Activated: 04/30/2018
Closed: 04/15/2022
Version Date: 04/19/2021
Amendment # 2

CHILDREN'S ONCOLOGY GROUP

ACCL1633

The Effectiveness of *Lactobacillus plantarum* (*LBP*, [redacted]) in Preventing Acute Graft-*versus*-Host Disease (GvHD) in Children undergoing Alternative Hematopoietic Progenitor Cell Transplantation (HCT)

IND Sponsor for *Lactobacillus plantarum*: Children's Oncology Group

A Groupwide Study

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SEE SECTION 7.4 FOR SPECIMEN SHIPPING ADDRESSES

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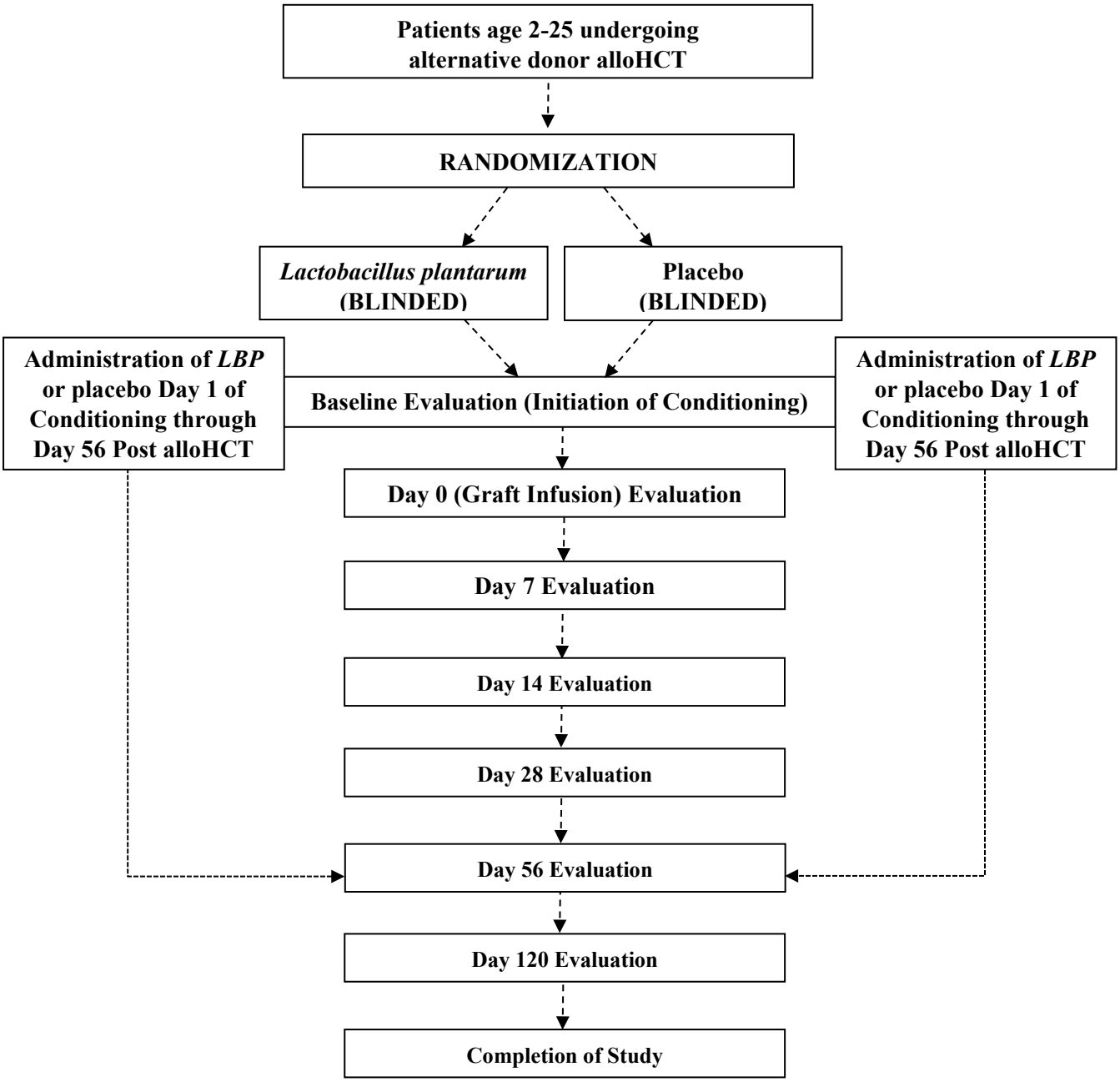
ABSTRACT

Despite prophylactic immune suppression, clinically significant (Grade II–IV) acute graft-versus-host disease (aGvHD) affects up to 45% of pediatric patients receiving allogeneic hematopoietic cell transplantation (alloHCT). As aGvHD is responsible for nearly 20% of deaths following alloHCT, the need for better prevention and therapy for aGvHD is readily apparent. Involvement of the gastrointestinal (GI) tract in the pathogenesis of aGvHD has been substantiated by the translation of pre-clinical and clinical studies. Emerging evidence suggests that perturbations in the microbiota diversity result in aberrant systemic immune response as well as pathogen colonization and mucosal invasion, fostering the development of aGvHD as well as increasing the risk for subsequent bacteremia with enteric pathogens. Pre-clinical studies also suggest that replenishing commensals like *Lactobacillus* prior to alternative donor alloHCT may substantially decrease aGvHD severity and intestinal insult. Our pilot data suggests that probiotics are safe to administer prior to and after alternative donor alloHCT in children and adolescents (IND#108,977).

The proposed study is a randomized, double-blinded, placebo-controlled intervention trial to determine the benefit of probiotic therapy in preventing the development of GI aGvHD in children undergoing initial alternative donor alloHCT. Importantly, correlative studies will be performed to elucidate how probiotic therapy affects the microbiome. Discovering such benefits of probiotic therapy may have significant impact on morbidity, mortality, and cost of care in children and adolescents alternative donor undergoing alloHCT.

EXPERIMENTAL DESIGN SCHEMA



1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aim

To determine efficacy of orally-administered *LBP* in preventing the development of GI aGvHD in children and adolescents undergoing alternative donor alloHCT.

1.2 Exploratory Aims

1.2.1 To determine whether orally-administered *LBP* decreases the incidence of Grade II–IV aGvHD following alternative donor alloHCT.

1.2.2 To determine whether *LBP* administration maintains intestinal integrity as measured by mean serum citrulline levels and reduction in mucosal barrier injury (MBI) bacteremia.

1.2.3 To measure the effects of *LBP* on the intestinal flora phylogenetic composition during and after alternative donor alloHCT using 16S rRNA gene deep sequencing.

1.2.4 To measure effects of *LBP* on intestinal flora function during and after alternative donor alloHCT using metagenomic and metabolite profiling.

1.2.5 To measure proposed immunomodulatory effects of *LBP* in mean serum levels of alloreactive-induced inflammatory cytokines (IL-2, IL-6, IL-12p70, IFNγ, TNFα, etc.) in patients receiving *LBP* compared to placebo.

1.2.6 To determine whether *LBP* administration reduces the incidence of *Clostridium difficile*-associated diarrhea in alternative donor HCT patients.

1.2.7 To determine whether *LBP* administration reduces hospital days within the first 120 days post hematopoietic cell transplant (HCT).

1.2.8 To define the safety of orally administered *LBP* strains 299 and 299v in alternative donor HCT patients as measured by incidence of *Lactobacillus plantarum* bacteremia.

2.0 BACKGROUND AND HYPOTHESES

2.1 Acute graft-versus-host disease (GvHD): Clinical impact

Allogeneic HCT (alloHCT) is the infusion of hematopoietic stems cells from another person or from another person's umbilical cord blood into a recipient. Alternative donor alloHCT is the infusion of hematopoietic stems cells from a donor who is not an HLA-matched related family member. Acute GvHD develops during the first 100 days post HCT as a result of the donor stem cells recognizing the recipient's body as a foreign substance and subsequently the donor cells attack the recipient's tissues and organs. The three main areas of the body affected by GvHD are the skin, liver and gut.

Despite prophylactic immune suppression, clinically significant (Grade II–IV) aGvHD afflicts up to 45% of pediatric patients receiving alternative alloHCT.[1] As aGvHD is

responsible for nearly 20% of deaths following alloHCT,[2] the need for better prevention and therapy for aGvHD is readily apparent. Preventative regimens for aGvHD incorporate immunosuppressive therapy (IST), which largely targets T-cell activation, function and cytokine production,[3] causing delays in donor-derived immune recovery[4-6] that result in increased risk for life-threatening infections.[7-9] Unfortunately, the rate of aGvHD has remained the same for nearly two decades despite the use of current immunosuppressive agents. Therefore, new approaches to prevent the development of severe aGvHD are critically needed.[10,11]

2.2 GvHD pathophysiology: Focus on the gastrointestinal tract (GI)

The pathophysiology of aGvHD is complex and involves donor T-cell activation by host alloantigens and the subsequent secretion of cytotoxic cytokines including TNFα and IFNγ.[12] In short, experimental and clinical data support the hypothesis that cytokine dysregulation associated with aGvHD occurs in three distinct phases.[13,14] In Phase I, HCT conditioning regimens instigate damage to the GI epithelium, leading to translocation of pro-inflammatory factors like endotoxin from the gut lumen into the bloodstream[15] and activation of host tissues to secrete inflammatory cytokines.[16] The resultant inflammatory environment activates host antigen-presenting cells (APCs) as well as induces chemokines that recruit additional donor leukocytes into host target organs, including skin, liver and gut.[17] In Phase II, activated host APCs present alloantigens to donor T cells infused within the hematopoietic cell graft.[18] In addition to antigen presentation, APCs provide co-stimulation, further enhancing T-cell activation and clonal expansion. Phase III involves T cell-mediated apoptosis of epithelial cells, leading to target organ damage and dysfunction, further compromising intestinal epithelial integrity and perpetuating inflammation. Thus, the breakdown of GI barrier function serves as both a major instigator and propagator of the pathophysiology of aGvHD.[19,20]

The field of proteomics has enabled biomarker discovery and validation for aGvHD[21] risk stratification. Specifically, cytokines like ST2 (suppression of tumorigenicity 2) and their associated receptors like TNFR1 (tumor necrosis factor receptor 1) have been validated as early markers of aGvHD onset and severity.[22] Furthermore, involvement of the GI tract in the pathogenesis of aGvHD has been substantiated by the translation of preclinical observations into validated serum biomarkers of GI tract insult. Specifically, aGvHD has been shown to lead to histological loss of Paneth cells and intestinal epithelial stem cells. Paneth cell destruction results in decreased production of antimicrobial peptides like α-defensins[23] and the release of the intestinal epithelium-derived C-type lectin, regenerating islet-derived IIIα (REG IIIα), into the circulation.[23] Clinically, Paneth cell loss is associated with aGvHD severity and non-relapse mortality.[24] Similarly, plasma elevations in REG IIIα also associate with aGvHD severity as well as overall response IST used to treat aGvHD.[25,26] Lastly, strategies to preserve intestinal epithelial stem cells via either intracellular signaling activation[27] or endogenous IL-22[28] reduce aGvHD severity in mouse models. The predominant source of IL-22 in the intestine is thought to be RORγt+ innate lymphoid cells (ILCs).[29] Clinically, activated ILCs have been shown to reduce aGvHD severity in acute leukemia patients undergoing alloHCT; as those patients with decreased pre-transplant ILC levels experienced more severe subsequent aGvHD.[30]

2.3 An emerging role for the intestinal microbiota in GI aGvHD: Probiotic therapy

Commensal organisms within the GI tract, collectively known as the intestinal microbiota, are predominantly comprised of two phyla, *Bacteroidetes* and *Firmicutes,* the latter of which includes the microbial order *Lactobacillales.*[31] The intestinal microbiota critically

functions in maintaining the physical, functional, and immunologic barriers within the GI tract such that perturbations in microbiota diversity or dysbiosis result in aberrant systemic immune response[32] as well as pathogen colonization and mucosal invasion.[31] For example, dysbiosis induced by broad-spectrum antimicrobial therapy causes life-threatening infections from *Clostridium difficile*[33] and vancomycin-resistant *Enterococcus*[34] in alloHCT recipients. In addition, aGvHD markedly alters the intestinal microbiota in mice and in human alloHCT recipients, decreasing intestinal microbial diversity and increasing predominance of enteric pathogens.[35] As a result, aGvHD increases the risk of bacteremia with enteric pathogens resulting from mucosal barrier injury (MBI).[36,37] Furthermore, loss of microbial diversity is an independent risk factor for mortality following alloHCT. Specifically, lower microbial diversity at the time of engraftment significantly associates with decrease in overall survival (OS) and increase in transplant-related mortality (TRM), particularly from aGvHD and infection.[37]

Replenishing commensals like *Lactobacillus* prior to HCT in recipient mice substantially decreases aGvHD severity and intestinal insult.[35,38] However, such attenuating effects of probiotic administration on aGvHD severity was limited to transplant recipients; as donor microbiota does not seem to influence alloreactive T-cell function and aGvHD course.[39] Dual effects of probiotics in maintaining intestinal cell function and inhibiting secretion of pro-inflammatory cytokines from intestinal mucosa suggest their beneficial role in the prevention and attenuation of aGvHD (Figure 1).[35,38] Evidence from clinical studies further supports the hypothesis that altering the microbiota may affect clinical outcomes. For example, the provision of enteral feeding, in contrast to total parenteral nutrition, is associated with an increase in survival and reduced rates of aGvHD.[40,41] Jenq et al also found that poor oral nutrition appears to more directly alter the composition of the microbiota compared to the conditioning regimen. It is hypothesized that administration of probiotic therapy will restore microbial diversity and maintain epithelial cell integrity, which together will preserve immune tolerance and prevent aGvHD. In addition, it is hypothesized that probiotic therapy will decrease susceptibility to enteric pathogens by maintaining intestinal luminal integrity.

