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Study Title:

Fecal MicrobiotaTransplantation in Cirrhosis and Hepatic Encephalopathy

IND #18578

Date: October 14, 2020

Amendment 4

Protocol Number: BAJAJ0024

Product: Fecal Microbiota Preparations vs Placebo From Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota.

Proposed Indication: Cirrhosis and Hepatic Encephalopathy

SCIENTIFIC BACKGROUND

Indication: Cirrhosis and hepatic encephalopathy

Study Objectives: To evaluate the safety and tolerability of fecal transplant in patients with cirrhosis and hepatic encephalopathy

Rationale and Supporting Evidence:

Hepatic encephalopathy affects 30-45% of patients with cirrhosis and adversely affects survival in these patients[1]. The mainstay of treatment for hepatic encephalopathy (HE) has long been the manipulation of the gut flora through antibiotics, prebiotics or probiotics[2]. The current first and second line therapies for HE in the US are lactulose and rifaximin respectively that uniquely act within the confines of the gut lumen with encouraging clinical results. However, there is a subset of patients with HE that continues to recur despite being on both treatments[3]. This patient group is at a higher risk of poor outcomes because HE has now been removed from liver transplant priority and multiple episodes of HE can result in cumulative brain injury which may be irreversible[4]. Therefore, the prevention of recurrent HE is an important therapeutic goal. Our group and other reports have shown that patients with HE and cirrhosis are more likely to have overgrowth of potentially pathogenic bacterial taxa such as *Enterobacteriaceae* and reduction of autochthonous species such as *Lachnospiraceae* and *Ruminococcaceae* in the stool and the colonic mucosa[5-8]. This has been linked to poor performance on cognitive tests that are a hallmark of HE and with increased systemic inflammation in these patients[6, 7].

Therefore, a gut-based therapeutic option that can potentially improve the recurrence rate and the overall prognosis is needed. Fecal transplant has been shown to be

effective in conditions with predominant gut-bacterial overgrowth or alteration such as recurrent *Clostridium difficile* and inflammatory bowel disease[9]. Safe protocols have been developed across the world and studies are being performed in the US under FDA-monitored INDs. Limitations to performing fecal transplant include identifying and screening appropriate donors, which is time consuming and costly, with the cost typically falling to the patient or donor as the required screening is generally not covered by insurance. For this reason, we had worked with OpenBiome and have now partnered with Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota, whose protocols are used by OpenBiome. We have obtained their collaboration towards performing this FMT by cross-referencing of their DMF (Drug Master File).

Our preliminary data suggest that a one-time administration of an FMT-enema using a rationally-selected donor is safe in patients with cirrhosis and recurrent HE. However, given the small bowel overgrowth and the predominantly small bowel location for bacterial translocation in cirrhosis, which is out of the reach of an enema, an upper GI route for FMT needs to be explored. The FMT capsule supplied by Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota acts on the small and large intestine and is available for *C. difficile*. It is potentially more acceptable to patients for repeated administrations and in cirrhosis has the advantage of acting on the small bowel in addition to the large bowel. We will be using fecal material from all available donors in the University of Minnesota donor pool, in consultation with facility scientists, and as noted in cross-referenced IND #15071. All of their donor have high relative abundance of taxa in their stool such as *Lachnospiraceae* and

Ruminococcaceae. In our published experience, a single enema from a rationally-derived donor was associated with significantly lower total and HE-related hospitalizations compared to patients who were randomized to standard of care, with a stable long-term course over >1 year[10]. Our data show that FMT was associated with favorable changes in fecal bile acid (BA) profile with a decrease in proportions of fecal secondary BAs, conjugated BAs and increase in sulfated BAs, indicating a healthier milieu. We also have preliminary data defining the safety of oral FMT capsules in patients with cirrhosis and HE in a current trial led by us[11]. *The use of combined oral and rectal routes of FMT, which can potentially alleviate both small bowel and colonic translocation are likely to be better than either alone*.

Details of the team's experience with FMT in cirrhosis using enema[10]:

In the trial conducted by us under IND 16452, we have recruited 20 subjects with cirrhosis and recurrent HE (the same population proposed for this trial). All subjects completed the 5-month follow-up without SAEs that were associated with FMT. The baseline subject characteristics are:

	No-FMT group (n=10)	FMT group (n=10)	P value
Age	62.9±9.8	64.5±5.1	0.65
Gender (Men/Women)	10/0	10/0	1.0
Race (Caucasian, African-American, Hispanic, others)	6/3/1/0	7/2/1/0	0.9
MELD score	13.2±3.7	12.0±2.9	0.36

None of the subjects reached a grade of 3 or change in >2 of any of their GI symptoms throughout the study. Similarly, none of the subjects developed clinically relevant hepatic encephalopathy or significant changes in liver chemistries or labs (WBC

>12,000 or <1000/ml, MELD >8 increase, AST/ALT/ALK>10 times ULN). As shown in the table below, there was a significant improvement (reduction in the MELD score) in the FMT group compared to the no-FMT group at day 1 post-FMT (day 6) and day 30 post-FMT (day 35) compared to baseline. No changes in the WBC count were observed.

	No-FMT group (n=10)	FMT group (n=10)	P value
MELD score			
Day 0 minus day 6	0.6±1.4	-1.5±1.4	0.004
Day 0 minus day 12	0.4±1.3	-0.2±1.3	0.3
Day 0 minus day 35	1.2±2.4	-0.9±1.7	0.04
Serum WBC count			
Day 0 minus day 6	-0.1±0.6	0.1±1.2	0.60
Day 0 minus day 12	-0.53±0.9	0.2±1.0	0.10
Day 0 minus day 35	0.22±0.5	0.2±0.9	0.95

There was a significant reduction in hospitalizations and improvement in brain function in patients randomized to FMT+ antibiotics compared to those randomized to SOC[10].

Effect of FMT on antibiotic-associated microbial diversity and function collapse[12]:

Since the trial above had antibiotics followed by FMT, we could study the impact of antibiotics on the microbial function (bile acid metabolism, short-chain fatty acid production) and microbiota diversity. There was a collapse of microbial diversity post-antibiotics that recovered after the FMT. In addition, after antibiotics, there was a reduction in deconjugation, desulfation and conversion of primary to secondary bile acids in the stool. These were reversed with FMT. Most importantly, the collapse in beneficial short-chain fatty acid production was reversed by one FMT in this population.

Experience with oral FMT in *C. difficile* and in cirrhosis:

In studies of capsules and nasojejunal/nasogastric infusion of the fecal contents in *C. difficile*, there was an excellent safety profile without serious adverse events

(Youngster et al JAMA 2014, Youngster et al Clin Infect Dis 2014). A pooled analysis of data from trials comparing oral vs. colonoscopic administration showed that both routes were safe and effective for C. difficile (Postigo et al Infection 2012).

There has been one study in the literature of nasojejunal infusion of fecal transplant from several donors in patients with severe alcoholic hepatitis, most of whom have cirrhosis. This case series also demonstrated a good safety profile and was able to show a survival benefit compared to historical controls (Phillips et al Clin Gastroenterol Hep 2016).

