NCT03720392

DF/HCC Protocol #: 18-293

TITLE: A Phase 2 Study of Fecal Microbiota Transplantation (FMT) in Recipients after Allogeneic Hematopoietic Cell Transplantation (HCT)

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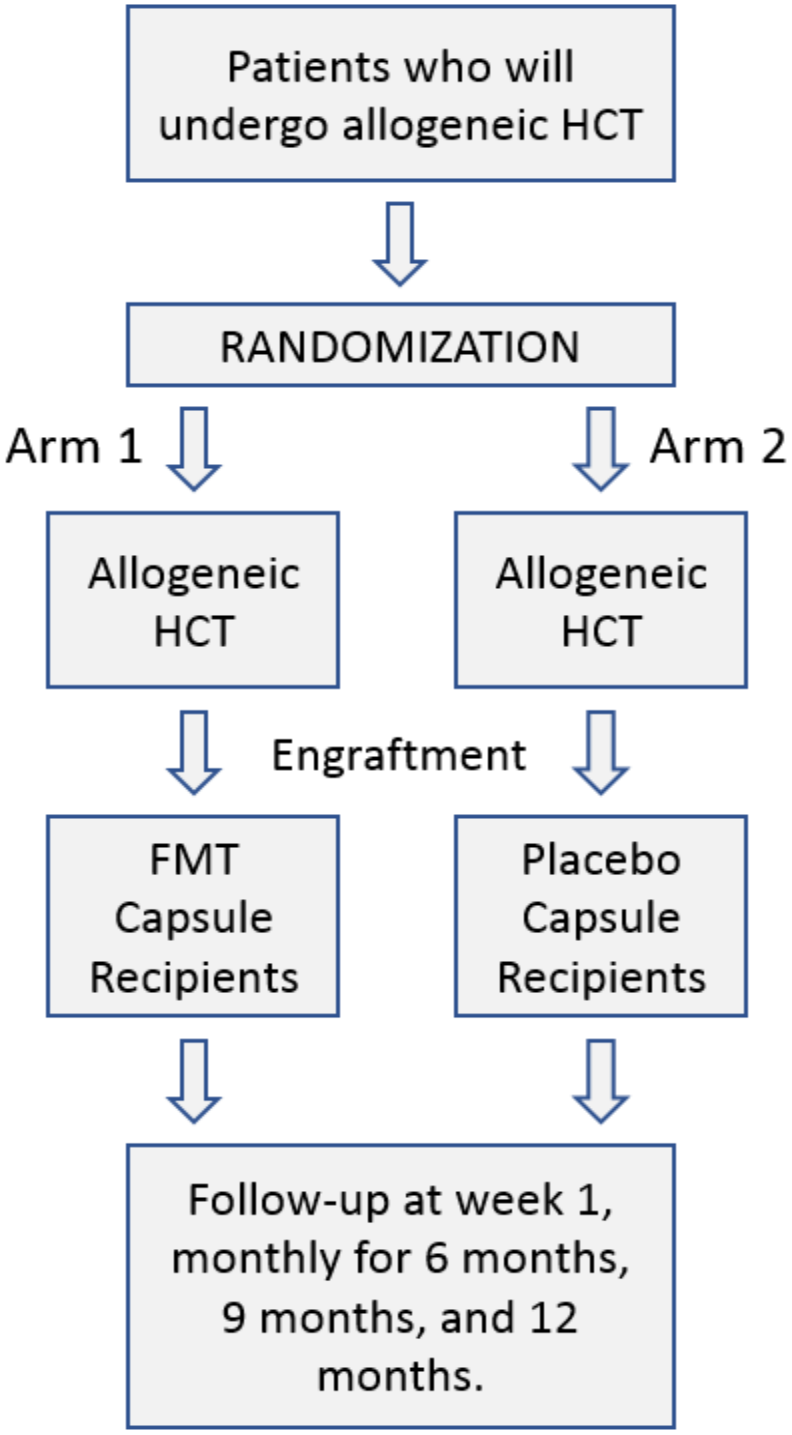
SCHEMA


Patients who will undergo allogeneic HCT
RANDOMIZATION
Arm 1
Arm 2
Allogeneic HCT
Allogeneic HCT
Engraftment
FMT Capsule Recipients
Placebo Capsule Recipients
Follow-up at week 1, monthly for 6 months, 9 months, and 12 months.

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1. OBJECTIVES

1.1 Study Design

This is a randomized, double-blind, placebo controlled, phase II study evaluating the ability of third-party oral fecal microbiota transplantation (FMT) to improve microbiome diversity in adult recipients of allogeneic hematopoietic cell transplantation (HCT). FMT or placebo capsules will be administered prior to and following HCT.

A total of 48 evaluable patients will be randomized between the FMT arm and the placebo arm (24 evaluable patients per arm).

1.2 Primary Objective

To determine if FMT administration increases microbiome diversity – measured by urinary 3-indoxyl sulfate (3-IS) levels at one month following the final post-HCT FMT – in recipients after allogeneic HCT as compared to patients receiving placebo. The primary endpoint is the proportion of participants who achieve gut microbiome diversity in each arm, measured at one month following the post-HCT FMT by urinary 3-IS levels, with 35 umol/mmol crea as the cutoff (≥ 35: diverse; < 35: not diverse).

1.3 Secondary Objectives

Timepoints for all objectives are from the last dose of FMT or placebo given post-HCT.

- Cumulative incidence of acute graft-versus-host-disease (GvHD) at 6 months
- Cumulative incidence of non-relapse-mortality (NRM) at 6 months and at 12 months
- Cumulative incidence of infection at 100 days
- Progression-free survival (PFS) and overall survival (OS) at 6 months and at 12 months
- GVHD-free/relapse-free survival (GRFS) at 12 months

2. BACKGROUND

2.1 HCT and the Microbiome

Allogeneic hematopoietic cell transplantation (HCT) is a potentially curative treatment for a number of malignant and non-malignant hematologic conditions. Non-relapse mortality (NRM) is a major barrier to allogeneic HCT, complicating 10-40% of all transplants, depending on patient- and transplant-related factors [Jagasia 2012, Deeg 2007]. Despite substantial advances in HLA-typing, immunosuppressive therapy, infectious disease management and supportive care, the leading causes of NRM remain GVHD and opportunistic infection. Thus, novel HCT approaches that can potentially limit such complications are of utmost importance. The recipient microbial flora may impact the development of acute GVHD [Peled, Blood 2016]. Recent focus on intestinal mucosal biology and advances in molecular techniques to simplify the complex mixtures of microorganisms have provided a better understanding of how a patient's fecal microbiome may be

associated with certain complications and overall outcomes after HCT [Teshima 2016, Morgan 2014, Taur 2015]. In HCT, the gastrointestinal mucosa is damaged, leading to an impaired intestinal microbiota with significantly reduced diversity. An analysis of fecal specimens taken from 80 recipients of allogeneic HCT at the time of engraftment showed that reduced intestinal microbiome diversity was associated with significantly worse survival outcomes (OS at 3 years was 36%, 60%, and 67% for low, intermediate and high diversity groups, respectively (p=0.019) [Taur 2014]. A subsequent study showed in 64 patients that increased bacterial diversity at 12 days after HCT was associated with reduced GVHD-related mortality. Furthermore, increased amounts of fecal bacteria belonging to the genus *Blautia* was specifically associated with reduced GVHD lethality [Jenq 2015]. More recently, a link between disease relapse after HCT and the abundance of several bacteria in the intestinal flora was demonstrated. In this study of 160 patients prospectively enrolled in a fecal collection protocol, *Eubacterium limosum* abundance was associated with less relapse and *Enterococcus* abundance was associated with more relapse [Peled, BBMT 2016]. While these associations between microbiome diversity and clinically important outcomes after HCT do not prove causality, the building pre-clinical evidence is compelling enough to investigate if these relationships can be altered to actually influence outcomes for patients.