2.4 Probiotic Strain

LBP was selected as the ideal single-species probiotic for several reasons. First, *LBP* is the only probiotic that has been studied during the HCT period and has an established safety profile when used in immunocompromised adults and children.[42-45] Furthermore, the investigators have direct experience with its use in the alternative donor alloHCT setting.[45] Secondly, pre-clinical and clinical studies suggest that *Lactobacillus* may prevent transplant-related complications. In a murine BMT model, *Lactobacillus* was administered beginning on the first day of conditioning therapy and continued for several weeks after transplant. *Lactobacillus* administration was associated with significantly reduced mortality, aGvHD score, and inflammation (lower number of inflammatory cells and no evidence of ulcerations or abscesses) as well as less translocation of microorganisms into mesenteric lymph nodes.[38] In clinical studies performed among adults in the post-transplant period, alloHCT patients experienced

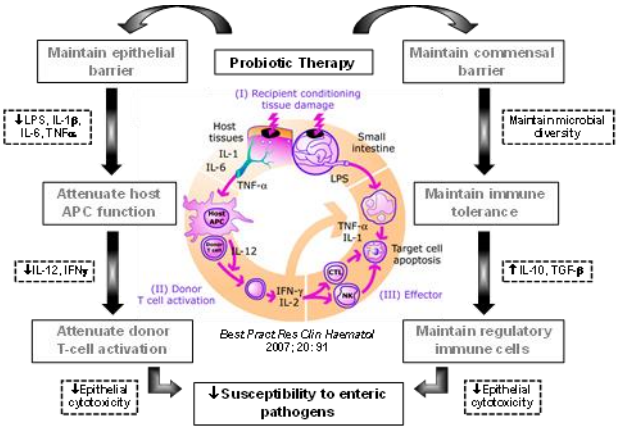


Figure 1. Putative effects of probiotic therapy in attenuating aGvHD.

decreases in GvHD-associated histopathology and mortality that were associated with *Lactobacillus*. The protective effect was attributed to the ability of *Lactobacillus* preventing *Enterococcus* domination in the GI tract.[35] Finally, *LBP* has an inherent ability to tolerate many different conditions.[46] Its growth requirement for manganese enables it to accumulate high intercellular levels of manganese, which provides protection against oxygen toxicity by reducing oxygen free radicals attributed to hydrogen peroxide.[47] *LBP* also has a high tolerance to low pH thereby surviving passage through acidic conditions like the human stomach.

The current study will use the same probiotic, *LBP,* which was tested in our pilot study. *LBP* is a combination of two genetically similar strains belonging to the *LBP* species **[*Lp*299v (DSM 9843) and *Lp*299 (DSM 6595)].** In preclinical studies, *Lp*299v improves mucosal barrier function, resulting in reduced permeability and decreased bacterial translocation.[48,49] Improved gut barrier function associated with *Lp*299v has been reported for patients in intensive care units[44] and patients with obstructive jaundice undergoing biliary drainage.[50] *Lp*299v also reduces gut permeability and subsequent bacterial translocation in methotrexate-induced enterocolitis.[51] In the clinical setting, *LBP* has been used in immunocompromised patients, including solid organ transplants,[52,53] HIV patients[42], and children undergoing HCT[45] and found to be safe.

*Lp*299v increases CD25 surface expression on CD4+ and CD8+ cells[54] and activates innate immunity (CD56+, CD16+ cells), which together exert immunomodulatory properties. *Lp*299v can produce nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2) via down-regulation of nuclear factor kappa B (NFκB) pathway, revealing a partial molecular basis for its anti- inflammatory properties, which have been exploited in clinical trials involving Crohn's disease and irritable bowel syndrome. [43,55,56] In a randomized, double-blind, placebo-controlled cross-over study involving healthy adult volunteers, subjects ingested either living or heat-killed *LBP* (strain WCFS1). Patients consuming live *LBP* had biopsies of the intestinal duodenal mucosa, which revealed altered mucosal gene expression patterns and cellular pathways supporting induction of immune tolerance.[56]

2.5 Preliminary Data

2.5.1 Biomarkers of Intestinal Integrity

Plasma citrulline is a plasma biomarker of intestinal integrity in adults undergoing HCT and in pediatric conditions.[57-61] To determine the association of plasma citrulline and intestinal integrity in children undergoing alternative donor alloHCT, we measured plasma citrulline levels in 10 children and adolescents beginning at the start of conditioning therapy and then every three to seven days.[62] The mean citrulline value prior to the initiation of treatment was 20.1 ±7 μmol/L. The greatest decrease in mean plasma citrulline occurred between the initiation of conditioning and the day of hematopoietic graft infusion (Day 0, 7.1 ±4.9 μmol/L) and reached a maximal mean nadir at Day 7 (6.6 ±5.3 μmol/L). Levels did not begin to recover to pre-transplant levels until Day 21 (10 ±3.7 μmol/L). Significant associations were found between plasma citrulline levels and the odds of developing mucositis (OR = 0.88, 95% CI=0.79–0.99, p=0.036) and diarrhea (OR=0.70, CI=0.59–0.84, p<0.0001). These results lend further support that plasma citrulline is a marker of GI integrity following alternative donor alloHCT. Moreover, we have identified a therapeutic window in which an intervention may prevent the decline in GI integrity as measured by plasma citrulline levels during HCT.

2.5.2 Pilot Study of *LBP*

A previous pilot study was completed that evaluated the safety and feasibility of *LBP*, the probiotic for the proposed trial, in 31 children and adolescents (mean age 7.7 ±4.7y) undergoing HCT (IND # 108,977).[45] Of the 31 patients, one patient was removed due to the inability to tolerate daily *LBP* dosing, which resulted in 30 evaluable participants. No patients suffered a serious adverse event (SAE) associated with *LBP* and no cases of *LBP* bacteremia were observed, surpassing the safety stopping criterion (≥1 case of *LBP* bacteremia among 30 evaluable patients). Of note, one patient with severe aplastic anemia developed acute appendicitis on Day 28. Pathologic examination of the appendix revealed a predominance of gram-negative enteric organisms and only a small number of gram-positive organisms that were morphologically inconsistent with *Lactobacillus* (no culture or PCR testing performed). The patient tolerated the appendectomy well, and investigators reported that the appendicitis was not related to *Lactobacillus*.

Three patients died prior to Day 100 and none of these deaths were attributed to *LBP*. Causes of death included veno-occlusive disease, idiopathic pneumonia syndrome, and multi-organ system failure in one patient, disease progression in the second patient, and complications due to aspiration pneumonia in the third patient. The observed deaths were within rates observed in the published literature.[2]

Incidence of non-*Lactobacillus* bacteremia (20%) and *Clostridium difficile*-associated diarrhea (CDAD, 20%) did not exceed published rates in pediatric alloHCT recipients.[63-66] Microbial pathogens associated with bacteremia or fungemia included: *Staphylococcus epidermidis* (1), *Serratia marcescens* (1), *Enterococcus faecium* (1), *Klebsiella pneumoniae* (3), *Streptococcus viridans* (1), and *Fusarium species* (1). Up to four weekly stool samples were collected from 22 patients receiving *LBP*. Of these patients, the majority (96%, 21/22) had at least one stool specimen that was positive for *Lactobacillus*. In this restricted analysis, 75% stool specimens (77/102) were positive for *Lactobacillus*, suggesting *LBP* administration colonized patient stool. The pilot study also found *LBP* administration was feasible during alloHCT. *LBP* was administered successfully to 97% (30/31, 95% CI (83%-100%) of enrolled children whom received at least 50% of the total intended dose of probiotic. Among the 30 evaluable patients, the mean and median percent of doses taken were 92 ±14% and 97% (range 50-100%), respectively.

Nine patients (9/30, 30%) developed clinically significant (Grade II–IV) aGvHD. Five patients (5/9, 56%), experienced Grade II aGvHD, and 4 patients (4/9, 44%) experienced Grade III aGvHD. No patient experienced Grade IV aGvHD. Overall incidence of GI aGvHD was 23%, which is lower than some published literature.[1] Importantly, the pilot study provides reasonable evidence that *LBP* administration did not increase the incidence of aGvHD and legitimates further investigation into defining its effects on GI aGvHD.

2.6 Summary

The proposed trial would be the only aGvHD prevention trial that is not cell-or drug-based in children and adolescents undergoing initial alternative donor alloHCT. As the majority of standard and investigational pharmaceutical therapies for preventing and treating GvHD

are globally immunosuppressive, these agents increase the risk for infection and potentially increase the risk of malignant relapse by delaying or inhibiting donor-derived immunity. Therefore, the major potential advantage for probiotic use as aGvHD prophylaxis is reduction in GvHD without inducing immunosuppression.

Initiation of probiotic administration in the proposed study (i.e., at the start of conditioning and through Day 56) will be instrumental in improving our understanding of the optimal timing of probiotic therapy in HCT recipients. We anticipate that the timing of probiotic administration will enhance the hypothesized benefits of probiotic therapy, potentially fortifying commensals prior to HCT-induced insult and replenishing commensal gradients faster after HCT-induced insult. The proposed study will also elucidate the mechanisms of probiotic-associated modulation of the intestinal microbiota composition and function that likely underlie anticipated clinical effects in reducing incidence of aGvHD. Gut decontamination with oral antibiotics is currently the only studied means to modulate the intestinal microbiota and has resulted in mixed clinical results.[21] The proposed study will increase our understanding in how alloHCT affects the microbiome itself as well as how probiotic administration might modify intestinal composition and metabolic activity. Furthermore, the use of a pure probiotic versus a composite mixture like fecal transplant has the ability to attribute proposed clinical benefit to the probiotic strain itself, simplifying mechanistic investigation into proposed immunologic effects. Finally, aGvHD presents a unique opportunity to study probiotic effects in a very high-risk patient population prior to development of inflammation. Taken together, the proposed clinical trial may significantly improve survival and quality of life in alloHCT patients and increase scientific understanding for how probiotics mediate their effects.

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number. This number is obtained via the Patient Registry module in OPEN once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help. For additional help or information, please contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

In order for an institution to maintain COG membership requirements, every patient with a known or suspected neoplasm needs to be offered participation in APEC14B1, *Project:EveryChild A Registry, Eligibility Screening, Biology and Outcome Study*.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

Please see Appendix I for detailed CTEP Registration Procedures for Investigators and Associates, and Cancer Trials Support Unit (CTSU) Registration Procedures including: how to download site registration documents; requirements for site registration, submission of regulatory documents and how to check your site's registration status.

3.1.2 IRB Approval

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. For CTEP and Division of Cancer Prevention (DCP) studies open to the National Clinical Trials Network (NCTN) and NCI Community Oncology Research Program (NCORP) Research Bases after March 1, 2019, all U.S.-based sites must be members of the NCI Central Institutional Review Board (NCI CIRB). In addition, U.S.-based sites must accept the NCI CIRB review to activate new studies at the site after March 1, 2019. Local IRB review will continue to be accepted for studies that are not reviewed by the CIRB, or if the study was previously open at the site under the local IRB. International sites should continue to submit Research Ethics Board (REB) approval to the CTSU Regulatory Office following country-specific regulations.

Sites participating with the NCI CIRB must submit the Study Specific Worksheet for Local Context (SSW) to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about

establishing site preferences can be addressed to the CTSU Regulatory Office by email or calling 1-888-651-CTSU (2878).

Sites using their local IRB or REB, must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation;
- IRB-signed CTSU IRB Certification Form; and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol Principal Investigator (PI) (i.e. the investigator on the IRB/REB approval) must meet the following criteria in order for the processing of the IRB/REB approval record to be completed:

- Holds an active CTEP status;
- Rostered at the site on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating roster;
- If using NCI CIRB, rostered on the NCI CIRB Signatory record;
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile; and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements
Additional requirements to obtain an approved site registration status include:

- An active Federal Wide Assurance (FWA) number;
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO); and
- Compliance with all protocol-specific requirements (PSRs).

For information about the submission of IRB/REB approval documents and other regulatory documents as well as checking the status of study center registration packets, please see Appendix I.

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

Note: Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review.

3.1.3 Study Enrollment

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the Lead Protocol Organization (LPOs) registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization

assignment. OPEN will populate the patient enrollment data in NCI's clinical data management system, Medidata Rave.

Requirements for OPEN access:

- A valid CTEP-IAM account;
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or participating organization roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type;
- If a Delegation of Tasks Log (DTL) is required for the study, the registrars must hold the OPEN Registrar task on the DTL for the site; and
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

- Patient has met all eligibility criteria within the protocol stated timeframes; and
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. You may print this confirmation for your records.

Access OPEN at https://open.ctsu.org or from the OPEN link on the CTSU members' website. Further instructional information is in the OPEN section of the CTSU website at https://www.ctsu.org or https://open.ctsu.org. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

3.1.4 Timing

Patients must be enrolled before taking the first dose of *LBP* or placebo. Participants may be enrolled up to 14 days prior to the planned start of the conditioning regimen and *LBP* or placebo administration.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated in the eligibility section below.

