PROTOCOL

A Phase I/II, double blinded, placebo controlled, single-center Study of Fecal Microbiota Transplant (FMT) for the Treatment of Active Pediatric Ulcerative Colitis

Protocol version: 11MAY2018

Stacy Kahn, MD
Boston Children's Hospital – Division of Gastroenterology & Nutrition
300 Longwood Ave. – Hunnewell 103
Boston, MA 02115
617-355-6058

CONFIDENTIAL

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Background

The history of fecal microbiota therapy and its success

Fecal microbiota transplantation (FMT) has been employed in the treatment of gastrointestinal disorders in humans since 1958, when it was first described by Eiseman to treat fulminant pseudomembranous enterocolitis.(1) Over the last several years, investigators have reported their experiences on over 300 patients who have received fecal microbiota therapy for treatment of *Clostridium Difficile* infection (CDI). (2) This 2012 meta-analysis reviewed over 27 studies using FMT for the treatment of refractory and often fulminant CDI associated colitis with an overall success rate of 92%. A more recent study on the treatment of CDI published in the *New England Journal of Medicine* was halted prematurely due to the overwhelming success of patients randomized to FMT for CDI as compared to standard antibiotic therapy. (3) Interestingly, Hamilton and colleagues have recently demonstrated that a frozen inoculum from a "universal screened donor" is equal in efficacy for CDI to a "fresh" donation from such an individual (90% vs. 92%; not significantly different). (4,5) They also note that the frozen approach allows for rigorous donor screening in a cost effective manner. Taking into account its well-documented clinical efficacy, apparent cost savings and safety, FMT for CDI, despite its unconventional nature, is now considered **standard-of-care therapy**. Research has recently shown oral frozen inoculum is non-inferior to colonoscopically delivered frozen donor FMT in treating recurrent CDI (6), thereby reducing substantial risks and costs associated with colonoscopic delivery of FMT.

The use of fecal microbiota therapy in inflammatory bowel disease (IBD), a natural next-step

IBD is a difficult to treat, chronic inflammatory disease, currently without a cure. IBD often requires life-long immune suppression and, at times, surgical intervention to keep under control. Even our most robust medical therapies are expensive, lack universal clinical efficacy, and are rife with significant risk of infection, malignancy and drug reactions. Given the strong microbial influence in the proposed etiopathogenesis of IBD, FMT has been used for the treatment of IBD in both children and adults for over a decade. Borody and colleagues pioneered the use of FMT in IBD as published in their initial case series in 2003, including six patients all of whom achieved a clinical remission following FMT. (7) A more recent retrospective meta-analysis reviewed 17 articles including 41 patients with IBD receiving FMT, many of whom also had CDI. (8) In patients treated for their IBD, the majority experienced a reduction of symptoms (19/25), cessation of IBD medications (13/17) and disease remission (15/24). While suggesting potential promise of FMT for the treatment of IBD, these heterogeneous retrospective reports are difficult to interpret.

Early evidence supports multiple FMT's

Early data suggests single administration of FMT may be inferior to multiple doses of FMT given over time. Vermeire prospectively delivered FMT by naso-jejunal tube over two days in 4 adult patients who suffered from refractory CD. While they reported no long-term

adverse effects, this group saw only temporary clinical response and microbial change in the gut ecosystem of these patients. (9) Kump found similar tolerability and safety in treating 6 Ulcerative colitis (UC) patients with a single FMT delivered colonoscopically but only temporary clinical improvement in some of the underlying IBD activity (11). Similarly, he reported no long-term engraftment of donor microbiota on recipient. Research has showed no apparent adverse events in 3 pediatric patients with IBD (2 of whom had CD) treated by single FMT all delivered colonoscopically for the primary indication of recurrent CDI but no improvement in underlying IBD clinical status. (12)

Multiple FMT administration seems to show more promising results. An adult case report by Kao showed complete resolution of severe Crohn's colitis after 2 colonoscopically delivered FMT's separated by 1 month with significant alterations/engraftment in the patient's gut microbial profile after FMT. (13) Similarly, Vandenplas reported improvement and eventually complete remission of infantile severe IBD activity with multiple FMTs from 2 separate donors over time. (14) Kunde and colleagues recently published a larger prospective safety and tolerability study of daily FMT enemas for 5 days in 10 children with IBD showing it was safe and well tolerated. (9) Seven of nine (78%) of subjects showed clinical response within 1 week, six out of the nine (67%) of subjects maintained clinical response at 1 month, and three out of the nine (33%) subjects achieved clinical remission at 1 week after FMT. In a large double-blind placebo controlled trial, Moayeddi suggests that 16/27 (60%) patients had subjective improvement of underlying UC activity after 6 weekly FMT therapies delivered by fecal retention enema. One third of these patients who showed initial subjective clinical response to an initial course of FMT eventually went into remission if FMT administration was maintained for a longer period of time. (15)

Best Mode of Delivery

There remains much uncertainty about the "best" mode of delivery of FMT. The most promising, prospective research using FMT for IBD to date utilized FMT delivery by retention enema (Moayeddi and Kunde, in adult and pediatric patients, respectively). Daily enema therapy for IBD colitis is routine in clinical standard practice for delivery of topical steroid and aminosalicylate therapy. Indeed, even in fragile, very sick adult populations with multiple co-morbidities and recurrent C. diff infection, where FMT is life-saving but any procedure is dangerous, FMT by retention enema is considered the safest and least invasive FMT delivery method available with minimal to no procedurally associated risk. Therefore, enema or colonoscopy represents the "traditional" method by which to perform FMT.

In the pediatric population, we are considerate of the invasive nature of enema therapy and the potential associated risks of anxiety and discomfort. An oral delivery would be an attractive, low risk and non-invasive alternative method of delivery for FMT therapy in this patient population. Based on our recent work in treating recurrent CDI successfully with oral inoculum by nasogastric tube delivery (16) and also by encapsulated oral inocula (work completed under FDA IND 15-199), the oral route of FMT seems to be non-inferior and obviates the need for persistent invasive delivery method. Vandenplas' case report showing ultimate remission of infantile IBD after 7 FMT (6 by an upper gastrointestinal delivery

route) provides "proof-of-concept" that this method of FMT delivery in a pediatric population is feasible, and potentially helpful in the treatment of IBD.

No studies to our knowledge have been done on FMT for pediatric IBD using frozen inocula collected from a universal donor. One might argue that given the known similarities of one's microbiome to their parents, a universal donor is more likely to introduce a more desirable commensal inoculum. (17,18) Moreover, given the frequency of IBD and other autoimmune conditions in first-degree relatives, it is common that a suitable healthy donor cannot be identified and an unrelated donor is required.

To best reflect and duplicate previous successes documented in the literature, we envision a hybrid FMT delivery method, using both FMT enema induction and an innovative oral encapsulated inoculum thereafter. We believe initial engraftment with donor stool is of paramount importance for eventual success of FMT therapy.

