collect pregnancy information (including the status of the newborn, if applicable).

*3. Reporting of Safety Observations by the Investigator*

Any occurrence of the following events or outcomes must be reported expeditiously by the Investigator or qualified designee to the local IRB and to the Sponsor, who will be responsible for reporting the event to the FDA:

1. SAE *related* to FMT material
2. Death of a subject *related* to FMT material

The site Investigator is to report any safety observations from the list above to the Sponsor within **72 hours** of becoming aware of the event, who in turn, will notify the FDA. Any observation that is also an AE will be recorded on the subjects case report form along with any actions taken. If not all information is available at the time of initial report, follow up SAE reports will be completed and submitted.

Any occurrence of the following events or outcomes must be reported by the Investigator or qualified designee to the local IRB and to the Sponsor, who will be responsible for reporting the event to the FDA:

1. SAE *unrelated* to FMT material
2. Death of subject *unrelated* to FMT material
3. Hospitalization related to CDI
4. New or worsening medical condition, or new medications

The Investigator is to report any safety observations from the list above to the Sponsor within **7 days** of becoming aware of the event. The Sponsor will notify the FDA in its *Annual Report*. Any observation that is also an AE will be recorded on the subjects case report form along with any actions taken. If not all information is available at the time of initial report, follow up SAE reports will be completed and submitted.

The site Investigator is required to follow SAEs until resolution, regardless of whether the subjects are still participating in the study. Resolution is defined as:

- Resolved with or without residual effects
- Return to baseline for a pre-existing condition
- Fatal outcome; if autopsy is performed, the autopsy report must be provided to the Sponsor.

VI-F. Monitoring the study database and submitting safety reports

The Sponsor will notify FDA of any unexpected fatal or life-threatening suspected adverse reaction *related* to FMT material as soon as possible, but no later than 7 calendar days after the Sponsor's initial receipt of the information. The Sponsor will notify the FDA and all participating Investigators in an IND safety report of potentially serious risks from this study as soon as possible, but no later than 15 calendar days after the Sponsor receives the safety information and determines that the information qualifies for reporting. Participating Investigators include all Investigators enrolling subjects under the Sponsor's IND. In addition, the Sponsor will identify in each IND safety report, all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction and will analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information. The Sponsor will evaluate a suspected adverse reaction in the context of other related reports or adverse events. The Sponsor will periodically review and analyze the entire safety database, for IND safety reporting purposes, and also to update Investigator brochures with new safety information. An IND safety report will be submitted when any of the following criteria are met:

**A. Serious and unexpected suspected adverse reaction related to FMT material**
A serious and unexpected adverse reaction is a SAE as determined by the Investigator or Sponsor, as defined previously, in combination with unexpected, defined as not being listed in the Investigator Brochure. At the request of the FDA, it will also include any documented infectious disease, defined as a new onset infection in the recipient after FMT and the infection present in the safety aliquot, which is a sample of the exact specimen that was transferred to the patient.

**B. Findings from other sources**
The Sponsor will also expeditiously report any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings that

suggest a significant risk in humans exposed to FMT and thought related to the material.

**C. Increased occurrence of serious suspected adverse reactions related to FMT material**
The Sponsor will report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol.

VI-G. Halting Rules

Specific safety findings will result in temporarily suspending enrollment until a safety review is convened, the objective of which is to determine whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment, for a particular group, such as colonoscopy group, or for the entire study, is another potential outcome of a safety review.

Subsequent review of serious, unexpected, and related AEs by the Data and Safety Monitoring Board (DSMB), IRB, the Sponsor, or the FDA or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of FMT at a site. The FDA and study Sponsor(s) retain the authority to suspend additional enrollment and study interventions/administration of FMT for the entire study, as applicable.

Findings that will trigger a safety review are

- Death related to FMT material
- Confirmed transmission of a clinically concerning infection from donor to subject via FMT
- If one or more subjects experiences a serious, unexpected adverse event related to FMT material
- Significant increase in frequency of events theoretically related to FMT material (specifically, new diagnoses of inflammatory bowel disease). Discussion on threshold will be discussed with DSMB and will be on a disease specific basis.

For individual subjects enrolled in the study: any subject who has an SAE related to FMT will be ineligible for a second FMT during the course of the study.

FDA/CBER will be notified by phone or fax within 48 hours if the study is halted for review.