Patients undergoing HCT have several reasons to have an altered microbiome, including: 1) prior and current use of antibiotics, 2) prior immediate hospitalizations, 3) use of chemotherapy and/or radiation, and 4) altered nutritional patterns. We hypothesize that approaches that restore a patient's microbiome diversity after HCT may be able to influence and improve outcomes after HCT. While therapeutic approaches to restoring microbiome diversity are still investigational, FMT from a healthy individual carries promise, after initial studies have shown this to be a remarkably effective therapy for recurrent *Clostridium difficile* colitis [van Nood 2013, Rohlke 2012]. At Massachusetts General Hospital (MGH), Dr. Elizabeth Hohmann has developed a novel protocol to produce and administer FMT via previously collected third-party frozen FMT capsules. The central hypothesis of our research is that empiric third party FMT after HCT can accelerate recovery of microbiome diversity and this may ultimately prevent infection and acute GVHD, thereby lowering NRM and improving overall outcomes after HCT.

2.2 FMT for *Clostridium difficile* infection

Clostridium difficile infection (CDI) is the predominant infectious cause of antibiotic-associated diarrhea, ranging in severity from mild diarrhea to death. Therapeutic approaches to restoring microbiome diversity have shown nearly complete cure in the treatment of recurrent *Clostridium difficile* colitis. The MGH Infectious Diseases Division has had a FMT program in place for recurrent CDI since 2012, initially demonstrating that frozen liquid fecal inocula from healthy volunteers can be administered by either the upper (NGT) or lower GI route (colonoscopy) with equivalent efficacy [Youngster CID 2014]. The FMT program subsequently transitioned to the generation of frozen, encapsulated inoculum from healthy unrelated donors to be delivered by the oral route. An initial pilot study of this novel third party FMT approach for recurrent CDI resulted in an overall 90% (95%CI, 68-98%) rate of clinical resolution of diarrhea (18 of 20 patients) [Youngster JAMA 2014]. To date, over 330 patients at MGH and partner institutions have been treated with third party freeze-dried capsule FMT for recurrent CDI. Overall cure rates in subjects for whom follow-up is available, as measured by resolution of diarrhea at 8 weeks, is 93%, with

only seven patients requiring two or more administrations.

Among the most vulnerable populations susceptible to CDI are HCT recipients, where the incidence of CDI is as high as 25%. Although FMT to treat CDI is becoming increasingly common in immunocompetent individuals, parts of the medical community have been reluctant to expand this therapy to include HCT patients, despite the increased risk of CDI- related morbidity and mortality. To date, nine HCT recipients (allogeneic= 7; autologous =2) referred from MGH and partner institutions with recurrent CDI have been treated with FMT with overall excellent success and limited adverse events. All patients were free of recurrent CDI at 8 weeks after FMT, although two patients eventually died of progression of their underlying malignancy, and one died of an intracranial hemorrhage. One patient did experience a spontaneous relapse of CDI at nine weeks after treatment. Side effects were primarily mild non-specific GI complaints after ingestion of capsules, which are common in patients with recurrent CDI. No transmission of infection was observed. CDI eradication in HCT individuals was associated with an increase in microbiome diversity, specifically an increase in *Firmicutes* and *Bacteroidetes*, and a decrease in *Proteobacteria phyla* [Zhu 2011]. These data provide preliminary evidence that FMT for HCT patients is mechanistically similar to CDI clearance in immunocompetent individuals, which supports clinical observations that FMT is safe and effective in HCT patients.

2.3 FMT to restore intestinal microbiome diversity following HCT

Following our experience with treating recurrent CDI with third-party cryopreserved FMT in HCT recipients, we conducted a pilot study administering empiric third party FMT after HCT. In this study, previously collected third party freeze-dried FMT was administered no later than 4 weeks after neutrophil engraftment and at least 48 hours after stopping systemic antibiotics. The absence of active acute GI GVHD was a pre-treatment criterion. Participants received a single standard dose of oral FMT, which was comprised of 30 FMT capsules administered over 2 consecutive days. The primary endpoint of this study was feasibility of empiric FMT in HCT recipients, which was defined as feasible if 80% or more of eligible participants were able to swallow 15 or more capsules. Correlative analyses were performed on serial stool (16s rRNA sequencing) and urine (liquid chromatography/tandem mass spectrometry for 3-IS) samples to measure microbiome diversity. We enrolled a total of 18 patients; 5 patients did not receive FMT capsules (aGVHD prior to planned FMT administration, n=3; patient withdrawn, n=2). All 13 participants who were

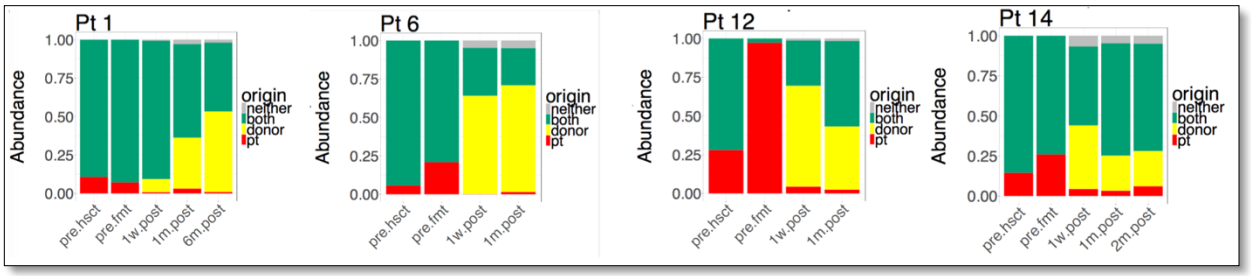

Figure 1. Microbiome dynamics in pilot FMT study. Depiction of 16s rRNA sequencing of stool samples collected from same two patients, emphasizing origin of microbiota taxonomic units, classified as either being patient-derived, donor-derived, both, or neither.

given FMT were able to swallow all 30 FMT capsules. Clinical follow up is still ongoing, but thus far, FMT has been well tolerated, with only 1 treatment-related significant adverse event (abdominal pain). Analysis of correlative stool samples indicate expansion of donor operational

taxonomic units following FMT (Figure 1) with additional studies (urine 3-IS, clostridia abundance, Simpson's Diversity Index) suggesting maintenance and recovery of intestinal microbiome diversity (Figure 2). The median pre-HCT 3-IS level in this study was 34.6 umol/mmol crea, a level that 57% of patients have thus far achieved one month post-FMT, indicating that FMT may be associated with restoration of pre-HCT microbiome diversity. Thus, the preliminary results of our pilot study indicate that empiric third party FMT following allogeneic HCT appears to be feasible, safe, and associated with expansion of recipient microbiome diversity.

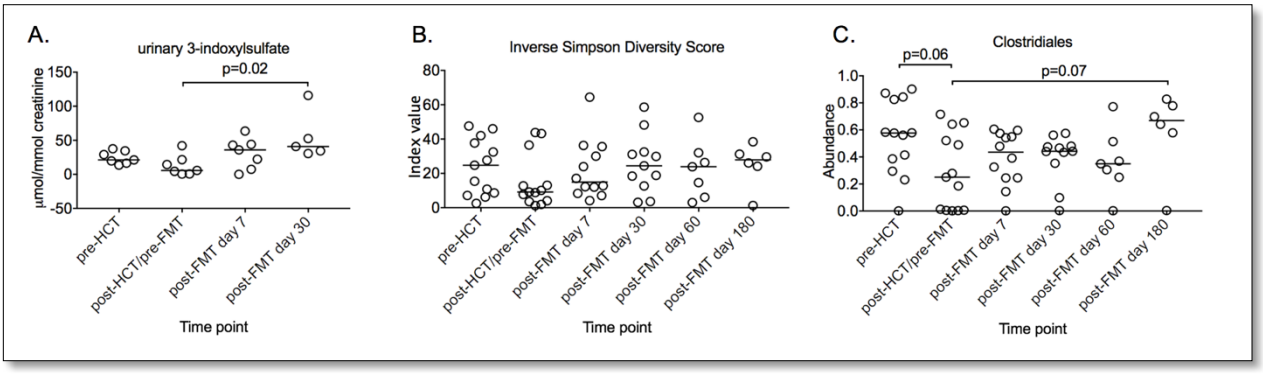

Figure 2. Longitudinal changes in A) urinary 3-IS, B) inverse Simpson index, and C) Clostridialis abundance prior to allo-HCT and following FMT administration in the early post-transplant period.

