NCT03339297

Clinical Study Protocol: JZP963-201

Study Title:	A Phase 2, Prospective, Randomized, Open-label Study on the Efficacy of Defibrotide Added to Standard of Care Immunoprophylaxis for the Prevention of Acute Graft-versus-Host-Disease in Adult and Pediatric Patients After Allogeneic Hematopoietic Stem Cell Transplant
Study Number	JZP963-201
Study Phase:	2
Product Name:	Defibrotide (defibrotide sodium)
[redacted]	
EUDRACT Number:	2017-003309-16
Indication:	Prevention of Acute Graft-versus-Host Disease (aGvHD)
Investigators:	Multicenter
Sponsor:	Jazz Pharmaceuticals 3180 Porter Drive Palo Alto, CA 94304
Medical Director:	[redacted]

Original Protocol:	19 September 2017
Amendment DE1:	07 March 2018
Amendment 1:	19 June 2018

Confidentiality Statement

This study will be conducted under Good Clinical Practice guidelines.

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SYNOPSIS

SPONSOR	Jazz Pharmaceuticals
PRODUCT	Defibrotide (defibrotide sodium)
TITLE	A Phase 2, Prospective, Randomized, Open-label Study on the Efficacy of Defibrotide Added to Standard of Care Immunoprophylaxis for the Prevention of Acute Graft-versus-Host-Disease in Adult and Pediatric Patients After Allogeneic Hematopoietic Stem Cell Transplant
STUDY NUMBER	JZP963-201
STUDY PHASE	Phase 2
LOCATION	This study is planned for conduct at approximately 60 enrolling study sites in North America and Europe.
PRIMARY OBJECTIVE	The primary objective of the study is to compare the efficacy of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone for the prevention of acute graft-versus-host disease (aGvHD) as measured by the cumulative incidence of Grade B-D aGvHD by Day +100 post-allogeneic hematopoietic stem cell transplant (HSCT) in adult and pediatric patients.
SECONDARY OBJECTIVES	Secondary objectives of the study are as follows: • To compare the efficacy of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone on additional variables, as follows: - Grade B-D aGvHD-free survival by Days +100 and +180 post-HSCT - Cumulative incidence of Grade B-D aGvHD by Day +180 post-HSCT - Cumulative incidence of Grade C-D aGvHD by Days +100 and +180 post-HSCT - Cumulative incidence of relapse by Days +100 and +180 post-HSCT Note: Disease relapse per local institutional guidelines • To evaluate steroid use in the treatment of aGvHD by Day +180 post-HSCT • To compare the health-related quality of life (HRQoL) using the following questionnaires: - Functional Assessment of Cancer Therapy-Bone Marrow Transplant-Trial Outcomes Index (FACT-BMT-TOI) (adults only) - EuroQoL-5D (EQ-5D; version dependent on age group) • To compare the safety of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone, including adverse event (AE) profile, serious adverse event (SAE) profile, laboratory abnormalities, neutrophil and platelet engraftment, graft failure, and infectious disease occurrence.