## VII. Minimizing Risks to Human Subjects

VII-A. Human Subjects Involvement, Characteristics and Design

1. IRB approval for stool donor program has been obtained; Local site IRB approval from *each* study center will be obtained subsequent to required FDA approval of IND.

2. Subjects will be given informed consent using the standard consent process for colonoscopy/enema. Additional theoretical risk for transmission of infectious agents, and other diseases and conditions will also be discussed.
3. Donors will be given informed consent and will tested for most common infectious agents. Individuals with communicable disorders or a history of high-risk behaviors will not be permitted to donate.
4. Protections against risk are provided in detail below.

A sample size of 150 patients will be recruited. These patients will have been treated at or referred to one of the study centers for management of recurrent CDI.

Additional sites may be asked to join this study as needed to expeditiously achieve the targeted enrollment. The initial study sites will include:

1. Brown University and Women's Medicine Collaborative
Providence, Rhode Island

2. Indiana University Health University Hospital
Indianapolis, Indiana

3. University of Virginia Health Systems
Charlottesville, Virginia

4. Edward Hospital
Naperville, Illinois

This will be an open-label, longitudinal cohort study with follow-up at Day 3, Week 6, Month 6 and Month 12. The estimated duration of this study is 24 months. No patient will be excluded from recruitment on the basis of ethnicity, race, or disability. Subjects are expected to range in age from 18-75. Subjects will all be outpatients and will likely have comorbidities. All subjects will be fully informed about the purposes of the study, and risks and benefits of the study, and informed consent for all phases of the study will be obtained. Patients who agree to participate will be free to withdraw from the project at any time.

The intervention, FMT, will be administered directly through the endoscope at time of colonoscopy/sigmoidoscopy or by retention enema at the discretion of the MD. The material, donor stool [redacted], will be given as a single dose of 250mL. Lower gastrointestinal delivery was chosen based on a systematic review and meta-analysis that in subgroup analysis suggested lower gastrointestinal delivery was superior to upper delivery.[21]

We are collaborating with Investigators at the Massachusetts Institute of Technology (MIT) who will perform microbiome analysis on the stools collected from donors and subjects as part of this study. The samples can be mailed via conventional mail to OpenBiome who will locally transport them to MIT on dry ice after sample inspection and logging.

VII-B. Potential Risks to Subjects

There are 3 areas of risk to subjects associated with participation in the proposed study.

These include:

1) Physical risks related to the colonoscopy/sigmoidoscopy
2) Theoretical risks (infectious and otherwise) related to FMT and
3) Psychological or other risks related to confidentiality and loss of privacy.

Risks of ingestion of the colon preparation include the risk of dehydration and minor electrolyte imbalances. Standard colonoscopy/sigmoidoscopy risks include the risk of bowel perforation, bleeding, and adverse cardiopulmonary events related to sedation. The infusion of the liquid fecal matter will prolong the colonoscopy by less than 5 minutes and adds no additional risk to the colonoscopy. Many adverse effects of colonoscopy/ sigmoidoscopy resolve shortly after the procedure has been completed, but in some cases abdominal discomfort and gaseous side pain can persist for several hours. The use of retention enema is widely accepted to be safe but may include the risk of bowel perforation or bleeding.

There have been no confirmed infectious complications directly attributable to FMT reported in the literature to date aside from a speculation about a norovirus case although the donor was untested for this.[29] There have been two FMT cases of bacteremia, although the strength of the association is unclear, and both patients did well on antibiotic therapy. However, since the process involves infusion of one person's "body fluids" into another person, transmission of an infectious agent or other disease or condition remains a theoretical possibility. From infusion of donor microbiome, the patient/subject could potentially acquire risk factors for chronic diseases such as diabetes or inflammatory bowel disease. Risks will be minimized by a rigorous donor selection process and evaluative studies on stool and blood of the donor to exclude transmission of infectious agents prior to FMT. Subjects will be informed that the treatment or procedure may involve these theoretical risks and additional risks that are currently unforeseeable. There have been reported cases of inflammatory bowel disease flares post-FMT, but it is unclear if this was attributable, and these patients have been excluded from this study. Infusion of fecal material containing a potential agent to which the subject is allergic is also a concern and so patients with a history of severe (anaphylactic) food allergy will be excluded from this study.

VII-C. Potential Risks to Donors

This study also involves individuals (donors) recruited to donate stool for FMT. There are 3 areas of risk to the potential stool donor.