2.4 FMT for treatment of GVHD

A few small case series to date have described the use of FMT for patients with acute GVHD of the GI tract. In one pilot study, a total of 4 patients with steroid-resistant (n=3) or steroid-dependent gut aGVHD (n=1) received FMT. No severe adverse events attributed to FMT were observed. All patients responded to FMT, with 3 complete responses and 1 partial response (Kakihana 2016). In a separate case series, 3 patients with steroid-refractory GVHD of the gut received FMT. There were 2 complete responses and 1 partial response (Spindelboeck 2017). These two reports represent the initial descriptions of FMT for GVHD. Further studies are planned to further investigate the role of FMT in this population.

2.5 Rationale

There is growing evidence that commensal intestinal microbiota is dysregulated following allogeneic HCT and that low microbial diversity is associated with increased non-relapse mortality (NRM) and inferior survival. The central hypothesis of our research is that empiric third-party FMT can accelerate recovery of microbiome diversity early after HCT and this may prevent infection and acute graft-versus-host disease (GVHD), thereby lowering NRM and improving overall outcomes after HCT. Initial results of our pilot study demonstrate this approach is feasible, safe, and associated with expansion of recipient microbiome diversity. We therefore plan to further this research by performing a placebo-controlled trial in this population.

2.6 Correlative Studies Background

In patients with *C. diff* infection and GVHD, the types of resident bacterial species or diversity in

their gastrointestinal flora is altered (de Castro 2015, Taur 2014). This dysregulated flora is restored to a more diverse composition following FMT [de Castro 2015, Youngster 2014]. In order to define the large number of unique bacterial and fungal species, ribosomal sequencing is used. Sequencing16S rRNA, a component of bacterial and fungal ribosomes, is unique to each species allowing phylogenic and taxonomic categorization. We will use16S rRNA sequencing to determine gastrointestinal bacterial (microbiome) and fungal (mycobiome) diversity in HSCT patients before and after receiving FMT. These data will be correlated with clinical outcomes.

Urinary concentration of 3-indoxylsulfate (3-IS) is a clinically relevant biomarker of intestinal microbiota disruption after allogeneic HCT. Low levels of urinary 3-IS in the first 10 days after allogeneic HCT have been associated with higher transplant-related mortality (TRM) and worse overall survival, with the majority of TRM being related to acute GI GVHD [Weber 2015)].

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Men or women ≥ 18 and ≤ 80 years old

3.1.2 Patients designated to undergo myeloablative or intermediate intensity allogeneic peripheral blood or bone marrow hematopoietic cell transplantation. Consent will be obtained prior to admission for HSCT. Patients receiving any donor source of stem cells are eligible. Eligible conditioning regimens are those defined as myeloablative by the ASBMT Consensus Criteria (Bacigalupo 2009) as well as the combination of fludarabine with melphalan (100-140 mg/mg^2)

3.1.3 Any GVHD prophylaxis regimen is allowed.

3.1.4 ECOG performance status ≤ 2 (Karnofsky ≥ 60%, see Appendix A)

3.1.5 Patients with adequate physical function as measured by

- Cardiac: Left ventricular ejection fraction at rest must be ≥ 40%, or shortening fraction >25%
- Hepatic:
  - Total Bilirubin ≤ 2.5 mg/dL, except for patients with Gilbert's syndrome or hemolysis
  - ALT, AST, and Alkaline Phosphatase < 5 x ULN
- Renal: Serum creatinine within normal range, or if serum creatinine is outside normal range, then renal function (measured or estimated creatinine clearance or GFR) ≥ 40mL/min/1.73m^2
- Pulmonary: DLCO (corrected for hemoglobin), FEV_1 and FVC ≥ 50% predicted

3.1.6 Women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Women of childbearing potential will have a urine pregnancy test, which must be negative, on Study Day 1, prior to receiving FMT. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study and for 3 months after FMT.

3.1.7 Ability to understand and the willingness to sign a written informed consent document, including the willingness to accept risk of unrelated donor stool.
3.1.8 Ability to swallow large capsules.

3.2 Exclusion Criteria

3.2.1 Prior allogeneic hematopoietic stem cell transplantation. (Patients may have received a prior autologous hematopoietic stem cell transplant.)
3.2.2 Participants who are receiving any other investigational agents.
3.2.3 Uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
3.2.4 Patients with active or uncontrolled bacterial, viral, or fungal infection(s) requiring systemic therapy.
3.2.5 Planned use of prophylactic donor lymphocyte infusion (DLI) therapy.
3.2.6 Delayed gastric emptying syndrome or large hiatal hernia
3.2.7 Known chronic aspiration
3.2.8 Participants with a history of significant allergy to foods not excluded from the donor diet (excluded foods are tree nuts, peanuts, shellfish, eggs)
3.2.9 Pregnant and breast-feeding women are ineligible because they are not eligible for hematopoietic stem cell transplantation.
3.2.10 HIV-positive participants are ineligible.
3.2.11 Participants who are unable to swallow pills.
3.2.12 Participants with end-stage liver disease (cirrhosis)
3.2.13 Participants with acute, active gastrointestinal infection (e.g., typhlitis, diverticulitis, appendicitis)
3.2.14 Participants with inflammatory bowel disease (e.g., ulcerative colitis, Crohn's)
3.2.15 Prior total colectomy

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. Given the small size of our study there is insufficient power to detect small effects between groups.

4. REGISTRATION AND RANDOMIZATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

An email confirmation of the registration will be sent to the Overall PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the registration.

Once registration is confirmed, the MGH FMT Core Lab will randomize per their "Randomization Scheme for FMT Clinical Trials."

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If the subject does not receive protocol therapy following registration, the subject must be taken off-study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

There will be two treatment arms. Arm 1 participants will receive **two doses of** FMT **after** HCT. Arm 2 participants will receive placebo capsules instead of FMTs at the same time points. Participants will be randomized in a 1:1 ratio. Double-blinding will be maintained.

An individual not involved in patient assessments will receive the randomization assignment, take appropriate capsules (either FMT (Arm 1) or placebo (Arm 2)) from relevant freezer locations, and place them on dry ice for administration. Vials will be labeled "STUDY Caps" for administration so that neither physician, research nurse, nor participant will be able to identify FMT vs. placebo capsules. The **certificate of analyses** identifying capsules as FMT or placebo will then be sealed in an opaque envelope and saved for review at the time of unblinding. This will serve as a "double-check" on actual assignment.

A total of 48 evaluable patients will be randomized between the FMT arm and the placebo arm (24 evaluable patients per arm).

Arm 1 will consist of **two (2)** doses of FMT **given after HCT,** starting within 4 weeks after engraftment after HCT.

Arm 2 will mirror Arm 1, using placebo capsules instead of FMT capsules. It will consist of **two (2)** doses of placebo, starting within 4 weeks after engraftment after HCT.

In this protocol, a standard dose of oral FMT **will be defined as** 15 capsules **administered during on a single day. Thus 2 doses of FMT will total 30 capsules.** In our previous study of FMT in patients undergoing allogeneic HCT, thirty capsules contained microbial content of a median of 38.6 g of feces (range, 24-56.7).

The FMT/placebo doses can be given within 4 weeks after engraftment after HCT. These 2 doses of oral FMT**/placebo will be administered as** 15 capsules per day for two days **(total 30 capsules)**; the two days must occur within a **14** -day span – they can be consecutive, but they do not need to be.

Participants will be asked to fast for 2 hours prior to and 1 hour following capsule intake (drinking water will be allowed) There are not specific prohibited foods in associated with FMT administration. Capsules will be individually handed to participants by a research nurse or physician. Each capsule will be taken with a sip of water. Participants will be asked to drink at least 12 ounces of water during capsule administration to facilitate dilution of stomach contents and transit into the small intestine. Participants should swallow the daily allotment of FMT capsules within 1 hour.

Capsules must not be crushed, chewed, or dissolved.

Capsules will be administered on an inpatient or outpatient basis. A record will be maintained of the time and date of each capsule administration. Participants will be evaluated for 15 minutes immediately following capsule administration. Immediate side effects have been very uncommon in our past experience in treating CDI or in our pilot study. Vomited doses will be counted and recorded. Substitute FMT capsules will not be administered to replace vomited doses.