Fecal Microbiota Transplant (FMT) as a therapy for Ulcerative Colitis

Ulcerative Colitis (UC), a chronic inflammatory bowel disease (IBD) of the colon has similar strong microbial drivers of inflammation to CDI. FMT has been studied as a therapy for IBD in a number of small case series over the last three decades. (7,9,19) We propose that FMT would be a safe and well-tolerated therapy for pediatric UC. We further hypothesize that subjects will demonstrate subjective measures of clinical improvement (i.e., in the Pediatric Ulcerative Colitis Activity Index), in patient related outcome variables such as abdominal pain and number of daily bowel movements, as well as in objective inflammatory biomarkers.

1.2. Rationale

Profiling studies of the intestinal microbiome have associated the pathogenesis of IBD to characteristic shifts of the intestinal microbiota, reinforcing the view that IBD results from altered interactions between intestinal gut microbiota and the mucosal immune system. It is known that the microbial community in IBD patients is less diverse; enriched in Gammaproteobacteria and invasive E. coli species; and deficient in Faecalbacterium prauznitzii, certain Clostridia, Bacteroidetes, and Firmicutes. Metabolically, there is increased oxidative stress in these individuals, decreased presence of short chain fatty acids and metabolites of short chain fatty acids, increased auxotrophy, sulfate transport, amino acid transport, and toxin secretion. (20)

Recent pediatric work in multiple immunomodulator-refractory acute severe colitis has shown promising results in treating ill IBD children successfully with aggressive antibiotic regimens. Seven of 15 (47%) of this retrospective cohort of patients achieved complete remission with only alteration of their microbial community via aggressive multiple antibiotic regimens. (21)

It is entirely reasonable to assume that repopulating the gut habitat with healthy bacteria may alter disease activity course and progression. It is also entirely reasonable to assume

depleting gut flora with broad-spectrum antibiotic would be beneficial in setting the stage for appropriate transplant engraftment.

2. OBJECTIVES

2.1. Primary Objective

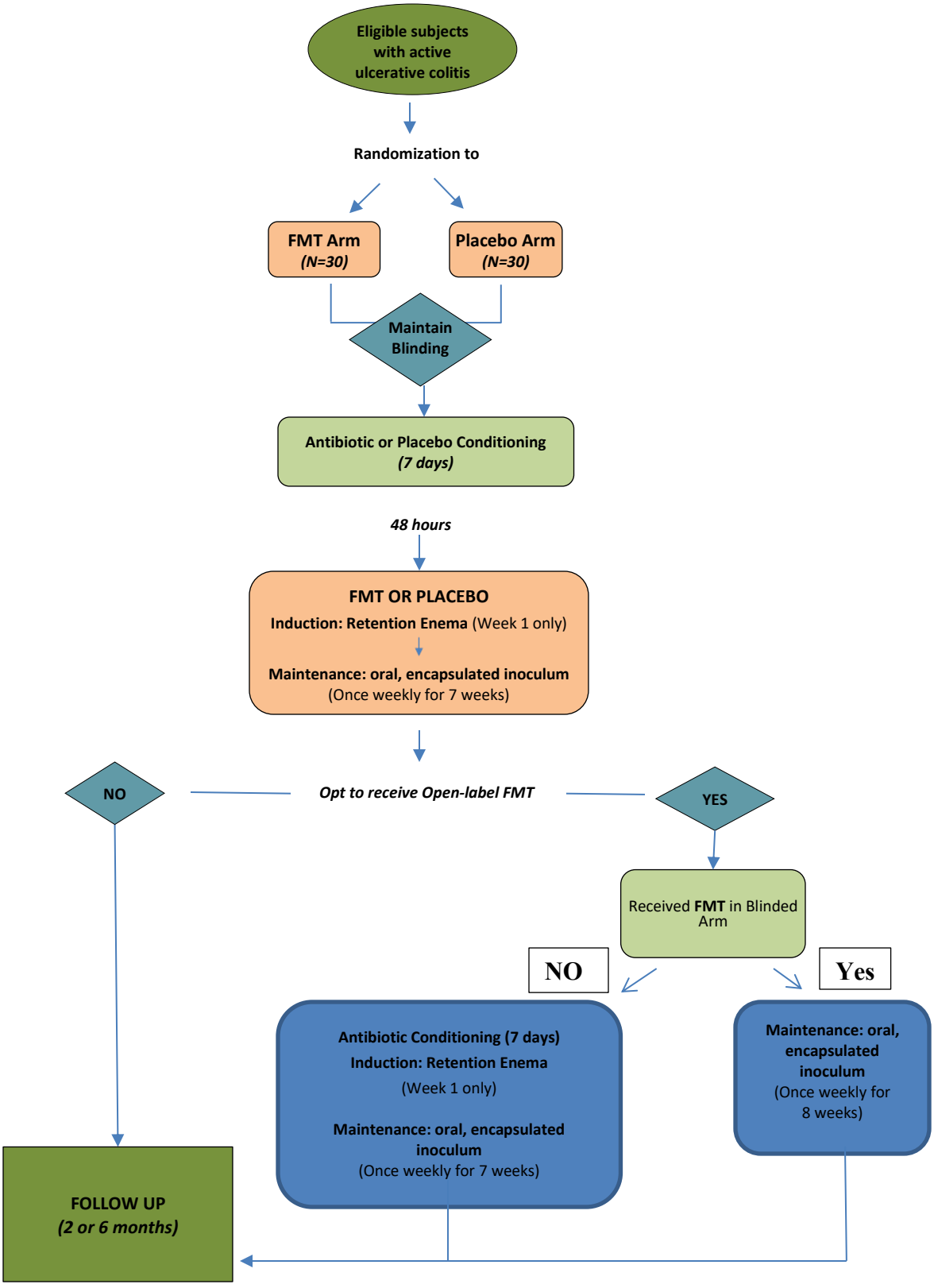
The primary aim of this phase I/II, randomized, placebo controlled study is the assessment of safety and tolerability of universal donor FMT compared to placebo in pediatric and young adult subjects (ages 5 years through 30 years) with Ulcerative Colitis, who have failed, are intolerant to, or have refused first-line maintenance therapy.

2.2. Secondary Objectives

1. Identify biomarkers in both donor and recipient that may confer a clinical response.
2. Establish whether or not ongoing FMT maintenance therapy is required for maintenance of clinical benefit in pediatric UC.

3. STUDY DESIGN

3.1. Study Schema



Description

This is a single-center, phase I/II, randomized, prospective, double-blinded, placebo-controlled study of FMT in the treatment of active pediatric UC. The primary aim is to assess safety and feasibility of a weekly FMT maintenance therapy. A total of 60 patients with active UC (as defined by PUCAI score of > 9) will be enrolled and randomized to first receive FMT or placebo (study treatment).