3.1.5 Randomization

Randomization will occur at the time of enrollment. Randomization will take place through the OPEN system. Upon enrollment, sites will receive notification of the kit number of study product that should be dispensed to the patient. All personnel at the site will remain blinded.

3.2 Patient Inclusion Eligibility Criteria

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived. All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

All Clinical and Laboratory studies, if applicable, must be obtained within 21 days prior to start of protocol therapy (repeat if necessary). Protocol therapy must begin within 6 months of study enrollment.

See Section 7.1 for required studies to be obtained prior to starting protocol therapy.

3.2.1 Age

Patient must be ≥ 2 years of age and ≤ 25 years of age at time of enrollment.

3.2.2 Diagnosis

Patient must have a diagnosis that is managed with an alternative donor allogeneic hematopoietic cell transplant.

3.2.3 Performance Level

Patients must have a Lansky (for patients ≤ 16 years of age) or Karnofsky (for patients > 16 years of age) performance status score of ≥ 70. Patients who are unable to walk because of a chronic underlying condition (such as paralysis), but who are up in a wheelchair, will be considered ambulatory for the purpose of assessing performance score. See https://members.childrensoncologygroup.org/prot/reference_materials.asp under Standard Sections for Protocols.

3.2.4 Hematopoietic Cell Transplant (HCT)

Patient must be receiving cells from alternative donor defined as one of the following:

a. Unrelated donor with a complete HLA match or a 1 or 2 HLA mismatch, considering only HLA-A, HLA-B, HLA-C, and HLA-DRB1.
b. Related donor with a 1 or more HLA mismatch (including haplo-identical).

Note: *history of HCT or other cellular therapy (e.g., CAR-T cells, donor lymphocyte infusions) is permitted.*

3.3 Exclusion Criteria

3.3.1 Patient plans on receiving stem cells from a matched (8/8) related donor.

3.3.2 Patient has used a probiotic dietary supplement within 30 days prior to enrollment. (Consumption of yogurt products is allowed.)

3.3.3 Patient has a history of severe GI tract insult including but not limited to previous bowel perforation, Grade 4 neutropenic colitis or typhlitis, inflammatory bowel syndrome, short small bowel syndrome (Crohn's disease, ulcerative colitis), history of gastrointestinal GVHD, or history of bowel resection.

3.3.4 Patient has a medical, psychiatric or social issue that would compromise patient safety or compliance with protocol therapy, or interfere with consent, study participation, follow up, or interpretation of study results.

3.3.5 Female patients who are pregnant are not eligible. Women of childbearing potential require a negative pregnancy test prior to enrollment.

3.3.6 Patient has diarrhea at the time of enrollment which is *Clostridium difficile* toxin positive.

3.3.7 Patient is receiving antibiotic therapy for an active bacterial infection.

3.3.8 Patient is allergic to all third or fourth generation cephalosporins, carbapenems, and all aminoglycosides, which are used to empirically treat LBP bacteremia.

3.4 Regulatory Requirements

3.4.1 All patients and/or their parents or legal guardians must sign a written informed consent.

3.4.2 All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PLAN

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

4.1 Overview of Treatment Plan

This will be a double-blind, randomized, placebo-controlled, intervention study that will equally allocate 454 children to either *LBP* or placebo for approximately two months. All patients, patient caregivers, direct healthcare providers, pharmacy and the institutional study team will be blinded to the randomization allocation. Sites will receive notification of the study product kit number to dispense to the patient via email at OPEN enrollment submission. The patient should receive the same study product kit number throughout the study. Study participants will receive a course of either *LBP* or placebo for approximately 64 days, starting at the initiation of conditioning regimen and ending at Day 56. However, participants will be followed until Day 120 to ensure capturing development of aGvHD. The table below provides a study overview.

	Start of conditioning	Day 0 (Graft Infusion)	Day 7	Day 14	Day 28 or discharge	Day 56	Day 120
Study Product Administration (LBP or placebo)	●	———	———	———	———	●	
Specimen Collection	-Blood -Stool	-Blood -Stool	-Blood -Stool	-Blood -Stool	-Blood -Stool	-Blood -Stool	-Stool

Please see Section 7.1 for specimen requirements.

4.2 LBP or Placebo Administration

4.2.1 Administration Schedule

Dosing will begin on the first day of the transplant conditioning regimen and continue through day 56 post HCT. The LBP/Placebo does not necessarily need to be given prior to receiving the first dose of the conditioning therapy as long as it starts on that first day. The first day of the transplant conditioning regimen is defined as the first day of chemotherapy or TBI, excluding any preceding anti T-lymphocyte antibody therapy.

4.2.2 Dosing and Administration Guidelines

Dose: The dose used in the study is 1 x 10^8 colony forming units (CFU)/kg/day. Patients will ingest 1 to 9 mL of reconstituted (mixed solution) *LBP* or placebo once each day. Please refer to the dosing chart below. All doses should be calculated based on the body weight at the start of the conditioning regimen. This should be the weight performed on the day of admission for HSCT (±72 hours). There will be no dose adjustment for weight gain or loss during the course of treatment.

See protocol Section 6.1.5 and the Pharmacy Manual (*available on the COG protocol web page*) for complete and current preparation instructions. The *LBP*/Placebo is supplied in capsules that must be opened and the contents mixed into the volume of liquid specified in the Pharmacy Manual. Once mixed, it is referred to as the "Mixed Solution" for administration as per the chart below.

Study Product Dosing Chart	
Subject's Weight (kg)*	**Total Amount of Mixed Solution to be Administered (mL)**
≤ 16 kg	1 mL
17-27 kg	2 mL
28-38 kg	3 mL
39-49 kg	4 mL
50-61 kg	5 mL
62-72 kg	6 mL
73-83 kg	7 mL
84-94 kg	8 mL
≥ 95 kg	9 mL
* Subject's Weight should be rounded to the nearest whole kilogram and total mixed solution selected accordingly. Any decimal < 0.5 round down, any decimal ≥ 0.5 round up. For example: a weight of 16.5 kg would be rounded up to 17 kg and 2 mL dose selected as per the Dosing Chart above.	

- Patients may receive *LBP* or placebo orally or through a nasojejunal, nasogastric or gastronomy tube. (Please note, an NJ, NG or G tube should not be placed solely for the administration of *LBP* or placebo.)
- Patient should ingest the dose of *LBP* or placebo within 30 minutes of mixing.
- *LBP* or placebo can be taken at any time during the day with or without cold or room temperature food.
- Liquids warmer than 98°F (36°C) are not allowed for mixing the product as high temperature may kill the bacteria.
- If vomiting occurs within 30 minutes of taking the dose, the dose may be repeated once on the same day.
- If a dose is missed, it should be taken immediately and only if there are at least 12 hours until the next scheduled dose. If there are less than 12 hours until the next dose, wait until the next scheduled dose.
- If several doses are missed due to inability to tolerate oral administration (e.g. mucositis or nausea/vomiting), therapy should resume as soon as the patient is able to tolerate oral administration. Please ensure all missed doses are documented in the patient medical record if the patient is in the hospital or patient diary (*available on the COG protocol web page*) if the patient is being treated at home.

  Most patients will receive the *LPB* or placebo in both the inpatient and outpatient settings. Adherence to the prescribed regimen will be measured by examination of medication administration records and as documented in the electronic medical record or other hospital source documentation. Patients will be given a diary to document the doses they take at home, which includes instructions for preparation and storage.

Adherence will be defined as administration of at least 80% of the prescribed doses. In the home setting, medication will be dispensed for a specified time period and medication diaries will be completed by families and delivered to the institution on a weekly basis at the time of weekly visits.

4.2.3 Discontinuation due to positive blood culture for *LBP*

Patients with a blood culture positive for *LBP* at any time during the study will discontinue the study agent (*LBP* or placebo) immediately and the patient will be removed from protocol therapy. *LBP*-bacteremia will be considered an **unexpected severe adverse event** and must be reported within 24 hours through the CTEP-AERS system. See Section 11.0 for reporting requirements.

For patients who test positive for *LBP* in the blood, empiric treatment should begin with either a third- or fourth-generation cephalosporin or carbapenem. If the clinical condition worsens after 24 hours, consideration should be given to adding an aminoglycoside. Empiric therapy should continue until sensitivity results are available. Prompt consultation with an Infectious Disease Specialist is recommended.

4.3 Concomitant Therapy

4.3.1 Supplementation with any additional probiotic dietary supplements is not permitted (food containing live cultures like yogurt is allowed) through Day 120.

4.3.2 Prophylactic use of both broad-spectrum and narrow-spectrum antibiotics is permitted. Information regarding antimicrobial therapy (agent, start and stop times, indication for use) will be collected throughout the study time period.

5.0 EMERGENCY UNBLINDING

Only in the event of a serious adverse event wherein the investigator feels that the patient cannot be adequately treated without knowing the identity of the study medication may the medication code be broken for a particular patient. If emergency un-blinding is required, the patient must stop taking the *LBP* or placebo and be removed from protocol therapy. The treating physician or their designee should use the contact information provided below to obtain the patient's un-blinded treatment information.

Emergency Unblinding Instructions

During normal business hours (9am-5pm Pacific Time)
Please submit unblinding requests using the procedure below:

1. Email to BOTH of the following:
   a. ACCL1633_unblinding@childrensoncologygroup.org
   b. Research Coordinator email listed on COG protocol webpage

2. Use email subject line: "ACCL1633 – Requesting treatment unblinding"

3. Provide the following info in the body of the email:
   a. COG Patient ID
   b. Treatment Assignment (Kit Number)
   c. Email address for site investigator
   d. Phone contact for any follow up questions/response to requesting institution

COG Contact Information

- Study Research Coordinator – Contact information listed on the COG Protocol Webpage
- COG Operations Office main line – (626) 241-1500

After hours
Please contact the Investigational Drug Service at the Perelman School of Medicine, University of Pennsylvania – Emergency pager (800) 670-3151. Be sure to provide a callback number.

When calling, be ready to provide (1) COG patient ID (2) patient's treatment assignment (kit number) and (3) investigator's callback information.

COG Operations or the Investigational Drug Service located at the Perelman School of Medicine, University of Pennsylvania, will contact the site investigator to provide the patient's unblinded treatment information.

Emergency Unblinding CRF
Once unblinded treatment assignment has been received, complete the Emergency Unblinding CRF in iMedidata Rave by accessing the patient's main page in Rave and using the Add Event dropdown.

6.0 AGENT INFORMATION

6.1 [redacted] (04/15/2021)

[redacted]

[redacted]

6.1.2 [redacted]

[redacted]

[redacted]

[redacted]

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[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

6.1.3 [redacted]

[redacted]

[redacted]

6.1.4 [redacted]

[redacted]

[redacted]

6.1.5 [redacted]

[redacted]

[redacted]

[redacted]

[redacted] [redacted]

[redacted]

[redacted]

7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable per COG administrative Policy 5.14 (except where explicitly prohibited within the protocol).

7.1 Required Clinical and Laboratory Evaluations

Studies	**Eligibility**[$]	First day of conditioning[@,&]	Day 0 (Graft Infusion)	Day 7	Day 14	Day 28 or Discharge[%]	Day 56	Day 120
History	X	-----	-----	-----	-----	-----	-----	-----
Physical examination, evaluation of GvHD	X	X	X	X	X	X	X	X
Performance status	X	-----	-----	-----	-----	-----	-----	-----
Pregnancy test*	X	-----	-----	-----	-----	-----	-----	-----
Screening for *Clostridium difficile* (only if diarrhea at enrollment or first day of conditioning)	X	X[&]	-----	-----	-----	-----	-----	-----
Review of *LBP* administration/patient diary	-----	-----	X	X	X	X	X	
Blood sample for citrulline and cytokine studies See Section 7.4.1	-----	X^	X	X	X	X	X[a,b]	
Stool sample for correlative studies See Section 7.4.2	-----	X^	X	X	X	X	X[b]	X[b]

$ All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment/randomization unless otherwise indicated.

^ Specimen collection on first day of conditioning or anytime within 72 hours before the start of conditioning (prior to chemotherapy). See Section 7.4.1 and Section 7.4.2 for overview of procedures and a Specimen Collection Manual available on the COG protocol web page with further technical detail.

@ The first day of the transplant conditioning regimen is defined as the first day of chemotherapy or TBI, excluding any preceding anti T-lymphocyte antibody therapy. See Section 4.2.1.

% Day 28 or day of discharge, whichever comes first.

* Women of childbearing potential, defined as a female who has had her first menstrual cycle.

& If the patient has diarrhea on the first day of conditioning and repeat stool culture shows *C. diff*, then the patient is ineligible and must be removed from protocol therapy. Reminder: patient condition(s) prior to initiation of protocol therapy must be within parameters required for eligibility. (See Section 8.1)

a Citrulline sample only.

b Blood and stool specimens may be collected within 14 days of the designated time point without being considered a protocol deviation.

7.2 Data Collection

The following table represents data that will be collected during the study.