The team's experience with FMT in cirrhosis using capsules:
Fecal transplant using the oral capsule route is safe: In a current ongoing safety trial, which is powered for 20 individuals (10 in placebo and 10 with FMT capsules using a rational donor) under FDA IND, we have currently enrolled all 20 patients (10 in placebo and 10 to FMT)[11]. All 10 FMT patients have tolerated the procedures and do not have any safety signal compared to the ones in placebo group. They have all safely undergone safety capsule testing, swallowed all the oral capsules required for placebo or FMT without any issues[11]. This study is being performed under an approved FDA IND 17239 and is a phase I study of capsules. There is good background information that this will be safe and well-tolerated, but we will aggressively monitor every patient as outlined below.

Concentration of bacteria in capsules and enemas in the oral and enema preparations used for the studies above:

CFUs per mL of Capsules G3 Microbial Emulsion Matrix

		Mean	St Dev
Aerobes	**Fresh**	2.53E+05	1.76E+05
	1 freeze-thaw	4.30E+05	4.43E+05
Anaerobes	**Fresh**	5.80E+07	3.96E+07
	1 freeze-thaw	6.20E+07	5.29E+06

CFUs per mL of stool homogenized 1:1 (w/v) with buffer containing 12.5% glycerol and 0.9% saline, after one freeze-thaw cycle

		per mL
Aerobes		4.53E+06
Anaerobes		1.47E+09
Spores		2.00E+06

These concentrations will be used for the upcoming trial to ensure that the material concentrations in our older trials are expanded.

- We anticipate providing 5 capsules at a time for the oral FMT and 60ml of the FMT enema.
- The placebo for the oral FMT will be made using identical capsules as the active drug, except it will contain 10%/weight trehalose (the cryoprotectant used in preparation of freeze-dried microbiota) and the rest carboxymethylcellulose. Since the capsules are not transparent and have no specific smell or taste, there should be no difficulty in maintaining double blinding.
- The placebo for the liquid formulation is non-bacteriostatic, sterile phosphate buffered saline containing 10% USP grade glycerol. Each dose will be packaged in individual cryobags, just as the active drug.

Study Design

Overall objective: *To determine the effect of dual oral and rectal administration of FMT from a rational donor on clinical outcomes (hospitalizations, brain function, quality of life) and host-microbiota interactions (microbial composition and bile acid composition with systemic and intestinal inflammation), compared to single route of administration and placebo, along with a second oral capsular FMT vs placebo administration in patients with cirrhosis and HE using a randomized, phase II clinical trial.*

Design overview:
Four groups of outpatients with cirrhosis will be randomized using random sequence generator into placebo and FMT groups and followed for 6 months under an FDA IND double-blind clinical trial.

Group 1: Dual oral and rectal FMT,
Group 2: Oral FMT and rectal placebo,
Group 3: Oral placebo and rectal FMT and
Group 4: Oral and rectal placebo

A repeat oral capsular FMT vs placebo based on the group assignments above will be provided to every subject in at day 30.

The primary aim is: *To evaluate the safety and tolerability of fecal transplant through rectal and oral capsular routes in cirrhosis and HE from a liver disease and symptom standpoint using analysis of serious adverse events, especially HE recurrence, in a placebo-controlled trial.*

Secondary aims are between the groups*:*

1. *Safety and tolerability of FMT compared to placebo*
    a. *Frequency, severity and relatedness of solicited and unsolicited AEs through day 30*
    b. *Occurrence of new potentially transmitted infections through six-months post FMT*
    c. *Occurrence of new onset or significant worsening of chronic medical conditions post-FMT*
2. *To define the changes in microbiota composition and function of the stool microbiota after FMT compared to pre-FMT baseline and donor compared to placebo*

3. *To evaluate changes in systemic inflammatory cytokines and endotoxin, quality of life and cognitive function compared to pre-FMT baseline and compared to placebo*
4. *To determine occurrence of new onset or significant worsening of chronic medical conditions through six months post-FMT and compared to placebo*
5. *To evaluate the changes in the microbiota after the second oral FMT vs. placebo compared to baseline values*

Inclusion/Exclusion Criteria:

Inclusion criteria:

A. Cirrhosis diagnosed by either of the following in a patient with chronic liver disease
    a. Liver Biopsy
    b. Radiologic evidence of varices, cirrhosis or portal hypertension
    c. Laboratory evidence of platelet count <100,000 or AST/ALT ratio>1
    d. Endoscopic evidence of varices or portal gastropathy
    e. Fibroscan values suggestive of cirrhosis
B. On treatment for hepatic encephalopathy (patient can be on lactulose and rifaximin)
C. Age: at least 21 years of age
D. Able to give written, informed consent (demonstrated by mini-mental status exam>25 at the time of consenting)
E. Women of child bearing potential must agree to use effective contraception for the duration of the study and for 10 days prior and 30 days after the study
F. Negative pregnancy test in women of childbearing age

Exclusion criteria:

A. MELD score >22
B. WBC count <1000 cells/mm3
C. Platelet count < 25,000/mm3
D. TIPS in place for less than a month
E. Currently on antibiotics apart from rifaximin
F. Infection at the time of the FMT (diagnosed by blood culture positivity, urinalysis, paracentesis as needed)
G. Hospitalization for any non-elective cause within the last 1 month
H. Patients who are aged >75 years
I. Patients who are pregnant or nursing (will be checked using a urine pregnancy test)
J. Patients who are incarcerated
K. Patients who are incapable of giving their own informed consent

L. Patients who are immuno-compromised due to the following reasons:
    a. HIV infection (any CD4 count)
    b. Inherited/primary immune disorders
    c. Current or recent (<3 mos) treatment with anti-neoplastic agent
    d. Current or recent (<3 mos) treatment with any immunosuppressant medications [including but not limited to monoclonal antibodies to B and T cells, anti-TNF agents, glucocorticoids, antimetabolites (azathioprine, 6-mercaptopurine), calcineurin inhibitors (tacrolimus, cyclosporine), mycophenolate mofetil]. Subjects who are otherwise immunocompetent and have discontinued any immunosuppressant medications 3 or more months prior to enrollment may be eligible to enroll.
M. Patients on renal replacement therapy
N. Patients with untreated, in-situ colorectal cancer
O. Patients with a history of chronic intrinsic GI diseases such as inflammatory bowel disease (ulcerative colitis, Crohn's disease or microscopic colitis), eosinophilic gastroenteritis or celiac disease
P. Major gastro-intestinal or intra-abdominal surgery in the last three months
Q. Patients who have received FMT within last 6 months

Other Exclusion Criteria:

Enema-related
(1) Platelet count<25,000
(2) Grade IV hemorrhoids

Safety-related:
(1) Dysphagia
(2) History of aspiration, gastroparesis, intestinal obstruction
(3) Ongoing antibiotic use (except for Rifaximin)
(4) Severe anaphylactic food allergy
(5) Allergy to ingredients Generally Recognized As Safe in the FMT capsules (glycerol, sodium chloride, hypromellose, gellan gum, titanium dioxide, theobroma oil)
(6) Adverse event attributable to prior FMT
(7) ASA Class IV or V
(8) Pregnant or nursing patients
(9) Acute illness or fever within 48 hours of the day of planned FMT
(10) Immunocompromised due to medical conditions
(11) Probiotics use within the last 48 hours of the day of planned FMT
(12) Any condition that the physician investigators deem unsafe, including other conditions or medications that the investigator determines puts the participant at greater risk from FMT

