Science Translational Medicine
AAAS

www.sciencetranslationalmedicine.org/cgi/content/full/10/460/eaap9489/DC1

Supplementary Materials for

Reconstitution of the gut microbiota of antibiotic-treated patients by autologous fecal microbiota transplant

Ying Taur, Katharine Coyte, Jonas Schluter, Elizabeth Robilotti, Cesar Figueroa, Mergim Gjonbalaj, Eric R. Littmann, Lilan Ling, Liza Miller, Yangtsho Gyaltshen, Emily Fontana, Sejal Morjaria, Boglarka Gyurkocza, Miguel-Angel Perales, Hugo Castro-Malaspina, Roni Tamari, Doris Ponce, Guenther Koehne, Juliet Barker, Ann Jakubowski, Esperanza Papadopoulos, Parastoo Dahi, Craig Sauter, Brian Shaffer, James W. Young, Jonathan Peled, Richard C. Meagher, Robert R. Jenq, Marcel R. M. van den Brink, Sergio A. Giralt, Eric G. Pamer*, Joao B. Xavier*

*Corresponding author. Email: pamere@mskcc.org (E.G.P.); xavierj@mskcc.org (J.B.X.)

Published 26 September 2018, *Sci. Transl. Med.* **10**, eaap9489 (2018)
DOI: 10.1126/scitranslmed.aap9489

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/10/460/eaap9489/DC1)

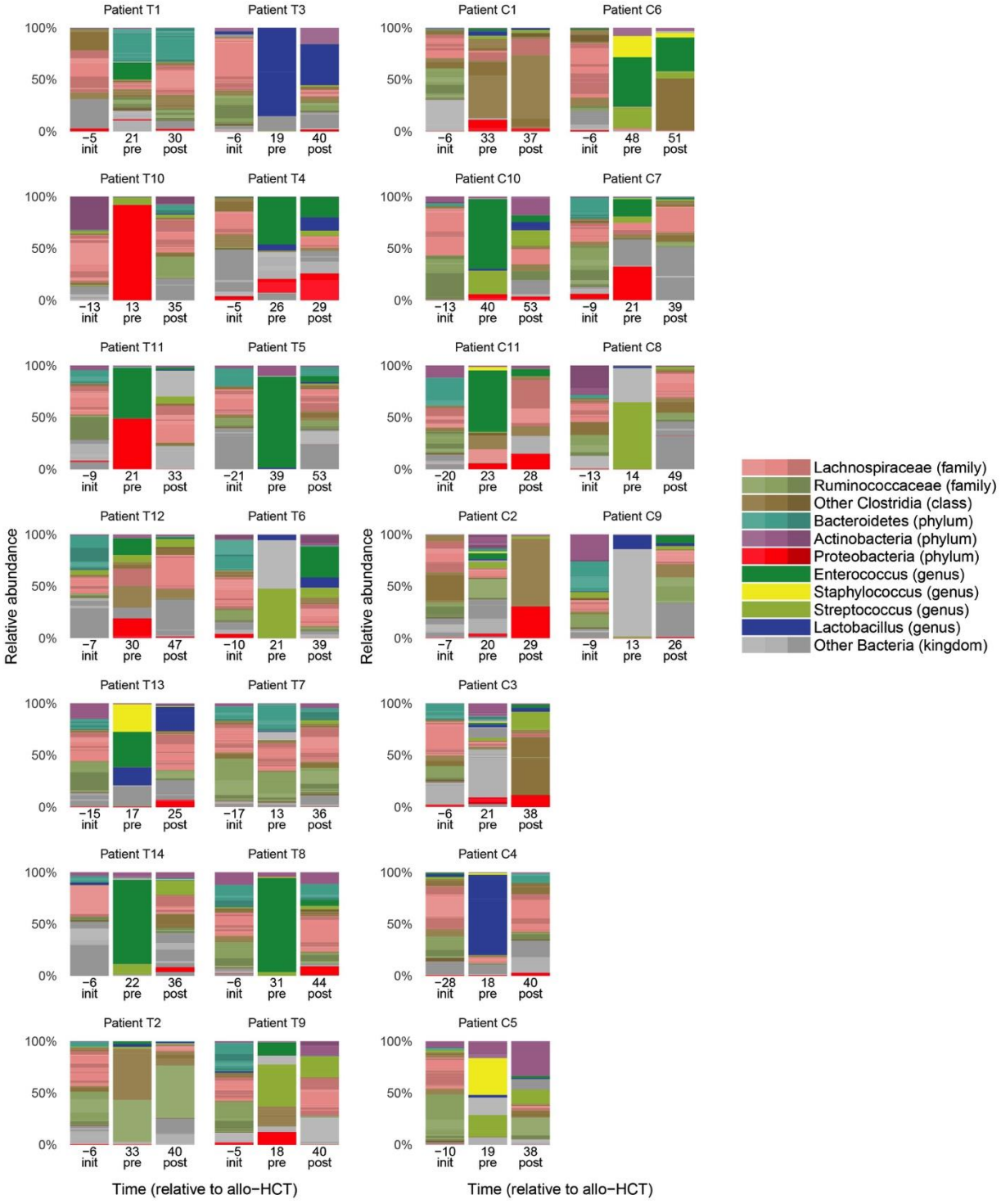


Fig. S1. Microbiota samples for the 25 patients (14 treatment and 11 control). Stacked bar plots showing microbial composition, determined by 16S rRNA gene abundances. The first sample is the initial sample taken from each patient (*init*), which was collected and stored for auto-FMT if the randomization assigned the patient to the treatment arm. The second sample is the latest sample before randomization (*pre*). The third sample is the first sample taken at least 24 hours after randomization (*post*).

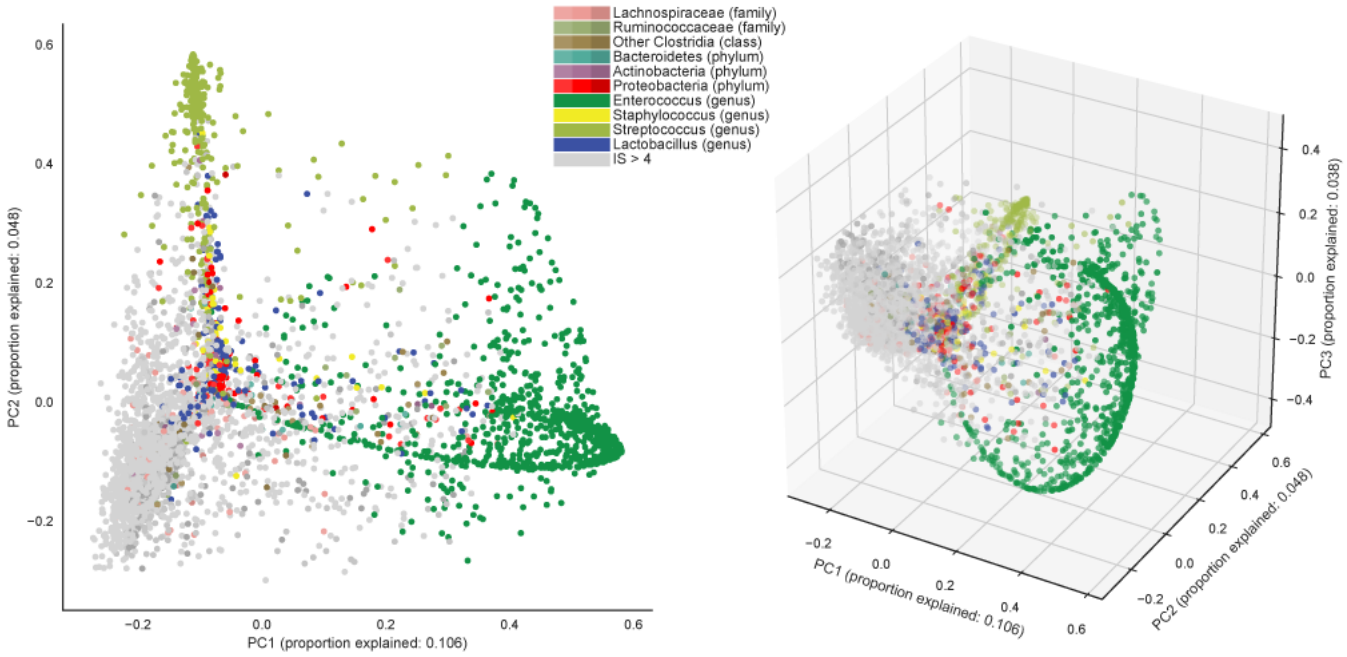


Fig. S2. PCoA of microbiota samples shown in Fig. 3. Here the data are plotted from Bray-Curtis distances. The PCoA method of dimensionally reduction, used by many other microbiota studies, fails to cluster the samples with the multiple domination states that characterize the dynamic microbiome of allo-HCT patients. The PCoA is shown here in 2-D for comparison with t-SNE (left-hand panel); the 2-D PCoA reveals, at best, the *Enterococcus*-dominated samples (in green) and the *Streptococcus*-dominated samples (in lime), while t-SNE shows other important dominated states. Including a third dimension to produce a 3-D PCoA (right-hand panel) does not reveals all the additional dominations, confirming again that t-SNE better captures the complex microbiota dominations seen in allo-HCT patients.