For therapy/placebo induction, a retention enema will be administered according to the treatment arm to which the patient is assigned. FMT by retention enema will afford us the best delivery method equivalent to that previously described by Moayyeddi and Kunde (see Background) for engraftment of donor stool using FMT materials available to us. Subsequent administrations will occur as oral, frozen encapsulated inocula/placebo for 7 weeks. After the first 8 weeks, subjects will have the option to continue on study treatment or switch to open-label FMT. Subjects will be followed by telephone to assess adverse events for a total of 6 months after their last FMT dose.

Patient metadata and stool samples will be collected at key time points. The patient-reported metadata collection technique will allow for numerous clinical correlations to be parsed out using the random forest machine learning capabilities of synthetic learning in microbial ecology (SLiME) to identify taxonomic features associated with important clinical parameters.(22)

An initial subset of 10 subjects will be enrolled in the study (will be limited to only those patients 12 years of age or older and to those who have mild to moderate ulcerative colitis) and randomized to receive FMT or placebo. We'd expect short term adverse events to occur within 7 days of FMT administration. Individual subject safety data will be reviewed by the PI to assess whether FMT appears to be safe in the subject before continuing the subject towards open-label use of FMT.

3.2. Study Population

The study will enroll 60 eligible patients with active UC (as defined by PUCAI score of >9). Patients from the Gastroenterology clinic at Boston Children's Hospital and outside patients referred or self-referring to the clinic will be considered for participation in the study.

3.2.1. Inclusion Criteria

Male and female children and young adults, aged 5 years to 30 years, who meet the following inclusion criteria, will be enrolled in the study.

An initial subset of 10 subjects will be limited to patients with mild to moderate ulcerative colitis (i.e., PUCAI < 65) and to individuals ≥ 12 years of age. If FMT appears to be safe in this subset of patients after 8 weeks in the study (to be assessed by a Data Safety Monitoring Board), expanded enrollment as is described above will occur.

All patients must satisfy below criteria:

1. Have UC (PUCAI >9) and have failed, are intolerant to, or have refused first-line maintenance therapy.
2. Have had visual or histologic evidence of inflammation confirmed through colonoscopy ideally within 90 days but no more than 105 days prior to randomization.
3. Have negative test results for Hepatitis B (HBV), Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV).
4. Have a negative urine hCG test if female of childbearing potential.
5. Able to swallow antibiotic, FMT or placebo capsules.
6. Able to give informed consent and/or assent as appropriate (patients 12-17 will be asked to provide written assent, patients 5-11 will be observed for assent or dissent behaviorally, or with verbal/written communication)
7. Willing and able to participate in the study requirements, including serial stool collection, survey completion and clinic visits.
8. Willing to undergo telephone follow-up to assess for safety and adverse events.
9. Must be free of any known food allergy.
10. Agrees and willing to have an enema for purposes of induction therapy.

Patients who have disease that has required other medications (including steroids, immunosuppressives, and biologics) will be included.

3.2.2. Exclusion Criteria

Subjects who fall into any of the following exclusion criteria at the time of screening are not eligible for enrollment into the study.

1. Patients in a clinical remission (PUCAI $\leq$ 9).
2. Patients with recent (within 4 weeks) dose change of biologics, 5-ASA, steroids or immunomodulators
3. Patients considered to have toxic megacolon.
4. Patients with a known drug allergy to vancomycin, metronidazole or polymyxin.
5. Patients with a history of aspiration, gastroparesis, surgery involving the upper gastrointestinal tract (that might affect upper gastrointestinal motility) or unable to swallow pills.
6. Patients with esophageal dysmotility or swallowing dysfunction.
7. Patients with known food allergies.
8. Patients with positive test results for HBV, HCV, or HIV.
9. Female patients with a positive test result on a urine hCG test.
10. Patients unwilling or unable to give consent or participate in all study requirements.
11. Patients unable or unwilling to receive a retention enema for purposes of induction therapy.

12. Patients with recent (within 6 weeks) systemic antibiotic use.
13. Patients who have testing consistent with active clostridium difficile.
14. Patients with known prior experience with donor FMT.

Research personnel and care providers will be educated about inclusion/exclusion criteria so that only appropriate patients are approached for informed consent. This will reduce the burden of worry for families and patients as to whether they are suitable candidates for the study. Subjects who are found to have no evidence of active disease by colonoscopy will be excluded from the study.

3.2.3. Subject withdrawal criteria

All subjects are free to withdraw from participation in the study at any time, for any reason, specified or unspecified. A subject may be withdrawn from the study under any of the following circumstances:

- Withdrawal of informed consent or assent when applicable.
- If, in the opinion of the investigator, IRB or DSMB, it is no longer in the subject's best interest to continue in the study.
- The subject no longer meets the inclusion criteria or meets any of the exclusion criteria.
- Lack of compliance with study procedures or study treatment, as determined by the investigator.

3.2.4 Screen failure

A subject who consented to participate in the study but who was disqualified during screening procedures and *never began Antibiotic/Placebo conditioning* will enter Passive follow-up, which means the subject may reach out to the study team for a second opinion for follow-up care or any other medically related reason. These subjects will not be included in the per protocol analysis.

4. STUDY TREATMENT

4.1. Investigational Agent

4.1.1. Fecal Microbiota Transplant (FMT) and placebo

The investigational agent consists of screened-donor inoculum of a biologically active human substance (FMT). FMT will be administered using either frozen screened-donor liquid stool (the investigational agent) in the form of retention enema (60 mL total for ages 5-10 and up to 120 mL total for ages greater than 10) or frozen capsules. Placebo will be administered using either placebo liquid or placebo capsules (both consisting of cocoa butter, glycerol, and brown food coloring). Donor material and placebo material will be obtained from Microbiome Health Research Institute (OpenBiome), a stool bank affiliated with the MIT-Broad Institute. All donors will be thoroughly screened for health and infectious status. (Cross ref. **BB-MF 15543**).

If randomized to FMT Arm, subjects will be matched with a specific stool donor and will then receive FMT induction enemas and FMT capsules containing this stool for the 8 week randomized portion of the trial. From each donor, sufficient stool to complete the study will be secured by OpenBiome and stored at their facility under appropriate storage conditions. The 8 week supply of the donor's inoculum will be maintained on-site at BCH and stored in accordance with supplier specifications and investigational agent guidelines. If subjects are eligible for open-label FMT and have previously been randomized to FMT, every effort will be made to use the same donor if possible. It is conceivable that enough stool to continue through open-label FMT treatment period(donors move or may become ineligible for stool donation) will not be practicably available and another appropriate donor may be selected for open-label FMT use if necessary.

4.1.2. Storage and Accountability

The Boston Children's Hospital (BCH) research pharmacy will be responsible for the receipt and accountability of study agent. The pharmacy will maintain accountability logs with quantities received and dispensed, specifying the corresponding dates, and the identification code of the study subject.

