DF/HCC Protocol #: 15-394 DF/HCC

TITLE: A Randomized Phase 2 Study to Examine the Impact of Gut Decontamination on Intestinal Microbiome Composition in Pediatric Allogeneic Hematopoietic Stem Cell Transplant Patients

NCT #: 02641236

Version Date: 6.0 / 10DEC2021

IRB Initial Approval: 10/19/2015

DF/HCC Protocol #: *15-394*

DF/HCC Pediatric Biomedical Protocol Template: June 30, 2014

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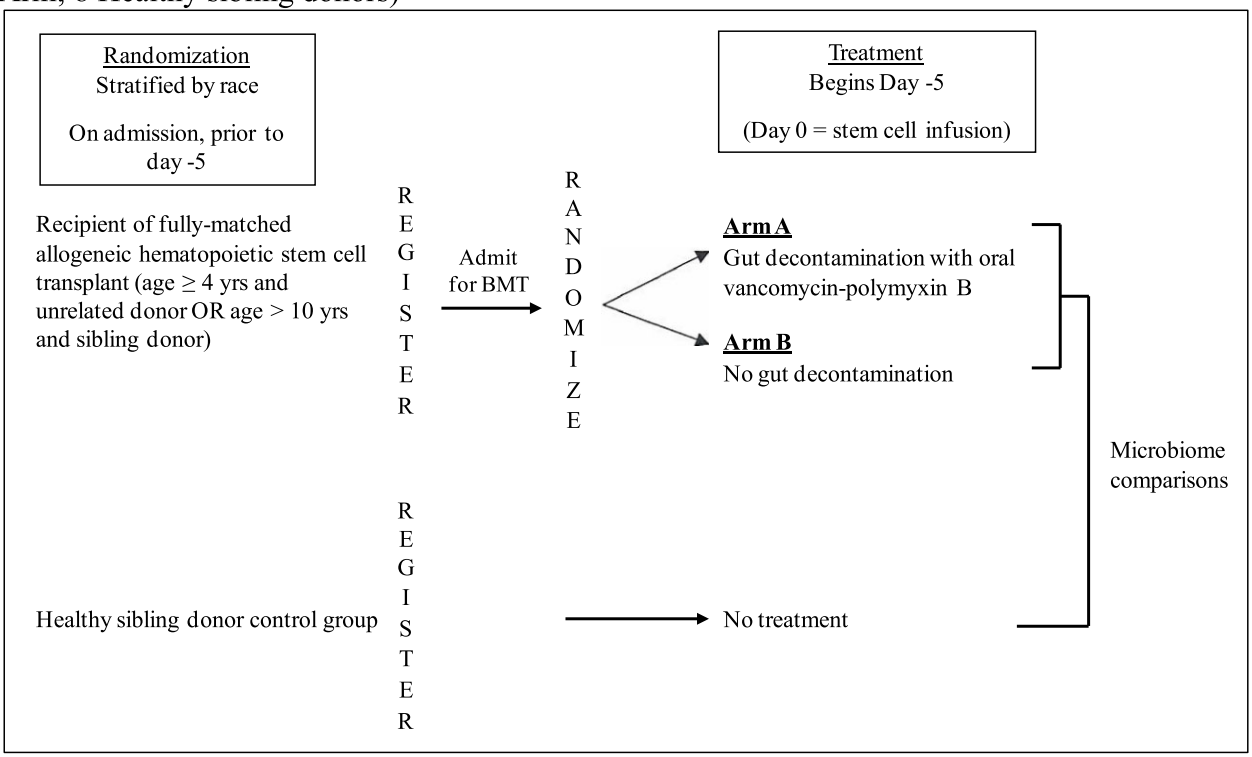
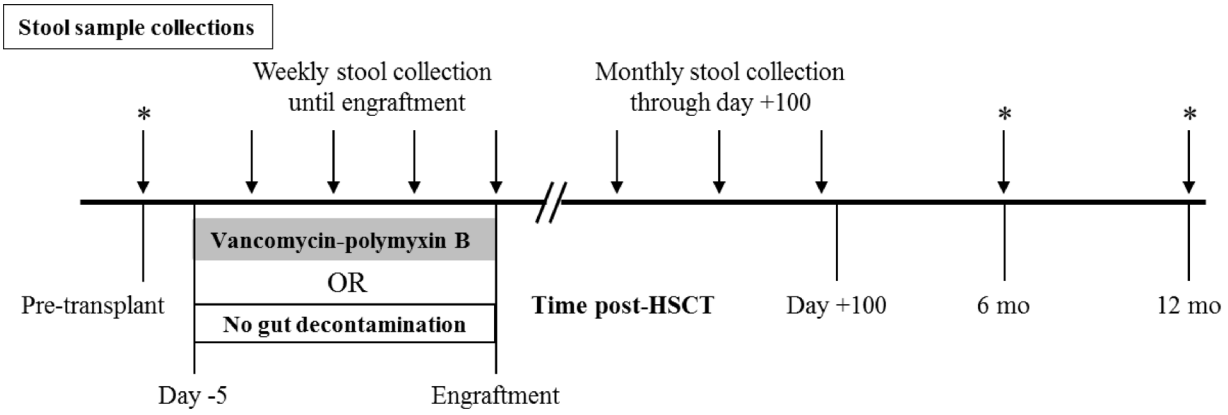
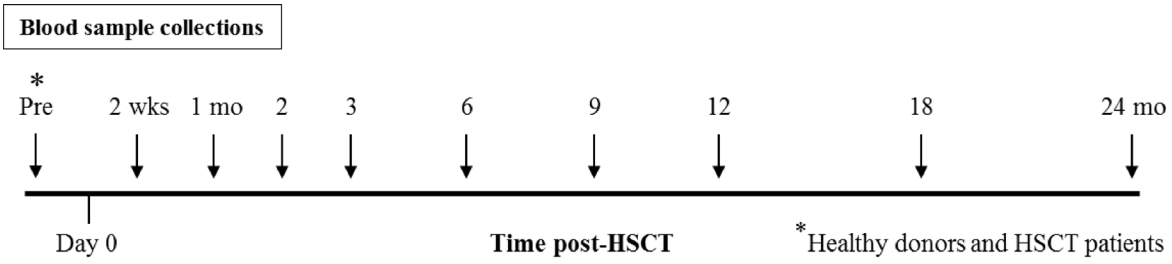
Study Exempt from IND Requirements per 21 CFR 312.2(b).

SCHEMA

Number of participants: 28 (10 in Gut Decontamination Arm; 10 in No Gut Decontamination Arm; 8 Healthy sibling donors)







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1. OBJECTIVES

1.1 Study Design

- Randomized (+/- gut decontamination) phase 2 trial to examine the impact of gut decontamination (GD) with oral vancomycin-polymyxin B on intestinal microbiota composition of allogeneic hematopoietic stem cell transplant (HSCT) patients. Patients will be analyzed as intention-to-treat. An additional cohort of healthy sibling donors will serve as a control comparison group.

1.2 Primary Objectives

- To describe longitudinal differences in the intestinal microbiota composition between the two randomized treatment arms of HSCT patients and a cohort of healthy sibling donors.

1.3 Secondary Objectives

- To describe the frequency of diarrhea in the two randomized treatment arms of HSCT patients.
- To determine the incidence of acute graft-versus host disease (GVHD) and bacteremia during the first 100 days post-transplant within the two randomized treatment arms of HSCT patients.
- To describe immune reconstitution and to estimate survival and malignant disease relapse at 2 years after study entry in the two randomized treatment arms of HSCT patients.
- To describe the practice of gut decontamination at pediatric stem cell transplant centers participating in the Pediatric Bone Marrow Transplant Consortium (PBMTC) in terms of the practice of gut decontamination, the antibiotic regimen used and the incidence of acute GVHD.