Intervention

Dosage:
FMT Procedure #1

5 FMT capsules or 5 placebo capsules *and*

60 ml FMT enema vs 60 ml placebo

FMT Procedure #2
5 FMT capsules or 5 placebo capsules

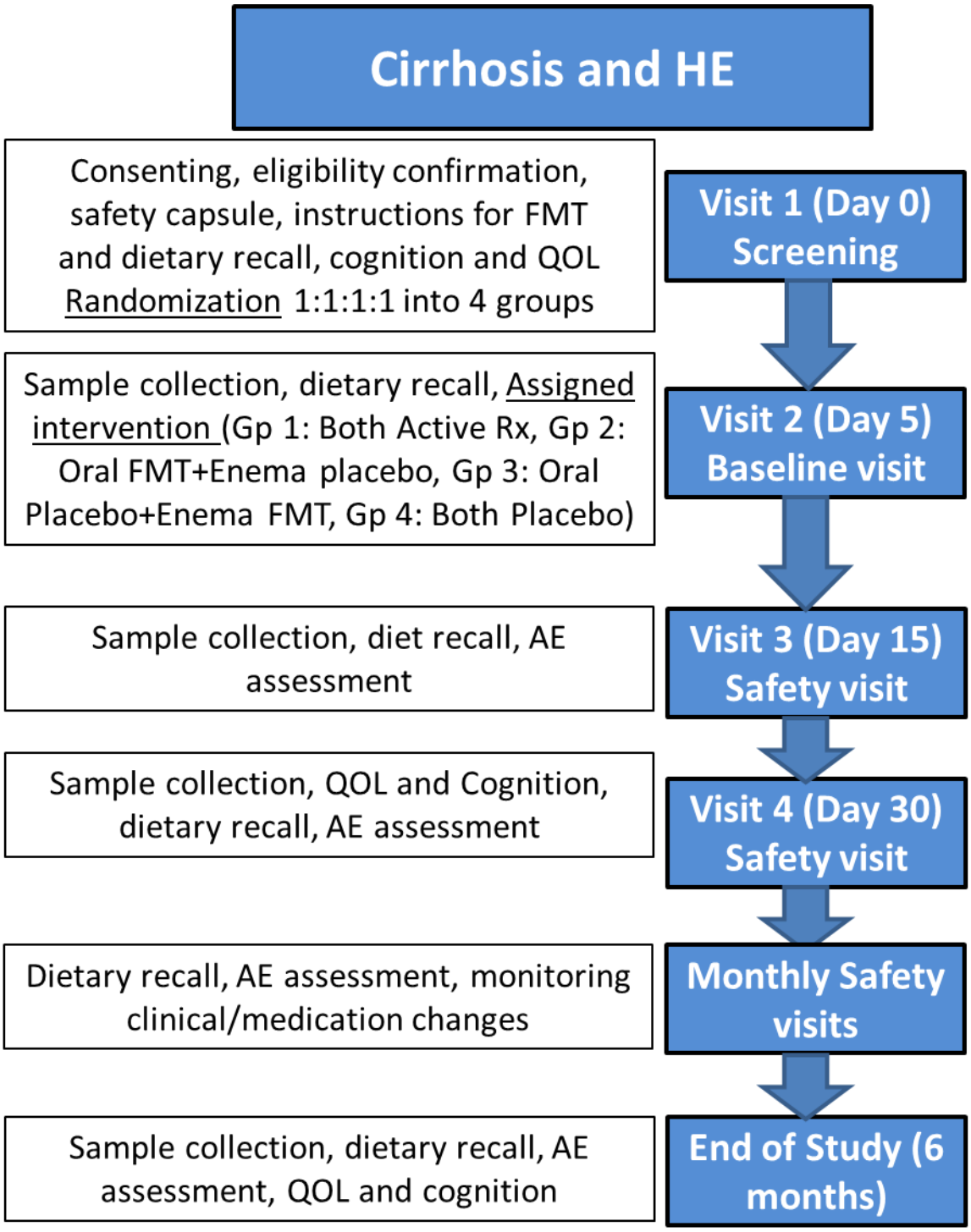
Route of Administration: Oral and enema for baseline, and oral only for Day 30

Timing of administration: Twice (at baseline and Day 30)

Procedures:
Once patients are randomized 1:1:1:1 into Group 1: Dual oral and rectal FMT, Group 2: Oral FMT and rectal placebo, Group 3: Oral placebo and Rectal FMT and Group 4: Oral and rectal placebo, both will be followed over 6 months with intensive follow-up in the first 30 days.

At the end of one month, we will provide another oral-route FMT or placebo to all subjects based on their initial assignment (Groups 1 and 2 will get oral FMT 5 capsules again, and Groups 3 and 4 will get 5 capsules of placebo again). All subjects will be followed with monthly visits till 6 months to assess SAEs.

An overview is shown below in Figure 1 (extended through 6 months)


Cirrhosis and HE
Consenting, eligibility confirmation, safety capsule, instructions for FMT and dietary recall, cognition and QOL
Randomization 1:1:1:1 into 4 groups
Visit 1 (Day 0) Screening
Sample collection, dietary recall, Assigned intervention (Gp 1: Both Active Rx, Gp 2: Oral FMT+Enema placebo, Gp 3: Oral Placebo+Enema FMT, Gp 4: Both Placebo)
Visit 2 (Day 5) Baseline visit
Sample collection, diet recall, AE assessment
Visit 3 (Day 15) Safety visit
Sample collection, QOL and Cognition, dietary recall, AE assessment
Visit 4 (Day 30) Safety visit
Dietary recall, AE assessment, monitoring clinical/medication changes
Monthly Safety visits
Sample collection, dietary recall, AE assessment, QOL and cognition
End of Study (6 months)

Procedures:

Visit 1: After initial review of the medical record is completed and informed consent is obtained, potentially eligible patients will be interviewed and their questions about the study will be answered by the study team. Vital signs (including heart rate, blood pressure, respiratory rate, temperature) and weight will be measured. Physical exam will be performed. We will administer the Mini-mental status exam (MMSE)[13 4]. For those <25, we will deem them not able to give consent and their clinicians will be notified. For the rest we will obtain blood and urine for further confirming eligibility (MELD score and complete blood count for platelet count/ANC). Patients will also be administered a safety test capsule orally to ensure they can swallow it without any issues. If the subject is eligible and can swallow the capsules and otherwise fits criteria, they will be randomized into one of the four groups (Groups 1-4, Table 2) and a date for visit 2 will be generated. A dietary recall will be performed and the patients will be asked to stay on the same diet throughout the trial. We will perform the validated lactulose/mannitol testing at baseline. Patients will undergo brain function tests and quality of life assessment during this visit to establish their baseline. Patients will be given a Stool Collection Kit to bring in the day of the baseline visit (Visit 2). They will also be given a symptom diary and instructions to complete it.

