Fecal Microbiota Transplantation for the Treatment of Primary Sclerosing Cholangitis.

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I. Background and Significance:

Primary sclerosing cholangitis (PSC) is a progressive, chronic cholestatic liver disease characterized by inflammatory and fibrotic destruction of the intrahepatic and/or extrahepatic bile ducts. PSC will progress to biliary cirrhosis, portal hypertension and liver failure (1) . It is a common indication for liver transplantation. In up to 90% of patients, ulcerative colitis or Crohn's disease will also be present (2) . Medications used for the treatment of ulcerative colitis such as sulfasalazine, corticosteroids and azathioprine or 6-mercaptopurine have not been effective in reducing inflammation or bringing about remission in PSC (3) . A number of studies of other anti-inflammatory agents have failed to demonstrate any benefit. In standard dosing, ursodeoxycholic acid (UDCA) may be of benefit in delaying the progression of disease, although a recent study showed that high dose UDCA was not only ineffective, it may also be harmful (4) . Currently there is no medical therapy that has been shown to be effective in PSC and no therapy has won FDA approval for this indication.

It has been postulated that bacterial components may stimulate an aberrant immune response resulting in the perpetuation of the biliary inflammation seen in PSC. Bacteria gain access to the liver and biliary tree through translocation across an abnormal and inflamed intestinal mucosa into the portal venous system (5) . Studies have shown an increased risk of portal venous bacteremia in patients with PSC. Animal models have demonstrated that enteric dysbiosis can lead to hepatobiliary inflammation with various features of PSC (6) . The pathways through which bacteria might then induce the pathology characteristic of PSC are speculative.

Bacteria might cause direct injury through colonization, though studies have not identified any particular pathogen or a consistent set of bacteria. Another potential pathway may be that a certain set of bacteria generate secondary bile acids, such as deoxycholic acid and lithocholic acid, which are injurious to the biliary system (7) . We recently found an altered serum bile acid composition in patients with PSC compared to non-cholestatic controls. Treatment with UDCA in PSC patients decreases the concentration of the toxic primary bile acid chenodeoxycholic acid but also increased the toxic secondary bile acid lithocholic acid (Abstract DDW 2014). Alteration of the gut microbiota may minimize or eliminate this injury.

There is limited experience in the use of antibiotics in treating PSC. Metronidazole has been shown to result in improvement in liver function tests (8) . Oral vancomycin has also been advanced as a potentially promising therapy (9) . An initial report of three pediatric patients and a subsequent small, uncontrolled series of oral vancomycin in 14 children showed improvement in liver tests and symptoms (10, 11) . We recently completed a study of oral vancomycin was given to 10 adults with PSC found mild improvement in serum alkaline phosphatase levels (Abstract DDW 2011).

Fecal Biotherapy (FBT) also known as fecal transplantation or fecal microbiota transplant—involves the transfer of a donor's fecal flora (bacteria) to a recipient's colon. It has become widely accepted as the standard of care for recurrent Clostridium difficile

infection, with a cumulative cure rate of >90% and minimal adverse events (12) . In C. diff infection, prior exposure to antibiotics diminishes the normal colonic flora, allowing C. diff organism to proliferate and release toxin (13) . This bacterial environment is similar to the major shifts in microbial diversity seen in patients with IBD. Interestingly, when patients with IBD receive FBT for C. diff. infections, their outcomes are excellent, reinforcing the notion that the enteric flora have a strong influence in the enteric immune system (14) . We currently have a robust FMT clinical program for recurrent and refractory c.difficle infections with a cure rate > 90%. We have also recently participated in an open label clinical trial for the use of FMT in crohn's disease.

We hypothesize that for patients with PSC, fecal microbiota transplantation will correct a dysbiosis that has led to hepatobiliary inflammation leading to improvement in LFTs and slow progression to cirrhosis.

II. Specific Aims:

Specific Aim 1: Determine the impact of fecal microbiota transplantation on the intestinal microbiome of patients with primary sclerosis cholangitis with and without inflammatory bowel disease via 16s ribosomal RNA sequencing comparing delivery modalities (colonoscopy and capsules).
Hypothesis: Fecal microbiota transplantation will result in a sustained repopulation of the patient's microbiome that corresponds to the bacteria from the donor stool.

Specific Aim 2: Assess for clinical response in patients with PSC receiving FMT comparing delivery modalities (colonoscopy and capsules).
Hypothesis: Fecal microbiota transplantation will lead to a 50% reduction in liver biochemistries in patients with PSC.

Specific Aim 3: Assess bile salt metabolomics as a therapeutic biomarker for clinical response to fecal microbiota transplantation.
Hypothesis: A decrease in the production of toxic secondary bile acids (lithocholic and deoxycholic acid) will correlate with clinical response to fecal microbiota therapy

III. Subject Selection

This is an open-label single-arm pilot study to measure the microbiological and clinical impacts of FMT in patients with PSC. We will prospectively enroll 16 PSC patients Stage 1-4. Donor Stool will be obtained from OpenBiome. OpenBiome is a nonprofit 501(c)(3) organization that provides hospitals with screened, filtered, and frozen material ready for clinical use (See Attachments for OpenBiome Quality and Saftey Program as well as donor screening examples).

Donors:
OpenBiome donors are rigorously assessed and monitored.

1. Donor candidates are screened with comprehensive evaluation of medical histories, behavioral risks, and current health status.

