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Table S1: Detailed eligibility criteria

Inclusion criteria:

1. Cirrhosis diagnosed by any one 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
2. At least two episodes of hepatic encephalopathy, one within the last year but not within the last month (patient can be on lactulose and rifaximin)
3. Age between 21 and 75
4. Able to give written, informed consent (demonstrated by mini-mental status exam>25 at the time of consenting)

Exclusion criteria:

1. MELD score >17
2. WBC count <1000 cells/mm3
3. Platelet count<50,000/mm3
4. TIPS in place for <one month
5. No HE episode within a month prior to the study
6. Patients allergic to ciprofloxacin, penicillins or metronidazole
7. Currently on absorbable antibiotics
8. Infection at the time of the FMT (diagnosed by blood culture positivity, urinalysis, paracentesis as needed)
9. Hospitalization for any non-elective cause within the last 1 month
10. Patients who are aged >75 years
11. Patients who are pregnant or nursing (will be checked using a urine pregnancy test)
12. Patients who are incarcerated
13. Patients who are incapable of giving their own informed consent
14. 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.
15. Patients with a history of severe (anaphylactic) food allergy
16. Patients who have previously undergone FMT
17. Patients on renal replacement therapy
18. Patients who are unwilling or unable to hold the enemas
19. Patients with untreated, in-situ colorectal cancer
20. Patients with a history of chronic intrinsic GI diseases such as inflammatory bowel disease (ulcerative colitis, Crohn's disease or microscopic colitis) , eosinophilic gastroenteritis, celiac disease or irritable bowel syndrome
21. Major gastro-intestinal or intra-abdominal surgery in the last three months
22. Unable to comply with protocol requirements
23. Patients who are American Society of Anesthesiologists (ASA) Physical Status

classification IV and V

24. Patients with acute illness or fever on the day of planned FMT will be excluded with the option of including that subject at a future date
25. Any conditions for which, in opinion of MD, the treatment may pose a health risk
26. *C. difficile* in the stool at baseline (qPCR)
27. Grade 2-4 or complicated hemorrhoids

Supplementary Methods:

Donor Selection:

To select a donor, data from 174 16s rRNA sequences generated from samples collected in prior studies from the study centers were analyzed. These sequencing data were from HE patients and healthy controls. This data was used to train a Random Forest Classifier, a machine learning classification technique to separate between HE and healthy among the OpenBiome donors. The resulting classifier had an AUC=0.94, which was then gave each donor a classification score (formally, the percentage of trees in the Random Forest Classifier that classify the sample as 'healthy'). To corroborate these, the relative abundances of Lachnospiraceae and Ruminococcaceae bacterial families in each donor (taxa found to be depleted in HE patients) was calculated. All this data was combined to rank the 28 stool donors at OpenBiome. Ultimately the donor with the highest aggregate ranking was selected as the FMT donor for FMT-randomized patients. This donor was a healthy 37-year-old male, whose material had been used for at least 280 patients for the treatment of recurrent C. difficile without serious adverse events. This donor also demonstrated the highest relative abundances of Lachnospiraceae, and Ruminococcaceae among the potential donors. The same stool sample from this donor was utilized for each of the aliquots for the FMT-assigned groups.

Cognitive testing:

PHES or psychometric hepatic encephalopathy score evaluates psychomotor speed, reaction time and visuo-motor coordination. It consists of five tests, the number connection test-A, number connection test B, digit symbol test, serial dotting test and line tracing test (has two components; time and errors). Population control norms are available for this battery and the added score of each standard deviation beyond norms is the total PHES score. A low total score indicates poor cognitive performance.

EncephalApp Stroop uses a validated smartphone App version of the Stroop test that has On and Off stages. The Off stage tests psychomotor speed and accuracy while the On stage also tests for cognitive flexibility. The easier "Off" Stage requires subjects to correctly identify the color of the pound-signs presented, while the more difficult "On" stage requires them to correctly identify the color of a discordant word presented. The App has two practice runs and requires 5 correct runs in the Off and On Stage. The total time required for 5 correct On and 5 correct Off stage runs is the "OffTime+OnTime" which is of relevance in HE and is recorded. A higher time required to complete the number of runs required indicates worse performance.

Study Design

Randomized, open-label Phase 1 study with exploratory endpoints and pathophysiological evaluation of the FMT

Two groups of outpatients with cirrhosis (Table S1) will be randomized using random sequence generator into standard-of-care and FMT groups.

Intervention Dosage: FMP-30 X 3 at the same time (27gm of stool)

Route of Administration: Enema

Procedures:

Once patients are randomized 1:1 into group 1 (FMT) and group 2 (standard of care), both will be followed over 35 days and will include a 5 month follow-up phone call to assess SAEs. An optional long-term extension will be performed as well for at least 12 months post-FMT administration.