Table 2: Patient Groups

Group 1: Oral and enema FMT, Group 2: Oral FMT+ enema placebo, Group 3: Oral placebo + enema FMT, Group 4: Both placebo

Visit 2 After re-confirming eligibility, the patient will be seen within 3±5 days of visit 1. Vital signs and weight will be measured. Targeted physical exam will be done based on any symptoms.
Their symptom diaries and dietary recall will be examined. Stool, blood and urine will be collected and processed (see section below), including urine pregnancy testing for women of child-bearing potential. After this we will administer the assigned intervention by enema and oral capsules. This has been tolerated without any issues in our current protocols. These will be dispensed from our VAMC Investigational Pharmacy, which has been handling these for our prior and current FMT trials. The

following will be recorded in all FMTs (dose, Unit ID/Lot# of each treatment, Expiration Date and storage condition) The patient will be observed for a further 1 hour and then given another stool kit to bring back 10±5 days from this visit (Visit 3). All subjects will be given contact information for the study team to call in case any issues arise.

Optionally, subjects may elect to combine Visits 1 and 2 to minimize potential COVID exposure. In this case, stool collection kit would be mailed to subject before visit, and the staff would provide further instructions to subject on their use. The subject may determine whether he/she will collect a stool sample to bring to the study clinic visit. In the event the subject decides not to participate in the study, the stool collection kit would be discarded.

Visit 3: We will see the patient in the McGuire VAMC at day 15, at which point, dietary recall will again be performed along with changes in GI or other adverse events using interview and review of the symptom diaries. If no adverse event (AE) or serious adverse events (SAE) occur (standard definitions and in the human subjects protection section per FDA specifications), the subject will continue in the study. Vital signs and weight will be measured. Targeted physical exam will be done based on any symptoms. The stool sample, blood and urine will be collected for microbiota and safety analysis. Changes in blood counts that prompt attention (MELD score increase by 8, WBC count change to >13,000 or <2,000) will be monitored. If all is well, then subjects will be discharged with another stool kit and instructions for the last visit 15±3 days later (visit 4).

Visit 4: All procedures as in visit 2 will be performed again, including dietary recall, targeted physical exam, and sample collection. All subjects will also get another oral FMT/placebo dose of 5 capsules each depending on their original assignment. All subjects in the study will get this intervention unless (a) they refuse the intervention (b) have undergone a liver transplant or have died/discharged to hospice (c) developed an infection or an SAE that was adjudicated to be related to FMT in the interim or (d) are currently undergoing antibiotic therapy for an infection that is not related to FMT.

For those who are infected due to an FMT-unrelated infection, we will approach the patients 2 days after the completion of the assigned antibiotic course. For those initiated on SBP (Spontaneous Bacterial Peritonitis) prophylaxis, we will provide the intervention after the initial SBP episode is treated. This may require delaying the intervention from day 30 to up to day 45 but this will still be counted as the second FMT.

All other subjects will be given the intervention and the blind will still be maintained. The brain function tests and QOL will be performed again. Groups 1 and 2 will get active FMT and groups 3 and 4 will get placebo FMT. No enemas will be given. The subjects will then be sent home.

Follow-up visits every month: The subjects will be followed monthly in person, or by phone or videoconference at Months 3, 4, 5 to evaluate dietary and medication changes and for safety analysis (hospitalizations, AEs or SAEs), targeted physical exam and for collection of samples at each study clinic visit. Mail-in kits for collection of stool, and urine will be provided along with instructions for use if visit is performed remotely. We will also perform the cognitive tests and QOL assessment at one month post-second intervention and at 6 months. We will continue to follow every patient for 6 months regardless of whether they developed an SAE or hospitalization in between. Only reasons not to follow them up for 6 months would be death, liver transplantation or patient withdrawal of consent.

End-of-Study visit: Per FDA mandates for FMT studies, we will see the patients at six months and we will evaluate AEs, interim hospitalizations, dietary recall, and perform targeted physical exam, QOL and brain function studies, and collect serum, and stool again. At this point the participation of the patient will end.

<u>Preparation and handling of FMT material</u>
<u>For enema material (60 ml)</u>

As per University of Minnesota guidelines

1. Frozen material will thaw for up to 4 hours in an ice bath. After thawing, material should be kept refrigerated/on ice for up to additional 8 hours.
2. Standard protocol for handling bio-hazardous material will be employed in order to avoid contamination and risk to healthcare handlers. Sterile microbiological technique will be employed during material transfer peri-procedure.

At that point we will provide 60 ml of the fecal material using universal precautions through a rectal tube. The procedure will be completed by a trained Registered Nurse, Nurse practitioner, or physician in an outpatient clinic, endoscopy recovery area, or standard endoscopy unit. We will ensure that patients are able to hold the enema for at least 30 minutes after the administration. This will be ensured by positioning patients in the left lateral decubitus position, and asked periodically to rotate 180 degrees to a right lateral position and back to the left lateral position, to promote movement of the FMT material throughout the colon. We can cut it down to 60ml in case the patient is uncomfortable with more volume although we do not expect that. The total retention time will be recorded.

The following will be recorded in all FMTs

- Dose
- Unit ID/Lot# of each treatment
- Expiration Date
- Storage Condition
- FMT retention time (in minutes)

<u>For capsules: As per University of Minnesota guidelines (5 capsules)</u>

1. Long-term storage of capsules can be done in -80^{o}C or -20^{o}C laboratory freezer (without automatic freeze-thaw cycles). Once thawed, the capsules may be kept at 4^{o}C for up to one month and may be kept at room temperature for up to 24 hours. If capsules are removed from frozen storage during an occasion in which they will not be administered to a patient, they must be returned to frozen storage within 10 minutes to

continue long-term storage or transferred to a refrigerator (4^{o}C) for temporary storage. The goal is to avoid repeat freeze-thaw cycles. Any product that has gone through more than one freeze-thaw cycle after its original release should be discarded.
2. Standard protocol for handling bio-hazardous material will be employed in order to avoid contamination and risk to healthcare handlers. Sterile microbiological technique will be employed during material transfer peri-procedure.
3. The following will be recorded in all interventions (dose, Unit ID/Lot# of each treatment, Expiration Date and storage condition)

Number of interventions: One administration of rectal placebo or FMT and two administrations of oral FMT or placebo

We will ensure that each subject receiving active FMT will receive material (oral/rectal) from the same donor.

Duration of Follow-up after FMT: 6 months

Samples collected at baseline (before FMT), and at the visits specified above will be:

a. Stool (microbiome, bile acid profile, metagenomics, metabolomics)
b. Blood (CBC, Basic metabolic panel, LFTs, INR, endotoxin, and storage for circulating microbiome, systemic inflammatory markers, bile acids and metabolomics)
c. Urine for metabolomics

Sample processing will be done using standard precautions at the McGuire VA Medical Center and samples will be stored for analysis of the secondary endpoints. Microbiota analysis will be performed at George Mason University and University of Minnesota, and metabolomics will be performed at the UC Davis NIH West Coast Metabolomics Center or Metabolon. We will perform the bile acid analysis at the McGuire VA Medical Center laboratories. Additional analyses at the Institute of Hepatology, London, UK,

and the University of North Carolina at Chapel Hill. We may also use other referral laboratories as needed.