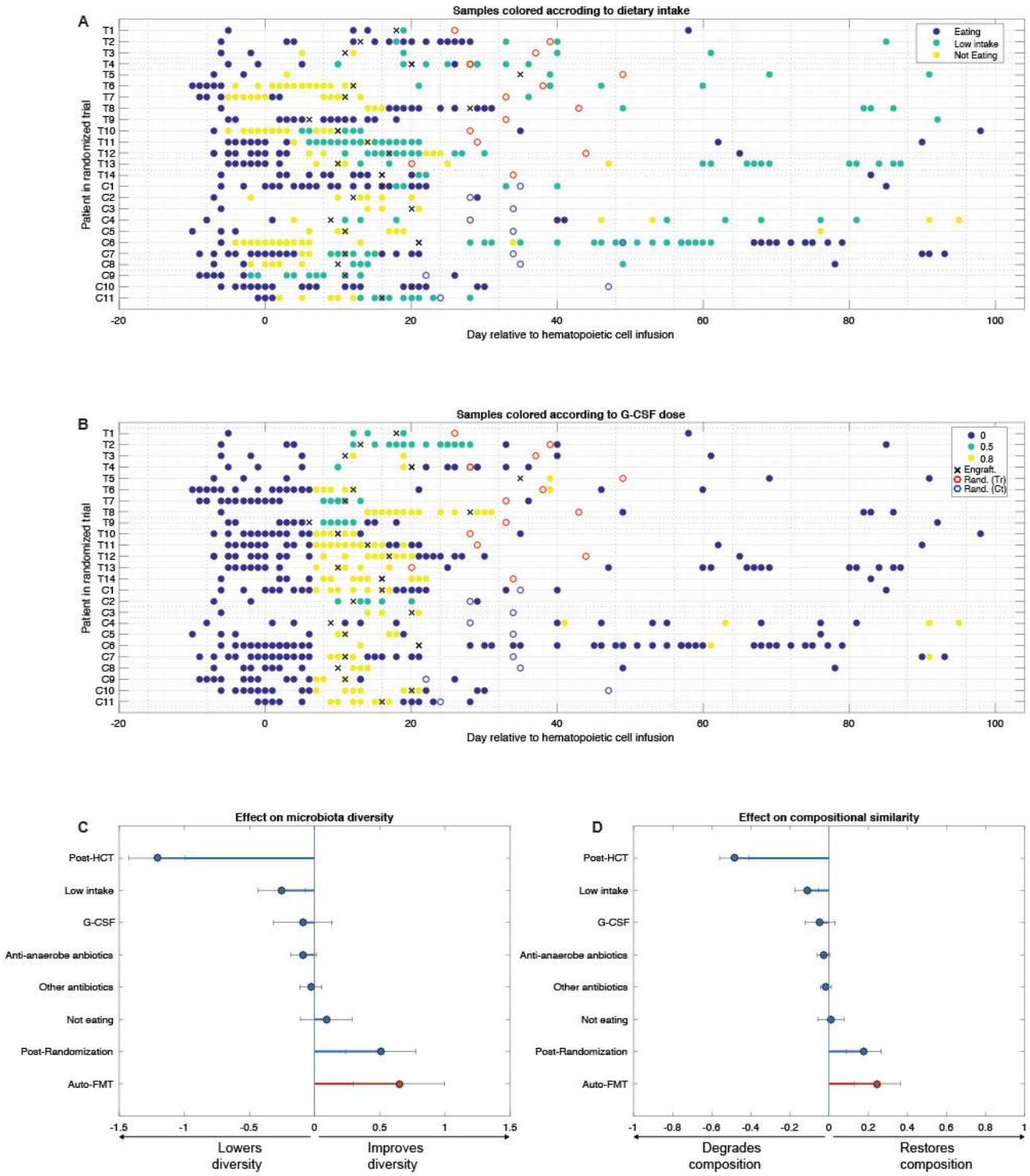


Fig. S3. Mixed-effects model that controls for other clinical parameters confirms the beneficial effect of auto-FMT in remediating the microbiota of allo-HSCT patients. Other clinical parameters include dietary intake and medication to boost the hematopoietic recovery. (**A**) Timeline of auto-FMT treated and control patient samples labeled according to the dietary intake. (**B**) Timeline of auto-FMT treated and control patient samples labeled according to the dose of G-CSF administered that day. (**C,D**) Effect sizes of clinical parameters quantified by a mixed-effects model (along with a 95% confidence interval) showing that auto-FMT brings significant improvements to the diversity (left-hand plot; P-value=$3x10^{-4}$) and recovery of original composition (right-hand plot; P-value=$4x10^{-5}$) of allo-HCT patients This model was expanded with dietary intake and G-CSF, but used only the samples from the 25 randomized patients, in contrast to the model presented in the main text which used hundreds of patients to compute the baseline.

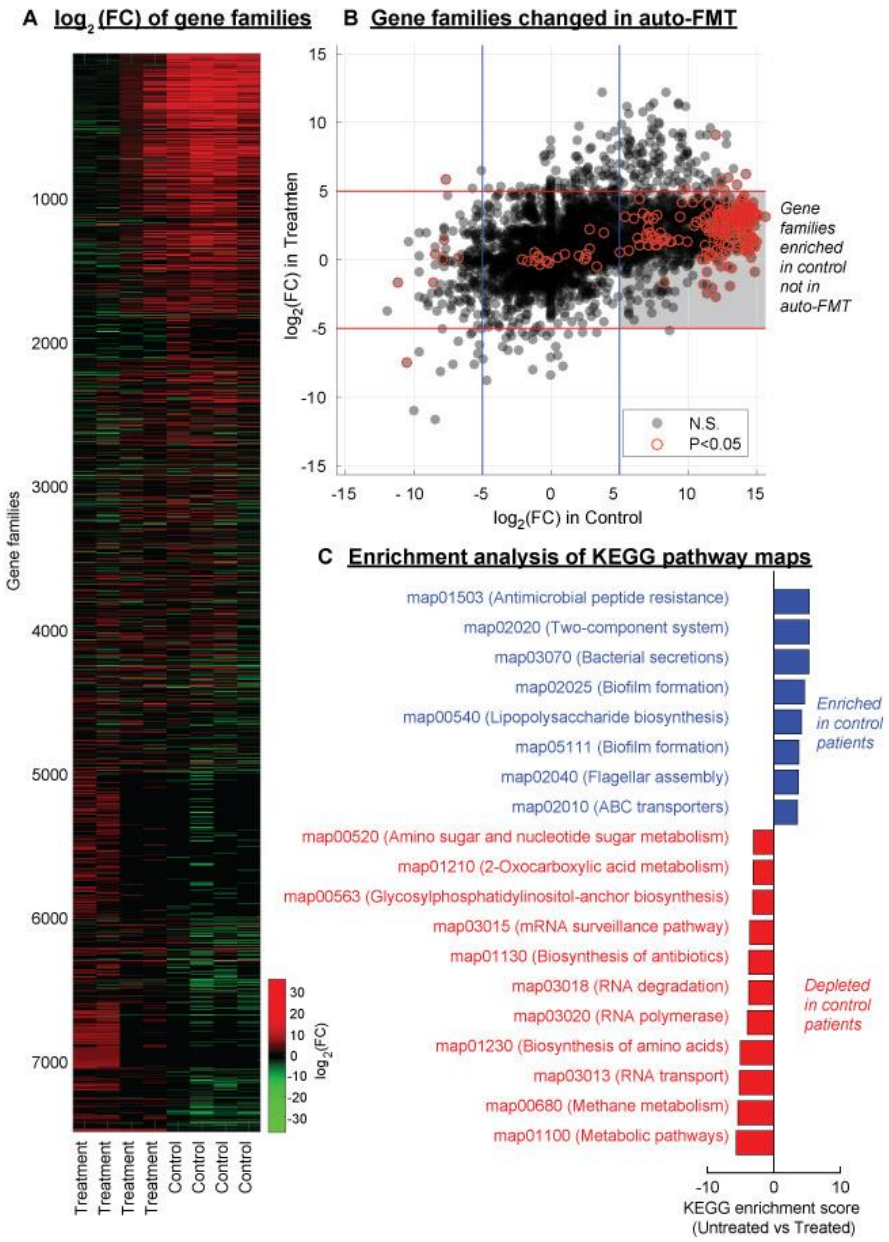


Fig. S4. Shotgun sequencing shows that auto-FMT remediates the perturbed microbiome. (**A**) A heatmap of base 2 logarithm fold-changes (log_2(FC)) of gene families ranked by their enrichment in control versus auto-FMT samples. Paired pre-HCT/post-randomization samples from eight patients were shotgun-sequenced. FMAP analysis of sequencing data revealed fold-changes in 7,496 gene families. (**B**) Scatter plot of KEGG gene family changes (log_2(FC)) reveals that the most significantly changed gene families (280 out of 309 that passed multiple hypothesis correction) are gene families enriched in control patients but unchanged in auto-FMT patients. (**C**) Enrichment analysis reveals eight KEGG pathway maps enriched in control samples, which suggest expansion of genes associated with bacterial virulence and antimicrobial resistance. Control patients remain significantly depleted in 11 KEGG pathway maps many of which may be relevant for proper microbiota function (metabolic pathways) and colonization resistance (biosynthesis of antibiotics).