These include:

1) Physical risks related to laboratory testing and the stool collection protocol
2) Psychological risks related to both revealing and potentially learning sensitive information during donor screening process
3) Risks related to confidentiality and loss of privacy.

OpenBiome volunteer donors will be utilized. In order to exclude donors at high risk of passing infection, a Donor Risk Factor Questionnaire (*Appendix 2*) will be administered. This questionnaire contains sensitive and potentially embarrassing questions [redacted].
Additionally, prospective donors will be asked questions about their baseline health status and co-morbidities. Laboratory tests drawn as part of the screening process will include testing for HIV, viral hepatitis, and syphilis. These serologic results, if found to be abnormal, could cause psychological distress to the donor. However, the benefit of being made aware of a previously undiagnosed infectious condition outweighs this risk. Potential donors may experience psychological distress if they are excluded from donating stool based on these screenings.

Physical Risks to Donor:
Very Likely
• Blood drawing: pain, bruising, feeling faint, slight risk of infection

Nonphysical Risks to Donor:
Less Likely
• Embarrassment or psychological distress from answering questions [redacted] asked during the donor screening process.
• Psychological distress (guilt and embarrassment) if screening questionnaire or laboratory test results exclude the person from donation.

Unlikely
• There is also risk of compromising donor privacy through breach of data confidentiality of sensitive protected health information such as results of HIV or viral hepatitis testing.

VII-D. Protection against Risks to Subjects and Donors

*Recruitment and informed consent*

Participants will be recruited from study sites previously outlined. After a patient has been referred for recurrent CDI and determined to meet inclusion criteria, the clinician will introduce him/her to the research assistant, if applicable. The research staff or clinician will carefully explain all aspects of the study to a potential recruit, including the risks and benefits, and obtain participant's written informed consent.

- The research assistant or clinician will orally describe the material written in the informed consent document and answer any questions the participant may have.
- Participants will be reminded that they are not required to participate in the study and that they will receive the standard care provided by their physician

regardless of whether or not they choose to participate. Participants who give their consent will sign a copy of the document and will be given a signed copy of the informed consent document.

Recruits will be informed of the treatment commitment, amount and general types of assessments, the follow-up telephone interview procedures, and clinic visits. They will be given detailed descriptions of the colonoscopy or enema with FMT procedure. They will be informed of the requirements to submit stool specimens up to 3 days before, as well as 6 weeks, 6 months, and 12 months after the FMT procedure.

Potential donors who are healthy will be recruited to serve in a pool through an IRB-approved process. Each potential donor will then be contacted by the research staff and asked to participate in the donor consent process. Research staff will carefully explain all aspects of the study to a potential donor, including the risks and benefits, and obtain participants' written informed consent. Recruits will be informed of the commitment including a detailed description of the donor screening process and a stool donation protocol. The research staff will explain that a thorough clinical assessment and screening questionnaire will be completed. Volunteers will be informed that the questionnaire asks potentially sensitive questions [redacted]. They will be informed of the requirements to have laboratory blood work, including HIV testing, and stool studies and to submit stool specimens. The research assistant will orally describe the material written in the informed consent document and answer any questions the potential donor may have. Potential donors will be reminded that they are not required to participate in the study. Participants who give their consent will sign a copy of the document and will be given a signed copy of the informed consent document.

*Protection against risk*

Data and safety monitoring will take place to assure the safety of subjects. All participants will be reminded that their participation is voluntary and that they can withdraw at any time without penalty.

Additionally, the risks described above will be minimized by the following procedures:

1. We will minimize the risk of potential coercion by following standard procedures for obtaining informed consent from both subjects and stool donors. We will begin this process during the intake, where we will clarify the nature of the study and possible alternatives upfront. Prior to enrolling subjects in the research, we will fully explain the study procedures, risks, benefits, and alternatives, emphasizing that the subject's participation has no impact on the other services they receive. Also, subjects who do not consent or who withdraw during the study period will continue to receive appropriate treatment if needed. All subjects and donors will be reminded that there is no penalty for those who choose to not participate or to withdraw from the study and that their decision to participate does not impact the standard services they receive through the hospital. Subjects will not receive financial compensation for their participation. Volunteer donors will be paid a small stipend of $40 per stool donation (to cover their time & burden of screening/stool collection).