Statistical analysis: These will be performed using ANOVA of delta changes after intervention/follow-up using Chi-square and Fisher exact for non-parametric (hospitalizations, HE episodes, covert hepatic encephalopathy etc.), for continuous variables, and specialized bio-informatics analyses for the microbiota (see details below). These will be adjusted for age, gender, MELD score and alcoholic etiology of cirrhosis.

Sample size: Based on the published study with rectal FMT[10], we found that standard of care patients had an 80% chance of hospitalization at 6 months, while FMT had a 20% hospitalization rate. Given that we had used antibiotics for this study, we will assume that rectal FMT alone will have a 30% chance of hospitalization rate without antibiotics. We will also assume that oral FMT alone will have a similar hospitalization rate, and their combination will have a 10% hospitalization rate. Using a significance level of $\alpha=0.05$, a sample size of 25 per group will provide a power of 100% between groups, 20 per group will give a power of 98% to define differences between the groups, while a sample size of 15 patients per group will give a power of 94% between the groups. Therefore, we will enroll 100 patients (25 in each group) for this trial. This sample size calculation will also be valid when we include the second oral FMT with a sample of 10 per group giving 88% power, 15 per group giving 97% power and 25 giving 99% power.

Specialized analyses:

Inflammatory milieu: We will perform the analysis for endotoxemia using the validated Limulus amebocyte lysate (LAL) assay and serum inflammatory cytokines (IL-6, TNF, IL-1b) using ELISA at Dr. Bajaj and Pandak's laboratory at the McGuire VAMC or at Assaygate[14].

Microbial Analysis: Bacterial community composition in stool will be characterized using OTU counts generated as described above. OTU counts will be converted to measures of relative abundance to account for variation in sequencing coverage between samples and compared to the donor sequence. Statistical analysis will be carried out using the statistical software package R. Alpha (α) diversity (richness and evenness of taxa within a population) will be reported using the Shannon Index[15] and Chao1 richness estimator[16]. Changes in abundance of individual taxa will also be analyzed using traditional univariate statistical methods. We will use LEfSe (Linear discriminant analysis Effect Size) to determine the features most likely to explain differences. All comparisons will be performed between treatment arms, to gauge donor engraftment in the three FMT-assigned arms, as well as within groups to compare to their baseline microbial composition[17]. These will be performed at George Mason University and University of Minnesota. Functional analysis using bile acids, SCFA and metabolomics will also be performed.

Brain function: These will be administered at visit 1, visit 4 (one month after the second FMT), and end-of-study by Dr. Bajaj and the coordinator/research assistant. Two validated testing strategies, Psychometric Hepatic Encephalopathy Score (PHES) and EncephalApp Stroop Test, which have norms for the Virginia population will be used[18]. The PI and his team are well-published in this field and have been administering these for several years to cirrhotic patients. Both these tests showed benefit in the FMT enema trial published by our group in HE[10]. PHES is a recommended battery that defines brain dysfunction in cirrhosis[19]. It consists of 5 paper and pencil tests (number connection tests A and B, digit symbol test, line tracing test and serial dotting tests), which form a composite score of standard deviations below an age/education-matched community healthy population. This gives a score ranging between -15 and +4. In addition to this continuous score, covert hepatic encephalopathy can be diagnosed with a score <-4 for our population[18]. This test battery takes 15 minutes to complete. EncephalApp Stroop[18, 20] is a freely available computerized test which consists of an easier “Off” state and a more difficult “On” state. The OffTime+OnTime is the output, which is the time taken to complete 5 correct runs in the Off and On states. The output here can be continuous (seconds in the

OffTime+OnTime) or covert hepatic encephalopathy percentage based on local norms[18]. This takes 5-7 minutes to complete.

Quality of Life: This will also be administered at Visit 1, Visit 4, one month after the second FMT, and End-of-Study visit. The Sickness Impact Profile (SIP)[21] is a validated profile for QOL in cirrhosis that the PI and his team have had several years of published experience[22-24]. SIP consists of 136 questions that pertain to any issues that patients have experienced in the last 24 hours. It has a composite score, two dimensions (physical and psycho-social) and twelve individual domains that are calculated based on norms. A higher score indicates a worse quality of life. This gives only a continuous score as the output. A copy is shown in the Appendix. Dr. Bajaj and the coordinator/research assistant will perform these.

Endpoints and Plan of Analysis:

Primary Endpoint: FMT-related SAEs including HE recurrence within 6 months

Secondary Endpoints (details below):

1. Safety-related (at 6 months): Liver-related hospitalizations, other serious adverse events (SAE) and adverse events related to interventions.
2. Microbiota composition: Stool 16SrRNA sequencing and engraftment of donor into recipients.
3. Inflammatory milieu: Endotoxemia (LAL), Systemic inflammatory cytokines (IL-6, TNF, IL-1b using ELISA)
4. Bile acid profile and gut-liver mediators expression: Bile acid profile using LC/MS in stool and serum and serum C4 (7α-Hydroxy-4-cholesten-3-one).
5. Urinary metabolomics to evaluate gut-microbial markers
6. Brain function: Two validated testing strategies, PHES and EncephalApp Stroop Test, and Quality of Life: Sickness Impact Profile (a validated profile for health-related quality of life in cirrhosis)

Adjudication: Data and safety monitoring for this study will be provided by the Clinical Science Research & Development (CSR&D) centralized Data Monitoring Committee (DMC). The DMC is provided by CSR&D to ensure independent oversight of the safety and integrity of the project. The DMC is an independent multidisciplinary group, whose members have collectively – through research, education, training, experience, and expertise – the requisite knowledge pertinent to the subject areas to be reviewed. Membership details are available on the CSRD website. The DMC will provide an ongoing independent evaluation of this study focused on safety and feasibility, including participant accrual and retention, adverse events monitoring, and data analyses. Meetings will be held three times per year at which recommendations will be made to the Director of CSR&D for endorsement. These recommendations will range from approval to continue (unconditionally or with conditions to be addressed) to probation or possibly termination, if there are problems with enrollment or safety concerns

All outcomes will be compared between groups as well as post-intervention to baseline.

Visits at VCU and VAMC:

None of the Veterans included in this study will have any visits at VCU. Non-Veterans will be recruited according the approved VCU protocol and will sign the VCU consent. These potential participants will also be shown the approved VA Consent form in order to provide them the full scope of the study. These non-Veterans will have a choice to perform all subsequent studies at VAMC or only the 2 treatment visits, which are visits 2 and 4. When they first arrive at the VAMC for their in-person visits, they will be asked to sign the VA IRB approved consent form before proceeding. Neither of the treatment visits will be performed at VCU even if the enrollee is a non-Veteran.

Dr Bajaj, who is the PI at both sites, will ensure that subjects meet criteria for enrollment and reporting to both IRBs as necessary. An MOU will be signed.

Safety and hospitalization analysis:

Using detailed FDA-approved protocols, patients will be adjudged based on adverse events and serious adverse events at every visit and during the 6-month follow-up. Any SAEs or deaths in an FMT-randomized group will require the DMC to define the "relatedness" of the event to the FMT. If the DMC votes that this is related to the FMT, then this will be sent to the FDA, and Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota to invoke stopping rules as detailed below. The safety endpoints will be all-cause hospitalizations, defined as unplanned, non-elective hospitalizations within the 6-month period. The reason for the hospitalization will be analyzed by the investigating team and divided into liver-related, liver-unrelated, planned or elective. The latter two categories will not count towards the study but the first two will be analyzed by the team and submitted to the DMC. Other SAEs will be analyzed per FDA definitions. AEs that do not meet the standards of SAE will be evaluated at each visit and be compiled to define trends in one group compared to the other.