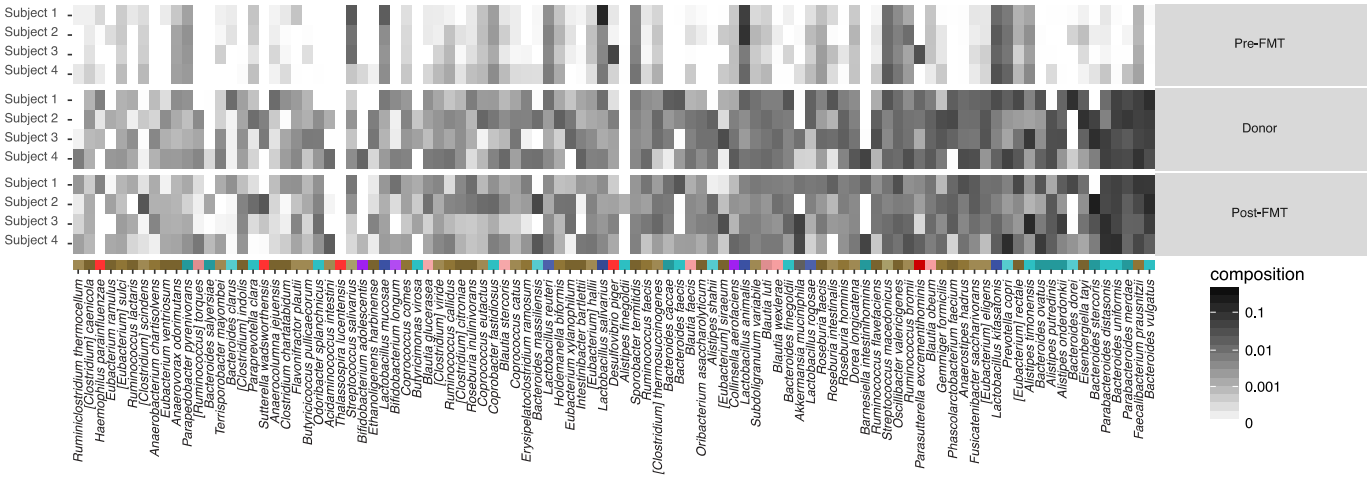


Fig. S5. Shown is an analysis of a heterologous FMT conducted in another study compared to the auto-FMT conducted in this study. Overview of the top 100 most abundant bacterial taxa in the pre-FMT, donor and post-FMT (2 week) microbiota samples from four patients in the other study (*20*) who had the data for all the three samples available.

Table S1. Characteristics of patients included in this study (14 treated and 11 control).

Patient	Randomization Arm	Age	Sex	Race	Disease	Disease Risk	Conditioning Intensity	HCT Type
T1	Treatment	53	F	White	AML	High	Myeloablative	Unmod BM
T2	Treatment	55	F	Black	AML	Low	Reduced intensity	Unmod Double cord
T3	Treatment	45	F	White	ALL	Low	Reduced intensity	Unmod Double cord/TCD Haplo
T4	Treatment	52	F	<No answer>	AML	Low	Reduced intensity	Unmod PBSC
T5	Treatment	57	F	White	AML	Low	Reduced intensity	Unmod Double cord
T6	Treatment	50	F	White	MM	High	Myeloablative	TCD PBSC
T7	Treatment	34	F	White	MDS	High	Myeloablative	TCD PBSC
T8	Treatment	55	M	White	AML	Low	Reduced intensity	Unmod Double cord
T9	Treatment	71	M	Asian	MDS	High	Myeloablative	Unmod PBSC
T10	Treatment	36	M	White	AML	Low	Myeloablative	TCD PBSC
T11	Treatment	49	F	White	CLL	Intermediate	Reduced intensity	Unmod PBSC
T12	Treatment	68	M	White	MDS	High	Myeloablative	Unmod BM
T13	Treatment	32	M	White	NHL	Intermediate	Reduced intensity	Unmod PBSC
T14	Treatment	57	M	White	AML	Intermediate	Myeloablative	Unmod BM
C1	Control	72	M	White	MDS	High	Reduced intensity	Unmod PBSC
C2	Control	55	F	White	AML	Low	Reduced intensity	Unmod Double cord/TCD Haplo
C3	Control	59	F	Black	AML	Low	Reduced intensity	Unmod Double cord
C4	Control	54	F	Black	MM	High	Myeloablative	CD34+ PBSC
C5	Control	62	F	White	ALL	Low	Myeloablative	TCD PBSC
C6	Control	57	F	White	Chronic NK-LGL Leukemia	Not Applicable	Reduced intensity	Unmod PBSC
C7	Control	62	M	White	MM	High	Myeloablative	TCD PBSC
C8	Control	28	M	White	ALL	Low	Myeloablative	TCD PBSC
C9	Control	44	F	White	AML	Low	Myeloablative	TCD PBSC
C10	Control	68	M	White	MDS	High	Myeloablative	Unmod BM
C11	Control	67	F	White	MDS	High	Reduced intensity	Unmod PBSC

TCD: T-cell depletion (ex-vivo) by CD34+selection; PBSC: peripheral blood stem cells; Unmod: unmodified (graft); Haplo: haploidential (graft); AML: acute myelogenous leukemia;

ALL: acute lymphocytic leukemia; CLL: chronic lymphocytic leukemia; MDS: myelodysplastic syndrome; NK-LGL: Natural killer cell large granular lymphocyte; NHL: Non-Hodgkin's Lymphoma

A Randomized Controlled Trial of Autologous Fecal Microbiota Transplantation (auto-FMT) for Prophylaxis of Clostridium Difficile Infection in Recipients of Allogeneic Hematopoietic Stem Cell Transplantation

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Ying Taur, MD, MPH	Medicine/ Infectious Disease
Co-Principal Investigator(s)/Department:	Eric G. Pamer, MD Richard Meagher, PhD	Medicine/ Infectious Disease Laboratory Medicine
Investigator(s)/Department:	Arthur E Brown, MD Kent A Sepkowitz, MD Michael Glickman, MD Genovefa A Papanicolaou, MD Susan Seo, MD Monika Shah, MD Anna Kaltsas, MD Peter A Mead, MD Cesar J Figueroa-Ortiz, MD Elizabeth Robilotti, MD Yeon Joo Lee, MD Gil Redelman-Sidi, MD Tobias Hohl, MD PhD Sejal Morjaria, MD Juliet Barker, MD Marcel Van den Brink, MD, PhD Hugo R. Castro Malaspina, MD Sergio Giralt, MD Boglarka Gyurkoza, MD Roni Tamari, MD Ann A Jakubowski, MD, PhD Miguel-Angel Perales, MD Doris Ponce, MD Craig Sauter, MD James Young, MD Brian Shaffer, MD Parastoo B Dahi, MD Robin Mendelsohn, MD Elyn Riedel, MA Sean Devlin, PhD Scott T. Avecilla, MD, PhD Gunnar Ratsch, PhD Joao Xavier, PhD Justin Cross, PhD	Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/ Infectious Disease Medicine/Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Adult BMT Medicine/ Gastroenterology Epidemiology & Biostatistics Medicine/ Cell Therapy Laboratory Sloan-Kettering Institute/Computational Biology Sloan-Kettering Insititue/ Computational Biology Sloan-Kettering Insititue/ Cancer Metabolism