Studies	First day of conditioning[@]	Day 0 (Graft Infusion)	Day 7	Day 14	Day 28 or Discharge[%]	Day 56	Day 120
Review of *LBP* or placebo administration #	X	X	X	X	X	X	-----
Use of TPN/EN (yes or no and days of administration)	X	X	X	X	X	X	X
Use of antimicrobial agents	X	X	X	X	X	X	X
Bloodstream infection	X	X	X	X	X	X	X
Clostridium difficile-associated diarrhea	X	X	X	X	X	X	X

[@]The first day of the transplant conditioning regimen is defined as the first day of chemotherapy or TBI, excluding any preceding anti T-lymphocyte antibody therapy. See Section 4.2.1.

[%] Day 28 or day of discharge, whichever comes first.

Total days product was administered will be collected for each reporting period. See Section 4.2.2.

7.3 Correlative Studies

7.3.1 Serum citrulline levels

Serum specimens will be evaluated for citrulline levels by the laboratory of Dr. Regina Santella at CUMC. Serum citrulline will be evaluated from the initiation of therapy to Day 56. Given previous experience, it is predicted that serum citrulline levels are expected to nadir at Day 7 and not begin to rebound until Day 21 in patients receiving placebo. In comparison, it is predicted that patients receiving *LBP* will have a less profound decrease in mean serum citrulline at Day 7 and earlier kinetic rebound in mean serum levels, reflecting less mucosal insult as hypothesized. Given the proposed effect in maintaining and/or attenuating injury to intestinal luminal integrity, it is hypothesized that maintaining higher serum citrulline levels in patients receiving *LBP* will be associated with less GI aGvHD and less mucosal barrier insult blood stream infections (MBI BSI).

7.3.2 Intestinal flora phylogenetic composition

Evaluation of the intestinal flora will be from the initiation of *LBP* therapy to Day 120. DNA from each stool specimen collected will be extracted and purified as we have performed previously.[34] Phylogenetic profiling using 16S rRNA gene sequencing will be performed on the Illumina MiSeq platform with 175 bp paired end reads of V4-V5 region amplicons. Sequence data will be compiled and processed using mothur[74] and QIIME[75], screened and filtered for quality,[76] then classified to the species level[77] using the Greengenes reference database.[78]

7.3.2.1 **Evaluating baseline colonization with LBP, loss of LBP during the course of hospitalization, re-colonization rate with LBP introduction and persistence of re-colonization**

Evidence of baseline colonization with *LBP* in our experience at MSKCC is uncommon. In 230 adult patients admitted for alloHCT, only 24 (10%) had detectable levels of *LBP* as measured by 16S deep sequencing. Pediatric patients will have a somewhat higher baseline colonization rate with *LBP*.[79] *LBP* strains isolated from the flora of healthy individuals have generally been reported to be antibiotic-sensitive with notable exceptions

of resistance to streptomycin, tetracycline, levofloxacin, and vancomycin being notable exceptions.[80] The vast majority of isolates are beta-lactam sensitive. Thus, we predict that during the course of alloHCT, patients who at baseline are colonized with *LBP* and require broad-spectrum antibiotic therapy for treatment of febrile neutropenia will demonstrate loss of commensal *LBP*. Because patients will continue to be administered *LBP* beyond transplant hospitalization (and after discontinuing antibiotic therapy often given during this hospitalization), we also predict that ***LBP introduction will lead to improved recovery of LBP abundance*** and that introduction will lead to persistent colonization.

7.3.2.2 **Evaluating the effects of LBP introduction on the abundance of other members of the intestinal flora**

LBP species are known to produce lactate via fermentation of a variety of sugars.[46] Lactate, in turn, can support other strains of bacteria, contributing to bacterial diversity. Bacterial strains with demonstrated lactate utilization can in turn produce butyrate,[81] which can mediate anti-inflammatory effects through recruitment of regulatory T cells (Tregs).[82-84] Species that can utilize lactate include relatives of Eubacterium halli and Anaerostipes caccae.[81] *LBP* species have also been found in animal models to mediate suppression of potentially pathogenic commensal bacteria, such as E. coli, within the intestinal tract,[85] perhaps via promoting augmented IgM and IgA responses against these pathobionts. Bacteria including E. coli and its relatives from the phylum Proteobacteria have been observed to commonly expand and dominate the intestinal tract in patients following allo HCT[86] and have been found to be associated with aggravated GvHD in animal models[23] and increased mortality in allo HCT patients.[37] In a similar manner, introduction of Lactobacillus species have also been observed in animal models to suppress the expansion of Enterococcus species,[35] leading to reduced GvHD. Enterococcal expansion, similar to Proteobacterial expansion, has been implicated in mediating aggravated GvHD in allo HCT patients.[87] Based on these prior results reported in the literature, we predict that *LBP* introduction will promote bacterial diversity by supporting the abundance of lactate utilizers and preventing domination by Proteobacteria and Enterococci. We will quantify microbial diversity using the Shannon diversity index [88] following closed-reference operational taxonomic unit picking.[75] We will also perform abundance comparisons evaluating for taxons modulated by *LBP* introduction using linear discriminant analysis (LDA) effect size (LEfSe) analysis.[89] We will compare relative abundances, determined by 16S deep sequencing, as well as absolute abundances by utilizing relative abundance data combined with quantitative total 16S gene levels using validated primers.[90]

7.3.3 Evaluating functionality of the intestinal flora

While isolates with near-identical 16S rRNA gene sequences tend to share many traits, they can often have variable gene content and as a result variable properties, even strains that are considered to be the same species.[91] Thus phylogenetic data can at best only provide an approximation of gene content and function. To further our understanding of how *LBP* can potentially reduce the risk of aGvHD in pediatric HCT recipients, we propose two targeted discovery approaches designed

to explore and identify mechanisms by which *LBP* may mediate a beneficial effect. These approaches include whole shotgun metagenomic sequencing and commercially available functional metabolite profiling.

7.3.3.1 **Evaluating the effects of LBP introduction on intestinal flora function via whole shotgun metagenomics sequencing to identify bacterial genes and gene pathways associated with modulation of GvHD**

Identifying mechanisms by which bacteria can modulate human phenotypes remains a formidable challenge in the microbiota field. One promising approach is metagenomic sequencing of whole community DNA,[91] or "shotgun sequencing." Whole shotgun metagenomic sequencing provides bacterial gene abundance data, which allows quantification of the abundance of individual genes and gene pathways. Following identification of bacterial DNA sequences, sequences are mapped against a reference database, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) database,[92] to identify bacterial enzymes in metabolic reference pathways. This approach has recently identified pathways that are enriched in the flora of patients with Type 2 diabetes mellitus,[93] obesity,[94] and newly-diagnosed Crohn's Disease.[95]

We will randomly select a subset of 30 samples from patients with similar overall 16S abundance, but 15 samples will have abundant *LBP* and 15 additional samples will have undetectable *LBP*. We will utilize the Illumina HiSeq2000 platform, aiming for a mean of 10 gigabases of 101 bp paired-end reads per sample. For comparative quantification of gene content at the individual gene level and the level of KEGG metabolic pathways, we will utilize linear discriminate analysis (LDA) effect size (LEfSe) analysis[89] for biomarker discovery. In addition to comparing samples with abundant and reduced *LBP* levels, we will also examine samples from patients who did or did not develop aGvHD. With this approach, we hope to identify novel bacterial genes and gene pathways, some of which may be *LBP*-derived, that are associated with modulation of aGvHD and may play a role in aggravating or attenuating inflammation. As all correlative assays will be performed on the backbone of a COG clinical trial, we anticipate high quality and very complete assessment laboratory findings.

7.3.3.2 **Evaluating the effects of LBP introduction on intestinal flora function via functional metabolite profiling**

While whole shotgun metagenomic sequencing provides information regarding the functional potential of the intestinal flora, a complementary approach is to assay directly for demonstration of substrate utilization and metabolite production. By determining bacterial metabolite abundances, one can confirm that increased abundance of individual genes and genes of metabolic pathways are associated with a corresponding increase in enzymatic activity. This method can also identify novel bacterial metabolites that are biologically active. One example of a group of metabolites that have garnered interest recently is short-chain fatty acids (SCFA), which are produced by many bacteria as a byproduct of carbohydrate fermentation. SCFA have been found to be important modulators of the immune system. In germ-free mice and vancomycin-

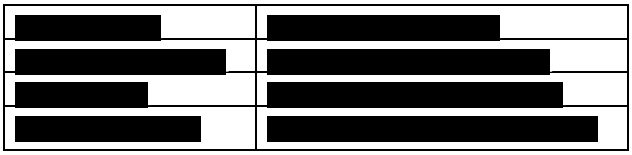
treated conventional mice, administration of SCFA (acetate, propionate, or butyrate) restored normal numbers of Tregs in the large intestine.[84] GPR43 (a SCFA receptor) has been identified as necessary for mediating the anti-inflammatory effects of commensal bacteria[96] and is required for restoration of large intestinal Tregs by SCFA.[84] We will quantitatively profile levels of enzymatic substrates and metabolites via UHPLC-MS services coupled with an extensive library with >2800 authenticated compounds provided by Metabolon (Durham, NC). We will profile aliquots of the same stool samples selected for the whole shotgun metagenomic sequencing approach detailed above as well as paired serum samples collected at the same time as the stool samples. A recent study linking intestinal flora with severity of an autism-like syndrome in mouse models similarly utilized Metabolon's services to identify a serum metabolite that induced anxiety-like behavior in these mice. Interestingly, the levels of this metabolite were modulated by intestinal flora composition.[97] Using this approach, we will ***identify novel bacterial metabolites, some of which may be LBP-derived, that are associated with modulation of aGvHD*** and may play a role in either aggravating or attenuating inflammation.

7.3.4 Serum cytokines

To measure proposed immunomodulatory effects of *LBP* as decreases in mean serum levels of alloreactive-induced inflammatory cytokines and of aGvHD-associated GI insult in patients receiving *LBP* prophylaxis compared to patients receiving placebo. Specifically, TNFR1, ST2 and REG3α will be analyzed given their previous validation as early biomarkers for aGvHD initiation and severity.[98] Furthermore, the concentrations of ST2 and REG3α on day 7 post-BMT strongly correlate with the development of GVHD-related mortality, steroid-refractory GVHD, grade III/IV GVHD and GI GVHD stage 3/4.[99] Serial serum biomarker levels will be evaluated from the initiation of therapy to Day 30. We expect that patients who do not develop GVHD will have lower levels of these biomarkers on Days 7 and 14 than patients who develop GVHD. Alloreactive-induced inflammation in the early post-transplant period activates recipient antigen-presenting cells, which, in turn, instruct donor T-cells in the allograft to produce cytotoxic soluble factors like TNFα and IFNγ that cause damage to the GI epithelium. As maintaining tolerance within the GI tract is an established function of the gut microbiota, *LBP* prophylaxis may induce tolerogenic effects, blunting allo-induced inflammation and subsequent tissue damage in transplant recipients. Therefore, we hypothesize that *LBP*-associated maintenance or restoration in the microbiota will attenuate serum levels of pro-inflammatory cytokines in the early post-transplant period. Specifically, patients receiving LBP prophylaxis will have reduced serum levels of inflammatory cytokines compared to control patients.

7.4 Specimen Collection, Processing and Shipping

7.4.1 Blood Specimens (Citrulline and Cytokines)

Sample Time Points:	• First day of conditioning or anytime within 72 hours before the start of conditioning (prior to chemotherapy) • Day 0 (graft infusion) • Day 7 • Day 14 • Day 28 or discharge, whichever is first • Day 56 (citrulline only) Sample acquisition can occur within 72 hours before the first day of conditioning. Sampling can occur within 24 hours before or after designated collection times for D0, D7, D14, D28 without being considered a protocol deviation. D56 sampling can occur within 14 days without being considered a deviation.
Blood Collection Procedure:	Draw 6 mL of whole blood in a red top tube (supplied by site).
Specimen Processing:	1. After collection, allow blood to coagulate at room temperature. 2. After coagulation (but within 1 hour after collection) centrifuge the blood samples at 4°C for 15 minutes at 3000 rpm. 3. Aliquot the serum (upper layer) into three 1.5 mL cryovials (0.5 mL of serum per aliquot). 4. Store frozen at -80°C (or -70°C) immediately after aliquoting.
Specimen Labeling:	Freezer-safe labels will be provided in specimen kits and must be used as specified in the Specimen Collection Manual. Each cryovial must be legibly labeled with: ACCL1633, COG Patient ID, Timepoint + Aliquot ID (to track individual vials), and Collection Date.
Specimen Packaging and Shipping:	Samples should remain frozen at -80°C (-70°C is permitted) until ready for shipping. Batched shipment after each participant's completion of all specimens is strongly encouraged. Be sure to include enough dry ice in the shipment to last 48 hours. Prior to shipment, the lab should be notified by e-mail with a copy of Specimen Transmittal Form(s) and Specimen Processing Worksheet (with FedEx tracking #) to:  Blood should be shipped priority overnight to: 

7.4.2 Stool Specimens

Please refer to the Specimen Collection Manual and video on the ACCL1633 page on the COG website for detailed instructions. Sites do not require special lab certification to process stool samples. However, local personnel collecting or processing study specimens should carefully review training references and forms.