Bottles (containing the FMT and placebo enema preparations) and FMT and placebo capsules will be delivered in temperature controlled packaging on dry ice with a time-temperature indicator. The bottles and capsules will be stored at -80°C in an access controlled freezer in accordance with the manufacturer's and BCH Infection Control specifications until dispensed for administration in a clinical research unit (CTSU).

In order to maintain the integrity of the FMT inoculum during storage, the study team will use temperature-controlled, limited access freezers. Each freezer is equipped with a temperature monitoring system that alerts members of the lab and study team if the freezer fails (e.g. power failure or abnormal temperature fluctuations). In the event that a failure does occur and the study drug becomes unusable, the affected items will be voided and discarded.

4.1.3. Administration and Dispensing

The first FMT or placebo dose will be administered as an enema 48 hours after completing antibiotic therapy (Week 1, Day 1). An un-blinded, independent pharmacy technologist will prepare the retention enemas or placebo which will be provided by OpenBiome. An un-blinded research study nurse will then dispense FMT or placebo (study treatment) retention enema according to the treatment arm to which the patient is assigned. The enema containers will be fully color-tinted from the tip to the bottom of the container. The container will be placed inside a paper bag, to prevent any odor detection. Both the patient and the study nurse will be required to wear a tightly fitted mask at all times during the infusion and retention of the enema.

Subjects will be put in left lateral decubitus position with elevated hips in a designated private study visit exam room for 60 minutes for FMT induction (or placebo) by retention enema. Each subject will receive FMT (or placebo) as either one or two retention enema(s) over 15-30 minutes. Each 60 mL enema will be infused over 5 minutes. Subjects will be asked to rotate 180° (from left lateral to right lateral position and then return to left lateral position) slowly over a 10 minute period. While 120 mL of fecal solution (or placebo) will be prepared for induction retention enema for patients over the age of 10, the final administered dose will be dependent on subject's comfort and willingness to proceed with the next enema which will be assessed after the first 60 mL enema infusion. Subjects will be monitored by a blinded study nurse for 60 minutes after enema administration for any immediate adverse events and discharged.

For the next 7 weeks of study treatment, an unblinded research pharmacist will dispense FMT (study treatment) or placebo capsules to be administered under observation in the CTSU. 15 capsules of study treatment (the equivalent of 7.5 grams of human stool) will be administered within 90 minutes of thawing once weekly for a total of 7 weeks. After the initial 8 weeks, participants who responded to FMT during the blinded portion of the study or participants who did not respond to placebo will have the option to continue treatment with weekly open-label FMT.

4.1.4. Blinding and Randomization

The first ten eligible subjects will be randomized in a 1:1 ratio according to a pre-determined block randomization procedure to receive either FMT or placebo. If safety allows, the remaining cohort of 50 eligible subjects will be randomized in a 1:1 ratio according to a pre-determined block randomization procedure to receive either FMT or placebo. An unblinded pharmacist or delegate will maintain the randomization list ensuring subjects are randomized appropriately and dispensed the correct treatment. The investigator, research personnel and study subjects will be blinded to treatment assignments.

4.1.5. Drug Related Risks

Theoretically, there is a small potential of transmission of an unknown pathogen when delivering human donor fecal material to a recipient. Additionally, there is potential concern that the recipient might have an allergic reaction to a food component in donor stool that they might not be aware of and the donor has not avoided. There is also a potential that

certain disease states might be inherently related to the gut microbial environment and a donor's risk for disease might be transmitted to a recipient. These risks will be mitigated by carefully screening donors for health status as well as relevant (known) infectious pathogens.

The literature to date comprises 100's of cases of FMT for CDI as well as for IBD. To date, no serious adverse events have been reported. Both Vermeire and Vandenplas reported systemic symptoms of fevers and abdominal pain in refractory IBD patients after FMT that resolved within 24-48 hours without intervention. (10,14) Pierog at Columbia University reported the development of granulomatous appendicitis two weeks after FMT for the indication of recurrent CDI in a pediatric patient with underlying ileocecal granulomatous Crohn's disease, but there is no clear reason to assume FMT is responsible for this otherwise Crohn's related outcome. (23) Quera reported a repeated sepsis episode with multi-drug resistant E. coli in a 61 year old male ill Crohn's patient treated by FMT for recurrent CDI who had had several episodes of similar bacteremia previous to FMT. (24) Dr. Hohmann reported a case of a 37 year old man who self-administered FMT at home for his ulcerative colitis who subsequently developed CMV colitis. (25) Additionally, De Leon reported a case of a patient who experienced a transient flare of ulcerative colitis that had been quiescent for twenty years after FMT for recurrent CDI. (26)

Retention enema is likely low-risk but may be less effective in some patients with inability to retain the FMT inoculum or placebo rectally. Mild discomfort and distress with anal manipulation are possible, though enemas are routinely used in our clinical practice to treat distal IBD colitis as well as constipation. Indeed in adult populations with advanced age, multiple comorbidities or limited life expectancy, this delivery mode is preferable. (27)

5. STUDY SCHEDULE AND PROCEDURES

Potential subjects will be assigned a unique identifier or study ID which will be used to identify the subject throughout the study period. Subjects who meet eligibility criteria will be consented and randomized to the FMT or placebo arm.

During the study period, no changes to the previous medical therapy of patients will be made unless clinically indicated. At all times during the study, progression of standard medical therapy will be advised and available if necessary regardless of study drug assignment. Weaning of steroids will be allowed of a patient by PI discretion

5.1. Schedule of Events

SCREENING		BLINDED TREATMENT								OPEN-LABEL FMT						FOLLOW UP[12]			Early Termination
	Day	Day			Visit Week (± 2 days)					Day			Visit Week (± 2 days)			Month			
Procedures	**-90 to -9**	**-8**	**-1**	**0 (Wk 1, D1)**	**2-3**	**4**	**5-7**	**8**		**-8**	**-1**	**0 (Wk 9, D1)**	**Weekly**	**Every 4 weeks**		**1,2,4,5**	**3**	**EOS 6**	
Informed Consent	x																		
Eligibility	x								UNBLINDING										
MD visit	x			x		x		x				x		x			x	x	
HBV, HCV, HIV[3]	x																		
Lavage	x																		
Colonoscopy (*up to 105 days)*	x																		
Randomization		x																	
Antibiotics[1]/placebo		X								X[11]									
hCG[3]	x	X[8]				x		x		X[8]				x					
Labs[2]	x			x		x		x				x		x			x	x	x
Disease activity surveys [5]	x	x	x	x	x	x	x	x		x	x	x	x			x	x	x	x
Stool sample[7,9]		x		x	x	x	x	x		x		x	x				x	x	x
AE assessment by RN/MD		x	x	x	x	x	x	x		x	x	x	x	x		x [6]	x	x	x
Telephone contact by RN/MD			x								x					x			
FMT/placebo dosing				X[4]	x	x	x	x				X[4,10]	X[10]						