2. BACKGROUND

2.1 Study Disease: Acute Graft-Versus-Host Disease

For many patients with severe benign and malignant hematologic disorders, allogeneic hematopoietic stem cell transplant (HSCT) offers the only opportunity for cure. Unfortunately, the development of acute graft-versus-host disease (GVHD) limits the success of allogeneic HSCT. Although factors such as the level of HLA matching, recipient age and conditioning regimen greatly influence the incidence, acute GVHD occurs in approximately 40% of transplants even where the donor and recipient are fully matched [1]. Patients with grades III and IV acute GVHD have poor outcomes with about a 30% and less than 5% probability of long term survival, respectively [2].

Acute GVHD is an inflammatory process within host tissues thought to be triggered by damage resulting from transplant conditioning regimens or infections. This tissue damage leads to the release of proinflammatory cytokines and enhanced expression of key receptors on antigen-presenting cells that, in turn, enhance presentation of unshared recipient tissue antigens to the

alloreactive donor T cells that mediate acute GVHD [1]. The intestinal microflora are also thought to play a role in mediating acute GVHD as gut injury caused by the conditioning regimen leads to the release of bacterial lipopolysaccharides that can activate innate immune receptors and cause a cytokine storm [3]. One approach to acute GVHD prophylaxis practiced by many HSCT programs is gut decontamination (GD) with non-absorbable oral antibiotics.

2.2 History of gut decontamination for acute GVHD prophylaxis

Early studies using experimental mouse HSCT models in the 1970's demonstrated that resident intestinal bacteria contribute to the pathogenesis of acute GVHD, and that growth suppression or eradication of intestinal bacteria prevented the development of acute GVHD even in MHC-mismatched transplants. For example, Van Beekum et al. induced acute GVHD by administering bone marrow cells or a mixture of bone marrow cells and spleen cells from C57BL donor mice into lethally irradiated CBA recipient mice [4].

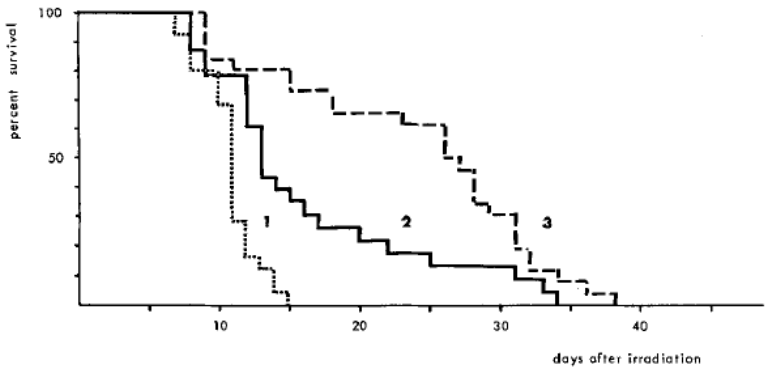


Figure 1. Influence of gnotobiotic state on mortality from acute GVHD in mice. CBA recipients were exposed to 900 rad of whole-body irradiation and received 107 bone marrow cells and 107 spleen cells from C57BL donors. *Curve 1* represents 25 conventional mice; *curve 2*, 23 germ-free mice; and *curve 3*, 26 completely decontaminated conventional mice (adapted from van Bekkum et al, JNCI 1974).

Using this model system, they demonstrated that conventional mice developed severe GVHD and had significantly decreased 90-day survival compared to gnotobiotic (germ free) mice. In addition, they were able to decrease the severity of acute GVHD and increase survival in conventional mice that were treated with antibiotics to achieve complete gut decontamination (Figure 1). Similar findings were later reported in a canine stem cell transplant model [5]. The practice of gut decontamination for acute GVHD prophylaxis in HSCT patients is based on these observations.

2.3 Trials of GD in allogeneic HSCT patients

Few human trials have examined the benefit of GD in lowering the risk of developing acute GVHD. One early study by Storb et al. showed significantly decreased rates of grades II to IV acute GVHD and increased overall survival in patients randomized to receive GD [6]. However, in addition to oral non-absorbable antibiotics, patients in the GD cohort were also placed in laminar airflow rooms and received "sterile" food and skin cleansing; thus, the reduction in acute GVHD risk and improvement in survival rate observed in this study cannot be attributed solely to oral non-absorbable antibiotics. Furthermore, sterile food and skin cleansing are not commonly used in modern HSCT programs. A more recent retrospective study examined stool culture results from pediatric patients transplanted between 1989 and 2002 [7]. Successful decontamination was defined as the absence of bacterial or fungal species in ≥ 3 of 5 stool samples obtained between days -10 to +30. Although the authors report that patients with

successful GD had a significantly lower rate of acute GVHD, only 8% (9/114) of all patients developed acute GVHD and nearly all cases were grade I with only 1 case of grade II. Several other studies have compared different antibiotic regimens for GD, but do not have a comparison group that did not receive any GD [8]. Thus, due to insufficient evidence, GD is not routinely recommended for the prevention of acute GVHD [9]. Yet, the practice of GD remains standard of care in many HSCT programs.

2.4 The gut microbiome and allogeneic HSCT

Gut microbiome studies in adult HSCT patients are beginning to elucidate correlations between the gut microbiome composition and clinical outcomes such as the development of bacteremia, acute GVHD and overall survival [10, 11]. For example, Taur et al. examined the microbial diversity present in fecal samples collected at the time of engraftment in 80 allogeneic HSCT patients. Patients in the high diversity group had 67% overall survival, whereas patients in the low diversity group had only 36% overall survival at 3 years [12]. More recently, Jenq et al. observed an association between lower bacterial diversity at day +12 post-transplant and an increased incidence of GVHD-related mortality ($p = 0.005$) in a cohort of 64 allogeneic HSCT patients who received conventional (non-T cell depleted) grafts [11]. In addition, they found that bacteria belonging to the genus blautia were associated with a significant reduction in GVHD-related mortality. The blautia genus includes anaerobic intestinal commensal organisms within the bacterial class Clostridia. Of note, in mouse models, Atarashi et al. showed previously that oral inoculation with a mixture of *Clostridium* strains promoted accumulation of colonic regulatory T cells (Treg), which function to decrease inflammation via their suppressive effects on effector T cells [13]. Additional mouse studies have shown that the gut microbiota produces immune modulatory metabolites that can regulate colonic Treg homeostasis and promote the generation of peripheral Tregs [14, 15]. Together, these recent findings suggest that the practice of GD should be carefully examined for its impact on the gut microbiome and immune reconstitution in patients undergoing HSCT.

2.5 Rationale

The current standard practice at our institution (Boston Children's Hospital) is to use an oral, non-absorbable antibiotic (vancomycin-polymyxin B) for GD in nearly all patients undergoing allogeneic HSCT, starting at day-5 through neutrophil engraftment. However, the use of GD for acute GVHD prophylaxis in patients undergoing allogeneic HSCT is controversial and is not practiced at all stem cell transplant centers. In addition, there is no consensus regarding efficacy or ideal choice of antibiotic regimen.

The recent correlations between higher bacterial diversity and better clinical outcomes following allogeneic HSCT raise concerns regarding the practice of intestinal decontamination. The impact of GD on diversity within the gut microbiome is unknown. Moreover, there are no published studies examining the gut microbiome in the pediatric HSCT population. Gut microbiome studies in humans and mice have demonstrated changes in bacterial composition and diversity with age [16, 17]. Thus, the correlations between gut microbiome composition and clinical outcomes observed in adults may not be extrapolated to the pediatric HSCT population. Furthermore, previous studies examining the gut microbiome in HSCT patients have only looked at early time points post-transplant (up to engraftment) when patients are still recovering from

the acute injuries caused by the conditioning regimen and may not have resumed full enteral nutrition. Longer term reconstitution of the gut microbiome post-HSCT and associations with clinical outcomes have not been examined. What is the time course of reconstitution of the gut microbiome following allogeneic HSCT? How long does it take to return to the pre-transplant diversity level or to the diversity level observed in normal healthy subjects? Do differences in the tempo of gut microbiome recovery correlate with clinical outcomes after HSCT?