Sample Time Points:	• First day of conditioning or anytime within 72 hours before the start of conditioning (prior to chemotherapy) • Day 0 (graft infusion) • Day 7 post-transplant • Day 14 post-transplant • Day 28 post-transplant or discharge, whichever is first • Day 56 post-transplant • Day 120 post-transplant Sample acquisition can occur within 72 hours before the first day of conditioning. Sampling can occur within 24h before or after designated collection times for D0, D7, D14, D28 without being considered a protocol deviation. If the patient is not able to produce a stool sample in the appropriate time frame, obtain sample as soon as possible. This will not be considered a protocol deviation. For D56 and D120 sample acquisition can occur within 14 days before or after the time point without being considered a deviation.
Supplies Required for Stool Collection & Processing:	The following stool specimens are required: A. 2 weighed Eppendorf vials (metabolomics) B. 4 cryovials (1.5 mL) for microbiome sequencing and long term storage Materials for Collection: A. Stool hats for collection B. Aliquot Vials - 2 Eppendorf vials - 4 cryovials C. Scale (*must weigh in increments of .001 grams*) for weighing specimen for Eppendorf vials only D. Pipette (for watery stool) or scoop and/or orange stick (for solid stool) E. Gloves – must be worn at all times when handling stool and the vials. F. Mask (optional) G. Black, fine-tip Sharpie pen and Freezer-safe Labels (provided in kits)
Specimen Labeling:	Reminder: freezer-safe labels will be provided in specimen kits and must be used as specified in the Specimen Collection Manual. Each specimen must be legibly labeled with: ACCL1633, COG Patient ID#, Timepoint + Aliquot ID, and Collection Date. For specimens placed in Eppendorf vials, weight before and after filling with stool must be documented on the Specimen Transmittal Form. Stool quality should be recorded on the Specimen Processing Worksheet. (See the Manual or Worksheet for stool quality classification guidelines.)

Specimen Processing:	Metabolomics (SCFA, bile acids, amino acids) For each sample: 1. Weigh and record weight of two (2) empty Eppendorf vials separately; indicate weight on specimen forms. Indicate one Vial A and the second Vial B. Make sure Vial A and Vial B are labeled clearly (e.g. label the 2 vials collected on Start of Conditioning as: SOCA, SOCB). 2. Aliquot using a pipette or scoop and/or orange stick **50-150 mg** of sample into each vial. Please do NOT exceed 150 mg of stool per aliquot. 3. Weigh and record weight of Vial A + sample, and Vial B + sample; enter each weight on specimen forms, separately. 4. Confirm that required labels are on each aliquot and that the details (i.e. the vial weights) are accurately recorded on the specimen forms. 5. Store aliquots at -80°C (-70°C is permitted). Microbiome Sequencing and Long-Term Storage (4 x 1.5 mL cryovials) For each of four (4) aliquots: 1. Fill the cryovial using a pipette or scoop and/or orange stick with stool. 2. Tighten the lid back on the vial. 3. Place label on specimen aliquots and complete the specimen forms. 4. Store vials at -80°C (-70°C is permitted).
Specimen Packaging and Shipping:	Samples should remain frozen at -80°C (or -70°C) until ready for shipping. Batched shipment after a participant's completion of **all seven time points** is strongly encouraged. Be sure to include enough dry ice in the shipment to last 48 hours. Prior to shipment, the lab should be notified by e-mailing electronic copies of the Specimen Transmittal Form(s) and Specimen Processing Worksheet(s) (with FedEx tracking #) to: [redacted] Single or multiple patient specimens may be bulk shipped to the Microbiome center at Columbia University Medical Center. If sending samples on multiple patients, be sure each patient's samples are bagged separately and clearly labeled. All specimen shipments must include Specimen Transmittal Form(s) and Specimen Processing Worksheet. [redacted]

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a. Completion of therapy.
b. Patient develops a *LBP*-associated bacteremia.
c. Patient relapses.
d. Patient develops another malignancy.
e. Refusal of further protocol therapy by patient/parent/guardian.
f. Physician determines it is in patient's best interest.
g. Patient requires emergency unblinding.
h. Patient never received transplant.
i. Patient experiences an adverse event requiring removal from protocol therapy.
j. Repeat eligibility studies (if required) or change in patient condition(s) prior to the initiation of protocol therapy are outside the parameters required for eligibility (see Section 3.2 and Section 3.3).
k. Patient develops graft failure (see Section 10.3).

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless patient is taken off study. Follow up time is defined as the duration of time between patient enrollment and assessment of primary outcome (120 days after enrollment).

8.2 Off Study Criteria

a. Death.
b. Lost to follow-up.
c. Withdrawal of consent for any further data submission.
d. Patient never received conditioning
e. Completion of follow-up through Day 120.

9.0 STATISTICAL CONSIDERATIONS

9.1 Sample Size and Study Duration

Based on estimates from Center of International Blood and Marrow Transplant Research (CIBMTR), approximately 1,400 allogeneic SCTs are performed at COG institutions each year with approximately 25% of them (350 per year) meeting the definition of "alternative donor" as defined in this study. If 30% of those eligible are enrolled, the accrual rate will be about 105 per year. Accounting for start-up time and possible delay of up to 4 months between the two stages of the study, we project accrual to the screening stage within 1.2 years and total accrual duration of 5 years. The sample size of 454 (the first 120 for the screening stage and an additional 334 for the confirmatory stage) may be increased to a maximum of 500 patients (up to 132 for screening stage and an additional 368 for the confirmatory stage) to conservatively account for up to 10% loss due to ineligible or not being randomized to a treatment regimen. Evaluable patients are defined as eligible (or meeting repeated eligibility criteria) randomized patients.

9.2 Study Design

This is a randomized, double-blind intervention trial evaluating the efficacy of *LBP* in preventing GI aGvHD in children and adolescents undergoing alternative donor HCT.

Patients will be randomized to receive daily *LBP* or placebo using a 1:1 allocation. Randomization will be stratified by stem cell source (cord vs. marrow or PBSC), HLA degree of match (complete match vs. 1 mismatch vs. 2 or more mismatches), and GvHD prophylaxis (ATG/CAMPATH vs. No ATG/CAMPATH). Haplo patients are those who have a related donor with 2 or more HLA mismatches. For stratification, Haplo would be included into the stem cell source. AGvHD outcomes will be assessed from day of stem cell infusion (Day 0) through Day 120. Accrual will be conducted in two stages: a randomized *screening* stage, and a larger *confirmatory* stage. The screening stage will compare the rate of GI aGvHD between the 2 arms at α=0.30 (1-sided). After recruitment of 120 patients (up to a maximum of 132 to account for up to 10% loss due to patients deemed to be ineligible or not being randomized to a treatment regimen), the evaluation of the outcome will be assessed. If the screening stage suggests efficacy ($p<0.3$), the study will proceed to the confirmatory stage (N=454). We will use a modified intention-to-treat analytic approach for all evaluable patients. Based on previous CCL studies, we expect the inevaluable rate to be very low.

The primary outcome measure is the incidence of Stage 1-4 GI aGvHD from the day of stem cell infusion (Day 0) through Day 120.

Endpoint incidence is assumed for evaluable patients who are lost to follow up including withdrawal of further data submission and death prior to determination of their outcome (stage 1-4 of GI aGvHD and grade II-IV overall GvHD) in the primary analysis.

Patients who never received conditioning are not at risk of aGvHD and will be considered invaluable for the primary outcome analysis. Patients who have graft failure are not at risk for graft versus host disease, therefore, they will contribute to the primary analysis up to date of graft failure. However, they can still be included in the analysis of toxicity data and laboratory data. See Section 10.3 for definition of graft failure.

Exploratory endpoints include the incidence of Grade II–IV overall aGvHD, blood stream infection (BSI), *Clostridium difficile*-associated diarrhea and assessment of laboratory correlative measures for serum levels of citrulline and GvHD biomarkers and blood/stool measures of intestinal flora and function and allo-induced inflammation.

Central Committee Review

The COG SCT Discipline has created consensus guidelines for GvHD determination that are based on modified Glucksberg criteria. These guidelines have been used in COG transplant trials for the past decade. We will collect information that includes the highest grade of overall GvHD (grades 1-4) as well as the highest stages of organ GvHD (skin, GI and liver). For acute GI GvHD, we will collect both stage (1-4) for lower GI GvHD as well as presence (yes/no) of upper GI GvHD. Both upper and lower GI GvHD are used to determine the stage of GI GvHD, but lower GI GvHD alone will be considered an event. Dates of onset and maximal GI GvHD stage and overall grade will be collected. The Study Committee will audit the GvHD data (assuring that the reported stages correlate with overall grade) for each patient every 3 months. Additional information will be requested from the reporting center to review completed GvHD forms if the overall Grade of GvHD does not appropriately correspond to the reported stages. This targeted centralized review will identify cases most prone to error, will be more efficient than review of all data, and will avoid what otherwise would be an enormous hardship for centers. All reviewers will be blinded to the patient's regimen in order to limit any bias in determining the primary and exploratory endpoints. The study chair will make the final determination for GvHD

organ staging and overall grading based on central review and may override staging and grading entered by the institution.

9.3 Data Analytic Plan

Preliminary Descriptive Analysis: Study variables will be summarized using simple descriptive statistics such as count/percentage for categorical variables and mean/standard deviation/median/interquartile range for continuous variables. Baseline patient demographics and clinical characteristics will be summarized. The two arms will be balanced on the factors included in the stratified randomization. Treatment arms will be descriptively compared for other potential confounders for possible inclusion in the multivariate models. Arm comparison will be performed using simple two-sample tests, such as χ^2 test of proportions for categorical variables and independent t-tests or Wilcoxon rank sum tests for continuous variables. Analyses for each of the aims is presented below:

Primary Aim: The primary aim is to determine whether oral *LBP* prevents the development of Stage 1-4 GI aGvHD incidence in children and adolescents undergoing alloHCT. We hypothesize that administration of oral *LBP* will reduce the incidence of Stage 1-4 GI aGvHD compared to placebo. As described above, accrual will be conducted in two stages, the screening stage and the confirmatory stage. The proportion of 120 centrally reviewed, eligible and evaluable randomized patients who received at least 1 dose of treatment having Stage 1-4 GI aGvHD incidence from Day 0 through Day 120 will be compared between the two arms after the screening stage via two-sample unpooled chi-square test of proportions at 1-sided α=0.3. If this liberal estimate of efficacy of *LBP* is demonstrated, accrual of the confirmatory stage will continue.

At the end of the confirmatory stage, the proportion of eligible patients having Stage 1-4 GI aGvHD incidence from Day 0 through Day 120 will be compared between the two arms (including patients randomized in both stages) via two-sample unpooled chi-square test of proportions at α=0.05 (1-sided). We will use a modified intention-to-treat approach for the primary analysis.

Secondary analyses will be performed on cumulative incidence of Stage 1-4 GI aGvHD using unconditional multivariate logistic regression with covariate adjustment. Covariates, to be considered in the statistical models are: use of total body irradiation, duration of broad spectrum antibiotic usage, incidence of *C. difficile* infection, Haplo designation, and duration of total parenteral nutrition. We expect few patients to be terminated from aGvHD observation prior to Day 120 due to death, loss to follow-up, or consent withdrawal before determination of GI aGvHD outcome. Nevertheless, we will also perform secondary analyses (*time from graft infusion to first Stage 1-4 GI aGvHD onset*) via competing risk modeling, where graft failure, relapse and death are treated as competing events, generating incident curves and comparing treatment arms with the Gray's test to accommodate censoring. Patients without Stage 1-4 GI aGvHD will be censored when they meet criteria to be off study. Actuarial analyses will be extended for covariate adjustment using Cox proportional hazards regression. If we observe moderate protocol non-compliance with the daily *LBP*, additional analyses, such as as-treated analyses, subset analyses including only patients adherent with protocol treatment, or sensitivity analyses, will be performed to assess whether treatment adherence influenced the primary outcome. Based on our pilot results, we not anticipate a significant adherence problem.

Exploratory Aim 1: This aim is to determine whether administration of *LBP* prevents the development of moderate to severe (Grade II–IV) overall aGvHD. We hypothesize that

administration of *LBP* will reduce the incidence of Grade II–IV aGvHD in alloHCT recipients compared to placebo. We will use a similar analysis approached as described above for the primary aim but using the dichotomous cumulative incidence of Grade II–IV aGvHD as the endpoint measure.

Exploratory Aim 2: This exploratory aim is to determine whether *LBP* administration maintains intestinal integrity as measured by serum citrulline levels and reduces the incidence of mucosal barrier bloodstream infections (MBIs). We hypothesize that administration of *LBP* will maintain serum citrulline levels in alloHCT patients compared to patients receiving placebo. As well, we expect a lower incidence of MBIs in the *LBP* arm compared to placebo.

The citrulline levels at each of the time points will be summarized and described by arm. The mean quantitative change in citrulline level from start of conditioning to each of the four later time points will be compared between the 2 arms to determine if *LBP* administration maintains citrulline level via two-sample t-test and repeated-measures general linear modeling which include adjustments for potential confounders. In such analyses, multiple comparison adjustments for the 4 parallel analyses (linear contrasts) will be performed with Bonferroni adjustment to maintain an overall fixed α level. Linear mixed models, which include data from all time points for longitudinal data analyses with adjustment for within-patient correlation, will also be considered.

Likewise, we will compare the incidence of MBIs between two groups looking at the time from start of protocol treatment to first infection as the primary endpoint. Patients without infection will be censored when they meet criteria to be off study. Cox proportional hazards regression will be used to compare the incidence density of infection for the two groups adjusted for confounders (use of total body irradiation, duration of broad spectrum antibiotic usage, incidence of *C. difficile* infection, and duration of total parenteral nutrition). Main analyses for this aim will be based on a modified intention-to-treat principle (inevaluable patients will be excluded), but additional as-treated analysis, subset analysis, or sensitivity analysis may be performed if we observe issues with adherence to the randomized treatment on the study.