Reasonably foreseeable significant risks:

Physical risks:

1. Infection or bruising during blood draw
2. Procedure-related adverse events from enema (e.g. perforation, bleeding etc)
3. Pill-associated dysphagia
4. FMT material-related adverse events (e.g. transmissible infection, allergic reaction)

Psychological/Social risks:

(b) Very small risk of loss of confidentiality and privacy

Specific issues to be captured will be

1. Study procedure-related adverse events from enema (e.g. perforation, bleeding, etc.)
2. FMT material-related adverse events (e.g. transmissible infection, allergic reaction)
3. Capsule ingestion-related adverse events (pill-associated dysphagia)

4. Short-term safety: Both solicited and unsolicited adverse events will be recorded by, clinical assessment at all in-person visits. The subjects will have the ability to telephone the study team at any point during the study.
5. Long-term safety: follow-up monthly till 6 months to evaluate for SAEs (listed below).

Solicited AEs throughout the trial will be
(a) fever:
(b) diarrhea
(c) vomiting
(d) constipation
(e) GI bleeding
(f) abdominal cramping and pain
(g) Development of HE (confusion/altered mental status):
(h) Development of infections, specifically spontaneous bacterial peritonitis
(i) New onset or significant worsening of chronic medical conditions
(j) New potentially transmitted infections

We do not expect the infections to occur at a higher rate than the natural history of cirrhosis at this stage. This is borne out by our enema study, in which the FMT group had no infections. Also, a nasojejunal study performed by Phillip et al in severe alcoholic hepatitis, whose MELD scores were much higher than our proposed patients, did not reveal a poor safety profile. We will monitor for all infections and specifically for spontaneous bacterial peritonitis (SBP).

All safety assessments in FMT will be compared to placebo groups given the advanced nature of the cirrhosis and the association of several of these symptoms in the natural history of the disease

<u>Stop-points or reportable adverse events</u>

Any hospitalization or event that is life-threatening will be deemed a serious adverse event in the FMT group and reported to the IRB, FDA, DMC and Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota within 72 hours (per usual protocol).

This will include the development of an infection that is gut-based/not gut-based:

Gut-Related

- Spontaneous Bacterial peritonitis
- Infectious diarrheal illnesses

Others

- UTI
- Respiratory
- Skin/soft-tissue
- Bacteremia

Overall Study Halting Rules

Specific safety findings will result in temporarily suspending enrollment until a safety review is convened, the objective of which is to determine whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment is another potential outcome of a safety review. This will be done if the assigned infection is in the FMT group since this patient population is prone to infections.

Subsequent review of serious, unexpected, and related AEs by the Data and Safety Monitoring Board, IRB, the PI, or the FDA or relevant local regulatory authorities may also result in suspension of further trial interventions/administration of FMT at a site. The FDA and PI retain the authority to suspend additional enrollment and study interventions/administration of FMT for the entire study, as applicable. The PI and the adjudication panel will make the initial decision. The subsequent safety review by the IRB and FDA will then decide if the study can resume.

Findings that will trigger a safety review are

Findings that will trigger a safety review are

- Death related to FMT material or study procedures in any subject;
- New potentially transmitted infection in five or more subjects assigned to FMT - i.e., cannot exdude transmission and/or attribution is

pending investigation/Data Monitoring Committee (DMC) adjudication;

- Any single potential transmitted infection assessed as possibly, probably, or definitely related to FMT by the investigator and DMC.
- One or more subjects assigned to FMT has a serious, unexpected AE assessed as possibly, probably, or definitely related to FMT by the investigator and DMC; Five or more subjects (per assigned group) develop a new diagnosis of a medical condition or immune-mediated disease considered probably, or definitely related to FMT.
- Five or more severe AEs of a similar nature occurring in different subjects and assessed as definite, possible or probable relatedness to study product in groups assigned to FMT (per assigned group)

FDA/CBER will be notified by phone or fax within 72 hours if the study is halted for review.

Individual study halting rules

(1) patient's choice

(2) change in GI adverse events with a magnitude of 3 increase over baseline

(3) fever and signs of infection (including WBC count >12,000/ml)

(4) SAE development including cirrhosis complications (OHE) and hospitalizations for any cause including bleeding, nausea, vomiting, or other GI conditions

(5) increase in MELD score by 8 or more

(6) Increase in AST, ALT or Alkaline phosphatase to 10X ULN

(7) Non-elective Hospitalization

Even if the subject is withdrawn before 30 days, they will still be followed till 6 months for safety.

Health risks (**please see adverse events table attached**):

Adverse events will be both for the FMT material, placebo as well as for the procedures. These risks are listed in the Risk Section of the informed consent.

These possible adverse events will be:

(a) Fever: yes or no answer with grading based on actual temperature on examination
(b) diarrhea (will be asked to grade them from 0-4 compared to baseline)
(c) abdominal cramping and pain (will be asked to grade them from 0-4 compared to baseline)
(d) vomiting (will be asked to grade them from 0-4 compared to baseline)
(e) Development of HE (Altered mental status) in every group: will be monitored by clinical examination through the West-Haven Criteria[25]. We will be closely monitoring all subjects for this development, including a clinical examination by a gastroenterologist at every visit.
(f) Development of infections specifically spontaneous bacterial peritonitis

-Fever will be reconfirmed by measuring by the study nurse and if it is confirmed, a detailed investigation into the cause of the fever will be performed as part of standard clinical care and the patient will be withdrawn from the study.
-Vomiting, constipation, GI bleeding, abdominal cramps and diarrhea: If the patient's diarrhea and abdominal cramps change in a magnitude of 3 over their baseline values per the AE chart (patients with cirrhosis often have these symptoms at baseline), they will be withdrawn from the study.

-Infections that occur in the FMT group will be thoroughly investigated according to standard clinical care to prove whether they were associated with the FMT.