2. **Laboratory Screening:** Donor candidates are screened for 20 stool and serological tests at a CLIA-certified laboratory. Less than 20% of those screened become qualified donors
3. **Continuous Requalification:** Qualified donors are under medical monitoring through the entire donation window and are fully rescreened every 60 days
4. **Quarantine Procedure:** Prior to release, donated material is quarantined for 60 days in between two full panel screens at a CLIA certified laboratory. After passing a first battery of tests, a donor may donate specimens for a 60-day window. All material made from these specimens is held in quarantine until a second battery of tests is administered. The material from this 60-day window is release only if and when the donor passes this second battery of tests.

Patients:
Patient enrollment will be done via referral of appropriate patients from the GI clinic. Patients with PSC evaluated at Brigham and Women's Hospital and the Massachusetts General Hospital will be enrolled in this protocol. All study procedures will take place at Brigham and Women's Hospital.

Inclusion criteria:
1. Age 18 or older
2. Confirmed diagnosis of PSC (with or without a concurrent diagnosis of inflammatory bowel disease) characterized by a cholestatic liver condition of greater than 6 months duration with confirmatory cholangiographic findings, as well as an elevation of Liver Function Tests of greater than 1.5 times the upper limit of normal.

Exclusion Criteria:
1. Decompensated liver disease
2. Patients who were pregnant or breastfeeding
3. Use of concomitant immune modulators including methotrexate, mycophenolate mofetil, tacrolimus, cyclosporine, thalidomide, Interleukin-10, or Interleukin-11 within 4 weeks prior to receiving the FMT
4. Patients who are unable to give informed consent
5. Patients who are unable or unwilling to undergo colonoscopy with moderate sedation (>ASA class II)
6. Patients who have previously undergone FMT Patients who have a confirmed malignancy or cancer
7. Patients who are immunocompromised
8. Treatment within last 8 weeks with infliximab, adalimumab, certolizumab, natalizumab, vedolizumab or thalidomide
9. Antibiotic use within 2-months of start date
10. Participation in a clinical trial in the preceding 30 days or simultaneously during this trial
11. Probiotic use within 30 days of start date
12. Congenital or acquired immunodeficiencies
13. Other comorbidities including:Diabetes mellitus, cancer, systemic lupus, must be able to tolerate conscious sedation with colonoscopy

14. Chronic kidney disease as defined by a GFR <60mL/min/1.73m2 44
15. History of rheumatic heart disease, endocarditis, or valvular disease due to risk of bacteremia from colonoscopy
16. Steroid dose >20mg/day
17. Ursodeoxycholic Acid within 4 weeks prior to receiving the FMT
18. 25. Patients with allergies to ingredients Generally Recognized As Safe (GRAS): glycerol, sodium chloride, hypromellose, gellan gum, titanium dioxide, cocoa butter
19. 26. Dysphagia: oropharyngeal, esophageal, functional, neuromuscular (e.g. stroke, multiple sclerosis, ALS)
20. 27. History of aspiration
21. 28. History of severe gastroparesis

Subject Withdrawal Criteria: Patients will be withdrawn under the following circumstances:

Patients – patients will be withdrawn under the following circumstances:

For patients in the colonoscopy Group:

1. Colonoscopy cannot be completed for technical reasons due to inability by endoscopist to reach terminal ileum, or patient intolerance of procedure.
    a. These patients will only be followed-up for week 2 adverse event screening, as they will not have received FMT. These subjects will be replaced, as they will not be considered "treated".

For patients in the capsule group

2. Patient is unable to successfully perform capsule test swallow.
3. These above patients will only be followed-up for week 2 adverse event screening, as they will not have received FMT. These subjects will be replaced, as they will not be considered "treated"

For all Patients

4. Patient withdraws their consent for follow-up visits or contacts.

IV. Subject Enrollment

Recruitment Procedures:

BWH or MGH gastroenterologists, including attendings and fellows, will be informed of the study's aims and inclusion criteria. These doctors will inform the principal investigator of patients who meet the study criteria and who may be good candidates for

the study. The treating gastroenterologist will introduce the study to the potential patient and request the patient's permission to be approached by study staff. We will not utilize advertisement material or other informational materials for patients beyond the consent form.
Patients will be given as much time as they need to decide. They will be given a copy of the consent form to take home, read, and consider, and they will be encouraged to discuss participation with family members and health care providers. Patients will receive parking vouchers at each visit to cover the cost of parking.

Consent Procedures:
The treating gastroenterologist will introduce the study to the potential patient and request the patient's permission to be approached by study staff. With the treating gastroenterologist's permission, either a physician investigator or study coordinator will describe the research study in detail, including participation and risks and alternative courses of treatment, and answer any questions or concerns that the patient may have. Patients will be given as much time as they need to consider participation before signing the consent form.

Subjects will be drawn from the investigators own practice. In order to avoid coercion, study staff will reinforce that participation is voluntary and that their decision will not affect the medical care that they receive now or in the future. If patients seek more time to consider participation, they will be given a copy of the consent form and encouraged to discuss the study with family, friends, PCP, or others. Study staff will follow-up to see if any questions or concerns have not been addressed. A physician investigator will obtain informed consent signatures.