2. We will minimize potential risks due to loss of confidentiality by having all information collected and handled by research staff trained to deal appropriately with sensitive clinical issues. Potential donors will also be informed about the risk of being ineligible to donate stool due to positive results on screening questionnaires or laboratory testing. Results of donor medical interview, screening and laboratory testing will be kept separate from subject data and only deidentified data will be available to the subject or his/her most responsible physician. All study information will be treated as confidential and will be available only to research staff. Hardcopies will be kept in locked file cabinets, and computer data files will be encrypted and available only to authorized personnel – with no storage of names or obvious identifying information. No participant will be identified in any report of the project. Further, when contacting participants for follow-up, no identifying information other than the first name of the research assistant will be used when leaving messages or speaking to anyone other than the participant him/herself. Written consent will be obtained to contact other persons for the purpose of locating the participant for follow-up and participants can refuse or revoke such consents. No information about participants will be released without their permission or where required by law.

3. We will minimize the theoretical risks of infectious disease or other conditions possibly transmitted through FMT by using donor screening protocols modeled after blood banks and organ transplant programs. Potential volunteer donors will undergo thorough screening to determine eligibility for donation. The primary purpose of the donor examination and interview is to ensure that the donor is in good health, and to identify risk factors for diseases transmissible by stool. The donor interview will be used to identify risks for diseases and conditions for which there are no laboratory tests, for which tests are not sensitive enough to detect infectious disease agents, and for tests unable to identify early stage or window period infections. Potential donors will be interviewed using a Donor Risk Factor Questionnaire (*Appendix* 2). Health status of donors will be monitored prospectively, and donors will be rescreened every 60 days.

4. We will minimize the risk of severe CDI recurrence by maintaining close clinical contact with all subjects. Subjects will be encouraged to contact the clinical team if they experience recurrence of diarrhea, fever, or abdominal pain – so that stool can be tested for *C. difficile* toxin if indicated, and antibiotics can be resumed if necessary. Subjects will be contacted via telephone by a Clinical Research Coordinator on Day 3, Week 6, Month 6 and Month 12. A clinical assessment will take place between Week 6 +/- 2 weeks. If any patient experiences recurrent CDI via FMP250 or FMP250E they would be eligible for a second FMT by colonoscopy using a different donor.

## VII-E. Safety Monitoring Protocol

*Safety monitoring plan*

An external Data and Safety Monitoring Board (DSMB) will be assembled to evaluate the data and the safety of subjects enrolled in the study. The DSMB will consist of 3 physicians with specialization in gastroenterology and/or infectious disease. Initially, the

Board will convene with the Principal Investigator to review the study protocol and guidelines for data and safety monitoring. This initial review will include establishing standard procedures for monitoring. At each quarterly meeting the DSMB will evaluate recruitment, the progress of the study, subject retention, data quality and confidentiality. The DSMB will review subjects' clinical status, adverse events and whether or not there have been any changes in risk to participating subjects. This quarterly review will ensure that subject risk does not outweigh study benefits. A report generated from each of these meetings will be retained at the study sites and will be forwarded to the IRBs and to the FDA (in accordance with Investigational New Drug (IND) regulations). The DSMB will be available to convene outside of the appointed meeting schedule if necessary, due to concerns regarding a particular subject, or due to any adverse events during the study. The DSMB will make appropriate recommendations for changes in the study protocol, if needed.

The safety of participants will be monitored for the duration of the study at each contact. Both anticipated and unanticipated adverse events and problems will be formally monitored and recorded. Unanticipated serious adverse events *related* to FMT material will be reported to the hospital and IRBs (as per local reporting requirements) and the FDA (within 15 days; or 7 days for unexpected fatal or life threatening events *related* to FMT material). Anticipated and less serious adverse events, as previously outlined, will be submitted annually in reports to the IRBs and FDA. The Principal Investigators will be responsible for monitoring the safety and efficacy of this study, executing the DSMB plan, and complying with the reporting requirements. The DSMB report will include the participants' sociodemographic characteristics, any quality assurance or regulatory issues that occurred during the past year, a summary of adverse events and serious adverse events, and any actions or changes with respect to the protocol.