To begin addressing these important questions, we propose the first randomized phase 2 study of GD with oral, non-absorbable antibiotics in pediatric allogeneic HSCT patients. We will randomize a cohort of allogeneic HSCT patients at Boston Children's Hospital (BCH) to receive or not receive GD and subsequently collect stool samples at multiple time points through 1 year post-transplant. We will extract genomic DNA from the stool samples obtained at each time point and describe the composition of the stool microbiota by high-throughput sequencing techniques. The primary endpoint will be measurement of bacterial diversity, quantified by the Shannon diversity index, at 2 weeks post-HSCT. Jenq et al. reasoned that this time point provides enough time for stool specimens to reflect changes induced by the conditioning regimen and antibiotic exposures, but precedes the development of GVHD [11]. As mentioned above, their study showed that decreased bacterial diversity around day +12 correlated with increased GVHD mortality.

The recent mouse studies described above showing a direct impact of the gut microbiome on immune cell generation and function suggest that there could be interesting correlations between the gut microbiome and immune reconstitution in HSCT patients. To explore the relationship between gut microbiome and immune recovery post-HSCT, we will prospectively collect peripheral blood samples for banking and immune cell profiling for 2 years post-HSCT. In order to perform comparisons with healthy gut microbiome compositions and healthy immune cell profiles, we will obtain stool and blood samples from healthy sibling bone marrow donors.

Future studies are necessary to establish whether GD has any clinical benefit in children undergoing allogeneic HSCT. A definitive study will not be possible at our institution or any single institution due to small patient numbers and relatively low incidence of moderate to severe acute GVHD in the pediatric HSCT population. For example, at our institution, the incidence of acute GVHD is approximately 25% for recipients of unrelated donor HSCT and lower for recipients of matched sibling donor HSCT. As part of this study, we will perform a survey of pediatric stem cell transplant centers that are members of the Pediatric Bone Marrow Transplant Consortium on their GD practices and their rates of acute GVHD. Results from this survey will be used to identify possible sites for participation in a future multi-center trial for the randomization of GD in pediatric HSCT patients.

2.6 Correlative Studies Background

Stool samples and peripheral blood samples obtained before, during and after therapy will be utilized to assess reconstitution of the gut microbiome and immune reconstitution post-HSCT. We will compare differences in reconstitution of the gut microbiome and immune system in patients with and without GD. The studies are summarized below.

Molecular profiling of the gut microbiota: Genomic DNA will be extracted from stool specimens using a phenol-chloroform extraction technique as described in a previously published protocol [18]. For profiling of the stool bacterial composition, purified DNA will be sent for bacterial 16S ribosomal RNA sequencing. Samples from selected time points will be sent for shotgun whole genome sequencing, which will enable identification of DNA viruses, fungi and novel bacteria within the gut microbiome. Bioinformatic analysis of the sequence data will be performed in the laboratory of Dr. Ami Bhatt (Stanford University). Dr. Bhatt has expertise in the use of next generation sequencing and bioinformatic methods for the characterization of the gut microbiome in HSCT patients [19].

Phenotypic analysis of lymphocyte subsets: Incubation of peripheral blood with monoclonal antibodies specific for lymphocyte markers will be used to identify functionally distinct lymphocyte subsets. After incubation of peripheral blood cells with directly fluorochrome-conjugated monoclonal antibodies, individual subsets will be enumerated by flow cytometry. Immune profiling will be performed at the same time points as for pediatric patients enrolled on DFCI protocol 13-257 (Tissue Collection for Research Studies in Children Undergoing Hematopoietic Stem Cell Transplantation and Normal Donors).

DNA and cytokine analyses: Additional genetic analyses (e.g. T cell receptor sequencing) can be considered to assess the immune cell repertoire, and banked DNA and cell samples would be accessed for such analyses. ELISA assays on banked plasma samples will be used to measure inflammatory and protective cytokines (e.g. IL-22) known to be involved in GVHD pathophysiology.

Taken together, these assays will provide molecular and cellular characterization of the effects of GD on participant gut microbiota and immune reconstitution following HSCT.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria for Patients Undergoing Allogeneic HSCT

3.1.1 Recipient of 9/10 or 10/10 (HLA-A, -B, -C, -DRB1, -DQB1) matched bone marrow allogeneic hematopoietic stem cell transplantation (HSCT) OR 4/6, 5/6 and 6/6 (HLA-A, -B, -DR) matched cord blood allogeneic HSCT.

3.1.2 Participants may have underlying malignant or non-malignant hematologic disease, except for primary immunodeficiency, as the indication for their allogeneic HSCT. Patients with immune dysregulation syndromes such as familial or secondary hemophagocytic lymphohistiocytosis (HLH) are eligible.

3.1.3 Participants may receive either a myeloablative or non-myeloablative (reduced-intensity) conditioning regimen.

3.1.4 Graft-versus-host disease (GVHD) prophylaxis with any of the following agents: calcineurin inhibitor, short-course methotrexate, steroids, mycophenolate mofetil, and sirolimus.

3.1.5 Age ≥ 4 years old and toilet-trained. Participants must be able to deposit stool samples directly into stool collection containers. Stool specimens from diapers are difficult to obtain and are prone to more sampling error, particularly for loose or liquid stools which are common in the peri-transplant period.

3.1.6 Lansky/Karnofsky performance status ≥60% (see Appendix A)

3.1.7 Ability to understand and/or the willingness of their parent or legally authorized representative to sign a written informed consent document.

3.2 Exclusion Criteria for Patients Undergoing Allogeneic HSCT

3.2.1 Patients undergoing allogeneic HSCT for correction of a primary immunodeficiency disorder (e.g. SCID).

3.2.2 Patients with age ≤ 10 years undergoing HSCT with a matched sibling donor. These patients are at very low risk of acute GVHD and do not receive gut decontamination per our institutional standard practice.

3.2.3 Participants receiving GVHD prophylaxis with drugs other than agents listed in 3.1.4 (e.g. abatacept).

3.2.4 History of allergic reactions attributed to oral vancomycin or oral polymyxin B.

3.2.5 Participants undergoing active therapy for immune-mediated or infectious colitis upon admission for allogeneic HSCT.

3.2.6 Participants receiving antibiotic therapy for treatment of a bacterial infection or bacterial prophylaxis upon admission for allogeneic HSCT. Use of any agent (e.g. sulfamethoxazole/trimethoprim) for prophylaxis of *Pneumocytis jirovecii* pneumonia is permitted. Concurrent use of anti-fungal and anti-viral therapies is also permitted.

3.2.7 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection or psychiatric illness/social situations that would limit compliance with study requirements.

3.3 Eligibility Criteria for Healthy Bone Marrow Donors

Healthy individuals, ages ≥ 4 years and toilet-trained, who have been identified by BCH or DFCI providers as 9/10 or 10/10 (HLA-A, -B, -C, -DRB1, -DQB1) matched, bone marrow donors for transplantation will also be eligible to participate in this study. Healthy donors may be related or unrelated to the bone marrow recipient.

3.4 Inclusion of Children and Minorities

Both male and female participants of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Office of Data Quality(ODQ) central registration system. Registrations and randomizations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy 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 ODQprotocol-specific eligibility checklist.

Following registration and randomization, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the DF/HCC Principal Investigator (PI).

4.2 Registration and Randomization Process for DF/HCC and DF/PCC Institutions

The ODQ registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time.

The registration procedure for HSCT patients and healthy sibling donors is as follows:

- Obtain written informed consent from the participant prior to the performance of any protocol specific procedures or assessments.