V. Study Procedures

Donor Procedures (OpenBiome):

Prior to enrollment, donors (age >18), receive informed consent with oversight from MIT's IRB and COUHES. Donors are interviewed by a healthcare professional to determine whether they meet the following exclusion criteria:
1. Infectious risk factors:
a. Known HIV, Hepatitis B or C infections or exposure within previous 12 months
b. High risk sexual behaviors
c. Use of illicit drugs
d. Tattoo or body piercing within previous 6 months
e. Incarceration or history of incarceration
f. Known current communicable disease
g. Risk factors for variant Creutzfeldt-Jakob disease
h. Travel within previous 3 months to countries where risk of infectious diarrhea is elevated
2. Gastrointestinal and systemic comorbidities:
a. History of IBD, irritable bowel syndrome, chronic constipation or chronic diarrhea

b. History of gastrointestinal malignancy or known polyposis
c. Antibiotic use within the previous 3 months
d. Immunosuppressive state or use of immunosuppressive medications
e. History of major gastrointestinal surgery (e.g., gastric bypass)
f. Metabolic syndrome with BMI >30
g. Systemic autoimmunity, e.g., multiple sclerosis, connective tissue disease
h. Atopic diseases including asthma and eczema, eosinophilic disorders of the gastrointestinal tract
i. Chronic pain syndromes, e.g., chronic fatigue syndrome, fibromyalgia

B. Donor Testing

Prospective donors that do not meet any of the exclusion criteria outlined above will then be subjected to a battery of serological and stool-based assays to determine whether common infectious agents are present. All tests will be 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 the results of these tests to the Microbiome Health Research Institute, in compliance with the Health Insurance Portability and Accountability Act (HIPAA). We will redact all personal identifying information from each report when we receive it. We will share copies of these raw diagnostic reports with our clinical partners. In the event that material from multiple donors is provided in a single shipment, results will be provided for all samples along with a file indicating which samples correspond to which donor screens. Documentation will be provided for the battery of tests prior to enrollment of a donor and for tests performed at the end of the collection window. Positive results for any of the following assays will be treated as exclusion criteria for all materials.

1. Serologic testing:
a. HIV antibody, type 1 and 2
b. Hepatitis A (IgM)
c. Hepatitis B panel (HBsAg & HBc [IgM])
d. Hepatitis C (HCV antibody)
e. *Treponema pallidum* screening cascade (EIA with reflex to RPR)
f. Liver function panel
g. Complete blood count
h. C-reactive protein assay
i. HTLV 1 and 2 antibodies
2. Stool testing:
a. EIA assay for *Clostridium difficile* toxins A and B
b. Culture-based assays for common enteric pathogens (including *Salmonella*, *Shigella*, *E. coli*, *Campylobacter* and *Vibrio*)
c. Fecal *Giardia* antigen EIA
d. Fecal *Cryptosporidium* antigen EIA
e. Ova and parasites exam
f. Tri-chrome stain for *Isospora*
g. *Cyclospora* smear test
h. *H. pylori* EIA

C. Collection Window and Donor Monitoring
Donors that meet the above criteria are enrolled to provide material for FMT. Donor material is collected for up to 60 days following the initial screening. During this collection window donors must not violate any of the risk factors identified in Part A above. During a collection window, all material is quarantined until the donor has passed a second battery of serological and stool tests as described in Part B above. Material will be released for clinical use only after the donor has successfully completed both sets of assays (before and after the collection window).

In the event that a donor is recruited to provide additional material beyond an initial 60 day collection window, additional testing will be performed at 60 day intervals. Repeat donors that have not been tested within 60 days will be treated as new donors subject to the same screenings described in Parts A and B above. This ensures that all donors (even long-term participants) are subject to regular health evaluation.

In the event that the donor passes a loose stool or has other symptoms of disease, donors will immediately meet with a healthcare professional to evaluate their continued suitability for participation in our program. If the clinician determines that the donor is not healthy,the donor will be un-enrolled from the program. In the event of such a diagnosis, all material contributed during the preceding collection window will be destroyed. In addition to this qualitative exclusion by clinician's discretion, we will also treat three or more loose stools passed in a 24-hour period as an absolute exclusion criterion, and all material from the affected donor's collection window will be destroyed, regardless of clinical evaluation. Although less frequent passage of loose stool is not medically indicated as a disease and is common among healthy individuals, in the interests of caution we will also destroy material collected near any loose stool movement. All material collected from within 48 hours of two loose stools will be destroyed. All material collected from within 24 hours of a single loose stool will similarly be destroyed.

D. Production and Process Controls
1. The donor deposits stool in a commode, seals the lid, and places the collection container in a plastic bag to serve as secondary containment. Donors receive training to ensure that samples are not contaminated during the collection process.
2. After passage, the sealed sample collection container is immediately transferred from the donor to a qualified technician to process the sample.
3. Samples are transferred to a UV-sterilized biosafety hood as quickly as possible, not to exceed 60 minutes from the time of collection.
4. Within the biosafety hood, the stool is transferred to a sterile filter bag. The filter bag fits around the collection commode entirely,
so there is no risk of material escaping during this transfer process. All stool material is added to the same side of the membrane in the filter bag.
5. An autoclaved dilutant consisting of 12.5% glycerol and a normal saline buffer (0.90% w/v NaCl in water) is added to the filter bag.
6. The sample solution sealed inside the filter bag is then introduced to a homogenizer blender for 60 seconds to mix the materials.

7. Samples are then aliquoted into sterile bottles using sterile, disposable serological pipettes.
8. The bottles are then capped, sealed and frozen immediately at -80°C. Any samples not fully processed and frozen within 120 minutes of passage will be destroyed.
9. Samples are sealed with a tamper evident shrink band as an additional level of containment and to ensure samples are not contaminated or tampered with during storage and distribution.
10. Samples will be delivered to clinicians on dry ice, in double-containment vessels, with temperature indicators to ensure that samples have not thawed during transportation.