## VIII. Data Monitoring Plan

Data will be collected using standardized paper forms and will only be identified with the study's ID of the subject. The codes that link the name of the subject and the study ID will be kept confidential by the Site Investigator in a secured cabinet. Collected forms will be transported to the Site Investigator data entry center. Data will be entered in the REDCap computer database independently by trained data entry staff, and discrepancies will be corrected by the Site Investigator, based on source documents. Data quality will be monitored once per month by random inspection of the completed forms by a Clinical Research Coordinator; any problems detected will be discussed with the Principle Investigator. Telephone follow-up will be conducted by a Clinical Research Coordinator with data directly entered immediately into the REDCap computer database. Descriptive statistics will be computed periodically and reported in aggregate as part of the study monitoring process.

## IX. Educational Training

Researchers and clinician Investigators involved in the study will need to have successfully completed an institutional human subjects research program/module. The principle Investigator will maintain documentation for all study personnel. For sites utilizing enema delivery, a Clinical Research Coordinator will be responsible for overseeing a brief enema session with site study personnel.

## X. Potential Limitations

1. Correlational Safety Data
   - This open-label, longitudinal cohort will not have an untreated arm. Thus, it will be difficult to compare the probability of a newly acquired adverse event compared to a control. Given that the patient population with recurrent CDI tends to be older with core comorbidities, they are at high risk for new medical conditions, new medications, and poor outcomes, which may or may not be related to FMT. Thus, it is difficult to discern if there is an increased probability over baseline risk. This study has purposefully limited the age to 50 and designed exclusionary criteria (e.g. severe underlying disease that the patient is not expected to survive for the next 1 year) to reduce noise and strengthen any signal. Where possible age matched, prognostic factor matched population data will be considered to determine if FMT material may present a statistically significant increase in risk of developing a serious adverse event. However, this will not be the primary aim, and the focus will be on capturing count data/rates for serious adverse events.

2. Stool Collection
   - Patients will be collect his or her own stool samples and there may be potential error carrying out this procedure. Our study minimizes this risk with clear instructions, simplified process, clear labels, significant contact with study representatives, and reminder phone calls.

## XI. Investigator Credentials and Associated Body Contact Information


Principal Investigator: Zain Kassam MD, MPH
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Co-Investigator: Mark Smith PhD
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Co-Investigator: Eric Alm PhD
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Co-Investigator: Colleen Kelly MD
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|---|---|
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| Co-Investigator: | Rachel Ann Hays MD |
| Address: | 1215 Lee St, Charlottesville, VA, 22908 |
| Co-Investigator: | Darren Kastin MD |
| Address: | 1243 Rickert Dr, Naperville, IL, 60540 |
| IRB Name: | MIT IRB/COUHES |
| IRB Address: | 77 Massachusetts Ave, E25-143B, Cambridge, MA, 02139 |
| Principal Investigator CV: | Appendix 7 |

## XII. ClinicalTrials.gov Requirements

As required by law, this trial will be registered in ClinicalTrials.gov no later than 21 days after the first subject is enrolled. The reporting of summary results information (including adverse events) will be done no later than 1 year after the study completion date. The principle investigator will be responsible for registering the trial and results reporting.

7. CHEMISTRY, MANUFACTURING, AND CONTROLS INFORMATION

Refer to the OpenBiome Master File (BB-MF 15543) for Chemistry, Manufacturing, and Controls Information. An accompanying authorization letter from OpenBiome can be found in *Appendix 1*.

**Addendum: Stability of fecal microbiota during long-term frozen storage**

We are able to integrate evidence from human trials, animal models and laboratory studies to inform our understanding of the long-term stability of gut microbial communities during long-term cryopreservation. This evidence supports the long-term stability of fecal microbiota preparations, potentially for periods of up to several years. However, direct human experience at these extended time scales is still lacking and a test of potency will be helpful for continuing to evaluate the limits of viability in these communities. As a result, in addition to current evidence supporting the investigational use of microbiota preparations subject to long-term storage, here we present our plans for future work that we hope will further address this question.