Exploratory Aims 3 and 4: These aims will examine the effects of *LBP* introduction on the intestinal flora of pediatric alloHCT recipients. The association between *LBP* administration and bacterial genes and pathways, and bacterial metabolites will be evaluated with Pearson's correlation coefficient with possible transformation to approximate normality. In addition to confirming the significance, a 95% confidence interval will be estimated for each marker. The total length of the interval depends on the observed correlation; the length will be 0.53 if the correlation is 0.40 and 0.24 if the correlation is 0.8. In addition to correlation, bivariate scatterplots will be used to display the association between *LBP* administration and each marker. Finally, we will perform a descriptive analysis examining the association between GvHD outcomes and bacterial genes, pathways, and metabolites using Cox regression. Additional regression models will adjust for other known GvHD risk factors.

Exploratory Aim 5: This aim will examine the effects of *LBP* on pro-inflammatory cytokines in alloHCT recipients. We hypothesize that *LBP*-associated maintenance or restoration in the microbiota will attenuate serum levels of pro-inflammatory cytokines in the early post-transplant period. We will use the same analytic approach as described for exploratory Aim 2 above.

Exploratory Aim 6: This aim will examine the effects of LBP on the incidence (yes/no) of *Clostridium difficile*-associated diarrhea in alternative donor HCT patients. Patients who have at least one incidence (positive) of *C. diff* during any reporting period is considered evaluable for this aim. We will compare proportion of *C. diff* during the study period between arms via the chi-square test.

Exploratory Aim 7: This aim will examine whether *LBP* administration reduces hospital days within the first 120 days post hematopoietic cell transplant. Total hospital days over the study period is calculated as the duration between the date of admission for conditional therapy and the date of discharge (or the study end date). Hospital days will be compared between arms by t-test (or Wilcoxon rank-sum test if assumptions for t-test are not met).

Exploratory Aim 8: This aim will examine the safety of orally administered *LBP* strains 299 and 299v in alternative donor HCT patients as measured by incidence (yes/no) of *Lactobacillus plantarum* bacteremia. Patients who have at least one incidence (positive) of *Lactobacillus plantarum* during any reporting period is considered evaluable for this aim. We will use the same analytic approach as described for exploratory Aim 6 above.

9.4 Power and Sample Size

Primary Aim: Review of the literature suggests that the incidence of GI aGvHD ranges from 15% to 45% across various studies; therefore a precise estimate of baseline GI aGvHD incidence for this study population is not available. The incidence of GI aGvHD was 27% in the control arm of the most recent COG trial (ASCT0431), which enrolled a patient population where 54% of patients received grafts from matched sibling donors. We anticipate a higher rate of GI aGvHD in the control arm of this trial, as we are including only alternative donor recipients (refer to eligibility criteria), and therefore project the baseline cumulative GI aGvHD incidence through Day 120 to be at least 30%. Success will be defined as a 40% relative reduction in the cumulative incidence of GI aGvHD (i.e., 18% in *LBP* arm vs. 30% in placebo arm). A sample size of 120 (60 per arm) for the screening stage and 454 (227 per arm) for the confirmatory stage (including patients randomized during the screening stage) will provide 85% power to detect an effect size of 40% relative reduction at 1-sided α=0.3 and 0.05, respectively in a two-sample test of binomial proportions. The overall power of the study, which is the probability of establishing efficacy in the primary endpoint in both analyses, is lower than the power for each component separately and higher than the product of two. Based on simulations, the overall power is at 80% in this scenario. The overall type I error for the design is also slightly reduced from the type I error of 0.05 for each individual component if they are analyzed separately. Based on simulations, the overall type I error is at 0.035.

Exploratory Aims 1 and 2: The sample size for the primary aim drives the overall accrual goal and thus the number of subjects available for the exploratory aims. These endpoints will be analyzed only at the end of the study, either at the end of the screening stage or confirmatory stage. Therefore, the power is estimated for the two situations separately based on sample size of 120 (60 per arm) or 454 (227 per arm) without any adjustment for power after the confirmatory stage. For Exploratory aim #1, a baseline rate of Grade II–IV aGvHD in the range of 30%–50%, the sample size after confirmatory stage is sufficient to detect a 15% reduction in aGvHD rate, while the sample size after screening stage is sufficient to detect a 20% reduction in aGvHD rate with *LBP* compared to placebo. Power estimation is based on 2-sample test of proportions at α=0.05 (2-sided). For Exploratory aim #2 on serum citrulline levels, the sample size after confirmatory stage is sufficient to

detect an effect size of 0.4 standard deviation (SD), assuming we obtain samples from 80% or more of the subjects; on the other hand, the sample size after screening stage is sufficient to detect an effect size of 0.7 SD if we obtain samples from ≥80% subjects. Power estimation is based on two-sample t-test at α=0.0125 (2-sided), where α is based on the Bonferroni adjustment for the 4 parallel comparisons with an overall α=0.05.

9.5 Interim Monitoring

Study data will be reviewed by DSMC twice a year during the first 2 years of study activation, and once a year thereafter if no concerns are identified.

The study will be conducted in two stages: a randomized *screening* stage, and a larger *confirmatory* stage. The screening stage will compare the proportion of Stage 1-4 GI aGvHD between the 2 arms. The proportion of 120 centrally reviewed, eligible and evaluable randomized patients having Stage 1-4 GI aGvHD incidence from Day 0 through Day 120 will be compared between the two arms via two-sample test of proportions at 1-sided α=0.3. If the screening stage suggests efficacy ($p<0.3$), the study will proceed to the confirmatory stage (N=454). If efficacy is not established at the screening stage, the data will be referred to the COG Data Safety and Monitoring Committee (DSMC) for consideration of early termination.

Amendment 1: Haploidentical transplantation in children has increased over the past few years. Although single institution studies have demonstrated a remarkably lower acute and chronic Graft vs Host Disease incidence and severity, these results have not been duplicated in larger multicenter trials. In these multicenter trials, the Grade II-IV Acute GvHD rate does not appear to be significantly different from that of other alternative donor trials. While we do agree that the rate of chronic GVH appears much less, our current study ends at Day + 120 and thus chronic GVH is not measured in this trial.

Therefore, it is presumed to have no impact on the power for the primary aim by having haploidentical patients enrolled in this study. The estimated primary outcome rate aGvHD and proportion of haploidentical patients will be monitored bi-annually. At each monitoring time, by assuming the observed aGvHD rate, proportion of haploidentical patients within the control arm, and using the same power simulation (that we've used to provide power analysis for the protocol), if the estimated power at the end of the study is less than 80%, we will adjust the sample size through an Amendment. All observed rates will be available only for members of DSMC to review.

9.6 Gender and Minority Accrual Estimates

DOMESTIC PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Hispanic or Latino		Not Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	16	16	32
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	16	63	111	190
White	95	16	47	95	253
More Than One Race	0	0	0	0	0
Total	95	32	126	222	475

INTERNATIONAL (INCLUDING CANADA) PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Hispanic or Latino		Not Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	0	0	1	1	2
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	0	1	3	6	10
White	5	1	2	5	13
More Than One Race	0	0	0	0	0
Total	5	2	6	12	25

This distribution was derived from the pilot study data, adjusted for Children's Oncology Group projections.

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 5.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v5.0' is understood to represent the most current version of CTCAE v5.0 as referenced on the CTEP website (i.e., v5.02 and all subsequent iterations prior to version 6.0).

10.2 Acute GVHD

See Appendix III protocol specific staging and grading criteria for acute GVHD.

10.3 Graft Failure

Primary Graft Failure

Failure to achieve an ANC ≥ 500/μL after 42 days, determined by three consecutive measurements on different days; or < 5% donor cells in blood or bone marrow by Day +42 (as demonstrated by a chimerism assay), without evidence of JMML.

Secondary Graft Failure

Initial engraftment followed by severe neutropenia (ANC ≤ 500/μL) that is not caused by recurrent leukemia, or < 5% donor cells in the blood or bone marrow (as demonstrated by a chimerism assay) without subsequent improvement occurring either spontaneously or after growth factor treatment. Improvement is defined as ANC > 500/μL consistently. Severe neutropenia with bone marrow cellularity ≥ 25% is not secondary graft failure.

Marrow, UCB or peripheral blood stem cell reinfusion carried out any time after Day 0 because of inadequate hematopoietic function will be taken as a definitive indication of graft failure regardless of AMC values and marrow cellularity. Donor lymphocyte infusion is not considered a stem cell reinfusion.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

11.2 Expedited Reporting Requirements – Serious Adverse Events (SAEs)

To ensure compliance with these regulations/this guidance, NCI requires that AEs be submitted according to the timeframes in the AE reporting tables assigned to the protocol, using the CTEP Adverse Event Reporting System (CTEP-AERS).

Any AE that is serious qualifies for expedited reporting. An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. A Serious Adverse Event (SAE) is any adverse drug event (experience) occurring at any dose that results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse drug experience.
3) An adverse event resulting in inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours). This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience 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.

11.3 Specific Examples for Expedited Reporting

11.3.1 SAEs Occurring More than 30 Days After Last Dose of Study Drug

Any Serious Adverse Event that occurs more than 30 days after the last administration of the investigational agent/intervention **and** has an attribution of a possible, probable, or definite relationship to the study therapy must be reported according to the CTEP-AERS reporting tables in this protocol.

11.3.2 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies or birth defects, must be reported via CTEP-AERS if it occurs at any time following treatment with an agent under a NCI, COG, or industry sponsor IND/IDE since these are considered to be serious AEs.

11.3.3 Death

Reportable Categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: Newborn death occurring during the first 28 days after birth.
- Sudden Death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.
- Death due to progressive disease should be reported as *Grade 5 "Disease progression"* in the system organ class (SOC) "*General disorders and administration site conditions*." Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Any death occurring ***within 30 days*** of the last dose, regardless of attribution to the investigational agent/intervention requires expedited reporting within 24 hours.

Any death occurring ***greater than 30 days*** after the last dose of the investigational agent/intervention requires expedited reporting within 24 hours **only if** it is possibly, probably, or definitely related to the investigational agent/intervention.

11.3.4 Secondary Malignancy

A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

All secondary malignancies that occur following treatment need to be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) must also be reported via the routine reporting mechanisms outlined in this protocol.

11.3.5 Second Malignancy

A ***second malignancy*** is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). Second malignancies require ONLY routine reporting via CDUS unless otherwise specified.

11.3.6 Pregnancy, Pregnancy Loss, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Death Neonatal", the Pregnancy Information Form, available at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf, needs to be completed and faxed along with any additional medical information to (310) 640-9193. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

11.3.6.1 **Pregnancy**

Patients who become pregnant on study risk intrauterine exposure of the fetus to agents that may be teratogenic. For this reason, pregnancy needs to be reported in an expedited manner via CTEP-AERS as **Grade 3 "*Pregnancy, puerperium and perinatal conditions - Other (pregnancy)*"** under the ***Pregnancy, puerperium and perinatal conditions"*** SOC.

Pregnancy needs to be followed **until the outcome is known**. If the baby is born with a birth defect or anomaly, then a second CTEP-AERS report is required.

11.3.6.2 **Pregnancy Loss (Fetal Death)**

Pregnancy loss is defined in CTCAE as "*Death in utero.*" Any Pregnancy loss should be reported expeditiously as **Grade 4 "*Pregnancy loss*"** under the **"*Pregnancy, puerperium and perinatal conditions*"** SOC. Do NOT report a pregnancy loss as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.3.6.3 **Death Neonatal**

Neonatal death, defined in CTCAE as *"Newborn death occurring during the first 28 days after birth*" should be reported expeditiously as **Grade 4 "*Death neonatal*"** under the **"*General disorders and administration*" SOC when the death is the result of a patient pregnancy or pregnancy in partners of men on study**. Do NOT report a neonatal death resulting from a patient pregnancy or pregnancy in partners of men on study as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.4 Reporting Requirements for Specialized AEs

11.4.1 Baseline AEs

Although a pertinent positive finding identified on baseline assessment is not an AE, when possible it is to be documented as "Course Zero" using CTCAE terminology and grade. An expedited AE report is not required if a patient is entered on a protocol with a pre-existing condition (e.g., elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the study and reported if it fulfills expedited AE reporting guidelines.

a. If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
b. If the AE resolves and then recurs, the investigator must re-assess the event to determine if an expedited report is required.
c. No modification in grading is to be made to account for abnormalities existing at baseline.

11.4.2 Persistent AEs

A persistent AE is one that extends continuously, without resolution between treatment cycles/courses.

ROUTINE reporting: The AE must be reported only once unless the grade becomes more severe in a subsequent course. If the grade becomes more severe the AE must be reported again with the new grade.

EXPEDITED reporting: The AE must be reported only once unless the grade becomes more severe in the same or a subsequent course.

11.4.3 Recurrent AEs

A recurrent AE is one that occurs and resolves during a cycle/course of therapy and then reoccurs in a later cycle/course.

ROUTINE reporting: An AE that resolves and then recurs during a subsequent cycle/course must be reported by the routine procedures.

EXPEDITED reporting: An AE that resolves and then recurs during a subsequent cycle/course does not require CTEP-AERS reporting unless:

1) The grade increases OR
2) Hospitalization is associated with the recurring AE.