Participants may not receive any anti-bacterial antibiotics (by any route) within 48 hours prior to the pre-HCT capsule administration and prior to the first day of the post-HCT administration. For subjects with renal dysfunction (CrCl < 60 ml/min/1.73m^2) participants may not have received antibiotics within 72 hours prior to pre-HCT capsule administration and prior to the first day of the post-HCT administration. Specifically, if vancomycin is being given for prophylaxis of *C. difficile* infection, it must be stopped at least 48 hours prior to the first day of the **FMT/placebo** administration. Administration of trimethoprim / sulfamethoxazole for prevention of Pneumocystis pneumonia is not forbidden although the use of alternative measures – i.e. oral atovaquone or IV pentamidine – is preferred for the time around and immediately after capsule administration.

Participants will undergo allogeneic hematopoietic stem cell transplantation according to institutional standards. Any donor source for stem cells and regimen for GVHD prophylaxis are allowed.

The doses of FMT or placebo will commence only after neutrophil engraftment has occurred**, defined as** ANC $\geq$ 500/μL for three (3) consecutive days. If the patient does not experience successful neutrophil engraftment, then the participant will not receive the second dose of FMT or placebo.

FMT/placebo **administration** must begin no later than 4 weeks after the date of neutrophil engraftment.

If the participant receives the first FMT placebo and then develops an infection requiring antibiotics, then the second dose of **FMT/placebo** will be delayed until 48 hours after the end of antibiotic dosing. In cases of infection requiring antibiotics, FMT or placebo must start within 2 weeks after stopping systemic antibiotics. Treatment with anti-viral agents, anti-fungal agents and/or trimethoprim / sulfamethoxazole is allowed at any time with no impact on FMT or Placebo administration.

A minimum of 15 (out of the usual total number of 30) capsules must be swallowed for a dose of FMT or placebo to be considered successful.

Subjects receiving any amount of FMT or placebo capsules will be followed for at least 6 months.

5.2 Pre-Treatment Criteria

Prior to administration of the FMT or placebo capsules, the participant must meet the following criteria:

- ANC must be **≥ 10**00/μL prior to each day of FMT/placebo**. Use of growth factor supplementation is allowed.**
- ECOG performance status ≤ 3 (Karnofsky ≥ 40%, see Appendix A)
- Participant must NOT receive any antibiotics within 48 hours prior to dose (72 hours for those with CrCl <60 ml/min/1.73m^2)
- Participant must NOT have a fever (temperature > 100.4 F) within 48 hours prior to FMT/placebo
- Participant must NOT have uncontrolled intercurrent illness including, but not limited to, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or acute psychiatric illness that could limit study compliance.
- Participant must NOT have acute, active gastrointestinal infection (e.g., typhlitis, diverticulitis, appendicitis)
- Participant must be able to swallow oral medications.

Additionally, this criterion must be met on day 1 only (does not apply on day 2):

- Adequate hepatic function (Total Bilirubin ≤ 5 mg/dL; AST (SGOT) and ALT (SGPT) ≤ 5 x institutional upper limit of normal). An exception may be made for patients in whom a diagnosis of hemolysis or Gilbert's is made.

Labs drawn on the day of FMT capsule/placebo administration must be reviewed and meet pre-treatment criteria prior to treatment. There are no planned dose delays allowed if these pre-treatment criteria are not met. Dosing delays are allowed for difficulty swallowing these many capsules as outlined in Section 6.

5.3 Follow Up

Participants will be seen one week after the second day of the FMT or placebo dose, and then monthly for 6 months (1 month, 2 months, 3 months, 4 months, 5 months, 6 months), at 9 months and at 12-months after FMT or placebo (see Section 10, Study Calendar). Subjects who receive

FMT or placebo as outpatients will receive a phone call at 24-48 h after the second FMT or placebo dosing day. Standard physical exam, vitals, KPS, blood testing (CBC and chemistries), interval history, and GVHD assessment will take place at each study visit along with research toxicity assessments. Additionally, stool and urine samples for correlative studies will be collected at the 1 week, 1 month, 2-month, 6-month and 12-month follow-up visits.

5.4 General Concomitant Medication and Supportive Care Guidelines

There are no required concomitant medications in this trial. Any concomitant medication administration is per the discretion of the treating physician(s). Participants may receive all concomitant therapy deemed necessary to provide optimal support.

Rarely, recipients of FMT have experienced fever after administration of FMT capsules. This is more common in younger individuals and children, and may represent an immunological reaction to a new intestinal flora. Participants who are febrile after FMT will have a clinical evaluation. Blood and other cultures will be performed as deemed clinically necessary. Such patients may be given antibiotics if they are hemodynamically unstable or clinically or microbiologically diagnosed with an active infection. Otherwise, it is recommended that they be observed.

Patients are allowed to be treated with any standard regimens for GVHD prevention as well as any post-HSCT therapies considered standard by the treating physician. Participants will not be allowed to be on any other investigational treatment protocol from admission for HCT until the final FMT vs placebo administration.

5.5 Criteria for Taking a Participant Off Protocol Therapy

After the second FMT or placebo administration (after HCT), the participant will be off protocol therapy. No further protocol therapy will be given.

A participant may not receive all doses of FMT or placebo if any of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the regimen
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy and the date the participant was removed must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the relevant Off-Treatment/Off-Study information will be updated in OnCore.

5.6 Duration of Follow Up

Participants will be followed for 12 months after the last day on which he/she received FMT or placebo capsules or until death, whichever occurs first.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death
- Completion of the 12-month follow-up period

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure Off Treatment/Off Study information is updated in OnCore in accordance with DF/HCC policy REGIST-101.

6. DOSING DELAYS/DOSE MODIFICATIONS

If a participant has a difficult time swallowing 15 capsules in one day, then capsule administration may be spaced out over three (3) days (10 capsules per day), instead of the standard dosing over two (2) days (15 capsules per day). This is a standard approach in capsule based FMT for recurrent *C. difficile* colitis, though it is noted that the vast majority of patients, including children and the elderly, are able to take 15 capsules in a single sitting. There will be no dose adjustment for participants unable to take 10 capsules per day. In the case of a dosing delay or dose modification, dosing and assessment schedules will remain unchanged (i.e., dosing day count will continue).

Administration of the capsules with applesauce or pudding rather than water is also a standard approach, for subjects who have difficulty swallowing large capsules. The capsules are size 00 (the size of a large pre-natal vitamin). Please note that subjects are fasting around the time of administration to minimize the risk and volume of potential emesis, which is rare. There is no reason food would alter the efficacy of FMT.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests. Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the CRF. If the abnormality was not a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE. If possible, the laboratory abnormality should be recorded as a medical term and not simply as an abnormal laboratory result (e.g., record thrombocytopenia rather than decreased platelets).

A qualified Investigator will evaluate all adverse events as to seriousness. A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or

Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- A standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.
- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- The administration of blood or platelet transfusion as routine treatment of studied Indication. However, hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.
- A procedure for protocol/disease-related investigations (e.g., surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
- Elective or pre-planned treatment for a pre-existing condition that did not worsen
- Emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- Respite care

For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to IP, action taken regarding IP, and outcome.

AE monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting in addition to routine reporting.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test, or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms as per instructions.

7.1 Expected Toxicities

In prior experiences, participants undergoing Fecal Material Transplantation experienced 24 – 48 hours of mild transient adverse events. Common events include fever, cramping, diarrhea, and gas.

Rarely participants experience bacteremia after FMT. In our institutional experience with over 400 FMT recipients (various clinical indications), there was a single incident where 2 subjects developed bacteremia from a relatively resistant organism (ESBL *E. coli*) that was later identified in the stool specimen from the same healthy FMT donor. This donor provided samples before specific screening for ESBL organisms was implemented. Additional screening will minimize this risk, but we don't believe it can be entirely eliminated.

Based on our previous experience with these subjects, special attention will be given to patients who develop bacteremia with enteric organisms within 4 weeks of FMT dosing and additional testing may be performed to assist with assessment of attribution.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  AEs that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information that is provided.