[1] 7-day induction regimen of 3 antibiotics (vancomycin 125 mg, polymixin 62.5 mg, and metronidazole)

BSA for vancomycin 125 mg, polymixin 62.5 mg **= SQRT [(cm*kg)/3600]**
<0.5 m²: PO 1 capsule, TID
0.5 – 1 m²: PO 2 capsules, TID
1 – 1.5 m²: PO 3 capsules, TID
1.5 –2 m²: PO 4 capsules, TID

Metronidazole weight-based dosing parameters
12.5 kg ≤ Patient Weight > 25.0 kg: 125 mg PO BID
25.0 kg ≤ Patient Weight > 37.5 kg: 250 mg PO BID
37.5 kg ≤ Patient Weight > 50.0 kg: 375 mg PO BID
Patient Weight ≤ 50 kg: 500 mg PO BID

[2] "Screening labs" are considered for research purposes only, and will be covered by the study. Routine laboratory assessments are per routine clinical care, and if you are a patient at BCH, these will be covered by the subject's insurance company. If the patient is not seen at BCH, then these are considered for research purposes and will be covered by the study.

[3] HBV, HCV, HIV, and hCG testing are research-only labs and are required to be obtained no earlier than 2 weeks prior to the 1st FMT dose.

[4] Initial FMT/placebo dose (Week 1 of blinded therapy will be administered as a retention enema). Week 1 of open label therapy will be a selective FMT induction enema or FMT weekly capsule.

[5] Disease activity surveys include the Patient Reported Outcomes Diary and Pediatric Ulcerative Colitis Activity Index

[6] Weekly telephone AE Assessments will also be conducted on a weekly basis for the 1st month of follow-up.

[7] IBD Biomarkers (quantitative fecal calprotectin) will be done after study completion from frozen samples taken from every study stool sample.

[8] A urine pregnancy test should only be obtained on day -8 if one has not been done in the past 30 days.

[9] The "X" location of stool samples indicates when the stool will be collected from the participant. I.e. stool will be collected from the subject on day -8 and Week 1, Day1 however collection kits will have been provided to the subject beforehand. Lack of stool sample collection will not constitute a protocol deviation.

[10]Subjects in the OL treatment will receive FMT (not placebo)

[11] If eligible, subjects in OL treatment will receive selective Antibiotics (no placebo).

[12] If a subject received at least 1 dose of FMP IP.

5.2. Study treatment and procedures

5.2.1. Screening and Enrollment

Patients with active UC that are part of the Boston Children's Hospital Gastroenterology and Nutrition clinic as well as referrals from outside of BCH will be pre-screened for study participation. Potential subjects who agree to participate in the study will have a medical exam with labs to assess eligibility. Participants will also undergo a baseline serologic assessment of HIV, HBV and HCV status within 2 weeks prior to the first FMT dose. Females who have achieved menses will undergo urine hCG testing. These four lab tests are considered for research purposes only.

It is expected that study participants will receive ongoing clinical care at BCH for the duration of the study. Study participants, who self-refer or are referred by a GI provider not at BCH, will have their primary GI provider notified of their participation in the FMT study and follow up clinical care at BCH during the course of the study at planned intervals. The study PI will provide the primary GI provider with contact information to relay any questions and concerns during the study period. At the completion of the subject's involvement in the study, the PI will provide a final written and/or verbal report to the primary GI provider for continuity of care.

Presence of active mucosal inflammatory bowel disease (active colitis) is an eligibility requirement for this study. Subjects with evidence of inflammation in a prior staging colonoscopy completed no more than 105 days will not be required to repeat a standard staging colonoscopy solely for the purposes of research. Subjects who have no evidence of active disease by endoscopic scoring of disease at the time of staging colonoscopy will not be randomized and will be excluded from the study.

An initial subset of 10 subjects will be limited to patients with mild to moderate Ulcerative colitis (i.e., PUCAI < 65) and to individuals $\geq$ 12 years of age.

Upon confirmation of eligibility and completion of randomization, subjects will receive an induction antibiotic regimen (or corresponding placebo if in placebo arm) for one week to decrease levels of indigenous microflora (Study Day -8 until Day -1).

The antibiotic regimen will include:

1) A double antibiotic capsule containing vancomycin 125 mg and polymixin 62.5 mg to be administered orally (PO) according to the following body surface area (BSA) parameters:

BSA* (m^2)	Capsule	Frequency
<0.5	1	3 times a day
0.5-1	2	3 times a day
1-1.5	3	3 times a day
1.5-2	4	3 times a day

*BSA will be calculated as **BSA = SQRT [(cm*kg)/3600]**

2) A third antibiotic metronidazole administered orally (PO) as a separate capsule according to the following weight-based dosing parameters:

Patient Wt (kg)	Dose (mg)	Frequency
12.5 - 25.0	125	2 times a day
25.0 - 37.5	250	2 times a day
37.5 – 50.0	375	2 times a day
>50.0	500	2 times a day

Corresponding placebo for each antibiotic will be supplied and distributed by the BCH Research Pharmacy.

Clinical data including symptom logs, medications and disease activity and stool samples will be collected at the time of study entry and after completion of the antibiotic regimen. Routine laboratory analysis will be done at the time of enrollment and before commencing study treatment.

Subjects who meet all eligibility criteria will be randomized to receive either FMT or placebo.

5.2.2. Fecal microbiota or placebo transplantation (8 weeks)

Approximately 48 hours after stopping antibiotics or placebo antibiotics, subjects will return for a clinic visit in the Clinical and Translational Study Unit (CTSU) for administration of either FMT or placebo. At the visit, they will undergo a medical exam by the investigator and laboratory samples will be collected. Subjects will then receive an induction retention enema of either FMT or placebo over a 15-30 minute period and will be encouraged to retain the inoculum for as long as possible. Subjects will be observed for 60 minutes after last enema and before discharge.

Subjects will return to the CTSU weekly, for a total of 7 weeks, for administration of blinded study treatment (FMT from the same donor or placebo). Prior to each weekly visit, subjects will provide stool and complete a survey (PUCAI) to monitor symptoms, medications and disease activity. At week 4 and week 8, subjects will also have routine laboratory assessments and a medical examination by the investigator. Adverse events will be assessed at each visit.

5.2.3. Open-label FMT or Follow-up

After completing 8 weeks of blinded study treatment, study subjects will be given the option to receive open-label maintenance FMT weekly for the remainder of the study if they initially received placebo and did not improve OR if they received FMT, improved, and wish to continue treatment. Subjects will be considered to have improved if their PUCAI score shows improvement of at least 10 points compared to baseline or a score of 10 points or less.

After a subject receives the last dose of blinded study treatment at week 8, the subject's treatment assignment will be unblinded to determine the appropriate course of action. Those subjects, who 1) are found to have improved after receiving 8 weeks of placebo or 2) are found not to have improved after receiving 8 weeks of FMT, will not be eligible to receive open-label FMT. Those subjects will be considered in follow up and will have disease status assessments conducted weekly for the 1st month and then monthly for the following month or 5 months. Subjects will also collect a stool sample and have medical examinations done at 3 and 6 months after their last dose of FMT.