Recipient Procedures

Overview. This is an open-label pilot study to assess the safety of FMT in patients with PSC. Additionally, the study will measure the microbiological and clinical impacts of FMT in patients with PSC. We will prospectively enroll 16 adults with PSC after providing written informed consent. If a subject qualifies for the study, they will be asked to stop taking their current PSC medications,which include azathioprine, methotrexate, and ursodeoxycholic acid for 4 weeks before starting the study. This "washout period" allows regular medications to leave the body before subjects begin taking the study drug. The first 10 study participants will receive a single FMT via colonoscopy. The last 6 patients will receive induction followed by monthly maintenance oral capsules for 6 months. Donor Stool and placebo material will be obtained from OpenBiome. OpenBiome, with whom we have previously collaborated with, is a nonprofit 501(c)(3) organization that provides hospitals with screened, filtered, and frozen material ready for clinical use.

For Colonoscopy Group:
Week -2: Screening – potential subjects will undergo the following screening procedures one to two weeks prior to FMT to determine if they meet the recipient selection criteria;

1. Medical record review to confirm diagnosis and treatment history
2. Symptom assessment and physical exam by study physician
3. Harvey Bradshaw Index (HBI) score for patients with Crohn's and the Simple Clinical Colitis Activity Scale for patient with Ulcerative Colitis to determine disease activity if IBD is present
4. Mayo Risk Score
5. PBC 40 Quality of Life evaluation. This is a QoL scale for PBC that has been validated in PSC(15).
6. stool sample to exclude current infections (bacterial culture, ova & parasites, Clostridum difficule toxin)
7. Stool will also be sent for bile salt metabolomics
8. Blood tests (CBC, LFTs, Renal profile, CRP, ESR, bile salt metabolomics)
9. Urine Pregnancy Test
10. Review of MRCPs done for clinical care.

Week -1: Baseline Microbiome – a stool sample will be taken for baseline microbiome profiling.

Week 0: Fecal Transplant - the following baseline assessments will be made in enrolled subjects on day of scheduled FMT;

1. Urine pregnancy test (HCG) for female patients.
2. Disease activity score will be assessed (HBI)
3. PBC 40
4. Stool transplant via colonoscopy
5. Tolerability questionnaire

Weeks 1, 4, 8, 12, 24, 52: Follow-up assessment of clinical scores and adverse event screening
Weeks 1, 4, 8 and 12, 24: stool sample for microbiome analysis and bile salt metabolomics, blood tests for (CBC, LFTs, Renal profile, CRP, ESR, bile salt metabolomics)

Week 52: Final Study Phone Call.

1. Final assessment of outcome of the FMT will be made; disease activity score will be assessed (HBI, PBC 40)
2. Outpatient MRCP will be scheduled.

Week 52: Patient obligations in the study will end at week 52 however the patient's medical record will be followed prospectively for evaluation of LFTs, MRCP and ERCPs done for clinical care through year 5 post-enrollment.

For Capsule Group:

Week -2: Screening – potential subjects will undergo the following screening procedures one to two weeks prior to FMT to determine if they meet the recipient selection criteria;

11. Medical record review to confirm diagnosis and treatment history
12. Symptom assessment and physical exam by study physician
13. Harvey Bradshaw Index (HBI) score for patients with Crohn's and the Simple Clinical Colitis Activity Scale for patient with Ulcerative Colitis to determine disease activity if IBD is present
14. Mayo Risk Score
15. PBC 40 Quality of Life evaluation. This is a QoL scale for PBC that has been validated in PSC(15).
16. stool sample to exclude current infections (bacterial culture, ova & parasites, Clostridum difficule toxin)
17. Stool will also be sent for bile salt metabolomics
18. Blood tests (CBC, LFTs, Renal profile, CRP, ESR, bile salt metabolomics)
19. Urine Pregnancy Test
20. Review of MRCPs done for clinical care.

21. Test swallow of an empty capsule

Week -1: Baseline Microbiome – a stool sample will be taken for baseline microbiome profiling.

Week 0: Fecal Transplant - the following baseline assessments will be made in enrolled subjects on day of scheduled FMT;

1. Urine pregnancy test (HCG) for female patients.
2. Disease activity score will be assessed (HBI)
3. PBC 40
4. Stool transplant via capsule (30 capsule dose)
5. Tolerability questionnaire

Weeks 1:

1. Follow-up assessment of clinical scores and adverse event screening
2. stool sample for microbiome analysis and bile salt metabolomics, blood tests for (CBC, LFTs, Renal profile, CRP, ESR, bile salt metabolomics)

Weeks 4, 8 and 12, 16, 20:

1. 5 Monthly maintance capsule dosing will be done in the clinic under direct observation
2. Follow-up assessment of clinical scores and adverse event screening
3. stool sample for microbiome analysis and bile salt metabolomics, blood tests for (CBC, LFTs, Renal profile, CRP, ESR, bile salt metabolomics)

Week 24:

1. Follow-up assessment of clinical scores and adverse event screening
2. stool sample for microbiome analysis and bile salt metabolomics, blood tests for (CBC, LFTs, Renal profile, CRP, ESR, bile salt metabolomics)

Week 52: Final Study Phone Call.

3. Final assessment of outcome of the FMT will be made; disease activity score will be assessed (HBI, PBC 40)
4. Outpatient MRCP will be scheduled.