- Complete the ODQ protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical record and/or research chart. **To be eligible for registration to the protocol, the participant must meet all inclusion and exclusion criterion as described in the protocol and reflected on the eligibility checklist.**

  Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for a treatment protocol. Registration to both treatment and ancillary protocols will not be completed if eligibility requirements are not met for all studies.

- Fax the eligibility checklist(s) and all pages of the consent form(s) to the ODQ at 617-632-2295.

- The ODQ Registrar will (a) review the eligibility checklist, and (b) register the participant on the protocol.

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

The randomization procedure for HSCT patients is as follows:

- The study team will contact the ODQ Registrar to request that a participant be randomized. This will occur on the day of admission to the hospital, which could be up to 1 month after study registration. If the day of admission falls on a Saturday, Sunday or holiday, then randomization should occur on the first business day before the scheduled admission day.
- The ODQ Registrar will randomize the participant.
- An email confirmation of the randomization will be sent to the DF/HCC PI, study coordinator(s) from the Lead Site, treating investigator and registering person immediately following the randomization.

4.3 General Guidelines for Other Investigative Sites

N/A.

4.4 Registration Process for Other Investigative Sites

N/A.

5. TREATMENT PLAN

5.1 Treatment Regimen

Assignment of allogeneic HSCT participants to treatment arms:
All eligible participants will be randomized to either **Arm A: "Gut Decontamination"** or **Arm B: "No Gut Decontamination"**. The Stem Cell Transplant Program at Boston Children's Hospital has discontinued use of gut decontamination in recipients of allogeneic HSCT who are ≤ 10 years old AND have fully-matched sibling donors as there is virtually no acute GVHD observed in this cohort. Thus, allogeneic HSCT recipients who are age ≤ 10 years old with fully-matched sibling donors are not eligible for this study.

Arm A: Gut Decontamination (GD)
Participants assigned to this arm will receive non-absorbable, oral vancomycin-polymyxin B as per our institutional standard practice. Participants will begin oral gut decontamination with vancomycin-polymyxin B on Day -5 relative to the stem cell infusion date (Day 0) and continue through neutrophil engraftment, defined as having an absolute neutrophil count ≥ 500 cells/mm^3 for 3 consecutive days.

Dosing for oral vancomycin-polymyxin B is based on body surface area:

Body Surface Area (m^2)	Dose of Vancomycin-Polymxyin B Capsules
< 0.5 m^2	1 cap PO TID
0.5 – 0.99 m^2	2 cap PO TID
1 – 1.49 m^2	3 cap PO TID
≥ 1.5 m^2	4 cap PO TID

Arm B: No Gut Decontamination (no GD):
Participants assigned to this arm will not receive oral vancomycin-polymyxin B, but all other HSCT supportive care will be the same as for patients in the GD Arm.

5.2 Pre-Treatment Criteria for Participants Undergoing Allogeneic HSCT

All allogeneic HSCT participants must meet eligibility criteria as specified in Section 3.1. The first stool and peripheral blood collection for all participants should occur prior to day -5.

5.2.1 **The following clinical information should be collected and recorded on the Medication and Diet History Form (see Appendix B) at each stool sample time point:**

- Antibiotic history: The names of all antibiotics being given to the participant at the time of the stool sample collection should be recorded.
- Immunosuppression history: The names of all immunosuppressive medications (e.g. cyclosporine, tacrolimus, prednisone) being given to the participant at the time of the stool sample collection should be recorded.
- Gastrointestinal (GI) prophylaxis history: The names of all GI prophylaxis medications (e.g. omeprazole, TUMS, Maalox, Carafate) being given to the participant at the time of the stool sample collection should be recorded.
- Diet history: The type of nutrition (e.g. parenteral vs. enteral, low bacteria) that the participant is receiving at the time of the stool sample collection should be recorded.

5.2.2 **The following clinical laboratory studies should be obtained at the time of each stool and investigational blood sample:**

- CBC with differential
- Chem 10
- Liver function panel (AST, ALT, alkaline phosphatase, total and direct bilirubin, LDH)

5.3 Additional Data Collection

5.3.1 **Stool Frequency Assessment**

The number of daily bowel movements during the first 7 days post-HSCT is charted by floor nurses and/or clinical assistants, and will be obtained from within each patient's electronic medical record in PowerChart. Diarrhea is defined as having greater than 3 bowel movements per day.

5.3.2 **Acute GVHD Assessment**

Acute GVHD assessment should be performed at each stool sample collection time point after neutrophil engraftment (see Appendix C for Acute GVHD Assessment Form) through day +100. Acute GVHD is defined by the NIH Consensus Criteria [20] as shown in Tables 1a and 1b below:

Table 1a: Clinical Stage of Acute GVHD According to Organ System

Stage	Skin	GI (stool output/day)	Liver (bilirubin)
1	Maculopapular rash <25% of body surface	Adults: 500 - 1000 mL/day Children: 10 - 19.9 mL/kg/day Or nausea, anorexia or vomiting with biopsy (EGD) confirmation of upper GI GVHD	2.1 - 3 mg/dL
2	Maculopapular rash 25-50% of body surface	Adults: >1001-1500 mL/day Children: 20 - 30 mL/kg/day	3.1 - 6 mg/dL
3	Maculopapular rash >50% of body surface or generalized erythroderma	Adults: >1500 mL/day Children: > 30 mL/kg/day	6.1 - 15 mg/dL
4	Generalized erythroderma with bullous formation and desquamation	Large volume stool with severe abdominal pain with or without ileus or stool with frank blood or melena	> 15 mg/dL

Table 1b: Overall Clinical Grading of Severity of Acute GVHD

Grade	Skin	GI	Liver
I	Stage 1-2	0	0
II	Stage 3 or	Stage 1 or	Stage 1
III	---	Stage 2-4	Stage 2-3
IV	Stage 4	---	Stage 4

5.3.3 Additional Stool Samples

Additional stool samples for banking and gut microbiome analysis should be obtained **within 48 hours of acute GVHD diagnosis or positive blood culture**.

5.4 Agent Administration

Per the institutional standard practice at Boston Children's Hospital, vancomycin-polymyxin B may be given orally or via enteric feeding tubes. Doses may be repeated if the participant vomits within 30 minutes of medication administration. This medication is given only in the inpatient setting and is administered by the floor nurses to the patient. Compliance with treatment is charted by nursing in the medication administration record (MAR) within each patient's electronic medical record in PowerChart.

For administration via enteric feeding tubes, vancomycin-polymyxin B capsules may be opened and the contents dissolved in 2-3 mL bottled or sterile water at room temperature.

Scheduling of the TID vancomycin-polymyxin B doses will be left to the discretion of the floor nurses. Forgotten or missed doses may be taken at any time within a 24 hour period after the first scheduled dose of the day. Although it is preferable for participants randomized to the Gut Decontamination Arm to take all of their prescribed doses, many stem cell transplant patients have difficulty taking vancomycin-polymyxin B capsules. The descriptive analyses of the randomized arms will be performed as intent-to-treat, regardless of the number of missed vancomycin-polymyxin B doses by participants in the Gut Decontamination Arm.

5.5 General Concomitant Medication and Supportive Care Guidelines

All participants will receive standard supportive care for allogeneic HSCT per our institutional guidelines.

5.6 Specimen Collection Procedures

5.6.1 Stool Collection for Participants Undergoing Allogeneic HSCT

Stool samples will be collected for banking and microbiome analysis from all participants. The first stool sample should be collected upon admission to the inpatient Stem Cell Transplantation Unit at BCH, prior to Day -5. During the inpatient stay, stool samples should be obtained on a weekly basis until neutrophil engraftment. After neutrophil engraftment, stool samples may be obtained on a monthly basis until day +100. Stool sample collection is also suggested at6 months and 1 year post-transplant. An additional stool sample will be collected if a diagnosis of acute GVHD is made or if a participant develops a positive blood culture.

