Montefiore Medical Center	
Official Study Title:	Multicenter, Randomized, Double-blinded, Placebo-controlled Trial of Fecal Microbiota Transplantation (FMT) for Diarrhea-Predominant Irritable Bowel Syndrome (IBS-D)
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Research Study Overview

A detailed research protocol, which is synonymous to the Investigator's Brochure that was approved by the FDA, has been included in this IRB submission. This brief document was drafted to facilitate the reviewers in obtaining a quick overview of the study since the detailed protocol is quite lengthy.

Title: Randomized, Double-blinded, Placebo-controlled Trial of Fecal Microbiota Transplantation (FMT) for Diarrhea-Predominant Irritable Bowel Syndrome (IBS-D)

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Background: Irritable bowel syndrome (IBS) is the most commonly diagnosed gastrointestinal (GI) disease in the United States, affecting 10-15% of the population,[1] and is estimated to account for up to $30 billion of the healthcare budget annually, both directly and indirectly.[2, 3] Existing therapies for IBS are often ineffective, underscoring the need for alternative treatment options. A previous study reported that despite medical therapy, 92% of patients with IBS remained symptomatic when followed for 10 to 13 years.[4] Alterations in the intestinal microbiota increasingly have been shown to play a significant role in the pathogenesis of IBS.[5] Fecal microbiota transplantation (FMT) is a microbiome-based treatment that targets and corrects intestinal dysbiosis and involves collecting stool from healthy pre-screened donors and after preparation, administering this fecal material to an individual with disease. Numerous studies have demonstrated that FMT is a safe and effective treatment for recurrent *Clostridium difficile* infection, with a reported overall cure rate of 92%.[6-24] Though numerous studies have been published on FMT for the treatment of recurrent CDI, studies on FMT for the treatment of IBS are lacking. One case series, consisting of 55 patients who underwent FMT for treatment of IBS and IBD, reported promising results.[25] In a recent study of 13 patients with refractory IBS, FMT resulted in symptomatic resolution or improvement in 70%.[26]

Objectives: The objectives of this study are (1) to determine the efficacy of FMT, given as oral capsules, compared with placebo for the treatment of refractory IBS-D; (2) determine the impact of FMT on the intestinal microbiome of patients with IBS-D; (3) assess the safety, feasibility, and tolerability of FMT for patients with IBS-D; and (4) determine patient factors that influence treatment preferences and patient attitudes toward FMT. An Investigational New Drug application (#16039) to conduct this study has been submitted to the Food and Drug Administration and was approved on 11/12/14 .

Design Overview: We propose a multicenter, prospective double-blinded, randomized, placebo-controlled trial to assess the efficacy, safety and tolerability of FMT for the treatment of IBS-D. Primary endpoints will include (1) disease severity and quality of life as determined by the IBS-SSS (see attached) and IBS-QOL (see attached) questionnaires, respectively; and (2) intestinal microbiota composition pre- and post-FMT. Secondary endpoints will recorded in a Subject Diary (see attached) and will include (1) adverse events; (2) satisfaction with FMT; (3) change in bowel habits and abdominal pain after FMT; (4) number of doctor or ED visits post-FMT for IBS-D-related symptoms; (5) initiation of new medications post-FMT for the treatment IBS-D symptoms; (6) patient attitudes towards FMT; and (7) tolerability of FMT.

Study Subjects: This is a multicenter study including Montefiore Medical Center, New York University Langone Medical Center and Yale University Medical Center. Patients with IBS-D will be