Week 52: Patient obligations in the study will end at week 52 however the patient's medical record will be followed prospectively for evaluation of LFTs, MRCP and ERCPs done for clinical care through year 5 post-enrollment.

Primary Physiological Endpoint:

1. Recipient's fecal microbial diversity at 12 weeks after FMT, when compared to baseline using 16s ribosomal RNA.

Primary Clinical Endpoint

1. The primary study end point is them mean change serum liver biochemistries after 3 months of treatment as compared with baseline. Treatment success was defined as an improvement in serum alkaline phosphatase, total bilirubin, alanine aminotransferase (ALT), or aspartate aminotransferase (AST) by 50 % or greater.

Secondary Endpoints

Metabolomics: Bile salt profiles of the samples and associated community structure of the fecal microbiome will be assessed as a measure of the interplay between host and gut microbiota. **Stool and Serum** will be analyzed using the metabolomics platform at the Broad Institute, targeting bile acids. Samples will be sent to the Broad Institute where we will use liquid chromatography tandem mass spectrometry (LC-MS) to measure endogenous bile salts and their metabolite levels in fecal supernatant. Water soluble metabolites will be extracted from feces as described by Saric et al while lipids will be extracted from lypophilized samples using isopropanol. Water soluble metabolites will be measured using ion pairing chromatography and hydrophilic interaction chromatography methods, and lipids and bile acids will be measured using C4 and C18 reversed phase chromatography methods. MultiQuant software (AB SCIEX) will be used for automated peak integration and manual review of peak quality prior to statistical analyses. The GenePattern (Broad Institute) and IPA (Ingenuity Systems) software will be used to analyze and visualize results.

Clinical Endpoints:

1) Mean change in Harvey Bradshaw Index (HBI) and PBC 40 score between week 0 and week 1, 4, 8, and 12. Percentage of patients in clinical remission (those with an HBI score at week 12 <5)
2) Mean change in Mayo Risk Score at week 12 compared to baseline. The Mayo Risk Score (MRS) for PSC is calculated based using the following formula: risk = (0.0295 * (age in years)) + (0.5373 * LN(total bilirubin in mg/dL)) - (0.8389 * (serum albumin in g/dL)) + (0.5380 * LN(AST in IU/L) + (1.2426 * (points for variceal bleeding))

    - If your Mayo Risk Score is less than or equal to 0 then you are in the "low" risk group.
    - If your Mayo Risk Score is greater than 0 but less than 2 then you are in the "intermediate" risk group.
    - If your Mayo Risk Score is greater than 2 then you are in the "high" risk group.

Safety Endpoints: Number and nature of adverse events at week 1, 4, 8, 12 and 24

<u>Treatment with FMT vial colonoscopy:</u>

The treatment for this trial will be a filtered solution of donor fecal microbiota (50 g) homogenized with sterile saline 90 mL. This will be administered as a single topical treatment administered via colonoscopy to the ileo-colonic mucosa. Follow-up after administration will be at 1,4,8,12,24 and 52 weeks

Trial Treatment with FMT via Capsule:

The treatment for this trial will be administration of an induction dose of 30 FMT capsules ingested orally. Treatment will occur after screening.

Subsequent FMT administration will be maintenance capsules of frozen FMT material ingested orally every 4 weeks (30 capsules). All ingestions will be undertaken in the clinic under supervision. A follow-up phone call will be undertaken 4 hours after each ingestion visit to screen for adverse events.

1. Initial dose - filtered solution of donor stool in 30 gelatin capsules at Day 0 (16500µl total).
2. Maintenance dose – thirty capsules of fecal material taken every 4 weeks (550µl of fecal material in each size 00 gelatin capsule)

Capsule preparations are delivered to clinical investigators in double containment shipping vessels packed with dry ice, as with non-encapsulated placebo preparations. Packaging is labeled with the same information included on non-encapsulated preparations, with the addition of a clause instructing clinicians to administer the material to patients within 90 minutes of removal from frozen storage. Patient instructions listed in Appendix 6.

Packaging

Enscapsulated fecal material in freezer safe bottles will be stored in Styrofoam boxes from the Microbiome Health Research Institute at Massachusetts Institute of Technology, as described in their Drug Master File (15543).

Adverse Event Reporting

For this trial, an adverse event (AE) is defined as any untoward medical occurrence in a patient or clinical investigation patient administered a biologic product; such an occurrence does not necessarily have to have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal product whether or not considered related to the medicinal product. A serious adverse event (SAE) is defined as any untoward medical occurrence that results in death, is life threatening, requires hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, is an important medical event 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 other outcomes previously mentioned. Adverse events will be determined by a toxicity grading scale.

The internal DSMB (Appendix 6) will include the following individuals: Dr. Ryou

(Gastroenterology- BWH), Dr. Walter Chan (Gastroenterology – BWH), and Dr. Andrew Courtwright (Pulmonary Critical Care). All events will be reported directly to the DSMB and the Institutional Review Board (IRB) in accordance with Harvard University policy: Investigators will promptly report to the IRB all unanticipated problems involving risks to human subjects or others under Title 21 of the Code of Federal Regulations (21 CFR) part 56 (Institutional Review Boards), part 312 (Investigational New Drug Application), and part 812 (Investigational Device Exemptions). Harvard Medical School policy is consistent with guidance set forth by the Office for Human Research Protections (OHRP) (presented January 15, 2007)
http://www.hhs.gov/ohrp/policy/AdvEvntGuid.htm and the FDA (presented January 14, 2009)
http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126572.pdf when determining what related events require review by the Institutional Review Board.
Any serious AEs also will be evaluated by the DSMB for review and determination of whether the trial should continue.
All subjects will be followed for an additional six months following the end of their treatment. Subjects that did not experience a AEs or SAEs will receive a telephone call to monitor their current symptoms and quality of life. For subjects that did experience a AE or SAE that was found to be connected to the FMT treatment, we ask that they return to our medical center for an exam with their gastroenterologist. All AEs will be followed to resolution or stabilization by the study physician.