The recommended windows for stool collection are as follows:

- +/- 3 days for weekly stool collection prior to neutrophil engraftment
- +/- 2 weeks for the monthly stool collection after neutrophil engraftment
- +/- 1 month for the 6 month and 1 year time points
- Within 48 hours of acute GVHD diagnosis or positive blood culture

Stool collection kits will be provided for both inpatient and outpatient sample time points. Once collected, stool samples should be stored immediately at 4^{o}C and should be frozen at -80^{o}C within 24 hours of collection.

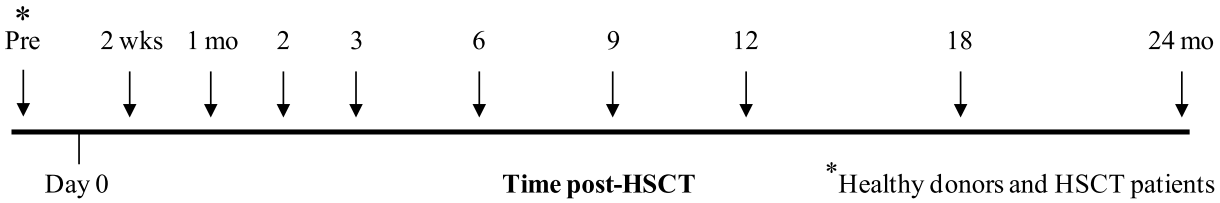
5.6.2 Stool Collection for Healthy Bone Marrow Donors

A stool sample will be collected for banking and microbiome analysis from healthy bone marrow donor participants around the time of donor evaluation or bone marrow harvest. This sample may be collected at an outpatient DFCI screening visit or during the inpatient stay following the bone marrow harvest. If feasible, additional stool samples will be collected from enrolled healthy bone marrow donors around the 6 month and 1 year post-transplant evaluations for their siblings (bone marrow recipient). These additional samples will not be required for donors who do not usually live in the same household with their sibling (i.e. donor is away at college), or if their sibling has died prior to the 6 month or 1 year follow up time point.

5.6.3 Peripheral Blood Collection for Participants Undergoing Allogeneic HSCT

Peripheral blood samples will be collected for both banking and immune cell profiling from all participants. Participants may be receiving inpatient services in the Stem Cell Transplantation Unit at BCH or outpatient care in the Jimmy Fund Clinic at DFCI at the time of the blood draws. Whenever possible, blood samples will be obtained at times when necessary clinical blood tests are being performed. Moreover, research samples will be collected after samples required for clinical management are obtained. Typically, blood samples will be collected 8-12 times/year

during the first year after HSCT and 2 times/year during the second year of follow-up. Peripheral blood mononuclear cells, plasma and DNA will be banked from most samples if cell numbers are adequate. In addition, immune cell monitoring by flow cytometry will be performed at specified time points. Suggested time points for banking and immune cell monitoring are:



The recommended windows for time point collections are as follows:

- +/- 3 days for the 2 week time point
- +/- 2 weeks for the 1 month to 3 month time points
- +/- 1 month for the 6 month to 12 month time points
- +/- 2 months for the 18 and 24 month time points

Time point collections may be deferred if, in the judgment of the treating physician, there are medical contraindications to the collection of additional blood or other samples for research. Additional samples for banking may be obtained outside of these time points if ≥ 1 month has passed since the last sample collection. The volume of blood collected at each blood draw will not exceed 30 ml or 3 ml/kg, whichever is less. This volume is within the maximum allowable total blood draw volume for research purposes as specified by the Dana-Farber/Harvard Cancer Center guidelines. Per institutional guidelines, blood draws for research purposes will not occur more frequently than 2 times per week. In addition, we will follow institutional guidelines for limits on the volume of blood that can be drawn for research purposes in a 28 day period, based on the weight of the patient.

5.6.4 Peripheral Blood Collection for Healthy Bone Marrow Donors

Healthy bone marrow donors will only be asked to provide a single research blood sample at the time of donor evaluation or bone marrow harvest. The volume of blood collected will not exceed 30 ml or 3 ml/kg, whichever is less.

5.7 Criteria for Taking a Participant Off Protocol Therapy

This section does not apply to participants in the "Healthy Bone Marrow Donor" group as they do not receive any protocol therapy. For participants randomized to the Gut Decontamination Arm, duration of gut decontamination with oral vancomycin-polymyxin B will be as per the schema outlined previously. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Intercurrent illness that prevents further administration of treatment.
- Hematologic malignant disease relapse

- Death
- 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.

The above criteria also apply for participants randomized to the No Gut Decontamination Arm. In addition, participants randomized to this arm will be removed from the protocol therapy if the participant decides to receive gut decontamination with vancomycin-polymyxin B per the institutional standard.

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.

A ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the ODQ website or obtained from the ODQ registration staff.

5.8 Duration of Follow Up

Allogeneic HSCT participants will be followed for 2 years from study entry or until death, whichever occurs first. Follow up through day +100 post-transplant will occur at DFCI for all participants. Participants will continue long-term follow up at DFCI if they live locally or with their local oncology providers if they live remotely. Participants living remotely will return to DFCI for post-transplant milestone time points (e.g. 6 mo, 9 mo, 12 mo, 18 mo and 24 mo) per our institutional recommendations.

5.9 Criteria for Taking a Participant Off Study

Allogeneic HSCT participants in either treatment arm will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Withdrawal of consent for stool and blood sample collection
- Death
- Two year follow-up on study completed

Participants in the “Healthy Bone Marrow Donor” group will be removed from the study if consent for stool and blood sample collection is withdrawn.

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

A ODQ Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the DF/HCC PI.

6. ADVERSE EVENTS: REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 6.1) and the characteristics of an observed AE (Section 6.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

6.1 Expected Toxicities

Oral vancomycin-polymyxin B is a medication compounded in the inpatient pharmacy at BCH from the commercially available powder forms of vancomycin and polymyxin B. The relevant side effects of oral vancomycin-polymyxin b in HSCT participants are described below:

- Bad taste
- Nausea and vomiting

Additional detailed toxicity information that relates to the injectable forms may be found in the vancomycin and polymyxin B package inserts. However, these listed toxicities are not relevant to HSCT patients receiving oral vancomycin-polymyxin B, which is given enterally and is non-absorbable.

6.2 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 for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **Attribution** of the AE:
  - 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.

6.3 Expedited Adverse Event Reporting

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

6.3.1 DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

6.3.2 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol only, the AEs/grades listed below do not require expedited reporting to the DF/HCC PI or the DFCI IRB. However, they still must be reported through the routine reporting mechanism (i.e. case report form). Grade 4 expected events related to stem cell transplantation that do not require reporting as SAEs include: neutropenia, neutropenic fever, thrombocytopenia, minor bleeding episodes (e.g. epistaxis), electrolyte abnormalities, rashes, diarrhea, infections (e.g. pneumonia, line sepsis, cellulitis), VOD, TMA and GVHD as these are expected events related to stem cell transplantation.

6.4 Expedited Reporting to Hospital Risk Management

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

6.5 Routine Adverse Event Reporting

For this study, routine reporting will include all SAEs and all Grade 4 and higher non-hematologic Adverse Events. For participants randomized to Arm A "Gut Decontamination", routine reporting will include Grade 3 Adverse Events that are Unexpected and with Definite, Probably and Possible attribution to vancomycin-polymyxin B. Grade 1 and Grade 2 Adverse Events will not be recorded. Adverse Events **must** be reported in routine study data submissions to the DF/HCC PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must also be reported in routine study data submissions.**

7. PHARMACEUTICAL INFORMATION

Vancomycin-polymyxin B capsules contain 125 mg of vancomycin and 62.5 mg of polymyxin B in each capsule. A list of the adverse events and potential risks associated with oral vancomycin-polymyxin B administered in this study can be found in Section 6.1.

8. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

8.1 Stool microbiome analysis

To determine the impact of gut decontamination on the composition of the gut microbiome, stool samples maybe collected prospectively from allogeneic HSCT participants on both treatment arms at the time points specified in Section 5.6.1. Healthy bone marrow donor participants will provide a stool sample at the time of outpatient screening or inpatient stay following bone marrow harvest. If feasible, additional stool samples will be obtained from healthy bone marrow donors at the time of their sibling's (bone marrow recipient) 6 month and 12 month follow up appointments.

8.1.1 Collection of stool specimen

Stool samples collected prior to engraftment will be collected on the inpatient Stem Cell Transplant Unit at BCH. Stool collection kits will be provided to the floor nurses. Stool samples collected after engraftment may be collected either on the inpatient Stem Cell Transplant Unit at BCH or as an outpatient if the participant has been discharged. At the time of discharge, the participant will be given a stool collection kit and instructions for home use. The participant will be instructed to collect a stool sample within 24 hours of a clinic visit. The participant will receive a new stool collection kit at each clinic visit for use at the next time point.

8.1.2 Handling of stool specimen

Once collected, stool samples should be placed immediately at 4^{o}C. Within 24 hours of collection, the stool sample should be aliquoted and frozen at -80^{o}C. For outpatient stool samples, participants will be provided with cooling gel packs and Styrofoam boxes for transport of their samples to clinic.

8.1.3 Site performing correlative study

Investigational stool samples will be processed and banked in the laboratory of Dr. Jerome Ritz at DFCI. Stool and/or extracted DNA will be sent to the laboratory of Dr. Ami Bhatt at Stanford University for either 16S rRNA sequencing or metagenomic whole genome shotgun sequencing. Bioinformatic analysis of the sequencing data will be performed in the laboratory of Dr. Ami Bhatt at Stanford University.

8.2 Immune cell profiling

To correlate immune reconstitution with reconstitution of the gut microbiome post-transplant, peripheral blood samples will be collected prospectively from participants on both treatment arms at the time points specified in Section 5.6.3. Healthy bone marrow donors will only be asked to provide a single research blood sample at the time of donor evaluation or bone marrow harvest.

8.2.1 Collection of blood specimen

Peripheral blood samples will be collected in 10 mL lavender top (EDTA) tubes. The volume of blood collected at each time point will be determined by the participant's weight, but will not exceed 30 mL.

8.2.2 Handling of blood specimen

Investigational blood samples will be processed and banked with the Pasquarello Tissue Laboratory at DFCI.

8.2.3 Site performing correlative study

Immune cell profiling by flow cytometry will be performed in the laboratory of Dr. Jerome Ritz at DFCI.

8.3 Survey of Centers Participating in the Pediatric Bone Marrow Transplant Consortium (PBMTC)

We will perform a survey to determine how many pediatric stem cell transplant centers currently use the practice of gut decontamination for acute GVHD prophylaxis and to determine the antibiotic regimen used. The survey will be sent by email to the director of each pediatric stem cell transplant center that is a full member of the PBMTC. Prior approval will be obtained from Dr. Michael Pulsipher, the chair of the Executive Committee of the PBMTC. See Appendix F for the survey instrument.

8.3.1 Survey eligibility criteria

Individuals eligible for participation in this survey include directors of pediatric stem cell transplant centers that are full members of the PBMTC. There are currently 82 centers that are full members of the PBMTC.

8.3.2 Survey administration

We will send an e-mail (Appendix D) to all eligible center directors informing them of the study and inviting them to participate. The e-mail will include an electronic link to the survey. The letter will explain that each survey respondent will be entered into a raffle upon receipt of the completed survey. The winner of the drawing will win a $100 gift certificate to Amazon.com. Additional reminder e-mails will be sent on a regular basis to all program directors who do not respond within 10 business days of the first e-mail. The reminder e-mails will contain different language (Appendix E) from the initial e-mail and will again contain an electronic link to an on-line survey.

9. STUDY CALENDAR FOR ALLOGENEIC HSCT PARTICIPANTS*

Table 2: Summary of Required Data

	Prior to Day -5	Day -5 to neutrophil engraftment: q Week (+/- 3 days)	Neutrophil engraftment to Day +100: q Month (+/- 2 weeks)	6 mo (+/- 1 mo)	9 mo (+/- 1 mo)	12 mo (+/- 1 mo)	18 mo (+/- 2 mo)	24 mo (+/- 2 mo)
Medical history	X		X	X	X	X	X	X
Physical exam	X		X	X	X	X	X	X
CBC with diff	X	X	X	X	X	X	X	X
Serum chemistry	X	X	X	X	X	X	X	X
Liver function panel	X	X	X	X	X	X	X	X
Stool sample	X	X	X^	X^		X^		
Immunology	X	X#	X$	X	X	X	X	X
Antibiotic history	X	X	X	X		X		
Immunosuppression history	X	X	X	X		X		
Gastrointestinal prophylaxis history	X	X	X	X		X		
Diet history	X	X	X	X		X		
Acute GVHD evaluation			X					

*Healthy bone marrow donor participants will provide a stool sample for banking and microbiome analysis and a single research blood sample at the time of donor evaluation or bone marrow harvest. Additional stool samples may be collected at the time of their sibling's (bone marrow recipient) 6 month and 12 month follow up appointments.

#Immunology: plasma banking and storage of additional mononuclear cells at 2 wks +/- 3 days post-transplant. Immune cell profiling by flow cytometry will not be performed at this time point due to low blood cell counts.

$Immunology: immune cell profiling by flow cytometry in addition to plasma banking and storage of additional mononuclear cells at 1, 2 and 3 mo +/- 2 wks post-transplant.

^Stool Sample Collection: collection of these time point stool samples is highly suggested and patients are strongly encouraged to collect these samples. Since patients often must collect these samples at home, within 24 hours of their clinic visit, collection is not always feasible. These time point samples will be collected as often as possible.

10. MEASUREMENT OF EFFECT

10.1 Primary Endpoint: Gut Microbiome Description

Genomic DNA will be extracted from stool samples at each time point. For most samples, 16S rRNA sequencing of genomic DNA will be used to profile the stool bacterial composition. For a subset of samples, metagenomic whole genome shotgun sequencing will be used to identify DNA viruses, fungi and novel bacteria within the gut microbiome, in addition to previously characterized bacterial species. Within each participant at each time point, the total number of species, the relative abundance of each species and a bacterial diversity index will be calculated.

The primary endpoint is the Shannon diversity index (range: 0-6), measured at 2 weeks post-HSCT.

10.2 Secondary endpoints

10.2.1 Assessment of Stool Frequency

We will define a patient as having diarrhea if the average number of bowel movements per day over first 7 days post-HSCT is > 3 (binary endpoint).

10.2.2 Immune Assessment

Participants on both treatment arms will undergo testing for immunologic function, performed prior to day -5, and at the post-transplant time points outlined in Section 5.6.3. Testing will include: immune cell profiling of T-, B-, NK- and dendritic cell subsets by flow cytometry, plasma banking, and storage of additional mononuclear cells.

10.2.3 Acute GVHD Assessment

Participants on both treatment arms will undergo assessment for development of acute GVHD at each stool collection time point after neutrophil engraftment. Refer to Appendix C for the Acute GVHD Assessment Form.

10.2.4 Overall and Progression-Free Survival

All participants will be followed for 2 years after study entry for survival and malignant disease relapse.