Consenting Professional(s)/Department:	Ying Taur, MD, MPH	Medicine/ Infectious Disease
	Eric G. Pamer, MD	Medicine/ Infectious Disease
	Arthur E Brown, MD	Medicine/ Infectious Disease
	Kent A Sepkowitz, MD	Medicine/ Infectious Disease
	Michael Glickman, MD	Medicine/ Infectious Disease
	Genovefa A Papanicolaou, MD	Medicine/ Infectious Disease
	Susan Seo, MD	Medicine/ Infectious Disease
	Monika Shah, MD	Medicine/ Infectious Disease
	Anna Kaltsas, MD	Medicine/ Infectious Disease
	Peter A Mead, MD	Medicine/ Infectious Disease
	Cesar J Figueroa-Ortiz, MD	Medicine/ Infectious Disease
	Tobias Hohl, MD PhD	Medicine/ Infectious Disease
	Elizabeth Robilotti, MD	Medicine/ Infectious Disease
	Yeon Joo Lee, MD	Medicine/ Infectious Disease
	Sejal Morjaria, MD	Medicine/ Infectious Disease
	Gil Redelman-Sidi, MD	Medicine/ Infectious Disease
	Juliet Barker, MD	Medicine/ Adult BMT
	Marcel Van den Brink, MD, PhD	Medicine/ Adult BMT
	Hugo R. Castro Malaspina, MD	Medicine/ Adult BMT
	Sergio Giralt, MD	Medicine/ Adult BMT
	Ann A Jakubowski, MD, PhD	Medicine/ Adult BMT
	Miguel-Angel Perales, MD	Medicine/ Adult BMT
	Doris Ponce, MD	Medicine/ Adult BMT
	Craig Sauter, MD	Medicine/ Adult BMT
	James Young, MD	Medicine/ Adult BMT
	Boglarka Gyurkoza, MD	Medicine/ Adult BMT
	Roni Tamari, MD	Medicine/ Adult BMT
	Brian Shaffer, MD	Medicine/ Adult BMT
	Parastoo B Dahi, MD	Medicine/ Adult BMT
	Robin Mendelsohn, MD	Medicine/ Gastroenterology

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

OneMSK Sites
Manhattan

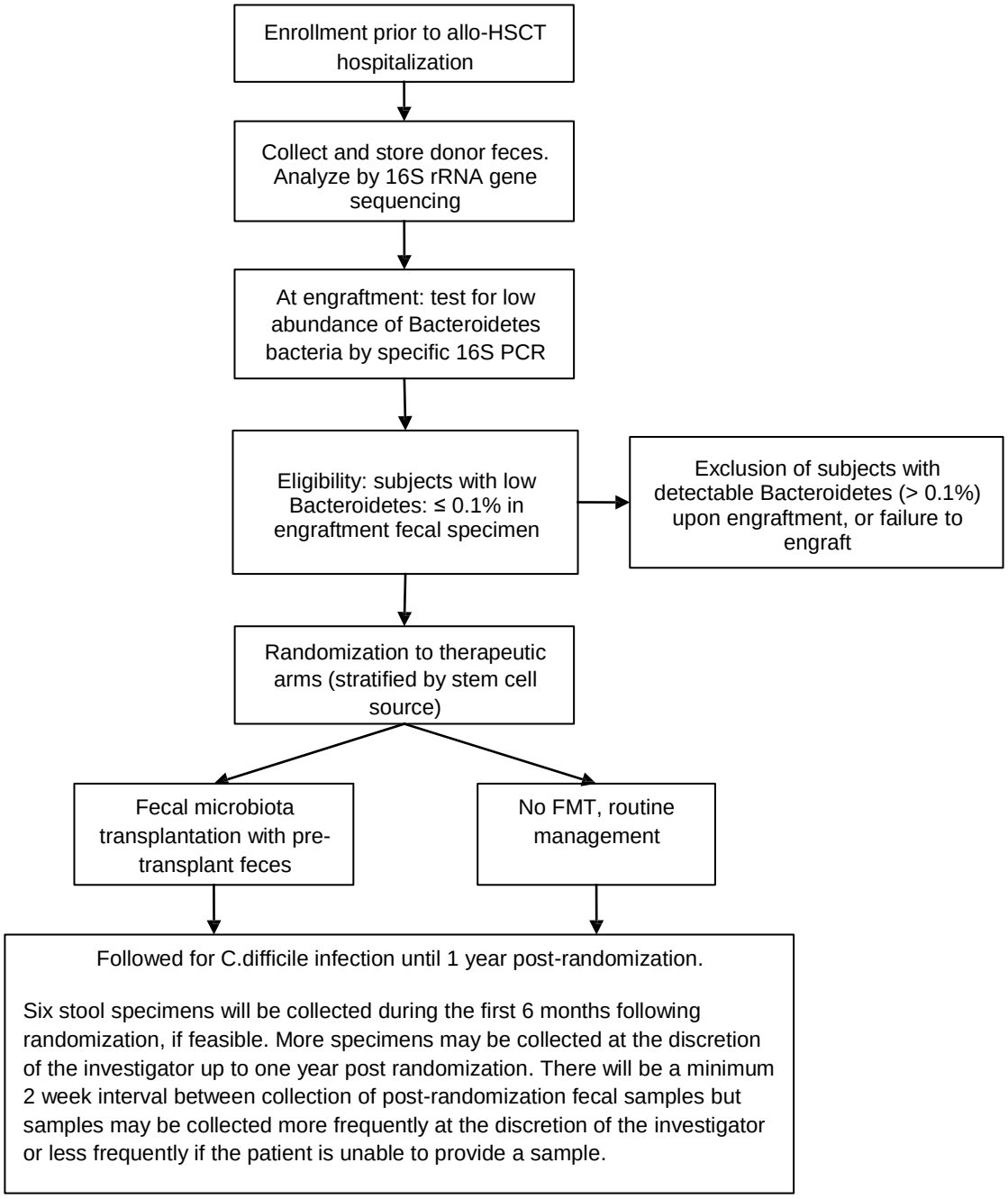
Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

Table of Contents

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a randomized, open-label, controlled study designed to assess the efficacy of an autologous fecal microbiota transplantation (auto-FMT) for prevention of *Clostridium difficile* infection (CDI) in patients who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT). Patients will be enrolled prior to allo-HSCT; feces will be collected and stored from all participating subjects prior to the initiation of conditioning regimens, analyzed by deep 16S rRNA gene sequencing, and tested by assay for intestinal pathogens including *Clostridium difficile*. Later in the course of transplantation, following engraftment (defined as the first day of three consecutive days, that the absolute blood neutrophil count is at or above 500 mm^3), subjects will undergo fecal testing for presence of Bacteroidetes by 16S PCR. Subjects will be eligible for study if they have a microbiologically diverse pre-transplant colonic microbiota and if the post-engraftment specimen contains Bacteroidetes at a prevalence equal to or below 0.1%.

Eligible patients will be randomized to undergo fecal microbiota transplantation with the subject's stored pre-transplantation feces, versus no fecal transplantation. The post-engraftment Bacteroidetes testing, randomization, and fecal microbiota transplantation procedure should all be performed within a 28-day window, beginning on the first day of engraftment. In the event that engraftment occurs prior to day +7, the 28-day window will start on day +7. Randomization will be stratified by stem cell source. Subjects from both arms will be followed for one year after randomization for development of CDI, which will be treated by their primary BMT clinician per the standards of care at MSKCC. Subjects from both arms will also be assessed by their BMT clinicians for infections and graft-versus-host disease. During the follow-up period, fecal specimens will be collected serially, if feasible, until one year post-randomization and analyzed for microbial diversity and composition.



2.0 OBJECTIVES AND SCIENTIFIC AIMS

[35][17] The primary objective of this study is to determine if prophylactic autologous fecal microbiota transplantation can reduce the incidence of post-engraftment *C. difficile* infection (CDI) in patients undergoing allo-HSCT.

[35][17] The secondary objectives are to (1) evaluate if auto-FMT can lead to decreased incidence of systemic and/or intestinalbacterial/viral infection(s), (2) evaluate for auto-FMT's effect on incidence of graft-versus-host disease (GVHD), (3) assess differences in intestinal microbiota between study arms, and(4) identify microbiota markers signifying increased or decreased risk for *C. difficile* infection and GVHD.

3.0 BACKGROUND AND RATIONALE

Clostridium difficile infection (CDI) is the most common cause of infectious diarrhea in the hospital setting, with a spectrum of illness ranging from mild diarrhea to life-threatening colitis, with risks of toxic megacolon, colonic perforation, and death.[1] The incidence of CDI is particularly high in allo-HSCT recipients; with rates of 15-30%,[2,3] compared with less than 1% in the general inpatient population.[1] Prior studies have observed increased mortality in association with CDI in these patients.[4,5] Studies of patients undergoing allo-HSCT have also shown an association between CDI and subsequent complications such as graft-versus-host disease (GVHD).[3–6]

Since the risk of CDI among recipients of allo-HSCT is affected by transplant parameters, rates of CDI vary in different studies and between transplant centers.[2,3,7] At MSKCC, CDI occurred in 20% of allo-HSCT recipients from the time of transplantation to 100 days post-transplantation (2005-2011). This rate matches the experience of other transplant centers, has increased over time, and positively correlates with the intensity of the conditioning regimen.[2,3]

CDI arises following perturbations of the intestinal microbiota.[8] Recipients of allo-HSCT are at particularly high risk for CDI due to the effects of intensive chemotherapy, radiation and antibiotic administration on the composition of the intestinal microbiota. Data from MSKCC demonstrates that allo-HSCT recipients undergo marked changes in microbial composition, with prolonged decreases in bacterial diversity and domination of the gut by bacterial species that are usually present at very low densities. Our studies of the intestinal microbiota of allo-HSCT patients have determined that the establishment of abnormal microbial communities markedly increase the risk of infectious and inflammatory complications, including bloodstream infections and GVHD.[9,10]