Stopping Criteria
Adverse events will be monitored at 0, 1, 4, 8, 12, 24 and 52. 12 weeks after FMT by symptom diaries and direct patient interviews. A data safety and monitoring board (DSMB, will review all patient data to ensure optimal patient safety and precautions for subjects with CD treated with FMT. A severity grade for adverse events (see Appendix) and subject symptom diary used (See Appendix). The DSMB will meet a minimum of 3 times: after treatment of the first 5 subjects and after treatment of 10 subjects and then after the first 3 capsule patients. Study will be stopped under the following circumstances:

1. Detection of new pathogenic intestinal infection in stool samples in any patient treated.
2. Any unexpected serious adverse event that that DSMB determines is of significant clinical impact, and probably related to FMT.

Accountability Procedures for the Investigational Product
The location, volume and number of FMT donor solutions will be maintained in a log by the research team. The principal investigator will be responsible for accurate record and tracking of all FMT solutions.

Medications Permitted
During the follow-up period after FMT, subjects may remain on their co-existing medications at stable doses. Rescue pathway for IBD subjects with worsening disease during the study period to be determined by the clinician investigator and based on standard of clinical practice.

This may include, but is not limited to the following;

i. Oral steroids (if not already receiving them or failing them)
ii. Rectal therapy (5-ASA or steroids)
iii. IV steroids
iv. Hospitalization
v. Anti-TNF therapy
vi. Surgery

VI. Biostatistical Analysis:

Spontaneous biochemical normalization occurs in less than 5% of patients with PSC. A response by natural history would be 0/5. This is an exploratory study as relatively little is known about the microbiomes of PSC patients. Thus, an objective of our study will be to characterize variability in PSC microbiomes to aid in estimating effect sizes for designing larger studies powered to detect differences between the microbiomes of PSC patients or to find associations between microbiome data and bile acid profiles.

Preliminary data from the colonoscopy cohort revealed that global engraftment in several patients decline during the first month post-FMT. While all patients' microbiomes increased in diversity and similarity to the donor at one week post-FMT, diversity declined in 4 out of 7 patients between one and four weeks post-FMT. Three of these patients also showed a decrease in microbiome similarity to the donor community between one and four weeks post-FMT. For this reason we have decided to test maintenance dosing as well at monthly intervals with capsules prior to a placebo controlled RCT.

VII. Risks and Discomforts:

Fecal Microbial Therapy:

Known Risks of FMT

- Altered bowel pattern (diarrhea, constipation)
- Cramping
- Belching

Potential Risks of FMT

- Transmission of pathogenic bacteria, viruses, fungi
- Transmission of allergens
- Alteration in intestinal metabolism

Privacy and Confidentiality:

This study involves the collection of personal health information. Accidental release of personal health information is a risk of participation in this study. Measures will be taken to protect the confidentiality of all subjects' information. These measures include keeping all information collected about the subjects' confidential, keeping information in locked rooms, and having physicians who are directly involved with a subject's clinical care involved in the study.

Colonoscopy:

Standard potential risks of the endoscopy procedure include discomfort, gastrointestinal bleeding either related or unrelated to biopsies, intestinal perforation, altered bowel habit. Complications of IV conscious sedation during the procedure include respiratory arrest, medication reactions, and aspiration.

Venipuncture:

Risks of having blood drawn include pain, bruising, or infection.

Pregnancy

The risks to fetuses and women who are pregnant are unknown.

VIII. Potential Benefits:

The potential benefits include:

- Restoration of fecal diversity
- Reduction in intestinal inflammation
- Improvement in clinical symptom scores

As this is a pilot study, it is difficult to quantify the expected benefits.

IX. Monitoring and Quality Assurance:

The Principal Investigator, Dr. Joshua Korzenik, will assure the validity and integrity of the data and adherence to the IRB-approved protocol.

Study staff will review completed CRF's before each visit to ensure completeness of previous entries. Entries that need clarification will be reviewed by the PI, and the subject and/or treating gastroenterologist will be consulted if needed.

Appendix 1.

Harvey-Bradshaw Index for Crohn's disease

Harvey-Bradshaw Index
General Well-Being (0=very well, 1=below par, 2=poor, 3=very poor, 4=terrible)
Abdominal Pain (0=none, 1=mild, 2=moderate, 3=severe)
Number of Liquid Stools per Day
Abdominal Mass (0=none, 1=dubious, 2=definite, 3=definite & tender)
Complications (1 per item)
Arthralgia
Uveitis
Erythema Nodosum
Apthous ulcers
Pyoderma Gangrenosum
Anal fissue

New Fistula
Abscess
TOTAL SCORE

Appendix 2: Simple Clinical Colitis Activity Index:

Ulcerative Colitis (SCCAI):