Adverse Event Grading Chart

Volunteer Study ID:_____Date:_____ Time: _____
Study Start Date:_____ Current Visit number:_____

**Volunteer's responses will be marked on grading sheet by circling the appropriate grade or indicating no symptoms.*

PARAMETER COMPARED TO BASELINE	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
ESTIMATING SEVERITY GRADE				
	Symptoms with minimal change from baseline causing no or minimal interference with usual social & functional activities	Symptoms with moderate change from baseline causing greater than minimal interference with usual social & functional activities	Symptoms with severe change from baseline causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
SYMPTOM SPECIFIC SEVERITY GRADE				
Fever (oral)	(99.9-100.5°F)	(100.6-101.5°F)	(101.6. -104°F)	> 104°F
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Distension/bloating, abdominal discomfort	Asymptomatic	Symptomatic, but not interfering with GI function	Symptomatic, interfering with GI function	---
Abdominal Pain	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Life-threatening consequences (i.e. acute peritonitis)

PARAMETER COMPARED TO BASELINE	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Dehydration	Increased oral fluids indicated; dry mucous membranes; diminished skin turgor	IV fluids indicated <24 hours	IV fluids indicated >24 hours	Life-threatening consequences (e.g. hemodynamic collapse)
Constipation	NA	Persistent constipation requiring regular use of dietary modifications, laxatives, or enemas	Obstipation with manual evacuation indicated	Life-threatening consequences (e.g., obstruction)
Procedural Enema	Rectal discomfort; minor bleeding <24 hours on toilet paper	Rectal discomfort; minor bleeding >24 hours on toilet paper	Gross hematochezia	Bowel perforation
Change in mental status (West-Haven Criteria)	No change in orientation and no asterixis	Disorientation to time, asterixis	Disorientation to place or person, asterixis, lethargy and stupor	Coma
MELD score increase (includes INR, bilirubin and creatinine)	<3	3-7	>8	NA
Serum WBC	NA	NA	>12,000/ml or <1000/ml	NA
AST	NA	≥ 5.0 to < 10.0 x ULN	≥ 10.0 x ULN	NA
ALT	NA	≥ 5.0 to < 10.0 x ULN	≥ 10.0 x ULN	NA
Alkaline phosphatase	NA	≥ 5.0 to < 10.0 x ULN	≥ 10.0 x ULN	NA

Specific investigation of the infection related to the FMT in those assigned to the FMT group:

1. Regular clinical care of the subject according to the infection developed will include the appropriate cultures and relevant antibiotics will be started

2. Stool tests from the patient will be compared to the donor safety sample when a CLIA-certified assay is available

3. Contact will be made with the FMT suppliers to evaluate this batch of the donor. Specifically, the cross-referenced drug master file has the means to track down the lots and batches of the donors. In the event of a suspected adverse event related to the potential transmission of an infection, an immediate initial consultation will be held between the researchers and the Chief Medical Officer of Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota (Dr. Alexander Khoruts) to gather further information. This will be followed by a detailed collaborative investigation as follows:

The investigation will entail:

a. Strict donor material quarantining during the investigation

b. Rescreening of the donor safety aliquot and the donor for infectious diseases as well as risk factors for the suspected infectious disease
c. Consultation with external experts in infectious diseases and gastroenterology with extensive experience in FMT.
d. Full Advisory board review at Molecular and Cellular Therapeutics (GMP) facility at the University of Minnesota to track the donors

Laboratory:

Lab values for WBC counts, serum AST, ALT, Alkaline phosphatase, serum bilirubin, INR and serum creatinine (all three are in MELD score) will be followed.

These criteria will be graded per the chart as well.

The lab values that will be followed from an adverse event perspective will be:

- WBC count: a new >12,000/ml or <1000/ml in the presence of fever will necessitate investigation of an infectious source
- Worsening of the MELD score by 8 or higher will be considered significant
- Increase in AST, ALT and alkaline phosphatase >10 ULN will be considered significant although this change is not expected in cirrhotic subjects

Dr. Bajaj asks subjects to allow him to keep a portion of their blood, urine and stool specimens collected during the study to be used for future testing. Subjects agreeing to this will have a portion of their blood, urine and stool specimens stored in a research laboratory at the McGuire DVAMC. The samples will be de-identified. This means that no information that can identify a subject will appear on the samples. Subjects do not have to agree to this in order to participate in the study. If at any time during or after the study, a subject decides they want their samples destroyed, the subject can contact Dr. Bajaj at 804-675-5021 and request that their samples be destroyed.

Adverse Events Reporting

Dr. Bajaj will function as both the Investigator and the Sponsor during the conduct of this study. Dr. Bajaj will be responsible for reporting all safety observations (adverse events and serious adverse events) to the FDA

A. Definitions

1. Adverse Event (AE)

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

• AEs may be new events or may be pre-existing conditions that have become aggravated or have worsened in severity or frequency.

• AEs may be clinically significant changes from baseline in physical examination, laboratory tests, or other diagnostic investigation (e.g. laboratory results, radiographic findings).

Pregnancy is not an AE; however, if a female subject becomes pregnant during the conduct of the study, the Investigator/Sponsor is responsible for notifying the FDA.

2. Serious Adverse Event (SAE)

A serious adverse event is any adverse experience occurring during or after FMT that results in any of the following outcomes:

• Death

• Life-threatening experience

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

• Requires inpatient hospitalization or prolongation of existing hospitalization

Note: Adverse events requiring hospital admissions that are less than 24 hours in duration do not meet this criterion. A scheduled hospitalization for a pre-existing condition that has not worsened during participation in the study does not meet this criterion. Pre-planned hospitalizations for an elective medical/surgical procedure or routine check-ups do not meet this criterion.

• Results in persistent or significant disability or incapacity

• Results in a congenital anomaly or birth defect

• Results in an important medical event

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

3. Planned Hospitalization

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

4. Adverse reaction

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

5. *Suspected Adverse Reaction*

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

6. *Unexpected*

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator brochure or is not listed at the specificity or severity that has

been observed; or is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

Solicited Adverse Events

According to the attached Adverse Event Grading Chart

Monitoring of Adverse Events

Each subject will be monitored for the occurrence of AEs, including SAEs. Each subject will be followed for safety monitoring at each follow-up interval and at the 6-month phone call

- Subjects will be questioned regarding stool patterns, abdominal pain, fevers, and subjective well-being. Subjects will be questioned about the possible occurrence of adverse events in a generalized way, such as, "How have you been feeling since your last visit?" Subsequent solicited questions and relevant details will be asked if there is a positive response. Site Investigators will be contacted by study representative if there is a suspected or reported AE
- Subjects having AEs will be monitored with relevant clinical assessments and laboratory tests, as determined by the site Investigator
- AEs, actions taken as a result of AEs, and follow-up results will be recorded as well as in the subject's source documentation. Follow-up laboratory results should be filed with the subject's source documentation
- Subjects will be seen every month till 6 months post-enrollment to record any SAEs, new onset of potentially transmitted infections, new medical conditions, or changes in pre-existing medical conditions (New onset or significant worsening of chronic medical conditions including, but not limited to, autoimmune diseases, inflammatory bowel diseases, metabolic syndrome, cardiovascular disorders, and neuropsychiatric disorders.)

Assessment of Adverse Events

1. Assessment of Severity will be performed compared to baseline symptoms that the patient has at enrollment and according to the adverse event table The severity of AEs will be assessed according to the following definitions:

•**Mild (1 change)**: the AE is noticeable to the patient and/or the Investigator, but does not interfere with routine activity

•**Moderate (2 change)**: the AE interferes with routine activity, but responds to symptomatic therapy or rest

•**Severe (3 change)**: the AE significantly limits the patient's ability to perform routine activities despite symptomatic therapy

- **Life threatening (4 change):** Potentially life-threatening

2. Assessment of Causality

The Investigator must assess the relationship of any AE (including SAEs) to FMT, as *related* or *not related*, based on clinical judgment and using all available information, and may include consideration of the following factors:

• Possible alternative causes of the AE, including the disease under treatment, pre-existing conditions, concomitant use of other drugs, and presence of environmental or genetic factors

• The temporal association between FMT exposure and onset of the AE

• Whether the manifestations of the AE are consistent with known actions or theoretical toxicity of FMT

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

Not Related: An AE is not associated with FMT if:

• Temporal relationship is lacking (e.g., the event did not occur within a reasonable time frame following administration of FMT); or

• Other causative factors more likely explain the event (e.g. pre-existing condition, other concomitant treatments);

Related: An AE is attributed to FMT if:

• There is a positive temporal relationship (e.g. the event occurred within a reasonable time frame following FMT); and

• The AE is more likely explained by FMT than by another cause

To aid this assessment, the Investigator/Sponsor will be helped by the VA Central DMC as mentioned above.