- **Expectedness.** Adverse events can be 'Expected' or 'Unexpected.'
  - Expected Adverse Event – An event that has been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list or is included in the informed consent document as a potential risk. Refer to section 7.1

for a listing of expected adverse events associated with the study agent.
    - Unexpected Adverse Event – An event that varies in nature, intensity or frequency from information provided in the current adverse event list or when it is not included in the informed consent document as a potential risk.

- **Attribution** is the relationship between an adverse event and the study treatment. Attribution will be assigned as follows:
    - Definite – The AE *is clearly related* to the study treatment.
    - Probable – The AE *is likely related* to the study treatment.
    - Possible – The AE *may be related* to the study treatment.
    - Unlikely – The AE *is doubtfully related* to the study treatment.
    - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Expedited Adverse Event Reporting

7.3.1 Reporting to the PI

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, and/or within 30 days of the last dose of treatment on the local institutional SAE form.

In the event of an unanticipated problem or life-threatening complications, treating investigators must immediately notify the Overall PI.

7.3.2 DF/HCC Expedited Reporting Guidelines

Investigative sites will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

The policy states that the following SAEs are required to be reported for all subjects enrolled and actively participating in the trial or when the SAE occurs within 30 days of the last study intervention:

- **Grade 2 (moderate) and Grade 3 (severe) events** - Only events that are Unexpected and Possibly, Probably or Definitely Related/Associated with the Intervention.
- **ALL Grade 4 (life-threatening or disabling) Events** – Unless expected AND specifically listed in protocol as not requiring reporting.
- **ALL Grade 5 (fatal) Events**

Grade 2 and Grade 3 laboratory abnormalities that are considered by the investigator to be clinically insignificant and do not require therapy, or adjustment in prior therapy, do not need to be reported to the DFCI IRB as an SAE.

7.4 Expedited Reporting to the Food and Drug Administration (FDA)

The IND holder / physician study sponsor, Dr. E. Hohmann, will be responsible for all communications with the FDA. She will report any serious adverse event that meets the FDA's

criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

Unexpected fatal or life-threatening experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 7 calendar days after initial receipt of the information.

All other serious unexpected experiences associated with the use of the study treatment will be reported to FDA as soon as possible but in no event later than 15 calendar days after initial receipt of the information.

Events will be reported to FDA b**y email and in hard copy as formal submissions to our IND (16895).**

7.5 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 7.1.

8.1 Fecal Microbiota Transplantation Capsules

8.1.1 Donor Screening

Donors are healthy, nonpregnant adults aged 18 to 50 years, taking no medications, and with a normal body mass index (18.5-25 [calculated as weight in kilograms divided by height in meters squared]). Volunteers are excluded for any significant medical history (with the exception of resolved traumatic injury) or for any use of antibiotics in the preceding 6 months, and cannot be healthcare workers active full time in a clinical setting. Candidates must pass the American Association of Blood Banks donor questionnaire, then undergo physical examination and general laboratory screening tests. Refer to Appendix C for a summary of the testing performed on donor blood and stool. Volunteers are asked to refrain from eating common allergens within 5 days of stool donation but otherwise not to alter their diets. At the time of donation, subjects have an interim health query for febrile, systemic, and gastrointestinal symptoms and were deferred for any change in health status. All donations are stored without use for an additional 4 weeks to allow retesting of donors for human immunodeficiency virus, hepatitis **<u>A</u>**, B and C**, and syphilis** prior to clinical use of the inoculum. Testing includes evaluation of donor's plasma viral load for EBV and

CMV before and after donations, and assessment of pre- **and post**-donation fecal samples for VRE, MRSA, **ESBL**, and carbapenemase producing Enterobacteraceae (CRE) using commercially available chromogenic agars. **A complete list and timing of screening tests is given in Appendix C, and approved by the FDA. See also the current IDB (Appendix B).**

8.1.2 Preparation of Frozen Inocula

Processing is carried out under aerobic conditions. A fecal suspension is generated in normal saline without preservatives using a commercial blender. Materials are sequentially sieved to remove particulate material. The final **microbial** slurry is concentrated by centrifugation and resuspended in saline at one-tenth the volume of the initial sample with **40%** glycerol added as a bacterial cryoprotectant. **The concentrated microbial** solution is pipetted into size 0 capsules (650 μL), which are closed and then secondarily sealed in size 00 capsules. Capsules are stored frozen at −80°C (−112°F). Capsules are transported to the clinic or bedside on dry ice. Commercially available acid-resistant hypromellose capsules (DRCaps, Capsugel) are used. Stability of capsules in an acid environment mimicking the stomach has been tested by evaluating trypan blue–filled capsules. At 37°C (99°F) and a pH of 3 or less, the capsules are stable for 115 minutes before dye is released. Each inoculum is prepared from the feces of a single donor and a full treatment of 30 capsules contains sieved, concentrated material derived from approximately 48 g of fecal matter (mean per capsule, 1.6 g; range, 1.0-2.05 g). Capsules are odorless and tasteless.

8.1.3 Storage and Stability

We do not expect, and have not previously observed a decrement in the viability or efficacy of the capsules, which will be stored at -80 C in an alarmed freezer. Capsules will be discarded if not used by their expiration date which is **9** months after encapsulation. Capsules which are not structurally intact cannot be used (see Certificate of Analysis). Representative FMT Capsules are saved for analysis in the event of infection transmission. FMT Capsule lots where numbers allow have assessment of aerobic colony forming units over time (0 and **9**months) as a measure of stability.

8.1.4 Administration

Participants will receive FMT capsules, 15 each on 2 days. Participants will be NPO for at least 2 hours prior to and 1 hour following capsule administration. Administration will be with at least 12 ounces of water.

8.2 Placebo Capsules

8.2.1 Capsule Preparation

Placebo capsules consist of a combination of food grade powdered cocoa and gelatin in normal saline (IV grade) / USP glycerol (same vehicle as FMT capsules). Gelatin is added to make the placebo solution more viscous and physically fill the capsules in the same way that the standard FMT inoculum does for appearance purposes. Placebo capsules are identical in appearance to FMT capsules, and are produced as described in IND #16011 (E. Hohmann MD).

8.2.2 Administration

Participants will receive placebo capsules, 15 each on 2 days. Participants will be NPO for at least 2 hours prior to and 1 hour following capsule administration. Administration will be with at least 12 ounces of water.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Laboratory Correlative Studies

9.1.1 Urinary 3-indoxyl sulfate levels

Urinary 3-indoxyl sulfate (3-IS) levels will be measured at baseline and at one month following the final post-HCT FMT administration. Analysis will be done via reverse-phase liquid chromatography – electrospray ionization - tandem mass spectrometry in negative ion multiple reaction monitoring mode as has been demonstrated previously [Weber 2015, Taur 2012].

9.1.2 16s rRNA Sequencing

To determine the fecal microbiome and mycobiome diversity, stool samples will be analyzed using a standard sequencing method that identifies unique signatures from each bacterial or fungal species. Specifically, the bacterial species are determined by sequencing 16S ribosomal RNA (rRNA) using V4-V5 PCR amplification. Fungal species will be identified using internal transcribed spacer sequences (ITS).

V4–V5 variable region of the 16S rRNA from isolated ITS DNA from each fecal specimen will be determined using PCR containing purified DNA, Taq DNA polymerase, and forward and reverse primers. Following amplification, the PCR product will be sequenced, analyzed and grouped into operational taxonomic units for classification.

Microbial diversity will be estimated from stool sequencing data by calculating the inverse Simpson index, an ecological estimate of α diversity, calculated to represent the reciprocal of the expected probability of two randomly selected bacterial sequences as belonging to the same operational taxonomic unit [Magurran 2004].

9.1.3 MAGIC GVHD biomarkers

In recent years, diagnostic and prognostic blood biomarkers have emerged for acute GVHD. In an exploratory analysis, we will evaluate GVHD biomarker profiles, using a validated algorithm of 2 Mount Sinai Acute GVHD International Consortium (MAGIC) biomarkers (ST2 and REG3α). Blood biomarker profiles will be evaluated for patients receiving FMT or placebo at multiple time-points post-transplant. We will also explore associations between biomarker profiles and microbiome diversity and composition, as evaluated from urine and stool specimens.