1) **Bowel frequency (day)**
   - ⓿ 1 to 3
   - ① 4 to 6
   - ② 7 to 9
   - ③ more than 9
2) **Bowel frequency (night)**
   - ⓿ None -->0
   - ② 1 to 3
   - ③ 4 to 6
3) **Urgency of defecation**
   - ⓿ None
   - ① Hurry
   - ② Immediately
   - ③ Incontinence
4) **Blood in the stool**
   - ⓿ None
   - ① Trace
   - ② Occasionally Frank
   - ③ Usually Frank
5) **General wellbeing**
   - ⓿ Very well
   - ① Slightly below par
   - ② Poor
   - ③ Very poor
   - ④ Terrible
6) **Extraintestinal manifestations of IBD (check all that apply; 1point for each):**
   - □ Arthralgia
   - □Erythema nodosum
   - □ Uveitis
   - □ Pyoderma gangrenosum

Appendix 3. Mayo Risk Score:

Mayo Risk Score (MRS) for PSC is calculated based using the following formula: risk = (0.0295 * (age in years)) + (0.5373 * LN(total bilirubin in mg/dL)) - (0.8389 * (serum albumin in g/dL)) + (0.5380 * LN(AST in IU/L) + (1.2426 * (points for variceal bleeding)).

Appendix 4:

Grading and Attribution Methods for Adverse Events

Grading Scale

0 No adverse event or within normal limits
1 Mild adverse event – did not require treatment
2 Moderate adverse event – resolved with treatment
3 Severe adverse event – resulted in inability to carry on normal activities and required professional medical attention
4 Life threatening or disabling adverse event
5 Fatal adverse event

Attribution Scale

Definite: The adverse event is clearly related to the study drug
Probable: The adverse event is likely related to the study drug
Possible: The adverse event may be related to the study drug
Unlikely: The adverse event is doubtfully related to the study drug
Unrelated: The adverse event is clearly not related to the study drug

Appendix 5: Record of Side Effects

Fecal Microbiota Transplantation - Record of Side Effects

This diary is one way researchers will get information from you regarding any possible problems or side effects in this study.

- **What you are going to do is simple.** Just keep a record of any unpleasant thing that happens to you while you are in the study, before, during, and after we have completed the stool transplant. We even want you to record things that do not seem to be part of the stool therapy, at all.
- **When do you start? When do you end?** You will record one entry 1-week prior and on the day of the transplant. You will then complete one entry per day for the first week following the treatment and then once a week thereafter for 12-weeks.
- **What do you look for? What do you report?** Any symptom or problem whether or not it may be from the medicine, stool therapy. This could include: fever, abdominal pain, a big belly, lots of gas, diarrhea, nosebleeds, and anything else you know is not quite right.
- **What will you do?** In the first 7 days after the transplant, you will report some of the specific things that have bothered you by checking the boxes in the diary (see below). You can also write any other problems that you may have had during that time. **Additionally, you will record your temperature once for each day for the first 7 days after the transplant, unless you feel hot. If you feel hot, please take your temperature again.** Please make sure to record the highest temperature taken that day if you take it more than once.

Continue to record any problem up to 6 months after the transplant.

How will you record it? Like this…

EVENT	DATE OF ONSET	INTENSITY	ACTION TAKEN	MEDICATION	DATE RESOLVED
Fever	3/1/12	3	Missed 2 days of school	Tylenol-200mg	3/3/12
Sore throat	3/5/12	1	None	None	3/6/12

OTHER SYMPTOMS
Record each symptom at its ***worst*** level for each day.
For example, a sore throat that starts at 'Grade 1" but increases to 'Grade 2' should be recorded as 'Grade 2".

Examples of Grades:
Grade 1 – Mild: I noticed the symptom. It did not keep me from doing my normal activities.
Grade 2 – Moderate: I noticed the symptom and it kept me from doing some of my normal activities.
Grade 3 – Severe: I really noticed the symptom and it kept me from doing activities that I wanted or needed to do.
Grade 4 – Very severe: The symptom made me unable to perform basic self-care functions such as washing myself **OR** medical or surgical intervention was needed to prevent serious consequences.

Subject ID:_____

Date:____/____/_____ Check here is no side effects present. ☐
Highest temperature of the day:_____^{o}F Total Number of Stools:_____

Check if symptom present	Event	Date of Onset	Intensity	Action taken	Medications	Date Resolved
☐	Fever					
☐	Abdominal Pain/ Stomachache					
☐	Abdominal distension /bloating					

☐	Flatulence/ excess gassiness					
☐	Diarrhea					
☐	Nausea/Vomiting					
☐	Blood in Stool					
☐	Other 1					
☐	Other 2					
☐	Other 3					

Grade 1 – Mild: I noticed the symptom. It did not keep me from doing my normal activities.
Grade 2 – Moderate: I noticed the symptom and it kept me from doing some of my normal activities.
Grade 3 – Severe: I really noticed the symptom and it kept me from doing activities that I wanted or needed to do.
Grade 4 – Very severe: The symptom made me unable to perform basic self-care functions such as washing myself **OR** medical or surgical intervention was needed to prevent serious consequences.

Appendix 6:

Charter, Data and Safety Monitoring Board for
Fecal Microbiota Transplantation (FMT) for the treatment of Primary Sclerosis Cholangitis
Version Date: January 2015

1. Introduction

This Charter is for the Data and Safety Monitoring Board (DSMB) for the study "Fecal Microbiota Transplantation (FMT) for the treatment of Primary Sclerosis Cholangitis"

The DSMB will consist of a team of clinical researchers who are unaffiliated with this project.
These individuals will not be investigators in this study and will be experienced in conducting and interpreting clinical trials; they will review the efficacy and safety endpoints at the below stated time points. There will be 3 members on the DSMB consisting of at least two gastroenterologists and one infectious disease specialist. The members of the DSMB are:

Dr Walter Chan (Gastroenterology- BWH)
Dr. Marvin Ryou (Gastroenterology – BWH)
Dr. Andrew Courtwright (Pulmonary/Critical Care- BWH) (Chair)

The Charter is intended to be a living document. The DSMB may wish to review it at regular intervals to determine whether any changes in procedure are needed.