Fecal microbiota transplantation (FMT) has become an increasingly accepted treatment of recurrent CDI. FMT achieves high rates of cure for recurrent CDI, having cured infections that failed conventional antibiotic treatment with metronidazole or oral vancomycin.[11–13] A randomized control trial of FMT for recurrent CDI demonstrated greater effectiveness compared with oral vancomycin.[14] This study demonstrated re-establishment of the Bacteroidetes phylum in recipient intestinal microbiota. Other studies of CDI and/or FMT have also noted a distinct negative correlation between recurrent Clostridium difficile and the presence of Bacteroidetes.[15–17] Furthermore, prior studies have provided strong support for the premise that Bacteroidetes bacteria play an important role in maintaining stability within the gut and serve to promoting overall intestinal health, with functions that include metabolism of complex carbohydrates, activation and regulation of T-cell immunity and other components of immunity, and interaction with intestinal epithelial cells.[18–23]

We have investigated *C. difficile* infection in recipients of double umbilical cord blood transplants (DUCB-Tx). Among 170 DUCB-Tx recipients, 56 (32.6%) were diagnosed with CDI. Of these, 23 were diagnosed with CDI prior to stem cell engraftment and 33 developed CDI between engraftment and the one-year anniversary of their transplant. We performed sequential microbiota analyses on 43 DUCB-Tx recipients at MSKCC, characterizing the

microbiota composition and diversity pre-transplantation and at multiple time points prior to and following engraftment. Among pretransplant samples obtained from 43 DUCB-Tx recipients, 36 contained bacteria belonging to the Bacteroidetes phylum. In contrast, in post-engraftment samples, 16/31 contained Bacteroidetes phylum bacteria and 15/31 had lost Bacteroidetes phylum bacteria. In the period between engraftment and the one year anniversary of the DUCB-Tx, the incidence of CDI in patients who maintained Bacteroidetes in their microbiota was 18.8% while the incidence of CDI who lost Bacteroidetes was 53.3%.

DUCB-Tx recipients who lose Bacteroidetes phylum bacteria from their microbiota have an exceptionally high risk of developing *C. difficile* infection and would likely benefit from the reconstitution of their microbiota with a diverse flora that contains Bacteroidetes. Because the risk of CDI exceeds 50% in Bacteroidetes-negative patients while the risk of CDI is only 15% in the Bacteroidetes-positive patients, the number of patients required to demonstrate a similar reduction in the incidence of CDI is achievable over a two to three year period of time.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

Subjects will be enrolled prior to allo-HSCT hospitalization. Pre-transplantation feces will be collected and stored for all subjects. Stored feces and a fecal specimen obtained following engraftment will be analyzed; subjects with evidence of a low relative abundance of Bacteroidetes bacteria from their fecal microbiota will be considered eligible for randomization. Subjects will be randomized to one of two arms within a 28-day window, which typically begins on the first day of engraftment:

1. Fecal microbiota transplantation with pre-transplantation feces
2. No fecal transplantation, routine standard care only.

In the event that engraftment occurs prior to day +7, the 28-day window will start on day +7. Randomization will be stratified by stem cell source (cord vs. non-cord blood donor). Patients receiving a combination of cord and haploidentical stem cell products will be stratified with the cord group for the purposes of this protocol. Following randomization, subjects will be followed for one year. Stopping rules are defined for consideration of early termination of the study. Evaluation for statistical futility may be performed once as an interim analysis. In addition, blood stream infections and severe GVHD (grade III or higher) will be assessed as safety endpoints by the subject's primary BMT clinician. An excess of events beyond these specified boundaries will result in consideration of early termination of the study. Inpatient fecal samples and outpatient fecal samples other than the intial and engraftment sample will be collected for follow up, if feasible, from all subjects during hospitalization and after discharge, and will be analyzed by 16S rRNA gene sequencing, at the investigator's discretion . The gene sequencing data obtained from these specimens will not be used to assess the eligibility of the subject nor will it influence the subject's treatment. Follow-up samples not collected at the expected time interval would thus not be considered violations.

4.2 Intervention

Donor Stool Collection

Prior to auto-FMT, the subject will be asked to provide a fecal (stool) sample that is typically between 50-100 g (a typical bowel movement) but may also fall out of this predicted range. The subject may be given either a bottle of magnesium citrate or a double dose of milk of magnesium to take during the clinic visit to mobilize a bowel movement and to ensure a soft stool sample. The subject will collect a fresh stool sample with or without aid, by placing a labeled, clean stool collection container (see Stool Collection Kit details below) on the toilet bowl before defecating. After defecating, the subject will seal the container, and deliver the sample to a treatment facility personnel. The treatment facility personnel will send the stool sample to the Department of Laboratory Medicine for processing and cryopreservation that must occur within 12 hours of stool collection.

Stool Collection Kit

The stool collection kit is designed to make the stool sample collection as clean and convenient as possible. It includes disposable gloves, a plastic tub with adaptor to hold the tub over the toilet bowl, and a sealable plastic tub lid. A zip lock biohazard bag will also be supplied.

Stool Sample Processing

As described by Hamilton et al.,[24] subject stool samples will be accessioned, weighed (in grams) and placed into refrigerated storage (2-4^{o}C) until processing materials and reagents are assembled. All open container procedures will be performed in a Class II Biosafety cabinet with nitrogen purging to prevent loss of function of anaerobic microbes. In brief, the stool sample will be diluted with approximately 6-8 volumes (250-500 mL) of nonbacteriostatic (preservative-free) infusion-grade saline and homogenized in a sterile blender for one-two (1-2) minutes at blend speed to prepare a stool slurry mixture. A two (2) mL sample will be obtained for microbial analysis and for deep 16S rRNA gene sequencing.

The stool slurry will then be passed through a series of sterile disposable meshes (2.0, 1.0, 0.5,and 0.25 mm) to remove large particulate matter and/or undigested food particles. Following stool suspension and filtration, the sample will be centrifuged at 6000 x *g* for fifteen (15) minutes in a centrifuge at 4^{o}-6^{o}C to pellet the stool slurry particulate matter. After centrifugation, the supernatant will be discarded and the homogenized stool pellet will be resuspended in 200 - 250 mL of a glycerol-nonbacteriostatic saline solution (10% final concentration of glycerol, v/v), transferred to a labeled cryobag, and frozen by dump freezing (place cryobag freezing container upright in a -86^{o}C ultralow mechanical freezer overnight (≥ 12 hours). The cryopreserved product will be stored frozen at ≤ -80^{o}C in a -86^{o}C freezer and may be stored for a period of time prior to use as determined by the investigator, but may generally be for up to two (2) months.

Stool Sample Infusion

The day before the infusion, the subject may be prepped using a split dosage polyethylene glycol purge (MoviPrep or equivalent), which is standard practice before GI procedures to wash out residual antibiotic and fecal material. This preparatory step can be foregone if it is deemed not necessary by the study investigator.

Frozen stool samples will be thawed over several hours in an ice bath. The thawed, diluted stool preparation will be delivered to the patient's room. The prepared auto-FMT sample will then be administered to the patient rectally by a retention enema. Fecal retention will be maintained for one (1) hour if possible, as tolerated by the patient in supine position. Subjects will be observed for up to two (2) hours following sample administration.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

The therapeutic agent in this study is the patient's fecal sample collected prior to allo-HSCT. This sample will be collected by the patient into a container with or without aid, and then stored on ice for less than 12 hours prior to delivery to the Cell Therapy Laboratory (CTL) of the MSK Department of Laboratory Medicine. These fecal samples will be prepared as described in section 4.2. In addition, an aliquot of the fecal sample will be provided to Clinical Microbiology Laboratory to screen for gastrointestinal pathogens such as *C. difficile.* A second aliquot will be provided to the Lucille Castori Molecular Microbiology Core Laboratory to extract DNA and perform PCR amplification of bacterial 16S rRNA genes for Illumina sequencing of the V4-V5 region. The diversity and composition of the fecal microbiota will be determined using mothur software version 1.30 (or latest version).[25]

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

In this study, subject eligibility for enrollment will be evaluated prior to transplant hospitalization. Criteria for study enrollment are as follows:

6.1 Subject Inclusion Criteria

- Planned to undergo allo-HSCT
- Age ≥ 18 years

6.2 Subject Exclusion Criteria

- As determined by the study investigators or consenting professionals, recent prolonged antibiotic treatmentas prevention or suppression of an ongoing infection, where treatment involves gut-perturbing antianaerobic antibiotics (see appendix).
- Has severe colitis of any etiology or a history of inflammatory bowel disease (IBD).