recruited from outpatient gastroenterology clinics at these institutions and referrals from the medical community. To date, FMT has been used for the treatment of predominantly diarrheal diseases including, CDI, IBD and IBS-D, therefore, we suggest that the first clinical trial of FMT for the treatment of IBS investigate the role of FMT in patients with IBS-D as opposed to IBS-C or IBS-M. The high prevalence of IBS and the large volume of patients seen in the GI practices at Montefiore Medical Center will allow for rapid recruitment and enrollment of patients. Patients ≥18 years-old, with an established diagnosis of IBS-D as determined by Rome III Criteria, moderate-severe disease activity (as determined by a score of >175 on the IBS-Symptom Severity Score [IBS-SSS]) and persistent symptoms despite conventional therapies, will be included in the study. Patients will be excluded if they are pregnant; severely immunocompromised or immunosuppressed (e.g., organ transplant recipients, severe neutropenia with an absolute neutrophil count of <500 cells/μL, current treatment or treatment within 3 months with anti- neoplastic agents and HIV-positive patients with CD4 counts <200 cells/mm^3); were treated with any antibiotics in the 3 months prior to FMT; if GI symptoms can be explained by the presence of an underlying organic disease including, underlying IBD, infectious enteritis, previously established and untreated SIBO or known motility disorder; or if they have a history of a severe (anaphylactic) food allergy.

Study Procedure:

Clinical Tests and Outcome Measures: Demographic data, including date of birth, gender, ethnicity, year of diagnosis, medical comorbidities, prior surgeries, current and past medications, will be obtained from each study participant. Patients who are enrolled in the study will undergo laboratory testing within 10 days prior to FMT. Testing will include complete blood count, comprehensive metabolic panel, HIV antibody, hepatitis A IgM, hepatitis IgG, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis C antibody, syphilis, stool culture, stool ova and parasites, *Giardia* stool antigen, and *C. difficile* toxin assay. All study subjects will also complete the previously validated IBS-SSS[27] and IBS-Quality of Life (IBS-QOL)[28] questionnaires up to 10 days prior to FMT to assess disease activity and quality of life. A list of attempted conventional therapies employed within 6 months prior to FMT will be obtained from each patient.

Donor Characteristics, Preparation of Fecal Suspension and FMT Procedure: Stool is a heterogeneous substance, composed primarily of bacteria and water, but also containing viral and fungal organisms, metabolic products of these organisms, undigested foods, bile, bilirubin, cholesterol, inorganic salts, dead cells, and mucus from the lining of the intestinal wall. The exact composition of human stool varies from person to person and from day to day. Frozen fecal material from a pool of standardized donors has been successfully used for FMT in patients with *C. difficile* infection. [29,30] Moreover, FMT capsules containing stool from a standardized donor have been used successfully treat patients with *C. difficile* infection (personal communication with OpenBiome). FMT capsules courtesy of OpenBiome, Medford, MA will be used for FMT in this study. Production and shipment of this product has been previously referenced in a prior Drug Master File (BB-MM15543) prepared by OpenBiome (document included in this IRB application). As delineated in this document, the encapsulated fecal product has been obtained from a pool of standardized donors who have undergone extensive testing to exclude an underlying diagnosis of IBS and functional GI disorders.

On day 1 of FMT or sham-FMT, the patient will be randomized to undergo FMT using fecal capsules (experimental group) or placebo capsules (control group) via a computer-generated program.

Patients in the experimental group will take 25 capsules, each containing approximately 0.67grams of donor stool (FMT) on days 0, 1, and 2. In order to successfully blind the investigators and the study participants, patients in the placebo group will take 25 placebo capsules containing saline and glycerol which have the same appearance as the fecal capsules (sham-FMT) on days 0, 1, and 2. All patients will cross-over into the other treatment arm of the study at week12.

In a prior retrospective study, that was done by the authors, a total of 13 patients were treated for refractory IBS with FMT administered via EGD by duodenal or jejunal infusion.[26] Symptomatic improvement ensued in 70% of these patients.[26] In this study, the fecal suspension that was infused via EGD consisted of approximately 36-38 grams of stool. This dose is comparable to the dose that would be administered by giving 25 OpenBiome FMT capsules for three consecutive days. As outlined in the included document drafted by OpenBiome in response to the FDA's comments, there is approximately 13.75 grams of stool in 25 OpenBiome FMT capsules, therefore, the 75 capsules given over three consecutive days would contain a total of 41.25 grams of stool which is similar to the dose used in our prior study of FMT for the treatment of IBS.

The FMT and placebo capsules will be shipped from OpenBiome and a third party who is uninvolved in data collection or analysis will give the patients FMT or placebo capsules after randomization. The patient will be instructed to take a proton-pump inhibitor (single 40mg dose) on the morning of the procedure in order to minimize bacterial killing by gastric acid. All patients will undergo 3 consecutive days of treatment with FMT capsules (in order to provide the maximal opportunity for successful re-colonization of the intestinal microbiome) or with placebo capsules.