EXPLORATORY OBJECTIVES	The exploratory objectives of the study are to evaluate the efficacy, hospital resource utilization and potential biomarkers associated with defibrotide treatment: • GvHD-free, relapse-free survival (ie, patients alive without relapse, without Grade B-D aGvHD and without chronic GvHD [cGvHD]) by Day +180 post-HSCT • Grade C-D aGvHD-free survival by Days +100 and +180 post-HSCT • Cumulative incidence of non-relapse mortality by Days +100 and +180 post-HSCT • Cumulative incidence of cGvHD by Day +180 post-HSCT • Overall survival by Day +180 post-HSCT • Cumulative incidence of veno-occlusive disease (VOD) with or without multi-organ dysfunction by Days +30 and +100 post-HSCT • Cumulative incidence of transplant-associated thrombotic microangiopathy (TA-TMA) by Day +180 post-HSCT • To compare the hospital resource utilization for defibrotide prophylaxis and standard of care patients • To evaluate plasma concentrations of potential predictive and prognostic biomarkers of aGvHD and cGvHD
DESIGN	This is a Phase 2, prospective, randomized, open-label study comparing the efficacy of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone for the prevention of aGvHD as measured by the cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT in adult and pediatric patients with acute leukemia or MDS who receive myeloablative conditioning (MAC) or reduced intensity conditioning (RIC) and a peripheral blood stem cell (PBSC) or bone marrow (BM) graft from an unrelated donor (URD). After informed consent and/or assent is obtained from patients, legal guardians, or representatives, as applicable, screening procedures will be performed within 28 days of the scheduled start of the patient's HSCT conditioning regimen. Eligible patients will be randomly assigned to receive defibrotide prophylaxis 25 mg/kg/day in addition to standard of care immunoprophylaxis ("defibrotide prophylaxis arm") or standard of care prophylaxis alone ("standard of care arm") in a 1:1 ratio. Standard of care immunoprophylaxis will consist of methotrexate (MTX) or mycophenylate mofetil (MMF) + calcineurin inhibitor (cyclosporine A [CSA] or tacrolimus [TAC]) +/- ATG, with dosing and treatment schedules per local institutional guidelines, physician preference, and patient need. Randomization will be stratified by age at screening (<17 years vs ≥17 years), geographical region (North America vs Europe), and use of anti-thymocyte globulin (ATG vs no-ATG) using an interactive web response system. ATG use is limited to 30% of patients. Patients randomized to the defibrotide prophylaxis arm will receive defibrotide prior to the start of conditioning therapy (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days (and stopping no later than Day +30 post-HSCT) at a dose of 25 mg/kg/day given as a 2-hour intravenous infusion every 6 hours.