*Evidence from studies in humans*

First, we have direct clinical experience using frozen fecal preparations for FMT with human subjects suffering from recurrent *C. difficile* infections. From this body of clinical experience, a few published reports in particular are worth highlighting. Hamilton et al provided the first published illustration of the clinical utility of frozen preparations.[28] In an open-label, non-randomized case series, they report that frozen material stored in glycerol for up to 56 days at -80°C was similarly efficacious for the treatment of recurrent CDI as freshly prepared material. Following up on this study, our group contributed to an open-label randomized trial comparing nasogastric with colonoscopic delivery using frozen material. This material was stored for up to 156 days at -80°C prior to successful use.[22]

*Evidence from in-vitro and animal studies*

In addition to this direct clinical experience using fecal microbiota for the treatment of recurrent CDI, we have a body of microbiological experience successfully culturing bacteria that have been cryopreserved for many years and in some cases decades.[29–31] For example, one group found less than 1 log-fold decrease in microbial viability after more than 4 years of storage at -70°C.[32]. Another group found that not only are cells viable after 2 years of storage at -80°C, they remain capable of causing infections in an animal model at similar rates to samples stored for shorter periods.[33] Standard microbiological practice and extensive laboratory experience supports the long-term viability of the gut microbiota during cryopreservation.

*Investigational plan*

Nonetheless, to supplement this data, we plan to conduct a small-scale clinical trial comparing the efficacy of material stored for either 6, 12 or 24 months at -80°C. We will supplement clinical experience, the most relevant indicator of viability in this case, with a differential fluorescent staining assay (Live/Dead, Life Technologies) to detect the relative ratio of intact versus ruptured cells in each sample. We have quarantined 60 samples for long-term storage and have saved additional aliquots from each sample that we can interrogate with our live-dead assay at 3-month intervals. By comparing measured viability with clinical outcomes at 6, 12 and 24 months, it will be possible to both establish the long-term stability of microbiota preparations stored under these conditions and to establish a surrogate marker of viability that does not require clinical evaluation. We hope that such a validated tool could facilitate the standardization of best practices for storing and processing of FMT.

To control for the extreme intra-subject and inter-subject variability that characterizes the gut microbiome, we will use treatments collected not only from the same donor, but from the same bowel movement for each treatment arm. For example, a single bowel movement that produces sufficient material for 3 treatments will have one unit included in the 6-month cohort, one in the 12-month cohort and one in the 24-month cohort. The average efficacy of material derived from each cohort will be compared to determine whether there are meaningful differences between the groups. This study will be powered to detect a difference of more than 25% between groups ($\alpha$ = 0.05 $\beta$= 0.2). Due to the need to store samples for an extended period of time prior to clinical work, we do not endeavor to include a formal proposal for this investigation at this time.

Nonetheless, given the time delay required, we felt it would be useful to include our plans in this application to solicit comments now. These comments can be used to inform the design of this study while it is still feasible to make modifications that will not substantially delay the investigation.

8. PHARMACOLOGY AND TOXICOLOGY INFORMATION

FMT grew from empirical clinical practice instead of a conventional drug development program. Accordingly, salient non-clinical data is not particularly informative, particularly in context that the human microbiome has co-evolved with its host since birth.

9. PREVIOUS HUMAN EXPERIENCE WITH THE INVESTIGATIONAL DRUG

## I. Indication

Recurrent *Clostridium difficile* infection

## II. Background of Disease and Rationale

*Background*

*Clostridium difficile* infection (CDI) has recently been reported as the most common healthcare-associated infection in the United States, and the emergence of community-acquired CDI is particularly concerning.[1 2] CDI is associated with a spectrum of symptoms ranging from diarrhea to abdominal pain, and severe infections may lead to toxic megacolon and death. The clinical burden of CDI is significant. In the United States, the incidence of CDI per 10,000 inpatient hospitalizations doubled between 2000 and 2005 (5.5 *versus* 11.2 cases).[3] A common management challenge is recurrent CDI, which occurs in 20% of patients after standard therapy. Patients with one recurrence have a 40% risk of further relapse and for those with two or more episodes of recurrence, the risk of subsequent relapse has been reported to exceed 60% with antibiotic treatment.[12 15 16] Current standard therapies for multiple recurrent CDI are limited and many patients become dependent on vancomycin, as they develop CDI relapse when this antibiotic is discontinued.[17] Counterproductively, ongoing antibiotic therapy is thought to continue perturbation of the microbial community and to establish an optimal environment for toxigenic *Clostridium difficile*.[18 19] Additionally, continued treatment may contribute to antibiotic-resistant *Clostridium difficile,* especially as the vanB gene, which confers vancomycin resistance, has been isolated in Clostridia.[7–10 34]