7.0 RECRUITMENT PLAN

Patients will be considered for this therapy and recruited by BMT Service physicians and study personnel during their pre-transplant evaluation for allo-HSCT at MSK. Patients screened for this study may have routine evaluations performed by their BMT clinician in preparation for allo-HSCT hospitalization. At the discretion of the BMT clinician, these may include a physical exam, vital signs, medical history evaluation, laboratory tests, radiographic scans, and other assessments but not necessarily all of them. Patients who fulfill the eligibility criteria listed in Section 6.0 will be recruited. Informed consent will be obtained by one of the participating investigators and/or consenting professionals authorized to obtain consent. A copy of the signed informed consent will be scanned to EMR by the primary study RSA and all consent forms will be tracked by the Clinical Trials Office. The study RSA will also maintain a screening log of all the patients approached.

While the majority of participants will be recruited during their routine clinical visit, for the purposes of increasing enrollment, the principal investigator may also screen the medical records of patients with whom they do not have a treatment relationship for the limited purpose of identifying patients who would be eligible to enroll in the study and to record appropriate contact information in order to approach these patients regarding the possibility of enrolling in the study.

During the initial conversation between the investigator/research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator/research staff may also review portions of their medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient turns out to be ineligible for the research study (in most cases, this will be because of ongoing cancer treatment), the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes.

In most cases, the initial contact with the prospective subject will be conducted either by the treatment team, investigator or the research staff working in consultation with the treatment team. The recruitment process outlined presents no more than minimal risk to the privacy of the patients who are screened and minimal PHI will be maintained as part of a screening log. For these reasons, we seek a (partial) limited waiver of authorization for the purposes of (1) reviewing medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) conversing with patients regarding possible enrollment; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

8.0 PRETREATMENT EVALUATION

Following enrollment, subjects will be evaluated at two stages of fecal specimen evaluation in order to determine whether they will proceed to randomization after stem cell engraftment

First, pre-transplant feces will be collected prior to the start of treatment with the pre-transplant conditioning regimen. This specimen can be collected at home and brought to clinic or hospital or at the start of the transplant hospitalization, so long as it collected prior to any pre-transplant conditioning or microbiota-perturbing antibiotics. Fecal samples will be tested for the presence intestinal pathogens (listed below). Fecal samples will also be analyzed by 16S rRNA gene sequencing using the Illumina platform. Barcoded primers will be used to amplify the V4-V5 region of the bacterial rRNA gene and approximately 10,000 to 50,000 sequences will be used to determine the diversity of bacteria and the relative abundances of commensal bacteria. Fecal samples will be considered suitable for rectal infusion if the inverse Simpson Diversity score exceeds ≥ 2.0.

Patients will undergo a second fecal evaluation following stem cell engraftment. In this step, a fecal sample will be collected from engrafted patients and evaluated for the abundance of bacteria belonging to the Bacteroidetes phylum. Patients with a relative abundance of Bacteroidetes less than or equal to 0.1% using Bacteroidetes PCR assay will be eligible to be randomized to FMT with autologous feces collected prior to conditioning or no FMT. The second fecal evaluation, subsequent randomization to arms, and possible FMT procedure must take place within a 28-day window after stem cell engraftment (first day of three consecutive days that the absolute neutrophil count is at or above 500 per mm^3). In the event that engraftment occurs prior to day +7, the 28-day window will begin on day +7.

The randomization eligibility criteria are summarized as follows:

1. <u>Pre-transplant stored feces (first fecal evaluation and subject criteria)</u>
   - ☐ At the time of providing the pre-transplant sample, subject is **not** taking antibiotic treatment, where treatment involves gut-perturbing antianaerobic antibiotics (see appendix)
   - ☐ Inverse Simpson diversity ≥ 2.0 by analysis of 16S bacterial rRNA sequences
   - ☐ No evidence of intestinal pathogens by:
     i. GI pathogens PCR panel (includes Salmonella, Giardia lamblia, Norovirus, etc.)
     ii. C.difficile by PCR assay*
     iii. Ova and parasites exam
2. <u>Post-engraftment fecal sample (second fecal evaluation and subject criteria)</u>
   - ☐ Subject has engrafted and sample has been collected within the 28-day window, described above.
   - ☐ Low abundance of Bacteroidetes bacteria via PCR assay (≤0.1%).
3. <u>Randomization Criteria (post both fecal evaluations)</u>
   - ☐ Subject is currently **not** taking antibiotic treatment as prevention or suppression of an ongoing infrection, where treatment involves gut-perturbing antianaerobic antibiotics (see appendix)
   - ☐ Subject has not exceeded 28 day window described above.

Note: tests with asterisk (*) are research tests that will not be shared with the patient and/or his/her provider.

9.0 TREATMENT/INTERVENTION PLAN

Patients who meet randomization criteria (described above) will be randomized to either undergo FMT afterstem cell engraftment, or will not undergo FMT. As stated in the prior section, randomization and FMT must be performed within the defined 28-day window.

Patients randomized to the control arm will receive standard transplant care alone. They will be monitored routinely during hospitalization and assessed by a BMT clinician. During this time, the study's investigator's and research team will follow the patient for any incidence of CDI, systemic infections, and GVHD; which are assessed by the patient's treating BMT clinician. During this time, the patient's are also receving routine care per MSK policy which may or may not be followed by the study team.

Randomization to arms can be delayed from the time of engraftment if the patient is completing a course of antibiotic treatment (see appendix, list of microbiota-perturbing antibiotics), is recovering from mucositis, is critically ill, or any other condition where, in the judgement of the study investigator and treating transplant clinician, warrants a delay in initiation of FMT, as long as it does not exceed the 28 day window. Subjects will not be eligible for randomization after delays exceeding this window. Within the FMT arm, fecal solutions will be prepared as described in section 4.2. A rectal tube used for retention enema will be placed. The filtered fecal solution will be administered over the course of approximately 0.5 to 5.0 minutes. The rectal tube will be removed following fecal infusion and the patient will be encouraged not to defecate for the next hour if possible.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Patients enrolled in this study may have radiographic exams that constitute routine post-allo-HSCT care during their initial hospitalization as performed by their treating BMT clinician. They will be followed in the BMT clinic for one year post randomization for any incidence of CDI systemic infections, and GVHD; as assessed by the patient's treating BMT clinician. Because allo-HSCT is associated with a prolonged period of immunocompromise, patients will be closely assessed and followed by the BMT clinic, for signs of infection, including fever, respiratory distress, abdominal pain, diarrhea, nausea and vomiting. In these settings, blood cultures, sputum analysis, urine cultures and stool cultures may be obtained in the BMT clinic which wouldn't relate to the study unless they reveal incidences of infection listed in the studies primary endpoints. The study team may acquire an aliquot from the stool samples for sequencing. Results of the BMT clinic studies will guide treatment decisions, including the initiation of antimicrobial treatment. The effects of these antimicrobial treatments will be analysed through outpatient fecal sample collections. The subsequent sequencing data obtained from these samples will not affect the patient's treatment nor will it lead to additional treatment post randomization to FMT.

Study Timeline

Period	Time	Study Events
Pre-hospitalization	Pre-admission visit	Enrollment

Transplant hospitalization	Admission	First fecal evaluation (microbial diversity ≥ 2.0 & none of the following: intestinal pathogens such as parasitic ova and parasites &C. diff.) Collect and store FMT feces
	Engraftment	Second fecal evaluation (prevalence rate of Bacteroidetes ≤0.1%) (If determined eligible prior to discharge) Randomize and administer treatment arms (FMT vs. no FMT)
	Discharge	
Outpatient visits (until 1 year post-post randomization)	Followup BMT clinic visits	(If determined eligible post discharge and patient is not randomized) Randomize and administer treatment arms (FMT vs. no FMT) Six stool specimens will be collected during the first 6 months following randomization if feasible. More specimens may be collected at the discretion of the investigator up to one year post randomization. There will be a minimum 2 week interval between collection of post-randomization fecal samples, unless otherwise specified by the investigator.

Clinical follow-up of study patients after transplant hospitalization and randomization will occur during outpatient evaluations by their treating clinician. Patients may also subsequently be followed inpatient if they were to be hospitalized post HSCT. BMT clinic follow-up may occur at approximately monthly intervals; but may be more frequent as deemed necessary by the treating clinician. The evaluations may include history and physical examination, blood counts and chemistries including liver function tests performed by the clinic but for the purposes of this study will, will not be tracked unless deemed relatable by the study investigator. Patients will be assessed for CDI, graft-versus-host disease, and toxicities/side effects (described in the next section) by their treating BMT clinician and will be followed by the FMT team to assess the outcomes of the study.

Research fecal samples will be collected according to the follow-up schedule described previously and analyzed at the investigator's discretion. These samples will be analyzed for correlative purposes, to evaluate the stability/instability of the intestinal microbiota, and for detection of *C. difficile* toxin gene by PCR assay. Clinical C. difficile PCR testing can be ordered by the treating clinician if CDI is suspected based on clinical symptoms including diarrhea, which are required for a C. diff PCR assay to be ordered.