Overall Survival: Overall Survival (OS) time is defined as the time from randomization to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) time is defined as the time from randomization to the earlier of progression of malignant disease or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

10.2.5 Survey on Gut Decontamination Practices

We will survey of all full members of the Pediatric Bone Marrow Transplant Consortium (PBMTC) on their GD practices. Full members of the PBMTC are FACT-accredited pediatric stem cell transplant centers capable of performing clinical trials. We will ask the following 6 questions of each participating center:

- Approximately how many pediatric allogeneic hematopoietic stem cell transplants does your center perform on an annual basis?
- What is your estimated incidence of Grade II-IV acute graft-versus-host disease (GVHD) among pediatric patients at your center for recipients of MATCHED SIBLING donor stem cell transplants?
- What is your estimated incidence of Grade II-IV acute GVHD among pediatric patients at your center for recipients of UNRELATED donor stem cell transplants?
- Does your stem cell transplant program practice gut decontamination or antibiotic prophylaxis for the duration of the peri-transplant period (e.g. > 7 days)?
- If your program practices gut decontamination, what is the antibiotic regimen used?
- For what reasons does your program practice antibiotic prophylaxis?

11. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 6.0 (Adverse Events: List and Reporting Requirements).

11.1 Data Reporting

11.1.1 Method

In conjunction with the Biostatistics Program of the Department of Pediatric Oncology, the ODQ will collect, manage, and perform quality checks on the data for this study.

11.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

11.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the DF/HCC PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher

unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12. STATISTICAL CONSIDERATIONS

This is a prospective, randomized (GD, no GD) phase 2 study of gut microbiome reconstitution post-HSCT. In addition, we will describe the microbiome of healthy sibling donors. Due to sample size limitations, there will be no inferential comparisons performed. The descriptive analyses of the randomized arms will be performed as intent-to-treat. The results of this phase 2 study will inform the design of a larger, multi-center randomized study of GD.

12.1 Study Design/Endpoints

Please refer to Section 10. The primary endpoint is the Shannon diversity index (range: 0-6), measured at 2 weeks post-HSCT.

12.2 Sample Size, Accrual Rate and Study Duration

12.2.1 Sample Size

There will be 10 HSCT recipients randomized to GD, 10 HSCT recipients randomized to no GD, and 8 healthy donors, for a total of 28 subjects enrolled on the study. The sample size is driven by our power to detect a difference in microbial diversity as measured by the Shannon diversity index between the two treatment arms. A previous gut microbiome study in adult HSCT patients has shown that the pre-HSCT mean diversity index ranges from 3-4 and decreases to a mean diversity index of approximately 2 after HSCT with a standard deviation of approximately 0.9 (Taur 2012 ref). We hypothesize that the patients in the No GD arm will have higher diversity (Shannon index ≥ 3) and patients in the GD arm will have lower diversity (index ≤ 2) following HSCT. Thus, assuming a standard deviation of 0.9, a diversity index of 4.0 for No GD and 2.8 for GD, our current sample size of 10 patients in each arm achieves > 80% power to detect a difference in Shannon diversity index of 1.2 with a one-side t-test and alpha = 0.05.

Although the power has been calculated based on a one-sided t-test, the difference between the randomized treatment arms will actually be tested using a Wilcoxon rank-sum test. The Wilcoxon rank-sum test will have slightly greater power (as an exact test within this small sample size) than the t-test to detect the same difference, and will not require an assumption of normality.

12.2.2 Accrual Rate

Our transplant program performs over 60 allogeneic HSCT per year, and approximately 20 of these transplants use matched sibling donors. If 50% of these patients enroll, then we estimate that the study accrual will be completed within two years.

12.2.3 Study Duration

Per Section 5.8, with a two-year follow-up period on the last patient enrolled, the total study duration will be 4 years.

12.3 Stratification Factors

The composition and bacterial diversity within the gut microbiome has been shown to differ significantly by age and geography [16]. The impact of race on composition and bacterial diversity of the gut microbiome is unknown; however, in a study of the healthy human microbiome, race/ethnicity was the host property that had the strongest association with the microbiome [21]. To protect the randomization from a potential bias, we will stratify the randomization by race (white, non-white),

12.4 Interim Monitoring Plan

We will have a one-stage stopping rule for the occurrence of acute GVHD. Within each treatment arm, if at any time there are 4 or more participants with acute GVHD, then we will stop the study for consideration of treatment modifications. The operating characteristics of this rule are: the null hypothesis is that the rate of acute GVHD is 15% (expected, desired), and the alternative is that it is 56%. With n=10, we will have 90% power with alpha = 0.05 to detect this 41% difference.

12.5 Analysis of Primary Endpoints

The comparison of randomized treatment arms, in terms of the Shannon diversity index at 2 weeks, will be performed using a Wilcoxon rank-sum test.

In order to identify predominating organisms and estimate the microbial species diversity in collected stool samples, taxonomic classification of each sequence will be determined and a relative abundance of the given species will be calculated. Microbiome diversity quantification and identification of microbial species will be performed using a customized computational analysis pipeline developed in the laboratory of Dr. Ami Bhatt at Stanford University. Dr. Bhatt and her laboratory have implemented several computational programs including PathSeq, SPAdes 3.1, Kraken and GAEMR; and they have verified that the computational pipeline is accurate and can be run within the limits of their cluster computing capacity [22-25]. Asymptotic richness (calculation of the estimated diversity of species given the underlying assumption of undersampling) will be calculated as described using the “Chao1” method [26]. Entropic measures, such as Shannon diversity, will also be calculated based on the microbial diversity present in these samples and will be compared both over time and between the two treatment arms and healthy controls to determine if overall microbial diversity is statistically different between these populations at various time points. Unsupervised hierarchical clustering will be performed with cases and controls using GENE-E in order to identify class-wise

microbiome differences between the two treatment arms and controls. Novel microbes will be identified using the metagenomic approach previously described by Dr. Bhatt [19, 27].

12.6 Analysis of Secondary Endpoints

The following analyses will be performed overall and within each treatment group.
We will calculate descriptive statistics, appropriate to the continuous or discrete nature of the endpoint, for the proportion of patients with diarrhea and for immune cell reconstitution post-HSCT. Graphical presentations will be generated for each factor, including box and whisker plots, and spaghetti plots, i.e. one line per patient of the values over time. The incidence of acute GVHD will be calculated including a 95% confidence interval. We will generate Kaplan-Meier curves of PFS and OS. We will not perform a log rank test comparison of the randomized treatment arms, as the study is not designed or powered for this comparison. For the survey of pediatric stem cell transplant centers regarding their practices of gut decontamination, we will descriptively summarize the survey responses.

12.7 Reporting and Exclusions

N/A.

13. 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 SCALES/SCORES

Performance Status Criteria

Karnofsky and Lansky performance scores are intended to be multiples of 10

ECOG (Zubrod)		Karnofsky		Lansky*	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
		90	Able to carry on normal activity, minor signs or symptoms of disease.	90	Minor restrictions in physically strenuous activity.
1	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.	80	Active, but tires more quickly
		70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity.
2	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	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
		50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities.
		30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities.
		10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed.

*The conversion of the Lansky to ECOG scales is intended for NCI reporting purposes only.

APPENDIX B MEDICATION AND DIET HISTORY FORM FOR PROVIDERS

Participant name: _____
MRN: _____
Stool sample collection date: _____
Time point: _____

Antimicrobials. Please list the names of all oral and IV antibiotic, antifungal and anti-viral therapies being given to the participant at the time of the stool sample collection:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

Immunosuppression. Please list the names of all immunosuppressive medications (e.g. cyclosporine, methotrexate, tacrolimus, MMF, prednisone) being given to the participant at the time of the stool sample collection:

1. _____
2. _____
3. _____
4. _____
5. _____

Gastrointestinal (GI) prophylaxis. Please list the names of all GI prophylaxis medications (e.g. omeprazole, TUMS, Maalox, Carafate) being given to the participant at the time of the stool sample:

1. _____
2. _____
3. _____
4. _____
5. _____

Diet. Please note the type of nutrition that the participant is receiving at the time of the stool sample collection (*check all that apply*):

- ☐ Regular oral diet
- ☐ Low bacteria oral diet
- ☐ Formula via nasogastric or gastric tube
- ☐ Total parenteral nutrition