All SAEs, possible transmission of infections, and new or worsening medical conditions possibly related to FMT will be referred to this panel, including ones elicited at the monthly visits. This panel will vote based on their analysis of the data regarding the "relatedness" of the AE.

Reporting Safety Observations by the Investigator /Sponsor

1. Reporting of Non-serious AEs

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

2. Reporting of FMT Exposure during Pregnancy

If a female subject becomes pregnant during the course of study, the Investigator/Sponsor is required to follow up on the pregnancy until it has completed. The outcome of the pregnancy and the status of the newborn (if applicable) will be reported to the local IRB within 24 hours of becoming aware.

3. Reporting of Safety Observations by the Investigator/Sponsor

Any occurrence of the following events or outcomes must be reported expeditiously by the Investigator/Sponsor to the local IRB (within 5 days of becoming aware) and will be responsible for reporting the event to the FDA:

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

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

Any occurrence of the following events or outcomes must be reported by the Investigator/Sponsor to the local IRB and for reporting the event to the FDA:
1. SAE *unrelated* to FMT material
2. Death of subject *unrelated* to FMT material
3. New or worsening medical condition, or new medications

The Investigator is to report any safety observations from the list above to the Sponsor within **7 days** of becoming aware of the event. The Sponsor will notify the FDA in its *Annual Report*. Any observation that is also an AE will be recorded on the subject's case report form along with any actions taken. If not all information is available at the time of initial report, follow up SAE reports will be completed and submitted. The investigator will report all new onset of potentially transmitted infections and new onset or significant worsening of chronic medical conditions to FDA regardless of the assessment of causality and according to time frames outlined in 21 CFR 312.32 (within 7 days for deaths and life-threatening SAEs; others within 15 days). Similarly, deaths and SAEs related to FMT procedures (not only FMT material) will be reported to the FDA expeditiously (within 7 days for deaths and life-threatening SAEs; others within 15 days.

The Investigator/Sponsor is to report any safety observations from the list above to the local IRB within **5 days** of becoming aware of the event. The Investigator/Sponsor will notify the FDA in its *Annual Report*. Any observation that is also an AE will be recorded on the subject's case report form along with

any actions taken. If not all information is available at the time of initial report, follow up SAE reports will be completed and submitted.

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

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

Monitoring the study database and submitting safety reports

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

A. Serious and unexpected suspected adverse reaction related to FMT material

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

B. Findings from other sources

The Investigator/Sponsor will also expeditiously report any findings from clinical, epidemiological, or pooled analysis of multiple studies or any findings that suggest a significant risk in humans exposed to FMT and thought related to the material.

C. Increased occurrence of serious suspected adverse reactions related to FMT material

The Investigator/Sponsor will report any clinically important increase in the rate of a serious suspected adverse reaction over that listed in the protocol.

Minimizing Risks to Human Subjects

Human Subjects Involvement, Characteristics and Design

1. Approval will be obtained from the McGuire VAMC IRB and get the required FDA approval of IND.
2. Subjects will be given informed consent using the standard consent process for enema. Additional theoretical risk for transmission of infectious agents, and other diseases and conditions will also be discussed.
3. Protections against risk are provided in detail below.

Protection against Risks to Subjects

Recruitment and informed consent

-Participants will be recruited after they are determined to meet inclusion criteria, the clinician will introduce him/her to the research assistant, if applicable. The research staff or clinician will carefully explain all aspects of the study to a potential recruit, including the risks and benefits, and obtain participant's written informed consent.

-The research assistant or clinician will orally describe the material written in the informed consent document and answer any questions the participant may have.

- Participants will be reminded that they are not required to participate in the study and that they will receive the standard care provided by their physician regardless of whether or not they choose to participate. Participants who give their consent will sign a copy of the document and will be given a signed copy of the informed consent document.

-Recruits will be informed of the treatment commitment, amount and general types of assessments, the follow-up telephone interview procedures, and clinic visits. They will be given detailed descriptions of study procedures. They will also be told of the possibility of being randomized to the non-FMT arm or an arm that receives one placebo.

Protection against risk

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

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

1. We will minimize the risk of potential coercion by following standard procedures for obtaining informed consent from subjects. We will begin this process during the intake, where we will clarify the nature of the study and possible alternatives upfront. Prior to enrolling subjects in the research, we will fully explain the study procedures, risks, benefits, and alternatives, emphasizing that the subject's participation has no impact on the other services they receive. Also, subjects who do not consent or who withdraw during the study period will continue to receive appropriate treatment if needed. All

subjects will be reminded that there is no penalty for those who choose to not participate or to withdraw from the study and that their decision to participate does not impact the standard services they receive through the hospital.

2. We will minimize potential risks due to loss of confidentiality by having all information collected and handled by research staff trained to deal appropriately with sensitive clinical issues. All study information will be treated as confidential and will be available only to research staff. Hardcopies will be kept in locked file cabinets, and computer data files will be encrypted and available only to authorized personnel – with no storage of names or obvious identifying information. No participant will be identified in any report of the project. Further, when contacting participants for follow-up, no identifying information other than the first name of the research assistant will be used when leaving messages or speaking to anyone other than the participant him/herself. Written consent will be obtained to contact other persons for the purpose of locating the participant for follow-up and participants can refuse or revoke such consents. No information about participants will be released without their permission or where required by law.

3. We will minimize the theoretical risks of infectious disease or other conditions by the following points:

(a) Infection or bruising during blood draw: We will minimize these risks by having the experienced nurse coordinator or phlebotomist draw the blood

(b) Procedure-related adverse events from enema (e.g. perforation, bleeding, etc.): will be reduced by conducting these procedures and post-procedure monitoring in the accredited Richmond VA Medical Center by trained certified practitioners. Patients will be monitored per standard protocols

(c) FMT pill-associated dysphagia: This will be minimized by using the safety capsule ingestion and also by excluding subjects who may be at risk for dysphagia. We can perform an endoscopy to retrieve any stuck capsules if needed urgently.

(d) FMT material-related adverse events (e.g. transmissible infection, allergic reaction): Using one rational donor as the source of all the capsules will greatly reduce these risks. These will be minimized by aggressive monitoring using a dedicated adverse event chart.

Data Monitoring Plan

Data will be collected using standardized paper forms and will only be identified with the study's ID of the subject. The codes that link the name of the subject and the study ID will be kept confidential by the PI in a secured cabinet. Data will be entered in the computer database independently by trained data entry staff, and discrepancies will be corrected by the PI, based on source documents.

Data quality will be monitored once per month by random inspection of the completed forms by the PI.

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