11.0 TOXICITIES/SIDE EFFECTS

FMT has been performed in hundreds of patients and has not been associated with adverse events. Of note, however, FMT has generally been performed in immunologically intact individuals with recurrent episodes of Clostridium difficile colitis. In published cases and

series of FMT, donor feces are obtained from a heterologous donor, which introduces the possible transmission of infectious agents that might not be detected by laboratory analysis. Our study represents the first study of autologous fecal microbial transplantation. Auto-FMT makes sense in the allo-HSCT population because patients generally come in with a diverse flora and lose microbial diversity during transplantation. Because the FMT proposed in this protocol is autologous, the risk of introducing undetected or unknown pathogens into a naïve host is markedly reduced.

On the other hand, allo-HSCT involves the introduction of a new immune system into the recipient, and the intestine's newly established mucosal immune system and the gut microbiota will establish a highly complex two-way relationship. Although it is most likely that the establishment of a diverse microbiota will enhance the redevelopment of the mucosal immune compartment, with the balanced development of effector and regulatory T lymphocyte populations, it is possible that auto-FMT may induce inflammatory responses in the gut that might lead to diarrhea, fever and even systemic infection. It will be important to determine whether the incidence of fever, diarrhea and systemic infection is increased following auto-FMT.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

The primary outcome measure is *Clostridium difficile* infection (CDI) up to one year following randomization after stem cell engraftment (defined as the first day of three consecutive days that the absolute blood neutrophil count is at or above 500 per mm^3). CDI is defined as diarrheal stool (unformed stool conforming to the shape of a specimen container), and a positive test for toxin-producing C. difficile (either by toxin B gene PCR or cytotoxicity assay). Although *C. difficile* PCR research assay may be performed in correlative specimens to determine colonization status, it cannot be used to diagnose CDI, the primary endpoint of the study. A diagnosis of CDI will be based on the presence of diarrhea and subsequent clinical-based microbiologic testing, where testing is sent based on clinical suspicion by the treating clinician.

Secondary outcomes will include systemic and/or intestinal bacterial and viral infections. Diagnosis of these infections will be made by blood culture or PCR assay of blood at the time that these type of infections are suspected by the treating clincian. These organisms include enteric gram negative bacteria (e.g. *Klebsiella, Escherichia coli, Pseudomonas aeruginosa*, viridans-group streptococci, Enterococcus, adenovirus, and cytomegalovirus).

The secondary outcome of GVHD will be evaluated and graded by the patient's treating BMT clincian using standard clinical criteria, and histological grading of skin, liver, or gastrointestinal pathology where possible. Transplant patients will be assessed by a transplant clincian for the development of GVHD approximately weekly. Data may be collected approximately every 1-2 weeks to characterize the severity of symptoms and signs caused by GVHD by the treating BMT clinician. The FMT research team will be following these evaluations to assess the outcome of FMT's role in GVHD. Evaluations for GVHD by BMT clinicians will continue until 100 days after transplant or sooner if relapse or recurrence occurs.

The microbiota will be characterized using the 16S rRNA gene sequence data obtained from serially-collected fecal samples. Characterizations of the microbiota will include a combination of metrics, including phylogenetic classification of sequences with calculation of relative abundances, grouping into operational taxonomic units (based on 97% similarity in sequence-based genetic distance) and calculation of microbial ecology measures (alpha diversity such as inverse Simpson index, and beta diversity measures such as Bray-Curtis dissimilarity). These will be used to describe the presence of Bacteroidetes bacteria in fecal samples before and after randomization to FMT, and to determine if any microbiota factors correlate with the development of CDI.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may be removed from the study at any point if they do not comply with BMT clinic followup, if they decide to withdraw from the study, or if there are any general or specific changes in the patient's condition that render the patient unacceptable for further evaluation in the judgment of the investigator.

14.0 BIOSTATISTICS

Our prior data estimated the incidence of CDI to be 53.3% one year post transplantation.- in recipients of DUCB-Tx patients lacking the Bacteroidetes phylum bacteria in their feces at the time of engraftment, while the incidence was 18.8% in patients with Bacteroidetes. Though this data specifically describes DUCB-Tx recipients, CDI rates are similar in patients undergoing myeloablative or reduced intensity allo-HSCT with other stem cell sources. Therefore it is likely that a benefit of similar magnitude exists for all allo-HSCT recipients of this conditioning intensity.

The primary endpoint for this study is CDI after randomization to arms during post-engraftment of allo-HSCT. We will compare the differences in CDI hazard using the Kaplan-Meier method with log rank testing. Analysis of primary endpoint will be by intention-to-treat. Our target accrual is 96 subjects, which would detect a hazard ratio of 0.5 reduction in CDI rate between arms with 80% power (total 68 CDI events, using two-sided alpha 0.05). This design also allows for an interim analysis halfway through enrollment using O'Brien-Fleming boundaries for futility. If $P \geq 0.73$ at the interim analysis, enrollment will stop with the conclusion that FMT does not decrease the incidence of CDI in patients meeting randomization criteria. If $P < 0.73$, the trial will continue to full enrollment.

Assuming approximately 105 consented subjects per year (131 patients undergoing myeloablative or reduced intensity allo-HSCT per year, with an 80% who consent to the study), with approximately 31% who will be found to have low Bacteroidetes bacteria on engraftment, we anticipate that approximately 33 patients will be eligible for randomization per year. Thus the target accrual would be completed in approximately 3 years' time.

A significantly lower incidence of CDI in the FMT arm compared with that of control will be considered a successful outcome. This may lead to a change in practice where FMT is given prophylactically to the subset of transplant recipients with abnormal intestinal microbiota with associated high risk for CDI.

In order to reduce patient risk and confirm the safety of the FMT arm, the study design includes early termination in event that patients either have high rates of bloodstream infections or have high rates of grade III-IV acute GVHD. If the FMT arm reaches either boundary, both arms will be stopped and the trial will be reviewed based on the patients who have accrued until that point. The calculations in the table below are based on marginal probabilities.

Failure Type	No. failures needed to stop the study	Failure rate in the population	Probability boundary is crossed
Bloodstream Infections to 50 days following fecal transplant	4 in the first 10 5 in the first 20 7 in the first 30	0.10	0.09
	8 in the first 40 9 at any point	0.25	0.91
Day +100 grade III-IV acute GVHD	3 in the first 10 4 in the first 20	0.05	0.06
	5 in the first 30 6 at any point	0.20	0.95

The baseline rate of 5% for grade III-IV acute GVHD and 10% for bloodstream infections is based on the anticipated accrual of cord and non-cord transplants. While there are differences in the baseline rates, the selected elevated GVHD rate of 15% and bloodstream infection rate of 20% represents an increased risk for both transplant types.

Secondary endpoints include systemic bacterial and viral infection up to one year post-allo-HSCT and GVHD (assessed and recorded by Adult BMT service at 100 days) Comparison of these endpoints between study arms will be done using survival analysis. Compositional differences in the intestinal microbiota will be determined by analysis sequenced stool samples during followup. Microbiota characteristics will include Shannon diversity index, and relative abundances of bacterial taxons. Microbial markers of CDI and GHVD will be assessed using LEfSe, a metagenomic computational method used for biomarker discovery with microbiome data.[26]

Based on data from prior years, we estimate that grade III-IV acute GVHD will occur at a rate of approximately 4% of subjects receiving T-cell depleted grafts, and 10% of subjects receiving non-T-cell depleted grafts. Bloodstream infection is estimated to occur at a rate of 10% in subjects within 50 days following stem cell engraftment. Overall mortality is estimated at 30% for subjects with underlying disease of leukemia or non-Hodgkin lymphoma, and multiple myeloma, and 21% for subjects with all other underlying diseases.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

Enrolled patients will be registered using the Clinical Research Database (CRDB). After eligibility is established after stem cell engraftment, enrolled subjects will be 1:1 randomized to the two arms using CRDB. Randomization will be stratified by stem cell source (cord blood vs. non cord blood donor). Randomization will be accomplished by the method of random permuted block.

16.0 DATA MANAGEMENT ISSUES

This is a single institution trial and all patients will be treated at Memorial Sloan-Kettering Cancer Center. A research study assistant (RSA) will be assigned to this study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of study protocol team activities. The data manager will also monitor laboratory compliance throughout the study. Laboratory data will be tabulated and summarized. Only the minimal data set will be entered into CRDB.