Blood sample collections (one red top tube with 5 mL blood for serum at each time point) for MAGIC GVHD biomarker evaluations will be collected at Day 0, Day +7, Day +14, Day +21, Day +28, Day +56, and Day +90. All these time points are in relation to BMT date. Participants are exempt from collection of these samples as part of this protocol if they are co-enrolled on the MAGIC Database and Bioreposity protocol.

9.1.4 Neutrophils functional analyses

An exploratory analysis of neutrophil function will be examined posttransplant. Blood will

only be collected at 2 months post second FMT/Placebo (+/- 14 days). One 10mL EDTA tube.

9.2 Sample Collection and Storage

Samples will be collected at the following timepoints:

- Baseline; between 14 days prior to first FMT/placebo until first stool after the first FMT dose
- 1 week post HSCT (+/- 4 days)
- 1 week following the second day of the post-HCT FMT or placebo administration (+/- 3 days)
- 1 month following the second day of the post-HCT FMT or placebo administration (+/- 14 days)
- 2 months following the second day of the post-HCT FMT or placebo administration (+/- 14 days)
- 6 months following the second day of the post-HCT FMT or placebo administration (+/- 28 days)
- 12 months following the second day of the post-HCT FMT or placebo administration (+/- 28 days)

Stool will be collected by the study participant using the instructions in Appendix E. Urine will also be collected at each of these timepoints.

If for any reason a participant does not receive the second day of capsules for FMT/**placebo,** then subsequent sample collections will be benchmarked from the first day of the post-HCT FMT.

Because bacteria and fungi require different analyses, the storage of the collected specimens differs. For fungal analysis, stool will be collected and then directly frozen at -80^{o}C. For bacterial analysis, 90% ethanol solution will be added to the stool, which will then be frozen. See appendix E for more information. Urine specimens will be frozen at -80^{o}C and stored for future analysis.

Universal precautions will be used when handling all specimens. All specimens will be labeled in a de-identified manner.

10. STUDY CALENDAR

	Baseline (within 42 days prior to admission for HSCT)	**1-week post HSCT**	**FMT/** placebo **Day 1**	**FMT/ placebo Day 2**	**1-week post 2nd FMT/ placebo (I)**	**Follow up (H)**	**12 months post 2nd FMT/ placebo (I)**

Informed consent	X						
Medical History	X						
Physical Exam	X	X	X	X	X	X	X
Vital Signs (A)	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X
CBC w/ diff, PLTs	X	X	X	X	X	X	X
Serum chemistry (B)	X	X	X	X	X	X	X
Pregnancy Test (C)	X		X				
Hepatitis B, hepatitis C, HIV testing	X						
Con Meds	X						
FMT or Placebo Capsule administration			X	X			
AE/Toxicity Evaluation		X	X	X	X	X	X
Stool and urine samples for correlatives (D)	X	X			X	X (D)	X
Blood sample collection for MAGIC GVHD biomarker evaluation (E)		**X**	**X**	**X**		**X**	
Blood for correlatives (F)						X	
Screen for AROs (G)	X						
Acute GVHD evaluation		X	X	X	X	X	X

(A) Must include temperature, heart rate, blood pressure, respiratory rate
(B) Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, creatinine, glucose, magnesium, phosphorus, potassium, total protein, SGOT (AST), SGPT (ALT), sodium.
(C) **Pregnancy test on FMT day 1 should be resulted prior to FMT administration**
(D) Stool and urine samples for correlatives are required only at the following visits:

- Baseline (between 14 days prior to first FMT/placebo until first stool after first FMT dose)
- 1 week post HSCT (+/- 4 days)
- 1 week post second FMT/placebo (+/- 3 days)
- 1 month post second FMT/placebo (+/- 14 days)
- 2 months post second FMT/placebo (+/- 14 days)
- 6 months post second FMT/placebo (+/- 28 days)
- 12 months post second FMT/placebo (+/- 28 days)

(E) Blood sample collections for MAGIC GVHD biomarker evaluations will be collected at Day 0, Day +7, Day +14, Day +21, Day +28, Day +56, and Day +90. All these time points are in relation to BMT date. If are exempt from collection of these samples as part of this protocol if they are co-enrolled on the MAGIC Database and Biorepository protocol. One red top tube with 5 mL blood for serum at each time point.
(F) Blood will only be collected at 2 months post second FMT/Placebo (+/- 14 days). One 10mL EDTA tube
(G) Screen for Antibiotic-Resistant Organisms including VRE, MRSA
(H) Follow up schedule: Participants receiving FMT/placebo will have a follow up visit at 1,

2, 3, 4, 5, 6, 9, and 12 months post last dose of study drug.
(I) Windows for visits / evaluations are as follows:

- 1-week post-FMT/placebo, 1-week post-HSCT: +/- 3 days
- **Follow up visits: months 1-6**: +/- 14 days
- **Follow up visits: months 9 and 12**: +/- 28 days

11. MEASUREMENT OF EFFECT

11.1 Microbiome Diversity

Microbiome diversity will be measured by urinary 3-indoxyl sulfate (3-IS) levels at one month following the final post-HCT FMT. The cutoff for gut microbiome diversity is a urinary 3-IS level of ≥ 35 umol/mmol crea (≥ 35 = diverse; < 35 = not diverse).

11.2 Survival

Overall Survival: Overall Survival (OS) is defined as the time from the first dose of FMT or placebo to death due to any cause, or censored at date last known alive. OS will be assessed at 6 and 12 months.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from first dose of FMT or placebo to the earlier of underlying disease progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation. PFS will be assessed at 6 and 12 months.

GVHD-Free/Relapse-Free Survival: GVHD-Free/Relapse-Free Survival (GRFS) is defined as the time from first dose of FMT or placebo to the earlier of diagnosis of GVHD or disease progression or relapse. Patients who have not experienced any of these events will be censored at date of last disease evaluation. GRFS will be assessed at 12 months.

Non-relapse mortality: Non-relapse mortality (NRM) is defined as the time from first dose of FMT or placebo to death without relapse or progression or underlying disease. Deaths from any cause without prior progression are considered as NRM events. NRM will be assessed at 6 and 12 months.

11.3 Infection

Infections (bacteremia, *C. difficile*, and others) will be diagnosed per clinical norms, and testing will be at the discretion of treating physicians. Data will be collected from medical records continuously, to assess possible concurrent infection. Bacteremia with organisms typically arising from the GI tract and C. difficile infection are diagnoses of special interest. There will be no specific microbiological screening tests ordered for the purpose of this study.

11.4 Acute GVHD

The cumulative incidence of overall grades II-IV and grades III-IV acute GVHD will be assessed through six months after last dose of FMT or placebo. Grading is as detailed in Appendix F.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Management

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Board (DSMB) will review and monitor study progress, toxicity, safety and other data from this study. The Board is chaired by a medical oncologist from outside of DF/HCC and its membership composed of internal and external institutional representation. Information that raises any questions about participant safety or protocol performance will be addressed by the Overall PI, statistician and study team. Should any major concerns arise, the DSMB will offer recommendations regarding whether or not to suspend the study.

The DSMB will meet twice a year to review accrual, toxicity, response and reporting information. Information to be provided to the DSMB may include: participant accrual; treatment regimen information; adverse events and serious adverse events reported by category; summary of any deaths on study; audit results; and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

13.1 Primary endpoint

The primary endpoint is the proportion of patients who achieve gut microbiome diversity at one month following the final post-HCT FMT, measured by a urinary 3-IS levels (≥35 umol/mmol crea: gut microbiome diversity achieved; <35: gut microbiome diversity not achieved). A total of 48 evaluable patients will be randomized between the FMT arm and the placebo arm (24 evaluable patients per arm). Patients who are able to swallow at least one capsule will be considered as evaluable. This will give 80% power to detect a 30% improvement (20% on the placebo arm vs. 50% in the FMT arm) in the proportion of patients who achieve gut microbiome diversity at one month, with one-sided type 1 error rate of 0.12. In the primary analysis, patients who are considered as evaluable but do not have one month urinary 3-IS data available due to any reason (e.g., early death) will be included in the denominator in calculating the proportion of patients who achieve gut microbiome diversity, and will be considered as failing to achieve gut microbiome diversity at one month. A secondary analysis will also be performed, which will only include those patients with one month 3-IS data available.