*Rationale*

The treatment options for recurrent CDI are limited. Clinical guidelines by the American College of Gastroenterology conditionally recommend, based on low-quality evidence, that first CDI recurrence is managed with metronidazole or vancomycin, and

second recurrence treated with a pulsed vancomycin regimen.[17] FMT is conditionally recommended for management of a third CDI recurrence; the recommendation is provisional in part because further clinical trials are ongoing and there is a lack of standardized longitudinal follow-up in patients post-FMT.[17] The rationale for this multi-center, open-label, longitudinal cohort study is to improve certainty about the safety profile of FMT, and complement the established literature and on-going studies of the efficacy of FMT. The current body of literature suggests that the risks of FMT are low, and are linked to complications associated with delivery such as colonoscopy/sigmoidoscopy or to theoretical infectious and non-infectious risks. Given the established benefits of FMT and the paucity of high-quality safety data, the potential benefits of this study outweigh the risks of using this investigational product.

## III. Summary of Previous Human Safety Experience

III-A Observational Data

A robust systematic review and meta-analysis of 11 observational studies with a total of 273 CDI patients did not report any adverse events attributable to the FMT material in variable follow-up from 3 weeks to 8 years.[21] However, 3 studies that used upper gastrointestinal FMT indicated that the nasogastric/nasojejunal tube itself could not be ruled out in contributing to a suspected adverse event: upper gastrointestinal bleed, peritonitis, and possibly enteritis.[21]

A multi-center retrospective review (n=77) reported no definite FMT adverse events in follow-up between 3-68 months (mean 17 months). The authors did report 4 patients developing diseases of potential interest after FMT (peripheral neuropathy, Sjogren's disease, idiopathic thromobocytopenic purpura, rheumatoid arthritis) and 7 non-FMT related deaths. Interestingly, the study noted post-FMT improvement in a patient with pre-existing allergic sinusitis and one with "arthritis", with the type unreported.[24]

Safety outcomes for FMT treatment of CDI have also been reported in immunocompromised patients in a multi-center retrospective review (n=80).[25] In this special group, 12 patients (15%) had a serious adverse event within 12 weeks of FMT, of which 10 were hospitalized. Specifically, 2 deaths occurred within 12 weeks, one a result of aspiration at the time of colonoscopy for FMT, and the other unrelated to FMT (progressive pneumonia). There were 3 deaths reported 6 months after FMT due to chronic, progressive medical conditions unrelated to FMT. There was another procedure-related complication as a subject sustained a superficial mucosal tear during colonoscopy. Importantly, no patients experienced an infection definitively ascribed to FMT, although 2 subjects sustained unrelated infections (influenza, catheter line infection). Self-limited diarrheal illness occurred in 5 patients but no causal organism was identified.[25]

III-B Randomized Clinical Trial Data

In a small randomized controlled trial (n = 42) for recurrent CDI assessing FMT via duodenal infusion compared to vancomycin and bowel lavage, or vancomycin alone, adverse event were minor and short-lived.[23] In the FMT arm (n=16), minor gastrointestinal symptoms occurred on the day of infusion including belching (n=3), nausea (n=1), abdominal cramps (n=5), and abdominal pain (n=2); however, these symptoms resolved in all patients within 3 hours. In follow-up, constipation occurred in three patients. There were no adverse events attributed to FMT at 10-week follow-up.[23]

In another small, open-label, randomized controlled trial (n=20) for recurrent CDI comparing FMT by nasogastric tube versus colonoscopy, no adverse or unexpected events were documented in the 6-month follow-up period.[22]

III-C Investigator Preliminary Data

OpenBiome is a 501(c)(3) non-profit organization that is the first public FMT stool bank in the United States for *C. difficile* infection non-responsive to standard therapy. In compliance with the current FDA regulatory framework, we have partnered with more than 100 health care institutions in over 32 states to expand safe, affordable access to FMT.[34] OpenBiome has sent out over 1000 treatments and has had limited adverse events.

To date, there have been two anecdotal cases of transient bacteremia potentially attributable to FMT, which was successfully treated with antibiotics. One case involved a 52 year old man with Hepatitis C and alcoholic liver cirrhosis admitted for pneumococcal bacteremia, that subsequently developed severe, complicated CDI including ileus that was non responsive to standard therapy. The patient underwent a FMT via nasoduodenal tube, and although diarrhea improved, 4 days after FMT was admitted to the intensive care for septic shock. Blood cultures grew 2 strains of pan-susceptible *E. coli*, and peritoneal fluid also contained *E. coli*. The patient was successfully treated with conventional sepsis therapy and improved.