16.1 Quality Assurance

Registration reports will be generated by the RSA on a regular basis to monitor patient accruals and completeness of the registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

16.2 Data and Safety Monitoring

MSKCC's Data and Safety Monitoring (DSM) Plan was developed to comply with the NIH/NCI policy guidance: "NCI's Essential Elements of a Data and Safety Monitoring Plan for Clinical Trials Funded by the NCI." The plan was reviewed and approved by the NCI in September 2001. It is established and monitored by the Office of Clinical Research. The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://inside2/clinresearch/Pages/protocol-review-committees/data-and-safety-monitoring-committee.aspx. During the protocol development and review process, each protocol will be

assessed for its level of risk and degree of monitoring required. Every type of protocol (i.e. NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

For this project, the Data Safety and Monitoring Committee (DSMC) will convene during this study to monitor study progression. Specific to this study, there are two defined stopping rules for safety, bloodstream infections, and severe GVHD (grade III or higher), and a third stopping rule for futility (see *Statistical Considerations*).

During the study period, if any new information becomes available regarding the safety and risk of FMT, such as from published literature or through clinical experience at MSKCC, all sub-investigators in the study will be notified and updated on this information through email and/or progress meetings with investigators.

17.0 PROTECTION OF HUMAN SUBJECTS

Consent process: Participation in this trial is voluntary. All patients will be required to sign a statement of informed consent, which must conform to MSKCC IRB guidelines.

Risks: Because the FMT proposed in this study is autologous (auto-FMT), the risk of introducing undetected or unknown pathogens into a naïve host is markedly reduced. The establishment of a diverse microbiota (new gut flora) may enhance the redevelopment of the mucosal immune compartment with a balanced development of effector and regulatory T-cell populations. However, it is possible that auto-FMT may induce inflammatory responses in the gut that might lead to diarrhea, fever and/or systemic infection.**Benefits**: Autologous FMT can potentially reduce the risk of CDI during post-engraftment of allo-HSCT. Other potential benefits include reduced risk of graft-versus-host disease, and reduced risk of systemic bloodstream infections.

Protocol Amendments and Study Termination: All protocol amendments will be reviewed and approved by the Institutional Review Board of Memorial Hospital before implementation.

Incentives: No incentives will be offered to subjects for participation in this study. Participation is voluntary.

Costs: Fecal microbiota transplantation, along with its corresponding biospecimen analysis, will be performed free of charge to patients.

Eligibility Exceptions: There will be no exceptions to the eligibility requirements for this protocol without the authorization of the Institutional Review Board of Memorial Hospital.

Adverse Reporting Requirements: Severe or unexpected adverse reactions will be reported to Ying Taur, M.D., M.P.H., principal investigator at MSKCC, the MSKCC IRB, and the FDA.

Inclusion of Children in Research: Children are excluded from this study as our initial analyses were performed on samples from adult BMT recipients only.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- Medical record number

- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI's signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office

17.2.1 Reporting of SAEs to FDA

Toxicities will be reported using NCI's Common Terminology Criteria for Adverse Events Version 4.0.

For IND/IDE protocols:

The CRDB AE report should be completed as above and the FDA assigned IND/IDE number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.

3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

19.0 REFERENCES

1. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). infection control and hospital epidemiology. 2010;31(5):431–55.

2. Leung S, Metzger BS, Currie BP. Incidence of Clostridium difficile infection in patients with acute leukemia and lymphoma after allogeneic hematopoietic stem cell transplantation. Infection Control and Hospital Epidemiology. 2010;31(3):313–5.

3. Alonso CD, Treadway SB, Hanna DB, et al. Epidemiology and Outcomes of Clostridium difficile Infections in Hematopoietic Stem Cell Transplant Recipients. Clinical infectious diseases. 2012;54(8):1053–63.

4. Dubberke ER, Reske KA, Srivastava A, et al. Clostridium difficile-associated disease in allogeneic hematopoietic stem-cell transplant recipients: risk associations, protective associations, and outcomes. Clinical transplantation. 2010;24(2):192–8.

5. Chakrabarti S, Lees A, Jones S, Milligan D, others. Clostridium difficile infection in allogeneic stem cell transplant recipients is associated with severe graft-versus-host disease and non-relapse mortality. Bone marrow transplantation. 2000;26(8):871.

6. Willems L, Porcher R, Lafaurie M, et al. < i> Clostridium difficile</i> Infection after Allogeneic Hematopoietic Stem Cell Transplantation: Incidence, Risk Factors, and Outcome. Biology of Blood and Marrow Transplantation. 2012;18(8):1295–301.

7. Tomblyn M, Gordon L, Singhal S, et al. Rarity of toxigenic Clostridium difficile infections after hematopoietic stem cell transplantation: implications for symptomatic management of diarrhea. Bone marrow transplantation. 2002;30(8):517–9.

8. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased Diversity of the Fecal Microbiome in Recurrent Clostridium difficile—Associated Diarrhea. Journal of Infectious Diseases. 2008;197(3):435–8.

9. Jenq RR, Ubeda C, Taur Y, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. The Journal of experimental medicine. 2012;209(5):903–11.

10. Taur Y, Xavier JB, Lipuma L, et al. Intestinal Domination and the Risk of Bacteremia in Patients Undergoing Allogeneic Hematopoietic Stem Cell Transplantation. Clinical Infectious Diseases. 2012;55(7):905–14.

11. Khoruts A, Dicksved J, Jansson JK, Sadowsky MJ. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent Clostridium difficile-associated diarrhea. Journal of clinical gastroenterology. 2010;44(5):354.

12. You DM, Franzos MA, Holman RP, others. Successful treatment of fulminant Clostridium difficile infection with fecal bacteriotherapy. Annals of internal medicine. 2008;148(8):632.

13. Bakken JS, Johnson S, Gerding D, others. Fecal bacteriotherapy for recurrent Clostridium difficile infection. Anaerobe. 2009;15(6):285–9.

14. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N. Engl. J. Med. 2013;368(5):407–15.

15. Bakken JS, Borody T, Brandt LJ, et al. Treating< i> Clostridium difficile</i> Infection With Fecal Microbiota Transplantation. Clinical Gastroenterology and Hepatology. 2011;9(12):1044–9.

16. Louie TJ, Emery J, Krulicki W, Byrne B, Mah M. OPT-80 eliminates Clostridium difficile and is sparing of bacteroides species during treatment of C. difficile infection. Antimicrobial agents and chemotherapy. 2009;53(1):261–3.

17. Tvede M, Rask-Madsen J, others. Bacteriotherapy for chronic relapsing Clostridium difficile diarrhoea in six patients. Lancet. 1989;1(8648):1156.

18. Van Vliet MJ, Harmsen HJ, de Bont ES, Tissing WJ. The role of intestinal microbiota in the development and severity of chemotherapy-induced mucositis. PLoS pathogens. 2010;6(5):e1000879.

19. Flint HJ, Scott KP, Duncan SH, Louis P, Forano E. Microbial degradation of complex carbohydrates in the gut. Gut Microbes. 2012;3(4):289–306.

20. Xu J, Bjursell MK, Himrod J, et al. A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science. 2003;299(5615):2074–6.

21. Gilmore MS, Ferretti JJ. The thin line between gut commensal and pathogen. SCIENCE-NEW YORK THEN WASHINGTON-. 2003;:1999–2001.

22. Guarner F, Malagelada J-R. Gut flora in health and disease. The Lancet. 2003;361(9356):512–9.

23. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. Clinical microbiology reviews. 2007;20(4):593–621.

24. Hamilton MJ, Weingarden AR, Sadowsky MJ, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent Clostridium difficile infection. The American

journal of gastroenterology. 2012;107(5):761–7.

25. Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Applied and environmental microbiology. 2009;75(23):7537–41.

26. Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. Genome biology. 2011;12(6):R60.

20.0 APPENDICES

Appendix 1. BMT Service SAE SOP

Appendix 2. Microbiota-Perturbing Antibiotics

FMT treatment and randomization to the treatment should not be performed if they are taking antibiotics with significant impact on the intestinal microbiota, as well as any microbiota from FMT administration. These antibiotics are specified below and include:

- Beta-lactam antibiotics
  - Piperacillin-tazobactam
  - Cefepime
  - Ceftazidime
  - Ceftriaxone
  - Oxacillin
  - Imipenem
  - Meropenem
  - Amoxicillin-clavulanate
  - Ampicillin-sulbactam
- Vancomycin (oral only)
- Metronidazole
- Clindamycin
- Tigecycline
- Linezolid
- Daptomycin
- Ciprofloxacin
- Levofloxacin

Antibiotics that CAN be administered during FMT include:

- Vancomycin (intravenous only)
- Aztreonam
- Amingoglycosides (gentamicin, tobramycin, amikacin; intravenous only)
- Polymyxin B (intravenous)
- Trimethoprim-sulfamethoxazole
- Pentamadine (aerosolized)
- Dapsone
- Atovaquone
- Doxycycline
- Azithromycin
- Clarithromycin
- Rifampin

Antivirals and antifungals can be given during FMT treatment. Non-systemic antibiotics such as those given topically or by aerosolized route can also be administered concurrently. For systemic antibacterials not specified here, the study investigator will make a determination as to whether it can be administered during FMT treatment.