Group 1 (FMT group) will undergo the following procedures

Pre-FMT preparation:

We will obtain written informed consent. After the patients are consented and are eligible, we will perform a detailed medical history and physical exam at day 0. We will then collect blood, urine and stool for baseline testing including *C.difficile* at day 0 using qPCR. If *C difficile* is positive, we will exclude these patients. We will perform urine pregnancy tests in women of childbearing age and only if it is negative and they agree to use effective contraception for the duration of the study and for 10 days before and 30 days after the study, they will be allowed to proceed. We will also perform cognitive testing. We will prescribe patients an antibiotic regimen

(ciprofloxacin 500 mg PO BID, amoxicillin 500 mg PO TID, metronidazole 500 mg PO TID) for 5 days to set up a clear baseline. The doses will be adjusted for end-stage liver disease (for metronidazole) and for creatinine clearance (for all three drugs). Drawing from ecological principles of microbial niche environments and data from recurrent *C.difficile*, pre-treatment antibiotics are likely to increase the probability of disrupting the host's intrinsic microbiota and creating an opportunity for a 'healthy' microbiota from the FMT to engraft.

After antibiotics and on the day of FMT:

At day 5, we will re-evaluate patients with a directed interval history and focused physical exam as needed. After ensuring that they are still candidates according to the inclusion/exclusion criteria, we will collect stool, urine and blood again for pre-FMT evaluation and urine for pregnancy tests from eligible women. Cognitive testing will be performed again.

Preparation and handling of stool for FMT infusion

As per Openbiome guidelines

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

At that point we will deliver 90 ml of the fecal material using universal precautions. The procedure will be completed by trained study personnel. We will ensure that patients are able to hold the enema for at least 30 minutes by positioning patients in the left lateral decubitus position.

Follow-up after FMT original trial:

We will see the patient in clinic the day following FMT (day 6 or FMT+1), day 12 (FMT+7), day 20 (FMT+15), and day 35 (FMT+30), at which point a detailed history regarding abdominal symptoms, evaluation of infectious complications, hospitalizations or complications of cirrhosis. Visits on day 6, day 12 and day 35 will be purely safety associated while, on the day 20 visit, we will repeat the pathophysiological studies. In order to ensure that we have enough samples, in case patients are not able to return at day 20, we will also collect all samples at day 12, but will only analyze them in case the day 20 visit does not occur. These visits, apart from the visit after FMT, will be ±2 days for patient convenience. At 5 months post-FMT, subjects will be followed up with

a phone call to evaluate potential SAEs, new onset of transmitted infections, new onset or significant worsening of chronic medical conditions or suspected unexpected serious adverse reactions that have occurred in between 35 days and 5 months for reporting purposes.

Group 2: Standard of care arm

The group 2 will undergo all sample collections, follow-up and cognitive testing as in group 1 but without the 5-day antibiotic therapy or the FMT. In addition, we will also not perform the sample collection that is done after 5 days of antibiotics in this group since no reasonable change in microbiota are expected over 5 days without antibiotics. The follow-up of this group will be same as that of the FMT group.

Primary Endpoint: Safety and Tolerability defined by the rate of development of FMT-related SAE and withdrawal from the study in FMT vs standard of care

Secondary Endpoints:

A. Microbiota composition and functional change

B. Cognitive function changes

Safety assessment

Specific issues to be captured will be

1. Antibiotic-related AE/SAEs
2. FMT procedure-related adverse events
3. FMT material-related adverse events (e.g. transmissible infection, allergic reaction)
4. Short-term safety: Both solicited and unsolicited adverse events will be recorded by, clinical assessment at FMT, (day 6 or FMT+1), day 12 (FMT+7), day 20 (FMT+15), and day 35 (FMT+30). The subjects will have the ability to telephone the study team at any point during the study.
5. Long-term safety: phone call at 5 months to evaluate for SAEs
6. Long-term extension (below)

Long-term extension:

We will aim to follow all subjects for at least 12 months post-enrollment in both groups after their initial study follow-up is completed. During this period, patients will be called at least or seen in person every 2 months, their clinical course monitored through active chart review and after at least 12 months, they will undergo stool collection, cognitive testing with PHES and

EncephalApp and an in-person evaluation for AEs and SAEs as defined in the original protocol.

Each intervening hospitalization will be evaluated and recorded for causes, changes in medications and outcomes. Specifically, infections will also be recorded.

Development of HE, defined as grade 2 or higher of West-Haven criteria which required admission, ER visits or changes to therapy will also be recorded in detail.

Patients will have an option to agree to this or participate in the follow-up via other protocols or ongoing studies or not to participate at all. They will also have an option to provide stool samples or not as they choose. We will stop follow-up after death or liver transplant or after at least 12 months based on the availability of the patient.