Study subjects who are eligible and interested in continuing on to open-label FMT can be enrolled within 14 days (± 7 days) following blinded treatment completion. For the open label phase, subjects who received active treatment (FMT) will proceed directly to open label treatment weekly capsules. However, subjects randomized to placebo (aka no antibiotics, no FMT) will undergo a 7-day antibiotic conditioning and a 2-day rest period prior to commencing open-label FMT treatment that will begin with a retention enema. Thereafter, all subjects will return weekly for observed FMT administration as previously described, AE assessment and survey and stool collection. Routine laboratory assessments and medical exams will be conducted monthly. At all times during this study, standard of care progression of medical/surgical therapy will be offered if clinical course dictates it is necessary for both FMT and placebo arms of the study.

5.2.4. Post-treatment follow up

All study subjects who received at least one dose of FMP will be followed by telephone to assess adverse events and disease status for a total of 6 months after their last FMT dose. Subjects will be contacted by phone weekly for the 1st month and then monthly for the following 5 months and will have stool sample collection and medical examination at 3 months and 6 months after their last dose.

5.2.5. End of Study or Early Termination Visit

If withdrawing early from the study for any reason, subjects will be invited to come in for a final visit to provide a stool sample and routine labs (if indicated).
Follow-up for subjects who signed the consent form, but did not complete the study (**does not include screen failures**) is as followed:

- **Active** (full follow-up schedule) if subject received at least one dose of FMP IP.
- **Active-abbreviated** (Subjects will be called weekly for one month and then once at the second month for a total of two months), for subjects that received at least one dose of antibiotics and/or placebo IP but did not complete blinded treatment period or receive any FMP.

5.3. Assessments

5.3.1. Stool analysis

Subjects will collect a maximum of 29 stool samples over the course of the study and complete a maximum of 36 disease activity surveys. All fecal samples will be stored at −80°C. DNA will be extracted, and the V4 region of the 16S gene will be sequenced using an Illumina MiSeq (Illumina, San Diego, California) as described previously. (28) The Shannon Diversity Index will be computed for each sample and a custom python script will be used to create summary plots illustrating the relationship between clinically relevant groupings and the diversity observed in the microbiome. We will use the Shannon diversity index as our primary measure of diversity because it takes into account both abundance and evenness of species present in the community and has been shown to most robustly accommodate the variation in sampling depth. (29)

5.3.2. Microbial analysis

A long-time collaborator, Eric Alm, PhD (MIT) and his group have pioneered novel approaches to analyzing complex microbial environments already leading to fruitful work together on IBD and the microbiome.(29) The Alm lab has a robust pipeline for processing, sequencing and analyzing this complex data and has already partnered with a leading biotechnology firm to study adult FMT for CDI. Our rich, patient-reported metadata collection technique will allow for numerous clinical correlations to be parsed out using the random forest machine learning capabilities of synthetic learning in microbial ecology (SLiME) to identify taxonomic features associated with important clinical parameters. These approaches are at the cutting edge of microbial ecology but are especially novel to the field of fecal transplantation, which has not yet been subjected to quantitative ecological analysis of any kind.

5.3.3. Clinical Outcomes

Primary Outcome Measures

1. Presence of FMT-related adverse events grade 2 or above.
2. Proportion of subjects with any FMT-related adverse events of grade 2 or above at 8 weeks post-FMT. Patient related outcomes of rectal bleeding and number of bowel movements. Rectal bleeding will be assessed using common language from PUCAI scoring. (See Figure 1)

Figure 1.

Rectal Bleeding

None
Small amount only, in less than 50% of stools
Small amount with most stools
Large amount (> 50% of stool content)

Secondary Outcome Measures

1. Remission as defined by PUCAI of ≤ 9
2. Improvement in inflammatory biomarkers (stool calprotectin, serum ESR/CRP, albumin, Hematocrit).
3. Changes in gut microbial composition.
4. Improvement of PUCAI of ≥ 20 points. (30)
5. Assessment of engraftment of donor microbes into recipients.

6. SAFETY ASSESSMENT

6.1. Adverse Event Assessment and Reporting

Comprehensive assessments of any apparent adverse event(s) (AE) experienced by the study subject will be performed throughout the course of the study.

The PI will submit reports of unexpected Serious adverse events (SAEs) and other unanticipated problems to the Institutional Review Board (IRB) at Boston Children's Hospital and to OpenBiome according to their policies.

The PI will report events that are both serious and unexpected and that are associated with FMT/study intervention to the FDA and other applicable health authorities within the required timelines as specified in 21 CFR 312.32 (IND Safety Reports).

The following unexpected AEs will be assessed, recorded and reported to the Data Safety Monitoring Board (DSMB) on an expedited basis: all SAEs, AEs grade 3 (according to CTCAE 5.0 (31) if applicable) or above, and any unexpected AEs grade 2 or above that is possibly or probably related to FMT (as determined by a blinded investigator). Only an AE that meets the above criteria will be recorded and reported. Each reported AE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, expectedness, suspected relationship to the study treatment, and actions taken (a blinded investigator will determine expectedness, severity and relationship to study treatment).

Individual subject participation will be temporarily halted until the DSMB determines appropriateness of re-initiation of therapy in any subject experiencing an adverse event resulting in: an Emergency Department visit that is possibly or probably related to FMT, significant worsening of their disease, or experiences 1 SAE Grade $\geq$ 3.

Individual subject participation in the study will be permanently stopped if a SAE Grade $\geq$3 is deemed possibly or probably related to FMT, including any hospital admission.

If 2 or more subjects experience 1 SAE grade $\geq$4, all study activities will be halted until the DSMB determines appropriateness of re-initiation of therapy in any subject.

6.2. Safety and Data Monitoring

A data and safety monitoring board consisting of three physicians and a biostatistician independent of the principal investigator will monitor this study. The DSMB will review all adverse events as previously described and will convene at least every four months or when needed to review initial limited cohort safety data as described previously. Additionally, the DSMB will be convened to evaluate subject data if the subject has been temporarily halted from participating in the trial in order to assess appropriateness of resuming participation in the trial.

If an SAE is thought to have been caused by the fecal transfer, the DSMB and IRB recommendation as to whether the protocol should be suspended or not will be followed.

6.3. Clinical Monitoring

Monitoring will be conducted according to a written monitoring plan to ensure the protection of the rights and safety of all participating human subjects, as well as to ensure the quality and integrity of data resulting from this investigation. Monitoring services will be performed by qualified monitors provided by Boston Children's Hospital.