DESIGN (continued)	Patients will be monitored for the primary endpoint through Day +100 post-HSCT and secondary endpoints through Day +180 post-HSCT. Clinical diagnosis of aGvHD will be based on clinical, laboratory, and histological assessments on site. Collection of data for determination of clinical diagnosis of aGvHD and clinical staging of aGvHD will be based on the recommendations of the Mount Sinai Acute GvHD International Consortium (MAGIC) (Harris et al 2016). Grading of aGvHD for assessment of the primary and applicable secondary efficacy endpoints will be based on the International Bone Marrow Transplant Registry Severity Index (Rowlings et al 1997). As sensitivity analyses, all aGvHD-related efficacy endpoints will also be analyzed using the modified Consensus Criteria detailed in the aGvHD grading system from MAGIC. Grading of cGvHD will be based on the National Institutes of Health (NIH) criteria (Jagasia et al 2015). For both treatment arms of the study, patients who develop aGvHD should be treated for aGvHD based on local institutional guidelines, physician preference, and patient need. Patients in the defibrotide prophylaxis arm diagnosed with aGvHD prior to Day +30 post-HSCT and receiving defibrotide prophylaxis at the time of diagnosis may continue to receive defibrotide at the discretion of their physician up to a maximum of Day +30 post-HSCT. Patients in the standard of care arm will not receive defibrotide for the treatment of aGvHD. If a patient in either the defibrotide prophylaxis arm or standard of care arm develops VOD during the course of the study, the investigator may treat with Defitelio® (commercially available defibrotide) if clinically indicated. Investigators should not use study drug for treatment of VOD. If patients in the defibrotide prophylaxis arm are diagnosed with VOD prior to Day +30 post-HSCT and are receiving defibrotide prophylaxis at the time of diagnosis, they may continue to receive defibrotide up to Day +30 post-HSCT and start Defitelio treatment thereafter. They will need to clearly demonstrate the diagnosis of VOD and the use of Defitelio should be clearly stated as for use in VOD. Details of VOD diagnosis will be captured as per the schedule of procedures and assessments. Safety will be assessed through monitoring of AEs, SAEs, vital signs, physical examinations, clinical laboratory tests, and Karnofsky/Lansky performance scales (PSs). In addition, the following parameters will be assessed for safety: neutrophil and platelet engraftment, graft failure, and infectious disease.
ESTIMATED DURATION OF STUDY	The study is expected to last approximately 24 months, with duration of participation for each patient of approximately 6 months.
STUDY POPULATION	A total of 150 patients are planned for the study.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION	Each patient must meet the following criteria to be enrolled in this study: 1. Patient must be ≥1 year of age at screening and undergoing allogeneic HSCT. 2. Patient must be diagnosed with acute leukemia in morphologic complete remission (CR1 or CR2) or with MDS with no circulating blasts and with less than 5% blasts in the BM.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION (continued)	Each patient must meet the following criteria to be enrolled in this study (continued): 3. Patient must have planned to receive either MAC or RIC regimen (patients who receive non-myeloablative regimens are not eligible) and have an unrelated donor who is human leukocyte antigen (HLA) matched or single-allele mismatched (7/8 or 8/8 match at HLA-A, -B, -C, -DRB; or 9/10 or 10/10 match at HLA-A, -B, -C, -DRB, and -DQB1 at high resolution using DNA-based typing). 4. Patient must receive the following medical regimen as part of standard of care immunoprophylaxis for GvHD in either study arm at doses and regimen determined by local institutional guidelines, physician preference, and patient need: MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (ATG use is limited to 30% of patients). 5. Graft must be a CD3+ T-cell replete PBSC graft or non-manipulated BM graft. 6. Patient has total bilirubin <2x the upper limit of normal (ULN; unless elevated bilirubin is attributed to Gilbert's Syndrome) and both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) <3x ULN. 7. Female patients of childbearing potential who are sexually active and male patients who are sexually active and have female partners of childbearing potential must agree to use a highly effective method of contraception with their partners during exposure to defibrotide and for 1 week after the last dose of defibrotide. Highly effective methods of contraception that may be used by the patient include abstinence (when this is in line with the preferred and usual lifestyle of the patient [periodic abstinence, eg, calendar, post-ovulation, symptothermal methods, and withdrawal are not acceptable methods]), combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (ie, birth control pills, patches, vaginal ring), progestogen-only hormonal contraception associated with inhibition of ovulation (ie, progestin implant or injection), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), surgical sterilization, and vasectomy (>6 months before study Day 1). Surgically sterile woman and men and post-menopausal women (ie, women with >2 years of amenorrhea) do not need to use contraception. 8. Negative serum pregnancy tests (for female patients) (performed locally) at Screening prior to treatment with study drug, and as required by local guidelines.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION (continued)	Each patient must meet the following criteria to be enrolled in this study (continued): 9. Adult patients must be able to understand and sign a written informed consent. For pediatric patients, the parent/legal guardian or representative must be able to understand and sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.
DIAGNOSIS AND MAIN CRITERIA FOR EXCLUSION	Patients who meet any of the following criteria will be excluded from the study: 1. Patient has had a prior autologous or allogeneic HSCT. 2. Patient has acute bleeding that is clinically significant within 24 hours before the start of study treatment, defined as either of the following (a or b): a. Hemorrhage requiring >15 cc/kg of packed red blood cells (eg, pediatric patient weighing 20 kg and requiring 300 cc packed red blood cells/24 hours, or an adult weighing >70 kg and requiring 3 units of packed red blood cells/24hours) to replace blood loss, or b. Bleeding from a site which, in the investigator's opinion, constitutes a potential life-threatening source (eg, pulmonary hemorrhage or CNS bleeding), irrespective of amount of blood loss. 3. Patient used any medication that increases the risk of bleeding within 24 hours before the start of study treatment, including, but not limited to, systemic heparin, low molecular weight heparin, heparin analogs, alteplase, streptokinase, urokinase, antithrombin III, oral anticoagulants including warfarin, and other agents that increase the risk of bleeding. Patients may receive heparin or other anticoagulants for routine central venous line management, and intermittent dialysis or ultrafiltration. Fibrinolytic instillation for central venous line occlusion is also permitted. Note: Heparin used to keep catheters open will be allowed (up to 100 U/kg/day). 4. Patient is using or plans to use an investigational agent for the prevention of GvHD. 5. Patient is receiving or plans to receive other investigational therapy. 6. Patient, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study. 7. Patient has a psychiatric illness that would prevent the patient or legal guardian or representative from giving informed consent and/or assent. 8. Patient has a serious active disease or co-morbid medical condition, as judged by the investigator, which would interfere with the conduct of this study. 9. Patient is pregnant or lactating and does not agree to stop breastfeeding. 10. Any other condition that would cause a risk to the patient if he/she participated in the trial.