11.5 Exceptions to Expedited Reporting

An expedited report may not be required for a specific protocol where an AE is listed as expected. The exception or acceptable reporting procedures will be specified in the protocol. The protocol specific guidelines supersede the NCI Adverse Event Reporting Guidelines. These special situations are listed under the CTEP-AERS reporting Table A for this protocol.

11.6 Reporting Requirements - Investigator Responsibility

Clinical investigators in the treating institutions and ultimately the Study Chair have the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the investigational agent/intervention. It is the responsibility of the treating physician to supply the medical documentation needed to support the expedited AE reports in a timely manner.

Note: All expedited AEs (reported via CTEP-AERS) must also be reported via routine reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database.

11.7 General Instructions for Expedited Reporting via CTEP-AERS

The reporting methods described below are specific for clinical trials evaluating agents for which the IND is held by COG, an investigator, or a pharmaceutical company. It is important to note that these procedures differ slightly from those used for reporting AEs for clinical trials for which CTEP holds the IND.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

An expedited AE report must be submitted electronically via CTEP-AERS at: https://eapps-ctep.nci.nih.gov/ctepaers

- Expedited AE reporting timelines are defined as:
  - **24-Hour; 5 Calendar Days -** The AE must initially be reported via CTEP-AERS within 24 hours of learning of the event, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
  - **7 Calendar Days -** A complete expedited report on the AE must be submitted within 7 calendar days of the investigator learning of the event.
- Any event that results in a persistent or significant incapacity/substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect, or is an IME, which based upon the medical judgment of the investigator may jeopardize the patient and require intervention to prevent a serious AE, must be reported via CTEP-AERS **if the event occurs following investigational agent administration.**
- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention requires expedited reporting **within 24 hours** via e-mail to the COG CTEP-AERS Coordinator and Study Chair.
- Any death occurring greater than 30 days of the last dose with an attribution of possible, probable, or definite to an agent/intervention requires expedited reporting **within 24 hours** via e-mail to the COG CTEP-AERS Coordinator and Study Chair.

CTEP-AERS Medical Reporting includes the following requirements as part of the report: 1) whether the patient has received at least one dose of an investigational agent on this study; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Fax or email supporting documentation **for AEs related to investigational agents** to COG: Fax # (310) 640-9193; email: COGAERS@childrensoncologygroup.org; Attention: COG AERS Coordinator.

- **ALWAYS include the ticket number on all faxed documents.**
- **Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.**

11.8 Reporting Table for Late Phase 2 and Phase 3 Studies – Table A

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (COG) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

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

1) Death.
2) A life-threatening adverse event.
3) Any AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours. This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) 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. (FDA, 21 CFR 312.32; ICH E2A and ICH E6.)

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	**7 Calendar Days**			**24-Hour Notification 5 Calendar Days**
Not resulting in Hospitalization ≥ 24 hrs	**Not Required**		**7 Calendar Days**	

NOTE: Additional Special Situations as Exceptions to Expedited Reporting are listed below.

Expedited AE reporting timelines are defined as:
"24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour notification.
"7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar days of learning of the AE.

[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

Expedited 24-hour notification followed by complete report within 5 calendar days for:

- All Grade 4, and Grade 5 AEs

Expedited 7 calendar day reports for:

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

11.9 Protocol Specific Additional Instructions and Reporting Exceptions

- *LBP*-bacteremia will be considered an **unexpected severe adverse event**. Patients with a blood culture positive for *LBP* will cease to receive the probiotic immediately and the patient will be removed from protocol therapy. However, data collection will continue on all patients through Day 120, the final data point of the study. A report of *LBP*-bacteremia will initiate a sequence of monitoring events. Centers must report *LBP*-bacteremia within 24 hours of the diagnosis through the CTEP-AERS system.
- Only Grade 3 or 4 Unexpected Serious Adverse Events that are attributable (possibly, probably or definitely related) to *LBP* or the placebo should be reported.
- Any death, regardless of attribution should be reported.

11.10 Routine Reporting of Adverse Events

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms and do not affect the requirements for CTEP-AERS reporting.

Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all CTEP-AERS reportable events and Grade 3 and higher Adverse Events that are possibly, probably or definitely attributable to the study product (*LBP* or placebo).

11.11 Syndrome Reporting

Unless otherwise specified in this protocol, syndromes should be reported as a single event using the CTCAE term for the composite syndrome, and not as the individual events that make up the syndrome. For example, Tumor Lysis Syndrome should be reported under the composite definition rather than reporting the component events (hyperkalemia, hyperphosphatemia, hypocalcemia, hyperuricemia) separately.

12.0 RECORDS AND REPORTING

See the Case Report Forms posted on the COG web site with each protocol under "*Data Collection/Specimens*". A submission schedule is included.

12.1 CDUS

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. This is not a responsibility of institutions participating in this trial.

12.2 Data Safety and Monitoring Committee

To protect the interests of patients and the scientific integrity for all clinical trial research by the Children's Oncology Group, the COG Data and Safety Monitoring Committee (DSMC) reviews reports of interim analyses of study toxicity and outcomes prepared by the study statistician, in conjunction with the study chair's report. The DSMC may recommend the study be modified or terminated based on these analyses.

Toxicity monitoring is also the responsibility of the study committee and any unexpected frequency of serious events on the trial are to be brought to the attention of the DSMC. The study statistician is responsible for the monitoring of the interim results and is expected to

request DSMC review of any protocol issues s/he feels require special review. Any COG member may bring specific study concerns to the attention of the DSMC.

The DSMC approves major study modifications proposed by the study committee prior to implementation (e.g., termination, dropping an arm based on toxicity results or other trials reported, increasing target sample size, etc.). The DSMC determines whether and to whom outcome results may be released

APPENDIX I: CTEP REGISTRATION PROCEDURES

INVESTIGATOR AND RESEARCH ASSOCIATE REGISTRATION WITH CTEP

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR — MD, DO, or international equivalent;
- NPIVR — advanced practice providers (e.g., NP or PA) or graduate level researchers (e.g., PhD);
- AP — clinical site staff (e.g., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges;
- Associate (A) — other clinical site staff involved in the conduct of NCI-sponsored trials; and
- Associate Basic (AB) — individuals (e.g., pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
GCP training	✓	✓	✓		
Agent Shipment Form (if applicable)	✓				
CV (optional)	✓	✓	✓		

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and Cancer Trials Support Unit (CTSU) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Addition to a site roster;
- Assign the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN;
- Act as the site-protocol Principal Investigator (PI) on the IRB approval; and
- Assign the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (investigator listed on the IRB approval), consenting/treating/drug shipment investigator in OPEN, or as the CI on the DTL must be rostered at the enrolling site with a participating organization.

Additional information is located on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov.

CTSU REGISTRATION PROCEDURES

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Protocol Specific Requirements for Site Registration:

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted)

Submitting Regulatory Documents:
Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU website.

To access the Regulatory Submission Portal log in to the CTSU members' website, go to the Regulatory section and select Regulatory Submission.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Checking Your Site's Registration Status:
Site registration status may be verified on the CTSU members' website.

- Click on *Regulatory* at the top of the screen;
- Click on *Site Registration; and*
- Enter the site's 5-character CTEP Institution Code and click on Go.
  - Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with NCI or their affiliated networks.

Data Submission / Data Reporting
Medidata Rave is a clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments. Requirements to access Rave via iMedidata:

- A valid CTEP-IAM account; and
- Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

Rave role requirements:

- Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type;
- Rave Investigator role must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR); and
- Rave Read Only role must have at a minimum an Associates (A) registration type.

Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

Upon initial site registration approval for the study in Regulatory Support System (RSS), all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Rave section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

APPENDIX II: YOUTH INFORMATION SHEETS

INFORMATION SHEET REGARDING RESEARCH STUDY – ACCL1633
(for children from 7 through 12 years of age)

A Study of a Probiotic to Prevent Graft versus Host Disease in Children Receiving a Stem Cell Transplant

1. We have been talking with you about a stem cell transplant. One problem from the treatment used in stem cell transplants is graft versus host disease (GvHD). GvHD happens when the donor cells attack parts of your body causing problems with your stomach, skin and liver.

2. We are asking you to take part in a research study because you are about to have a stem cell transplant that may cause GvHD. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to keep GvHD from happening.

3. Children in this study will drink water or juice mixed with a probiotic or placebo. Probiotics are made up of good bacteria that help keep your stomach healthy. The placebo in this study will be made of exactly the same ingredients as the probiotic but will not have the good bacteria mixed in. Both the probiotic and placebo will look the same and taste the same. When you join the study, you will randomly (like the flip of a coin) be assigned to the probiotic or the placebo. You and your doctor will not know which treatment you will get. You will be asked to drink your mixture once a day starting on the first day of chemotherapy to get ready for transplant up to 56 days after your transplant.

4. As part of this study, the doctors and nurses will collect blood and stool samples to run special tests. They will keep track of any GvHD you might have and whether you get any infections.

5. Sometimes good things can happen to people when they are in a research study. These good things are called “benefits.” We don’t know for sure if there is any benefit of being part of this study. We hope a benefit to you would be to have less GvHD during stem cell transplant.

6. Sometimes bad things can happen to people when they are in a research study. These bad things are called “risks.” We do not expect you will have any problems from the probiotic or the placebo. It is possible that you could get an infection from the good bacteria found in the probiotic, but we have not seen that happen in other children receiving the same treatment as you. Other things may happen to you that we don’t yet know about.

7. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. Make sure to ask your doctors any questions that you have.

INFORMATION SHEET REGARDING RESEARCH STUDY – ACCL1633
(for teens from 13 through 17 years of age)

A Study of a Probiotic to Prevent Graft versus Host Disease in Children and Teens Receiving a Stem Cell Transplant

1. We have been talking with you about a stem cell transplant. One problem from the treatment used in stem cell transplants is graft versus host disease (GvHD). GvHD happens when the donor cells attack parts of your body causing problems with your stomach, skin and liver.

2. We are asking you to take part in a research study because you are about to have a stem cell transplant that may cause GvHD. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to keep GvHD from happening.

3. Children and Teens in this study will drink water or juice mixed with a probiotic or placebo. Probiotics are made up of good bacteria that help keep your stomach healthy. The placebo in this study will be made of exactly the same ingredients as the probiotic but will not have the good bacteria mixed in. Both the probiotic and placebo will look the same and taste the same. When you join the study, you will randomly (like the flip of a coin) be assigned to the probiotic or the placebo. You and your doctor will not know which treatment you will get. You will be asked to drink your mixture once a day starting on the first day of conditioning up to 56 days after your transplant.

4. As part of this study, the doctors and nurses will collect blood and stool samples to run special tests. They will keep track of any GvHD you might experience and whether you get any infections.

5. Sometimes good things can happen to people when they are in a research study. These good things are called “benefits.” We don’t know for sure if there is any benefit of being part of this study. We hope a benefit to you would be to have less GvHD during stem cell transplant.

6. Sometimes bad things can happen to people when they are in a research study. These bad things are called “risks.” We do not expect you will have any problems from the probiotic or the placebo. It is possible that you could get an infection from the good bacteria found in the probiotic, but we have not seen that happen in other teens receiving the same treatment as you. Other things may happen to you that we don’t yet know about.

7. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. Make sure to ask your doctors any questions that you have.

APPENDIX III: ACUTE GVHD STAGING AND GRADING

Reporting Requirements for Acute GVHD in COG Studies

In an attempt to standardize reporting of acute GVHD, the COG Stem Cell Transplantation Committee has adopted a modification of guidelines that were originally developed at the University of Michigan.

Table 1 outlines standard criteria for GVHD organ staging. However, confounding clinical syndromes (such as non-GVHD causes of hyperbilirubinemia) may make staging GVHD in a given organ difficult. In addition, timing of organ specific symptoms affects whether that symptom is more or less likely to be true GVHD. Please refer to ***Tables 2 and 3*** to assist you in deciding whether to attribute these clinical findings to GVHD, especially in situations where a biopsy is not possible. For additional help, please see the text which follows the tables. ***Table 4*** reviews the approach to assessing GVHD as acute, chronic, or the overlap between the two.

Finally, *engraftment syndrome* will be reported separately from the GVHD scoring presented below.

Engraftment Syndrome

A clinical syndrome of fever, rash, respiratory distress, and diarrhea has been described, just prior to engraftment in patients undergoing unrelated cord blood and mismatched transplantation. If, in the judgment of the local investigator, a patient experiences this syndrome, details of the event should be reported when requested in the study CRFs.

Modified Glucksberg Staging Criteria for Acute Graft versus Host Disease

Table 1 Organ Staging (See tables and text below for details)

Stage	Skin	Liver (bilirubin)	Gut (stool output/day)
0	No GVHD rash	< 2 mg/dL	Adult: < 500 mL/day Child: < 10 mL/kg/day
1	Maculopapular rash < 25% BSA	2-3 mg/dL	Adult: 500–999 mL/day Child: 10 -19.9 mL/kg/day ***Or persistent nausea, vomiting, or anorexia, with a positive upper GI biopsy.***
2	Maculopapular rash 25 – 50% BSA	3.1-6 mg/dL	Adult: 1000-1500 mL/day Child: 20 – 30 mL/kg/day
3	Maculopapular rash > 50% BSA	6.1-15 mg/dL	Adult: > 1500 mL/day Child: > 30 mL/kg/day
4	Generalized erythroderma plus bullous formation and desquamation > 5% BSA	>15 mg/dL	Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).