13.2 Secondary endpoints

Secondary endpoints include acute GVHD at 6 months, NRM at 6 and 12 months, 100 day infection rate, PFS, OS and GRFS. All time to event outcomes will use the date of first administration of FMT (or placebo) as time 0. Definitions for these endpoints are given in section 11.

13.3 Analysis plan

The proportion of patients achieving gut microbiome diversity at one month will be compared between the FMT and the placebo arm, using a Fisher's exact test at one-sided significance level 0.12, The estimated proportions will be calculated along with 90% confidence intervals for each arm. This analysis will be performed for all evaluable patients, as well as within the subset of patients with one month 3-IS data available

The cumulative incidence of acute GVHD (using early deaths as competing risks), NRM (using relapse as competing risks), and infections (using early deaths as competing risks), will be compared between the two arms using the Gray test, The cumulative incidence for each type of endpoints will be estimated along with 90% confidence intervals for each arm. PFS, OS and GRFS will be compared using the Log-rank test, and will be estimated using the Kaplan-Meier method along with 90% confidence intervals for each arm.

13.4 Accrual

Accrual rate is expected to be approximately 24 evaluable patients per year, therefore it will take approximately 2 years to complete accrual of 48 evaluable patients.

13.5 Stopping Rule to do Death

If a participant dies due to a bloodstream infection with enteric organisms occurring within 4 weeks of final FMT dosing, the participant will be unblinded in order to determine if FMT or placebo capsules were administered. If the participant received placebo capsules, the death will not be attributed to study treatment. If the patient received FMT capsules, additional testing will be performed to assist with assessment of attribution of the infectious death. If 1 additional participant experiences a grade 5 treatment-related adverse event, the study will be terminated.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B FMT INVESTIGATOR'S BROCHURE

Investigators Brochure
MGH FMT Frozen Capsules

Hohmann Laboratory
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Infectious Disease Division

Department of Medicine
Massachusetts General Hospital

Principal Investigator: Elizabeth L. Hohmann MD

Version: 9.0
Date: June 4, 2019

The purpose of this document is to provide co-investigators with concise, current information about FMT capsules, direction for dosing, and active state of knowledge about their use. Information included is current as of June 4, 2019 and includes subjects receiving one or more doses of FMT capsules.

This document will be updated at least annually.

Capsule Overview
FMT capsules are made from stool donated by healthy individuals 18-50 who must pass a careful health screening, including blood banking questionnaire, medical history, physical exam, and numerous blood and fecal tests as shown below. Fecal material is blenderized in sterile preservative-free saline, and then sieved, pelleted, and re-suspended in sterile saline/40% glycerol and double encapsulated in pharmaceutical grade, nested vegan hypromellose capsules designed to open in neutral pH environments (Capsugel). Capsules are then frozen. One standard dose of thirty capsules contains soluble material harvested from approximately 40 grams of fecal material. Manufacturing details are in separate Clinical Manufacturing and Control documents.

Done at screening within 30 days of start of donations	**General Health Laboratory Tests**
	C-Reactive protein, high sensitivity
	Lipid panel
	Comprehensive metabolic panel
	CBC with differential
	ANA – sent out to Mayo
	HTLV I & II antibodies
	Pregnancy test by HCG if female
Done at screening within 30 days of start of donations ***re-done with stool samples from first and last donations**	**Stool Tests**
	Clostridium difficile testing by PCR
	Stool culture for enteric pathogens* – includes *Yersinia*, *Vibrio cholerae*, *V. parahemolyticus*, and *E. coli* O157
	Rotavirus antigen detection by ELISA
	Ova and parasites – includes giardia and cryptosporidium
	Norovirus
	Adenovirus
	VRE rectal screen
	MRSA nasal screen
	Helicobacter pylori – sent out to Mayo

	MRSA plating*
	Listeria plating*
	CRE plating*
	ESBL plating*
Done at screening within 30 days of start of donations **Repeated 28-40 days after last donation**	**Serologic Tests**
	Hepatitis A (IgG + IgM)
	Hepatitis B (surface Ag + surface Ab)
	Hepatitis C (IgG)
	HIV ½ (IgG)
	Syphilis TrepSure ELISA
Done at the time of the first and last donations	**Viral Loads**
	Epstein-Barr virus
	Cytomegalovirus
Optional – done at screening	**Oral Glucose Tolerance Test – Optional**
	Glucose (fasting)
	Hemoglobin A1C
	Glucose tolerance test, 2 hours post glucola ingestion

Appearance
Exemplary capsules are shown below.

Storage
Capsules are frozen and stored at -80°C and shipped on dry ice. A dated certificate of analysis which includes lot number, and manufacture date, acceptance of donor testing, and structural integrity of capsules at the time of shipment or in person delivery on dry ice to an investigator for a specific recipient will be filed. It is recommended that capsule be stored no longer than 30 days at -20°C, in a FROST-FREE freezer. Capsules should be discarded 9 months after manufacture. Capsules should be promptly transported on dry ice once removed from the freezer, for administration to subjects.

Animal testing
No animal testing has been performed on FMT capsules.

Solubility
FMT capsules and prototypes with aqueous dyes are demonstrated to be relatively stable in an acid environment and release payload under neutral conditions. It is

therefore NOT required nor recommended that subjects take antacids, bicarbonate or other acid- suppressing medications prior to administration. Taking these medications are not contraindications to administration of FMT capsules however.

Instructions for Use
Subjects must meet study-specified inclusion and exclusion criteria. Subjects should be NPO for 4 hours prior to and one hour after administration of capsules.

For treatment of *C. difficile* infection (CDI)
A single dose is considered 30 capsules when treating CDI. Capsules may be administered on 2 successive days (15 each day) or 3 successive days (10 each day), at the choice of subject/physician. For simple convenience most people prefer 2 days. Subjects or parents should sign a clinical consent form. Follow-up phone calls and completion of the 2-sided follow-up Case Report Form provided <u>are required</u> at the following intervals

in the 24-96 hours window after administration
at 7-14 days
at 8 weeks (+/- 5 days)
at 6 mo (+/- 2 weeks)

and subjects should be seen in person as dictated by a physician's clinical judgment. Physicians who do not complete and return follow-up forms in a timely manner will not be provided additional capsules.

For other applications, doses will be defined within specific clinical protocols, but will generally follow the above timing for safety assessments.

Acute reactions after dosing in 403 subjects treated for *C. difficile*
The capsules are tasteless and odorless. Five adult subjects and two children of 403 total have vomited within 8-12 h after capsule administration and **<u>not</u>** brought up feculent material or intact capsules. One cancer patient with chronic vomiting of medications vomited up all 15 capsules of his second dose, after tolerating the first dose well. Diarrhea and irritable bowel- like symptoms are commonly reported after FMT for CDI and often resolve over time. Subjects may be discharged after explanation of follow-up and advised re diet, relapse and AE's to monitor which usually takes about 20 minutes.

Total Adverse Events in 395 subjects reaching 8 weeks follow-up
Most subjects treated for CDI have some ongoing GI symptoms, typically minor (Grade 1). It is impossible to determine if these symptoms are related to resolving CDI, post-infectious irritable bowel syndrome, or the capsules themselves. See Chart 1 below. One IBD flare requiring intensified treatment has been noted and deemed related.

EVENT	Number	Percentage
Fevers - transient	9	3%
Hospitalizations	44	16%
Deaths	24	8%

Diarrhea	145	48%
Nausea/bloating	78	25%
Ulcerative colitis flare	3	0.01%

Expected resolution of symptoms of *C. difficile*.
This is variable. The occasional individual has fever, cramping, borborygmi and occasional diarrheal bowel movements after dosing. Patients with significant abdominal pain as part of their presenting or ongoing symptoms may have a component of Irritable Bowel Syndrome. Episodic pain or nonspecific abdominal discomfort may persist. Some subjects resolve diarrhea but have ongoing frequent formed stools compared with baseline. In the MGH experience, some subjects have cleared stool of *C. difficile* based upon repeated testing, but had slow resolution of other symptoms over weeks. Cure is defined as resolution of diarrhea at 8 weeks (3 stools or less per day).