	11. Patient has a known history of hypersensitivity to defibrotide or any of its excipients. Patient has a known hypersensitivity to agent(s) (or excipients of agent[s]) within the immunoprophylaxis regimens allowed by the protocol and also to be part of the patient's planned immunoprophylaxis regimen (refer to the approved product label and reference therapy section for additional details). 12. Patient has any contraindications to agent(s) (or excipients of agent[s]) within the immunoprophylaxis regimens allowed by the protocol and also to be part of the patient's planned immunoprophylaxis regimen (refer to approved product label and reference therapy section for additional details).
TEST PRODUCT, DOSE, AND MODE OF ADMINISTRATION	Defibrotide solution is administered intravenously by study site personnel at a dose of 25 mg/kg/day, divided into 4 equal doses of 6.25 mg/kg/dose given as 2-hour infusions every 6 hours.
REFERENCE THERAPY	Standard of care immunoprophylaxis should be administered according to local institutional guidelines, physician preference, and patient need, and include the following: • MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (rabbit-derived ATG-Fresenius or Thymoglobulin); ATG use is limited to 30% of patients. Reference therapy will not be provided by the Sponsor.
DURATION OF TREATMENT	The recommended treatment duration is ≥21 days beginning immediately prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) and ending no later than Day +30 post-HSCT.
EFFICACY ASSESSMENTS	Efficacy will be assessed through monitoring of patient symptoms, physical examinations, laboratory testing, imaging studies as needed, biopsy/pathology for development of GvHD, disease relapse, and survival. Other assessments include HRQoL, hospital resource utilization, and measurement of potential predictive or diagnostic GvHD biomarkers in blood.
BIOMARKER ASSESSMENTS	Blood samples (3 mL for pediatric patients; 7 mL for adult patients) to evaluate plasma concentration of potential GvHD biomarkers will be obtained from all patients (weighing >15 kg) who have provided consent or assent, at the following time points: • At baseline (prior to conditioning) • On Day +7 post-HSCT • On Day +14 post-HSCT • On last day of treatment with study drug • On Day +100 post-HSCT • On Day +180 post-HSCT • Upon diagnosis of aGvHD • 14 days following diagnosis of aGvHD
SAFETY ASSESSMENTS	Safety will be assessed through monitoring of AEs, SAEs, vital signs, physical examinations, clinical laboratory tests, and Karnofsky/Lansky PSs. The severity of AEs will be classified by the investigator using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. In addition, the following safety parameters will be assessed: • Time to neutrophil and platelet engraftment and cumulative incidence of

	graft failure by Day +100 post-HSCT • Incidence of infectious disease by Days +100 and +180 post-HSCT
STATISTICAL ANALYSIS	The sample size of 150 patients (75 patients per treatment arm) provides a 90% confidence interval (CI) of (-0.28, -0.03) for the treatment difference of the primary endpoint (ie, cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT), assuming the cumulative incidence of 28.6% and 44% for defibrotide prophylaxis arm and standard of care arm respectively. The calculation of the CI is based on the large sample normal approximation. The 44% cumulative incidence for standard of care arm is based on published studies and results from a previously conducted study (Study 2004-000592-33). The 28.6% cumulative incidence for defibrotide prophylaxis arm is projected based on a relative 35% improvement from standard of care. The primary efficacy endpoint of this study is cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT. It will be estimated by the cumulative incidence competing risk estimator, as described by Marubini and Valsecchi (1995). Death prior to Grade B-D aGvHD by Day +100 post-HSCT will be considered as a competing risk. The difference in the cumulative incidence between the 2 treatment arms will be estimated with 2-sided 90% CI (Zhang and Fine, 2008) and the treatment comparison will be based on the Gray's test (Gray et al 1988). The primary efficacy endpoint will be examined by the intent-to-treat analysis set. Randomization will be stratified by age at screening (pediatric <17 years old vs adults ≥17 years old), geographical region (North America vs Europe), and ATG (ATG vs no ATG; ATG use is limited to 30% of patients).
DATE OF ORIGINAL PROTOCOL	19 September 2017
AMENDMENT DE1	07 March 2018
AMENDMENT 1	19 June 2018