III-D Overall Summary Table

**Table 1: Efficacy and safety outcomes for FMT in CDI**

| Author & Year | Study size | CDI Type | FMT route & type* | Efficacy outcomes after up to 2 FMT** | Safety outcomes*** |
|---|---|---|---|---|---|
| **Clinical Trials** | | | | | |
| Youngster et al. 2014[22] | 20 | rCDI | CsC or NGT (frozen) | 90% (1-2x)<br>70% (after 1x only)<br>100% (3 children) | AE: abdominal discomfort, bloating in 20% of patients. |
| Van Nood et al. 2013[23] | 16 (FMT arm) | rCDI | NDT | 94% (1-2x)<br>81% (after 1x only)<br>Non-FMT arms stopped early for benefit. | AE: constipation (19%), unrelated urinary tract infection (6%) and culture-negative fever during hemodialysis (6%). |
| **Observational Data** | | | | | |
| Lund-Tonnesen et al. 1998[35] | 18 | mix | CsC, G-tube (frozen) | 83%(1x) | No "complications during or after" FMT. |
| Hamilton et al. 2012[28] | 43 | rCDI and IBD | CsC (fresh or frozen) | 95%(1-2x)<br>Patients who also had IBD: statistically same. | AE: irregular BM, more flatulence in ~33% of patients for ≤2 weeks. |
| Mattila et al. 2012[36] | 70 | mix | CsC | 94% (1x) | No related AEs. |
| Kelly et al. 2012[25] | 26 | rCDI | CsC | 92% (1x) | AE: loose or irregular BM in 11.5% of patients; mild & temporary effect. |
| Mellow et al. 2011[37] | 13 | mix | CsC | 92% (1x) | No comment made.<br>Note: 1 CDI recurrence at 7 months. |
| Garborg et al. 2010[38] | 40 | rCDI | CsC, gastroscopy | 90% (1-2x) | No comment made about responder AEs. |
| Rohlke et al. 2010[39] | 19 | rCDI | CsC | 100% (1-2x) | No AEs. |
| Yoon et al. 2010[40] | 12 | mix | CsC | 100% (1x) | No AEs. |
| Kassam et al. 2012[41] | 27 | mix | Enema | 93% (1-2x) | No AEs. |
| Silverman et al. 2010[42] | 7 | rCDI | Enema | 100% (1x) | One patient developed IBS symptoms. |
| Paterson et al. 1994[43] | 7 | mix | "rectal tube" | 100% (multiple infusions) | No comment made regarding AEs. |
| Kelly et al. 2014[25] | 80 | mix and | Mostly lower GI; | 89% (1-2x, IC), incl. 5 pediatric patients | SAEs: 1 procedure-related, 1 severe |

| | | | | | |
|---|---|---|---|---|---|
| | | also IC | varied | 94% (1-2x, sub-group with concurrent IBD) | abdominal pain, 4 IBD flares. See discussion. AEs: 4 related, 5 possibly related. See discussion. |
| Polak et al. 2011[44] | 15 | rCDI | NJT | 87% (1-2x) | SAEs: 1 procedure-related. No comment on other AEs. |
| MacConnachie et al. 2009[45] | 15 | rCDI | NGT | 73% (1-2x) | No material-related AEs. One upper GI hemorrhage, may be procedure-related. |
| Aas et al. 2003[46] | 18 | rCDI | NGT | 83%(1x) | SAEs related to procedure or co-morbidities only: peritonitis, pneumonia. |

**Notes:**
* FMT product is fresh as opposed to frozen unless stated otherwise.
**Note that follow-up time for examining efficacy was variable. Most studies continued for at least 1.5-2 months, and some for years, but at least one continued for only 2-3 weeks.
*** This column focuses on responder AEs. Note that patients who were unresponsive to FMT treatment died of rCDI or other non-FMT-related causes in several studies.

**Abbreviations:**
CDI form: rCDI = recurrent Clostridium difficile infection; mix = mix of recurrent (repeated), refractory (not responding to treatment), and severe (e.g., hospitalization) CDI.
CDI form and also Efficacy: IBD = inflammatory bowel disease; IC = immunocompromised.
FMT route: CsC = colonoscopy; G-tube = gastrotomy tube; NGT = nasogastric tube; NDT = nasoduodenal tube; NJT = nasojejunal tube.
Safety: SAE = serious adverse event; BM = bowel movement; IBS = irritable bowel syndrome.

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