7. STATISTICAL CONSIDERATIONS

7.1. Power Calculation

The primary aim of this phase I/II study is to establish whether or not FMT is a safe treatment for children and young adults with UC. Based on clinical experience, adverse events (AEs) in IBD patients are common and include abdominal pain, cramping, diarrhea, fever, bloody stool, vomiting, frequent defecation, joint pain, and eye pain. It's estimated that 70% of patients in any given year will experience a flare of their disease and have some or a combination of these symptoms. Safety, therefore, needs to be assessed relative to the high prevalence of AEs in this population. For the primary outcome, we will take any new reported SAEs or worsening of the existing AEs during the study period following enrollment into account.

The study will be powered for equivalence in the proportion of new SAEs or worsening of the existing AEs, assuming null (Ho) and alternative (Ha) hypotheses defined as follows:

Ho: $p1 - p2 \leq -\delta_L$ or $p1 - p2 \geq \delta_U$.
Ha: $\delta_L \leq p1 - p2 \leq \delta_U$

where p1 is the proportion of new SAEs or worsening of the existing AEs in the placebo group, p2 is the proportion in the FMT group, δ_L is the lower margin and δ_U is the upper margin. We expect to enroll a total of 60 patients into this study for a total of 30 FMT and 30 placebo patients. Assuming the rate of new incident SAEs or worsening of existing AEs in the placebo group is 10%, there will be 82% power to reject Ho that the difference in proportions is 0.20 or greater from zero in the same direction. Similarly, we will have an 85% power to reject a difference of 0.25 or greater if the rate of new incident SAEs or worsening of existing AEs increases to 15%.

7.2. Simon's two stage design

We will follow stopping rule in Simon's two-stage design if high incidence of new SAEs occurred in study participants. In the first stage, a subset of 5 patients will be enrolled. If all the 5 patients develop new SAE in the study period, we will declare the study is not safe for patients and will stop the trial immediately to check if FMT is related to these SAEs. If less than 5 patients develop SAEs in the first stage, we will proceed to second stage to enroll another 5 patients. If 7 out of the total 10 patients enrolled develop at least one SAE, we will stop again and check if FMT is related to these SAEs. Otherwise the enrollment will be continued until the sample size for the primary outcome analysis is met.

Assuming the probability of the new SAE is 76% among study participants, following the 5/5 and 7/10 two-stage stopping rule, we will have 25% chance to stop in the first stage, and 80% chance to stop in the second stage. If probability of new SAE increases to 0.96, we will have 82% chance to stop in the first stage, and 100% chance to stop in the second stage.

Table. The probability of stop following 5/5 and 7/10 stopping rule in two-stage design.

True probability of SAE	0.76	0.80	0.90	0.96	0.98
Probability to stop in the first stage	0.25	0.33	0.59	0.82	0.90
Probability to stop in the second stage	0.80	0.88	0.99	1.00	1.00

7.3. Data Analysis

All patients who are eligible, randomized, and have received at least one-dose of the study medication including antibiotics/placebo in the first week will be included into the analysis for primary outcomes to assess the safety and tolerability of FMT, as this will be a per protocol (and not intent-to-treat) analysis . Continuous variables will be presented as mean and standard deviation (SD) or median and interquartile range (IQR), depending on the distribution of the data. Categorical variables will be presented as number and percentage. Disease severity (PUCAI) and patient characteristics at baseline will be compared between the treatment groups to determine whether or not randomization resulted in balanced groups. Methods of 2-group comparison will include Student's t-test, the Mann-Whitney test, Pearson chi-square test, and Fisher exact test, depending on the distribution of the variable under consideration. The primary outcome of safety will be assessed via a 2×2 cross-tabulation, where the proportion of patients with new incident SAEs or upgrade of existing AEs in the FMT group will be compared to the proportion in the placebo group. Two one-sided tests for equivalence (32) will be constructed and the overall P value taken to be the larger of the lower and upper tests. Statistical significance will be assessed at $P<0.05$.

For the second outcome, we will use longitudinal data analysis methods to investigate changes in inflammatory biomarkers including stool calprotectin, stool lactoferrin, serum ESR/CRP, albumin, and hematocrit over the study period. Generalized Estimating Equations will be performed to examine the association between FMT and changes in these biomarkers.

8. DATA CAPTURE AND RECORD KEEPING

All study data will be captured in a 21CFR Part 11 compliant INFORM™ database. Data and source documents will be maintained in compliance with ICH-GCP and local and national regulatory requirements.

By conducting this study, the investigator affirms that all study results and information furnished will be maintained in strict confidence. Such information will be communicated to the investigator's review committee under an appropriate understanding of confidentiality. A published summary of the results of this study is not inconsistent with the preceding affirmation of confidentiality.

All subjects will be actively followed by an investigator at Boston Children's Hospital within the Division of Gastroenterology & Nutrition/ Inflammatory Bowel Disease Center for the duration of the study. The patients will undergo routine visits with clinical staff as is described and a running log of symptoms, medication(s)/medication change(s), and adverse events will be maintained. This information will be collected by case report forms for entry in the INFORM™ database and will be signed-off by the Principal Investigator. The subjects' progress in the study and any changes to their treatment plans will be reviewed with the study team at regularly scheduled weekly study team meetings while the patient remains on the study protocol. Others providing care to the patients will be included to the extent needed to provide appropriate medical care to the patients with patient permission.

9. PROTECTION OF HUMAN STUDY SUBJECTS

9.1. Declaration of Helsinki and Ethical Review

The study will be conducted in accordance with the protocol, applicable ICH Guidelines, Good Clinical Practice and the World Medical Association (WMA) Declaration of Helsinki and its amendments concerning medical research in humans.

In accordance with guidelines and U.S. Code of Federal Regulations applicable to clinical studies, the protocol and informed consent/assent forms will be reviewed and approved by the Boston Children's Hospital Institutional Review Board (IRB). The investigator will inform the IRB and FDA of subsequent protocol amendments and reportable events as defined by IRB policy and FDA regulation.

9.2. Informed Consent

In accordance with applicable guidelines, informed consent/assent will be documented by the use of a written informed consent approved by the IRB and signed by the subject and/or subject's parent or guardian before any screening and protocol specific procedures are performed.

The investigator (or designee) will explain to the subject and/or the subject's parent or guardian the nature of the study, the action of the study treatment, and any risks and benefits. The subject and/or subject's parent or guardian will be informed that participation is voluntary and that the subject can be withdrawn from the study at any time without prejudice to their subsequent care.

The subject and/or the subject's parent or legal guardian will be given a copy of the fully executed consent/assent and the original will be maintained with the subject's records.

9.3. Inclusion of Women, Minorities, and Children (Special Populations)

Eligible male and female subjects who are members of minority groups and their subpopulations will not be excluded from the study. Children 5 years of age or older will be included in the study.