2. Responsibilities of the DSMB

The DSMB is responsible for safeguarding the interests of study participants, assessing the safety and efficacy of study procedures, and for monitoring the overall conduct of the study.

The DSMB is an independent group advisory and is required to provide recommendations about starting, continuing, and stopping the study. In addition, the DSMB is asked to make recommendations, as appropriate, to the about:

- Selection, recruitment, and retention of participants
- Adherence to protocol requirements
- Completeness, quality, and analysis of measurements
- Amendments to the study protocol and consent forms
- Participant safety
- Notification of and referral for abnormal findings

3. Scheduling, Timing, and Organization of Meetings

Data and Safety Monitoring Meetings

The DSMB will meet a minimum of 3 times: after treatment of the first 5 subjects, after treatment of 10 subjects and then after the first 3 capsule patients. In addition the DSMB may convene additional meetings if necessary to ensure the ongoing monitoring and safety of the subjects treated with FMT. Any serious AEs also will be evaluated by the DSMB for review and determination of whether the trial should continue. An example of the DSMB meeting minutes are at the end of this document. The study will not proceed at each of these time points until the DSMB gives approval to continue.

Safety Reporting

In accordance with applicable policies of the BWH Institutional Review Board (IRB), the investigator-sponsor will report, to the IRB, any observed or volunteered Unanticipated Problem that is determined to be 1) unexpected); 2) related or at least possibly related to study participation; and 3) suggests that the research places subjects or others at a risk of unknown harm or addition/increased frequency of harms (including physical, psychological, economic, legal, or social harm) than was previously known or recognized. Unanticipated problems may be adverse events, protocol deviations, noncompliance or other types of problems, but MUST meet all of the criteria listed above. Unanticipated problem reports will be submitted to the IRB in accordance with the respective IRB procedures.

Applicable unanticipated problems will be reported to the IRB as soon as possible and, in no event, later than 10 calendar days following the investigator-sponsor's receipt of the respective information. For Internal Fatal/Life-Threatening Unanticipated Problems, the PI should notify the IRB Chair by phone immediately and consider voluntarily halting subject enrollment.

Follow-up information to reported adverse event will be submitted to the IRB as soon as the relevant information is available. If the results of the investigator's follow-up investigation show that an adverse event that was initially determined to not require reporting to the IRB does, in fact, meet the requirements for reporting; the investigator will report the unanticipated problem to the IRB as soon as possible, but in no event later than 10 calendar days, after the determination was made.

4. Grading and Attribution Methods for Adverse Events

Grading Scale

6 No adverse event or within normal limits
7 Mild adverse event – did not require treatment
8 Moderate adverse event – resolved with treatment
9 Severe adverse event – resulted in inability to carry on normal activities and required professional medical attention
10 Life threatening or disabling adverse event
11 Fatal adverse event

Attribution Scale

Definite: The adverse event is clearly related to the study drug
Probable: The adverse event is likely related to the study drug
Possible: The adverse event may be related to the study drug
Unlikely: The adverse event is doubtfully related to the study drug
Unrelated: The adverse event is clearly not related to the study drug

Data and Safety Monitoring Meeting Minutes

Date:

Title of Protocol/IRB Number:

Principal Investigator/Designee:

Recommendations:

□ **Continue the trial without modification**

□ **Accrual:**

□ Recommend study be closed because of slow accrual

□ Continue to monitor study, but consider closure because of slow accrual

□ **Recommend study is amended/changed:**

□ For patient safety reasons

□ Rate of adverse events

□ Early stopping of inferior therapy

□ To extend accrual because of an event rate slower than expected

□ **Other:** _____

Signature/Principal Investigator or Designee:

_____

DSMB meetings will be held at the Brigham and Women's Hospital. The purpose of the first meeting is to review and discuss this charter and process.

The DSMB also will review adverse event data, other safety data, quality and completeness of study data, and enrollment data at each meeting to ensure proper trial conduct. At intervals, as noted above, the DSMB will also review formal interim analyses of the primary end point.

5. Reports of DSMB Deliberations

- Initial summary: The Director or designee will review this summary and approve or disapprove the recommendation(s), or request additional information. The recommendations will then be sent to the DCC, and the clinical investigators.

- Action plan: If the DSMB's recommendations require significant changes or follow-up, the BWH IRB staff will prepare an action plan outlining the steps required to implement the recommendations.

- Formal minutes: The DSMB Chair is responsible within 30 days of the meeting or call to present minutes to the IRB. These minutes are subject to FOIA requests and are prepared accordingly to summarize the key points of the discussion and debate, requests for additional information, response of the investigators to previous recommendations, and the recommendations from the current meeting. These minutes will be reviewed by IRB staff, key study personnel before being forwarded to the DSMB Chair for final review and approval. The DSMB Chair may sign the minutes or indicate approval electronically via email. Then, the minutes are sent to the BWH IRB for approval. Subsequently, the minutes are sent back to the IRB and the relevant investigators, and included in the materials for the subsequent DSMB meeting to be approved by voice vote at that meeting. Once they have been voted and approved by the Board, they are considered Final.

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