Patient Visits and Post-FMT Follow-up: Patients will be seen by one of the research investigators prior to FMT, at which time they will be enrolled in the study. Enrolled patients will then be seen on days 1, 2, and 3 of FMT or sham-FMT and day 10, week 4, week 8 and week 12 after FMT/sham- FMT. All patients will cross-over into the other treatment arm at 12 weeks. After cross-over, patients will undergo a second course of FMTs or sham-FMTs. Patients will be seen on days 1, 2, and 3 of FMT or sham-FMT #2, and day 10, week 4, week 8 and week 12 after FMT #2. Therefore, the overall duration of the study will be 6 months. The IBS-SSS questionnaire will completed prior to FMT or sham-FMT and at specified follow-up visits (enrollment visit, day 1, day 10, week 4, week 8 and week 12) to determine symptom severity over time. The IBS-QOL will be completed prior to FMT or sham-FMT and at monthly intervals (visits at week 4, week 8 and week 12), since the IBS- QOL is based on a 30-day recall. AEs will be solicited at each patient visit. Additionally, patients will be asked to maintain a daily diary of AEs, in addition to weekly assessments of satisfaction with FMT, degree of improvement in bowel habits and abdominal pain, number of doctor or ED visits and new medications or therapies used for IBS-D symptoms. The research team has developed a specifically dedicated diary to facilitate standardized reporting. All findings will be de-identified and recorded in a notebook which will be kept in a secure location. A detailed timeline of patient visits is included in the Investigators' Brochure and complete research proposal which are included in this application.

Intestinal microbiome analyses using DNA sequencing and non-cultivation-based approaches (16S DNA technology) will be performed in all patients in the experimental and control groups prior to FMT and after FMT at day 10, week 4, and week 12 in order to assess stability of the microbiome over time. Metabolomic profiles and inflammatory cytokine panels will be performed on all patients in the experimental and control groups prior to FMT and after FMT at day 10. All of these

analyses will be performed by OpenBiome who has extensive experience performing these analyses.

Statistical Analyses: Based on the results published by Drossman, *et al* in 2007 [30], if the true population values for the IBS-QOL questionnaire are assumed as a mean improvement of 0 for the placebo group and a mean improvement of 12 for the experimental group, 45 patients would be needed in each arm of the study in order to achieve a power of 80%. Based on a results published by Johanneson, *et al* in 2011 [31], if the true population values for the IBS-SSS questionnaire are assumed as a mean decrease of 5 points with a range of -101 to 113 for the placebo group and a mean decrease of 50 points with a range of -130 to 49 for the experimental group, 45 patients in each arm would yield a power of >80%. A total of 55 patients will be recruited in each arm of the study in order to account for potential dropouts. Of the 110 patients, 37 will be recruited at Montefiore Medical Center, 37 at New York University Langone Medical Center and 36 at Yale University Medical Center. A primary analysis will be performed at 3 months and a secondary analysis will then be performed at 6 months in the FMT and sham-FMT groups. For the secondary analysis, FMT group data will be combined and will include data collected at the end of 3 months in those who were initially randomized to FMT and data collected at the end of 6 months in patients who were initially randomized sham-FMT and then crossed over to FMT. Changes in score on the IBS-SSS and IBS-QOL will be compared using the Student's t-test for normally distributed data or the Mann-Whitney U test for non-normally distributed data. Within group changes will be analyzed using the paired t-test for normally distributed data or the Wilcoxon Rank-Sum test for non- normally distributed data. Categorical variables will be compared using the Chi-Squared test or the Fischer's Exact test when appropriate. Intention-to-treat and per-protocol analyses will be performed. In the intention-to-treat analysis, all those randomized will be included whether or not they completed all FMT or sham-FMT treatments. In those patients who dropout of the study, the last recorded data point will be used for statistical analyses. The per-protocol analysis will include only those who complete all FMT or sham-FMT treatments and have complete data at the end of the study.

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