10. REFERENCES

1. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. *Surgery* [Internet]. 1958/11/01 ed. 1958;44(5):854–9.
2. Gough E, Shaikh H, Manges AR. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. *Clin Infect Dis* [Internet]. 2011/10/18 ed. 2011;53(10):994–1002.
3. Van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, De Vos WM, et al. Duodenal Infusion of Donor Feces for Recurrent Clostridium difficile. *N Engl J Med*. 2013; 368 (5): 407-15.
4. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. *The American Journal of Gastroenterology* [Internet]. Nature Publishing Group; 2012 May [cited 2013 Mar 1];107(5):761–7.
5. Hamilton MJ, Weingarden AR, Unno T, Khoruts A, Sadowsky MJ. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. *Gut microbes* [Internet]. 2013 Jan 18 [cited 2013 Feb 23];4(2).
6. Youngster I, Sauk J, Pindar C, Wilson R, Kaplan J, Smith M, Alm E, Gevers D, Russell G and Hohmann E. Fecal Microbiota Transplant for Relapsing *Clostridium difficile* Infection Using a Frozen Inoculum from Unrelated Donors – a Randomized, Open Label, Controlled Pilot Study. *Clinical Infectious Disease*. 2014: [5]8(11): 1515-1522.
7. Borody TJ, Warren EF, Leis S, Surace R, Ashman O. Treatment of ulcerative colitis using fecal bacteriotherapy. *J Clin Gastroenterol* [Internet]. 2003/06/18 ed. 2003;37(1):42–7.
8. Anderson JL, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Aliment Pharmacol Ther* [Internet]. 2012/07/26 ed. 2012;36(6):503–16.
9. Kunde S, Pham A, Bonczyck S, et al. Safety, Tolerability, and Clinical Response after Fecal Transplantation in Children and Young Adults with Ulcerative Colitis. Journal of Pediatric Gastroenterology and Nutrition. 2013; 56(6): 597-601.
10. Vermeire S, Joosens M, Verbeke K, et al. Pilot Study on the Safety and Efficacy of Faecal Microbiota Transplant in Refractory Crohn's Disease. *Gastroenterology*. 2012; 142(5): s360.
11. Kump P, Grocehnig H, Lackner S, et al. Alteration of Intestinal Dysbiosis by Fecal Microbiota Transplantation Does Not Induce Remission in Patients with Chronic Active Ulcerative Colitis. *Inflamm Bowel Dis*, 2013; 0:1-11.

12. Russell GH, Kaplan JL, Winter HS. Fecal Microbiota Transplant to treat Recurrent *C. difficile* Infection in Children with and without Inflammatory Bowel Disease. *Journal of Pediatric Gastroenterology & Nutrition.* 2014; 58(5): 588-592.

13. Kao D, Hotte N, Gillevet P, et al. Fecal Microbiota Transplant Inducing Remission in Crohn's Colitis and Associated Changes in Fecal Microbial Profile. *J Clin Gastroenterol.* 2014; 48(7): 625-8.

14. Vandenplas Y, Veereman G , van der Weerf ten Bosch J, et al. Fecal microbial transplantation in a one-year old girl with early onset colitis - caution advised. *JPGN*. Published Ahead of Print. 2014. 10.1097/MPG.0000000000000281.

15. Moayeddi P, Surrette M, Wolfe M, et al. A randomized, placebo controlled trial of fecal microbiota therapy in active ulcerative colitis. *Gastroenterology.* 2014; 146 (5): s-159.

16. Brandt LJ, Borody TJ, Campbell J. Endoscopic fecal microbiota transplantation: "first-line" treatment for severe clostridium difficile infection? J Clin Gastroenterol [Internet]. 2011/07/01 ed. 2011;45(8):655–7.

17. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. Nature [Internet]. 2008/12/02 ed. 2009;457(7228):480–4.

18. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. The application of ecological theory toward an understanding of the human microbiome. Science (New York, N.Y.) [Internet]. American Association for the Advancement of Science; 2012 Jun 8 [cited 2013 Feb 28];336(6086):1255–62.

19. Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. *Nat Rev Gastroenterol Hepatol* [Internet]. 2011/12/21 ed. 2012;9(2):88–96.

20. Kostic A, Xavier R, Gevers D. The Microbiome in Inflammatory Bowel Disease: Current Status and the Future Ahead. Gastroenterology 2014; 146:1489-1499

21. Turner D, Levine A, Kolho, Kaija-Leena, et al. Combination of oral antibiotics might be effective in severe pediatric ulcerative colitis: a preliminary report. Journal of Crohn's and Colitis. 2014; Jun 20. pii: S1873-9946(14)00183-4. doi: 10.1016/j.crohns.2014.05.010. [Epub ahead of print]

22. Caporaso JG, Lauber CL,WaltersWA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012; 6:1621–4.

23. Pierog A, Mencin A, Reilly N, et al. Fecal Microbial Transplantation for Children with Recurrent Clostrodium difficile Infection. *The Pediatric Infectious Disease Journal.* Published Ahead of Print. 2014; 33 (11): 1198-200.

24. Quera R. Letter to the Editor – Bacteremia as an adverse event of fecal microbiota transplantion in a patient with Crohn's disease and recurrent Clostridium difficile infection. *Journal of Crohn's and Colitis*. 2014; 8 : 252-3.

25. Hohmann EL, Ananthakrishan AN, Deshpande V. Case 25-2014: Case records of the Massachusetts General Hospital. *New England Journal of Medicine*. 2014; 371(7): 668-675.

26. De Leon LM, Watson JB, Kelly CR. Transient flare of ulcerative colitis after fecal microbiota transplantation for recurrent *Clostridium difficile* infection. *Clinical Gastroenterology & Hepatology*. 2013; 11(8): 1036-8.

27. Kassam Z, Hundal R, Marshall J, Lee C. Fecal Transplant via Retention Enema for Refractory or Recurrent Clostridium difficile Infection. Arch Intern Med 2012; 172(2):2012-14)

28. Preheim SP, Perrotta AR, Friedman J, et al. Computational methods for high-throughput comparative analyses of natural microbial communities. Methods Enzymol 2013; 531:353–70.

29. Papa E, Docktor M, Smillie C, Weber S, Preheim SP, Gevers D, et al. Non-invasive mapping of the gastrointestinal microbiota identifies children with inflammatory bowel disease. Ravel J, editor. *PLoS ONE* [Internet]. Public Library of Science; 2012;7(6):e39242.

30. Turner D, Otler A, Mack D, et al. Development, Validation, and Evaluation of a Pediatric Ulcerative Colitis Activitis Index: A Prospective Multi-center Study. Gastroenterology. 2007; 133: 422-432.

31. National Cancer Intitute's Common Terminalogy Criterial for Adverse Events (CTCAE version 5.0 [cited 2017 Nov 27];0:0–135.

32. Schuirmann, D. J. (1987), "A Comparison of the Two One-Sided Tests Procedure and the Power Approach for Assessing the Equivalence of Average Bioavailability," Journal of Pharmacokinetics and Biopharmaceutics, 15, 657–680.