APPENDIX C ACUTE GVHD ASSESSMENT FORM FOR PROVIDERS

Participant name: _____
MRN: _____
Stool sample collection date: _____
Time point: _____

Please circle appropriate code (see below for code descriptions):

Skin	0	1	2	3	4	% body rash:__ _____
Liver	0	1	2	3	4	Total bilirubin (mg/dL):_____
Lower GI	0	1	2	3	4	Volume stool (mL):_____
Upper GI	0	1	2	3	4	

Differential diagnosis (please circle all that apply):

GVHD Drug reaction Infection VOD Other: _____

Systemic agents (check all that apply):

☐ CSA	☐ Prednisone/prednisolone	☐ Pentostatin
☐ Tacrolimus	☐ Methylprednisolone	☐ ECP
☐ MMF	☐ Infliximab	☐ Other: _____
☐ Sirolimus	☐ Etanercept	☐ Other: _____

Current steroid dose: Predniso(lo)ne _____ mg/kg/day; Methylprednisolone _____ mg/kg/day

Code	Skin	Liver (bilirubin)	Lower GI (stool output/d)	Upper GI
0	No rash	≤ 2 mg/dL	Adult: < 500 mL/day Child: < 10 mL/kg/day	No protracted nausea and vomiting
1	Maculopapular rash <25% of BSA	2.1 - 3 mg/dL	Adult: 500 - 1000 mL/day Child: 10 - 19.9 mL/kg/day	Persistent severe nausea/vomiting with a positive upper GI biopsy
2	Maculopapular rash 25-50% of BSA	3.1 - 6 mg/dL	Adult: 1001 - 1500 mL/day Child: 20 - 30 mL/kg/day	
3	Maculopapular rash >50% of BSA or generalized erythroderma	6.1 - 15 mg/dL	Adult: >1500 mL/day Child: > 30 mL/kg/day	
4	Generalized erythroderma with bullous formation and desquamation	>15 mg/dL	Severe abdominal pain with or without ileus, or stool with frank blood or melena (regardless of stool volume)	

APPENDIX D INITIAL E-MAIL TO PEDIATRIC STEM CELL TRANSPLANT PROGRAM DIRECTORS

Date
Dear Dr. _____:

We would like to invite you to participate in a research project. You have been included in this study because we have identified you as a director of a pediatric hematopoietic stem cell transplantation (HSCT) program, and your center is a full member of the Pediatric Blood and Marrow Transplant Consortium. We have invited all such directors across the U.S. and Canada to complete our survey.

The practice of gut decontamination for acute graft-versus-host disease (GVHD) prophylaxis in patients undergoing allogeneic HSCT is controversial, and there is no consensus regarding efficacy or ideal choice of antibiotic regimen. We are performing a study to examine the effect of gut decontamination using an oral, non-absorbable antibiotic as per our institutional standard practice on the composition of the gut microbiome in pediatric HSCT patients. As part of this study, we are conducting a survey to learn how many pediatric stem cell transplant centers practice gut decontamination for acute GVHD prophylaxis and what antibiotic regimen is used. This project has been approved by the Institutional Review Board at Dana-Farber Cancer Institute.

Participation in our study is entirely optional and should carry no risk to you. Your identity will be linked to your response only by a coded identifier. While participation carries no direct benefit to you, it will increase our understanding of the range of gut decontamination practices among pediatric stem cell transplant centers. This information may be helpful in the design of future multicenter trials examining the efficacy and/or benefit of gut decontamination.

Please access the survey by clicking on the link below and completing the survey electronically. The survey should take less than 15 minutes to complete.

[will insert electronic link to on-line survey here]

If you do not wish to participate, you can opt out by replying to this email or by calling Dr. Lehmann at the telephone number listed below. In this case, we will not attempt to contact you again. Should you decide to complete the survey, your name will be entered into a raffle and its winner will receive a $100 Amazon gift certificate.

If we have not heard from you within 10 business days of this e-mail, we will e-mail you again. In the meantime, if you have questions about this research project, feel free to call Dr. Lehmann at 617-632-4882 . You can also reach the Institutional Review Board at Dana-Farber Cancer Institute by calling 617-632-3029.

Thank you in advance for your assistance with this survey.

Sincerely,
Leslie Lehmann, MD

APPENDIX E FOLLOW UP E-MAIL TO PEDIATRIC STEM CELL TRANSPLANT PROGRAM DIRECTORS

Date
Dear Dr. _____:

We recently sent you an e-mail inviting you to participate in a research project. You have been included in this study because we have identified you as a director of a pediatric hematopoietic stem cell transplantation (HSCT) program, and your center is a full member of the Pediatric Blood and Marrow Transplant Consortium. We have invited all such directors across the U.S. and Canada to complete our survey.

As described in the first e-mail we sent you, we are performing a study to examine the effect of gut decontamination using an oral, non-absorbable antibiotic as per our institutional standard practice on the composition of the gut microbiome in pediatric HSCT patients. As part of this study, we are conducting a survey to learn how many pediatric stem cell transplant centers practice gut decontamination for acute GVHD prophylaxis and what antibiotic regimen is used. This project has been approved by the Institutional Review Board at Dana-Farber Cancer Institute.

Participation in our study is entirely optional and should carry no risk to you. Your identity will be linked to your response only by coded identifiers. While participation carries no direct benefit to you, it will increase our understanding of the range of gut decontamination practices among pediatric stem cell transplant centers. This information may be helpful in the design of future multicenter trials examining the efficacy and/or benefit of gut decontamination.

Please access the survey by clicking on the link below and completing the survey electronically. Completion of the survey should take less than 15 minutes.

[will insert electronic link to on-line survey here]

If you do not wish to participate, please opt out by replying to this email or by calling Dr. Lehmann at the telephone number listed below. In this case, we will not attempt to contact you again. If we do not hear from you in 1-2 weeks, we will contact you again by mail. Should you decide to complete the survey, your name will be entered into a raffle and its winner will receive a $100 Amazon gift certificate.

In the meantime, if you have questions about this research project, feel free to call Dr. Lehmann at 617-632-4882. You can also reach the Institutional Review Board at Dana-Farber Cancer Institute by calling 617-632-3029.

Thank you in advance for your assistance with this survey.

Sincerely,
Leslie Lehmann, MD

APPENDIX F SURVEY ON GUT DECONTAMINATION PRACTICES

1. Approximately how many pediatric ALLOGENEIC hematopoietic stem cell transplants does your center perform on an annual basis?
   - ☐ Less than 10 per year
   - ☐ 10-25 per year
   - ☐ 26-35 per year
   - ☐ 36-50 per year
   - ☐ Greater than 50 per year

2. What is your estimated incidence of Grade II-IV acute graft-versus-host disease (GVHD) among pediatric patients at your center for recipients of MATCHED SIBLING donor stem cell transplants?
   - ☐ Less than 5%
   - ☐ 5-10%
   - ☐ 10-15%
   - ☐ 15-20%
   - ☐ Greater than 20%

3. What is your estimated incidence of Grade II-IV acute GVHD among pediatric patients at your center for recipients of UNRELATED donor stem cell transplants?
   - ☐ Less than 5%
   - ☐ 5-10%
   - ☐ 10-15%
   - ☐ 15-20%
   - ☐ Greater than 20%

4. Does your stem cell transplant program practice gut decontamination or antibiotic prophylaxis for the duration of the peri-transplant period (e.g. > 7 days)?
   - ☐ Yes ✂ Proceed to Questions 5 and 6.
   - ☐ No

5. If your program practices gut decontamination for GVHD prophylaxis, what is the antibiotic regimen used?

   Antibiotic(s):_____

   Schedule (e.g. from day -5 to neutrophil engraftment):_____

6. For what reasons does your program practice antibiotic prophylaxis? Please check all that apply:
   - ☐ GVHD prevention
   - ☐ Bacteremia prevention
   - ☐ Empiric coverage for fever and neutropenia
   - ☐ Other: _____