Definition of Relapsed CDI
A relapse qualifying for re-dosing must meet the following criteria:

- Relapsed or ongoing diarrhea (more than 4 bowel movements of Bristol Grade 3 or higher AND
- A positive stool test for *C. diffile* (toxin or PCR) on day 5 or later (Day 0 = initial dose day). Routine rescreening of stools in the absence of significant symptoms is not recommended.

Clinical Results (as of June 4, 2019)
Of the total 395 patients currently followed to the 8-week time frame 89% were cured with a single dose and an additional 9% were cured with a second dose (98% total). Notable other observations are below.

- One 62 y.o. man with a history of 2 prior small bowel obstructions had another, 1 month after successful FMT, which resolved with conservative management and no operation. This was deemed unrelated. There was no relapse.
- A total of 19 patients over the age of 90 have been treated (successfully).
- A total of 19 children (age range 7-17) have been treated with capsules.
- One man with chronic inflammatory arthritis on immunosuppression was treated and did well for weeks, but then relapsed shortly after more rituximab was given (with our and others agreement). He was readmitted with severe ileus and relapsed *C.difficile*. He was subsequently retreated with enema FMT by flexible sigmoidoscopy but ultimately came to operation for the ileus, where Crohns was considered based upon gross findings but not confirmed on pathology. These findings were deemed unrelated to FMT. Intestinal TB was recommended as a possible diagnosis to the treating clinicians.
- 13 patients with prior bone marrow transplant were treated, though two subsequently developed relapsed malignancy and died of that, and one died of an intracranial hemorrhage.
- 10 solid organ transplant patients were treated successfully, though some required more than a single dose.
- One 67 y.o. woman with massive polycystic liver disease and a history of bacteremia of unclear origin with GI organisms was admitted with the same

(Enterococcal bacteremia) 3 weeks after FMT. The presumed source was biliary/liver.

- There has been NO transmission of infection or suspicion of that.
- Two immunocompromised adults (one who received chemotherapy and one with cirrhosis) who received FMT products as part of clinical studies developed bloodstream infection caused by extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli (E.coli). One of the individuals subsequently died. The FMT products used in these two individuals were prepared from stool obtained from the same donor. The donor stool and resulting FMT products used in these two individuals were not tested for ESBL-producing gram-negative organisms prior to use. After these adverse events occurred, stored preparations of FMT product from this stool donor were tested and found to be positive for ESBL-producing E. coli identical to the organisms isolated from the two patients.
- Caution is warranted in individuals with immunocompromise.

APPENDIX C FECAL DONOR TESTS

(All performed at MGH Clinical Labs unless noted below)

General Health Laboratory Tests (within 1 mo. of donation)
C-Reactive Protein, high sensitivity
Lipid Panel
Comprehensive Metabolic Panel
CBC w. Differential
ANA (Send out to Mayo – Approved by Kent Lewandrowski
HCG (if female)
Serologic Testing (within 2 weeks of donations; repeated 28 to 40 days after completing donations)
Hep A (IgG + IgM)
Heb B Surface Ag
Hep B Surface Ab
Hep C Ab
HIV Antibody
Syphilis Ab Screen
HTLV 1&2 ab
Swabs (within 1 mo. of donations)
VRE Rectal Screen
MRSA Nasal Screen
Stool Testing (within 1 mo. of donations; repeated with first and last donations)
Clostridium difficile toxin by PCR
Stool Culture (include vibrio cholerae, V. parahaemolyticus, yersinia, E coli O157)
Rotavirus Antigen Detection ELISA
Ova & Parasites (include Giardia & Cryptosporidium)
Norovirus
Adenovirus
H. Pylori (Send out to Mayo – Approved by Kent Lewandrowski)
Hohmann Lab Plates (within 1 mo. of donations; repeated with first and last donations)
MRSA Plating
Listeria Plating
CRE Plating
ESBL Plating

APPENDIX D INSTRUCTIONS FOR STOOL SAMPLE COLLECTION

Sample collection supplies you will receive:

- Stool collection device including collection frame, containers and lids
- Black sharpie pen

Step 1: Stool sample collection preparation

You should wear disposable gloves during the following steps.

Set up the Stool Collection system as follows:

- Raise the toilet seat and place the collection frame on the bowl.

- Then place the collection container in the frame and lower the toilet seat.

Step 2: Collection of Stool Sample

- Please collect a stool sample on the morning of your visit to the clinic.

- You should urinate before collecting the stool.
- Pass your entire stool into the clean, dry collection container.
    - Please collect a minimum of 10 grams of stool (approximately ½ stick of butter). If this is not possible, please provide as much as possible.
- Do not mix toilet paper, soap, urine or water with your sample.
- After collection place the collection container on a flat surface and then close the lid tightly to ensure that the sample does not leak during transit.
- Discard the gloves and wash hands with soap and water

Step 3: Post Stool Sample Collection

- Using the Sharpie pen, write your initials, the date and the time (including am/pm) of your bowel movement on the container.
- Put the closed collection container into a plastic bag, zip or tie it closed, and bring it to the lab **as soon as possible (preferably within 3 hours of sample production)**.
    - There is no need to refrigerate or freeze the sample.
    - Transport the sample in the sealed container, and place it in a plastic bag additionally.
- Repeat hand washing and if necessary.

Our research coordinator can be reached at 617-724-8625 Monday through Friday between 9:00 a.m. and 5:00 pm, if you have any questions about this process.

Thank you for your participation. We would appreciate if you bring in samples on the following days:

APPENDIX E STOOL COLLECTION PROCESSING FOR SEQUENCING ANALYSES, INCLUDING FUNGAL MICROBIOME

Stool samples will be aliquoted and stored frozen in two formats to facilitate varied analytical methods. Some processors prefer neat stool frozen with no preservatives (fungal and shotgun sequencing), some use RNA later and some utilize 95% ethanol. We will store samples neat and in 95% ethanol. Duplicates of each sample are made. Samples are aliquoted in a biosafety hood and immediately frozen.

Protocol
Supplies: 2 mL microfuge tubes with a screw cap or locking cap for long-term freezer storage.

1. Fill 4 (four) 2 mL microfuge tubes about half full with stool. These specimens do not need to be weighed.

2. Fill 4 (four) 4 ml microfuge tubes with 2 ml 95% ethanol. Add stool such that vials are approximately ¾ full, allowing space for expansion during freezing.

3. Place stool specimens at -80^{o}C until needed for sequencing at which point specimens will be shipped on dry ice. It is absolutely critical that these specimens stay frozen during the entire shipping duration.

4. Fungal ribosomal DNA will be prepared from all specimens simultaneously to ensure uniform procedure and quality of the prep from 2 of the 4 samples.

5. The remaining 2 stool specimens will be held in -80^{o}C storage as back up.

APPENDIX F MAGIC CRITERIA FOR STAGING AND GRADING FOR ACUTE GVHD

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (Stool output/Day)
0	No active (erythematous) GVHD rash	< 2 mg/dL	No or intermittent nausea, vomiting or anorexia	< 500 mL/day or less than 3 episodes per day
1	Maculopapular rash < 25% BSA	2 – 3 mg/dL	Persistent nausea, vomiting, or anorexia	500 – 999 mL/day or 3-4 episodes per day
2	Maculopapular rash 25-50% BSA	3.1 – 6 mg/dL	-	1000-1500 mL/day or 5-7 episodes per day
3	Maculopapular rash > 50% BSA	6.1 – 15 mg/dL	-	> 1500 mL/day or > 7 episodes per day
4	Generalized erythroderma (> 50% BSA) plus bullous formation and desquamation > 5% BSA	> 15 mg/dL	-	Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

Overall clinical grade (based on most severe target organ involvement)
Grade 0: No stage 1-4 of any organ.
Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement
Grade II: Stage 3 rash and/or Stage 1 liver and/or Stage 1 upper GI and/or Stage 1 lower GI
Grade III: Stage 2-3 liver and/or Stage 2-3 lower GI, with Stage 0-3 skin and/or Stage 0-1 upper GI.
Grade IV: Stage 4 skin, liver or lower GI involvement, with Stage 0-1 upper GI.