For GI staging: The "adult" stool output values should be used for patients > 50 kg in weight. Use 3 day averages for GI staging based on stool output. If stool and urine are mixed, stool output is presumed to be 50% of total stool/urine mix (see Section 3.2).

For stage 4 GI: the term "severe abdominal pain" will be defined as:
(a) Pain control requiring institution of opioid use, or an increase in on-going opioid use, PLUS
(b) Pain that significantly impacts performance status, as determined by the treating MD.

If colon or rectal biopsy is +, but stool output is < 500 mL/day (< 10 mL/kg/day), then consider as GI stage 0.

There is no modification of liver staging for other causes of hyperbilirubinemia

Overall Clinical Grade (based on the highest stage obtained):
Grade 0: No stage 1-4 of any organ
Grade I: Stage 1-2 skin and no liver or gut involvement
Grade II: Stage 3 skin, or Stage 1 liver involvement, or Stage 1 GI
Grade III: Stage 0-3 skin, with Stage 2-3 liver, or Stage 2-3 GI
Grade IV: Stage 4 skin, liver or GI involvement

Table 2 Evaluating Liver GVHD in the Absence of Biopsy Confirmation (See Table 3.0 below)

Establishing liver GVHD with no skin or GI GVHD

No Skin/GI GVHD Day 0-35	**Assume no liver GVHD,** unless proven by biopsy	
No Skin/GI GVHD Day 36-100	If NO other etiology identified, NO improvement with stopping hepatotoxic medications/TPN: **Stage as liver GVHD**	If other etiology identified or improves with stopping hepatotoxic drugs/TPN: **Do not stage as liver GVHD**

Establishing liver GVHD with skin or GI GVHD and other cause of hyperbilirubinemia

Skin and/or GI GVHD present	Worsening bilirubin level (includes worsening just prior to onset of skin or GI tract GVHD) OR stable elevated bilirubin despite resolution of non-GVHD cause of increased bilirubin: **Stage as liver GVHD**	Stable or improving bilirubin after diagnosis of skin or GI GVHD, irrespective of treatment: **Do not stage as liver GVHD**

Changing liver GVHD stage with other cause of hyperbilirubinemia

Skin and GI GVHD stable, improving, or absent	Liver GVHD staging is carried forward without increase in stage until other disease process resolves (e.g., if TTP is diagnosed in the presence of stage 2 liver GVHD, the liver GVHD stage 2 is carried forward despite rising bilirubin level until TTP is resolved. If there is no liver GVHD – stage 0 – and new onset TTP, the stage 0 is carried forward until TTP is resolved).

Skin and/or GI GVHD worsening	**Liver GVHD is staged according to the Glucksberg criteria. The elevated bili is attributed to GVHD alone.** Thus, when skin or GI GVHD is worsening, there is no downgrading of liver GVHD staging for other causes of hyperbilirubinemia. (e.g., if TTP is diagnosed in the presence of stage 2 liver GVHD and worsening skin or GI GVHD, the liver is staged according to the actual bilirubin level even if some of the rise in bilirubin is attributed to TTP). Similarly, even if there is no liver GVHD at onset of a new process, (such as TPN cholestasis), but skin or GI GVHD worsen during that process, then liver GVHD is diagnosed and staged according to the height of the bilirubin. **There is one exception to this**: the diagnosis of TTP, with high LDH and **unconjugated** bilirubin precludes the diagnosis and staging of new liver GVHD in the absence of a confirmatory liver biopsy.

Table 3 Evaluating GI GVHD in the Absence of Biopsy Confirmation (See Table 4.0 below)

Establishing GI GVHD with new onset diarrhea and no skin or liver GVHD

No Skin/liver GVHD Day 0 through engraftment	Assume no GI GVHD, unless proven by biopsy	
No Skin/liver GVHD Engraftment through day 100	NO other etiology of diarrhea identified: **Stage as GI GVHD**	Any other etiology of diarrhea identified: **Do not stage as GI GVHD**

Establishing GI GVHD with pre-existing diarrhea and skin or liver GVHD

Skin and/or liver GVHD present	Worsening diarrhea (includes worsening just prior to onset of skin or liver GVHD) OR persistent diarrhea despite resolution of non-GVHD cause: **Stage as GI GVHD**	Improving diarrhea after the diagnosis of skin or liver GVHD (irrespective of treatment) OR persistent diarrhea without resolution of underlying non-GVHD cause: **Do not stage as GI GVHD**

Differentiating Acute GVHD, Chronic GVHD, and Overlap Syndrome

There is often confusion differentiating acute from chronic GVHD, especially in the setting of reduced intensity transplants, DLI and new prophylactic treatments. The NIH Working Group recently published new classifications for GVHD:

Table 4 Acute GVHD, Chronic GVHD, and Overlap Syndrome

Category	Time of Symptoms after HCT or DLI	Presence of Acute GVHD features	Presence of Chronic GVHD features
Acute GVHD			
Classic acute GVHD	≤100 d	Yes	No
Persistent, recurrent, or late-onset acute GVHD	>100 d	Yes	No
Chronic GVHD			
Classic chronic GVHD	No time limit	No	Yes
Overlap syndrome	No time limit	Yes	Yes

- Scoring of acute GVHD may need to occur past day 100. In particular, patients should continue to be scored for acute GVHD when classic acute GVHD symptoms (maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea - particularly if bloody and ileus) persist past day 100 or if identical symptoms previously scored as acute GVHD resolve and then recur within 30 days during immunosuppression taper but past day 100.

- Those patients being scored as having acute GVHD should NOT have diagnostic or distinctive signs of chronic GVHD.

- **Patients with both acute and chronic symptoms should be diagnosed as having Overlap Syndrome and scored according to their chronic GVHD score**.

Further Explanation of Criteria presented in Tables 2 and 3

1.0 Assessment of Skin GVHD

1.1 Presence or Absence of Skin GVHD: Skin GVHD will be considered present if a rash characteristic of acute GVHD develops after allogeneic marrow transplantation involving more than 25% of the body surface not clearly attributable to causes such as drug administration or infection. The extent of the body surface area involved can be estimated by the "Rule of Nines". In estimating the extent of skin GVHD, the area involved is calculated for individual anatomic areas, such as the arm or leg, and then the total is derived from a simple summation. Areas that are non-blanching should not be considered involved regardless of the overlying color of the rash (red, brown, etc.). Limited distribution erythema (with the exception of palms and soles) in the absence of associated rash elsewhere on the body will not be considered GVHD.

2.0 Assessment of Liver GVHD

2.1 Assessing for the Presence or Absence of Liver GVHD

A. Hyperbilirubinemia (total bilirubin ≥ 2.0 mg/dL) in the **absence** of other signs of acute GVHD in the skin or GI tract:

i) Day 0-35: If hyperbilirubinemia alone is present with no other signs of acute GVHD in other organ systems, acute GVHD will not be diagnosed based solely on laboratory abnormalities. Acute GVHD will be diagnosed if findings on histopathology studies of liver from a biopsy or autopsy are confirmatory.

ii) Day 35-100: If hyperbilirubinemia (must be conjugated bilirubin) is not improving or is exacerbated (especially if serum alkaline phosphatase is increased), in the absence of acute GVHD in other organ systems, no other etiologies are identified, and does not improve with discontinuation of hepatotoxic drugs, acute GVHD will be diagnosed. However, it is distinctly unusual to develop ascites or a coagulopathy in the early stages of acute GVHD of the liver alone. In the absence of histopathology studies of liver from a biopsy or autopsy specimen, ascites or a coagulopathy secondary to liver dysfunction will be considered to indicate the presence of another disease process (e.g. veno-occlusive disease). Recommended non-invasive studies to define an etiology for hyperbilirubinemia are:

a. Imaging of liver (CT or ultrasound)
b. Hepatitis screen (only if ALT is elevated)
c. PT
d. Blood cultures
e. Review of medication list for potentially hepatotoxic drugs
f. Review of risk factors for viral liver infection (HSV, CMV, VZV, adenovirus, EBV, HBV, and HCV)
g. Hemolysis screen

B. Pre-existing hyperbilirubinemia clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems.

i) If pre-existing non-GVHD liver disease (documented clinically, by lab assessment, or by imaging studies) is stable or improving at the onset of signs of acute GVHD in other organs, then acute GVHD of the liver will not be considered to be present unless proven by liver biopsy or autopsy.

ii) If hyperbilirubinemia worsens several days before or at the time of onset of signs of acute GVHD in other organ systems, GVHD will be considered to be present unless histopathology studies of liver are available and negative on a biopsy during that time interval or autopsy results exclude GVHD.

iii) If hyperbilirubinemia persists and is not improving after resolution of a pre-existing non-GVHD liver disease process (e.g. localized infection of liver, systemic sepsis, biliary tract obstruction) when signs of acute GVHD are present in other organ systems or no other intervening cause has been diagnosed, then acute GVHD will be considered to be present in the absence of a new, clearly identifiable cause of non-GVHD liver disease or unless a liver biopsy or autopsy specimen is negative.

C. Prior acute GVHD in liver with new onset of a disease process that exacerbates pre-existing or recently resolved hyperbilirubinemia:

i) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia and acute liver GVHD has been diagnosed and has been stable, improving, or resolved, then the liver will not be restaged for acute GVHD until the resolution or stabilizing of the concurrent disease process (i.e., the liver stage prior to the onset of the new disease process will be carried forward until the new disease process resolves). Example: Acute GVHD of the liver and gut is diagnosed on day 20. Treatment of acute GVHD results in falling bilirubin levels to liver stage 1. Sepsis or TTP develops with transient worsening of the hyperbilirubinemia. The liver stage is not increased, despite a higher bilirubin level, because the cause of worsening hyperbilirubinemia is attributed to sepsis or TTP.

ii) If an etiology other than acute GVHD is clearly identified as causing or exacerbating hyperbilirubinemia in the presence of already worsening acute liver GVHD **or** GVHD of the skin or GI tract is simultaneously worsening, then the liver GVHD will be staged according to the actual bilirubin level, even though another cause of hyperbilirubinemia is present.

3.0 Assessment of GVHD of the Gastrointestinal Tract

3.1 Assessing for the Presence or Absence of GVHD of the Gastrointestinal Tract

A. Diarrhea (≥ 500 mL/day in adults or > 10 mL/kg in pediatric patients) in the absence of other signs of acute GVHD in other organ systems

i) Day 0-engraftment: If diarrhea alone is present without other signs of acute GVHD in other organ systems, acute GVHD will not be considered present. Diarrhea will be attributed to acute GVHD if histopathology studies of gastrointestinal tract from a biopsy or autopsy are diagnostic.

ii) Engraftment-day 100: If diarrhea persists and is not improving, is exacerbated, or develops de novo in the absence of acute GVHD in other organ systems, histopathology studies of gut biopsies or from autopsy specimens are not available, and no other etiologies are clearly identified, acute GVHD will be considered to be the cause. A stool specimen should be examined to rule out infectious causes (e.g. rotavirus, adenovirus, and *C. difficile* toxin). It is recommended, if at all possible, that biopsies be obtained for diagnostic purposes.

B. Pre-existing diarrhea clearly attributed to an etiology other than acute GVHD in the presence of signs of acute GVHD in other organ systems:

i) If pre-existing diarrhea caused by a process other than GVHD has been documented clinically or by lab assessment and is stable or improving at the onset of signs of acute GVHD in the skin or liver, then acute GVHD of the intestine will not be considered to be present in the absence of biopsy confirmation or autopsy report.

- ii) If diarrhea or gastrointestinal symptoms are already present, but worsen significantly at the time of onset of signs of acute GVHD in the skin or liver, GVHD will be considered present, unless biopsy or autopsy are negative.

iii) If diarrhea persists after resolution of a pre-existing disease process with signs of acute GVHD present in other organ systems, GVHD will be considered present, unless biopsy or autopsy are negative.

C. Prior or present acute GVHD in other organ systems with new onset of diarrhea:

If diarrhea is **clearly** attributable to an etiology other than acute GVHD (e.g., infection) and a history of acute GVHD exists or acute GVHD is present in other organ systems and is stable, then the gastrointestinal tract will not be evaluable for acute GVHD until the resolution or stabilizing of the other disease process (e.g., infection) in the absence of biopsy or autopsy confirmation.

D. Persistent anorexia, nausea or vomiting in the absence of signs of acute GVHD in other organ systems:

Persistent anorexia, nausea or vomiting in the absence of other known causes of these symptoms will be considered stage 1 acute GVHD if confirmed by endoscopic biopsy.

If a biopsy is not possible (e.g. secondary to thrombocytopenia) but the clinical findings are compatible with acute GVHD, then the patient will be treated and recorded as having acute GVHD.

3.2 Staging of the Gastrointestinal Tract for the Severity of Acute GVHD

The severity of gastrointestinal tract GVHD will be staged according to modified Glucksberg criteria. To minimize errors caused by large day-to-day variation, diarrhea volume is measured as an average over 3 days and reported as the volume in milliliters per day. When urinary mixing is noted the stool volume will be considered half of the total volume unless nursing staff is able to give a better estimate from direct observation. Abdominal cramps are considered significant for staging if the severity results in a clinical intervention (e.g. analgesia, fasting, etc.). Blood in the stools is considered significant if the blood is visible or hematochezia/ melena is present and not clearly attributed to a cause other than GVHD (e.g. epistaxis/ hemorrhoids).

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