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Abbreviation or Specialist Term	Explanation
AE	Adverse event
aGvHD	Acute graft-versus-host disease
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATG	Anti-thymocyte globulin
ATIII	Antithrombin III
BM	Bone marrow
BSA	Body Surface Area
CBC	Complete blood count
CEC	Central ethics committee
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
cGvHD	Chronic graft-versus-host disease
CI	Confidence interval
CIBMTR	Center for International Blood and Marrow Transplant Research
CSA	Cyclosporine A
CTCAE	Common Terminology Criteria for Adverse Events
D5W	5% Dextrose in water
DNA	Deoxyribonucleic acid
EC	Endothelial cell
eCRF	electronic Case Report Form
EQ-5D	EuroQol-5D
EQ-5D-5L	5-Level EuroQol-5D health questionnaire
EQ-5D-Y	EuroQol-5D health questionnaire for Youth

Abbreviation or Specialist Term	Explanation
EU	European Union
FACT-BMT-TOI	Functional Assessment of Cancer Therapy-Bone Marrow Transplant-Trial Outcome Index
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GvHD	Graft-versus-host disease
GvT	Graft-versus-tumor
HepA	Hepatitis A
HepB	Hepatitis B
HepC	Hepatitis C
HLA	Human leukocyte antigen
HRQoL	Health-related quality of life
HSCT	Hematopoietic stem cell transplant
IBMTR	International Bone Marrow Transplant Registry
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICU	Intensive care unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	Interactive response technology
ITT	Intent-to-treat
MAC	Myeloablative conditioning
MAGIC	Mount Sinai Acute GvHD International Consortium
MedDRA	Medical Dictionary for Regulatory Activities
MMF	Mycophenylate mofetil
MTX	Methotrexate
PBSC	Peripheral blood stem cell

Abbreviation or Specialist Term	Explanation
PS	Performance scale
QTc	Corrected QC interval
RIC	Reduced intensity conditioning
SAE	Serious adverse event
SOS	Sinusoidal obstruction syndrome
SUSAR	Suspected unexpected serious adverse reaction
TAC	Tacrolimus
TEAEs	Treatment-emergent adverse events
t-PA	Tissue plasminogen activator (alteplase)
ULN	Upper limit of normal
US	United States
URD	Unrelated donor
VOD	Veno-occlusive disease
WBC	White blood cell
WHODrug	World Health Organization Drug Dictionary

1. INTRODUCTION

1.1. Background and Rationale

Defibrotide (Defitelio®) is proposed to be indicated for the prevention of acute graft-versus-host disease (aGvHD) in adult and pediatric patients undergoing hematopoietic stem cell transplant (HSCT) and at high risk of aGvHD. Defibrotide is a highly complex polydisperse collection of predominantly single-stranded polydeoxyribonucleotides prepared by controlled depolymerisation of DNA from porcine intestinal tissue. While the mechanism of action of defibrotide has not been fully elucidated, nonclinical and clinical data have shown that defibrotide protects endothelial cells (ECs) and has the potential to modulate early alloreactivity and subsequent end organ damage post-HSCT. Defibrotide is postulated to reduce the incidence of aGvHD without an increase in opportunistic infections or disease relapse by: 1) potentially ameliorating the onset and propagation of the alloreactivity seen immediately after conditioning and HSCT, 2) protecting ECs in GvHD target organs from donor alloreactive T-cell infiltration and damage, and 3) not directly depleting T-cells involved in the graft-versus-tumor (GvT) effect. Defibrotide (defibrotide sodium; brand name Defitelio) has been approved in the United States (US) and Canada for the treatment of adult and pediatric patients with hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), with evidence of renal or pulmonary dysfunction following HSCT. Defibrotide is also currently marketed in Europe, Israel, and South Korea with the brand name Defitelio for the treatment of severe hepatic VOD post-HSCT therapy.

Graft-versus-host disease is a life-threatening complication following allogeneic HSCT. Graft-versus-host disease occurs when immune cells transplanted from a non-identical donor (the graft) recognize the transplant recipient (the host) as foreign, thereby initiating an immune reaction that causes multisystem disease in the host. Allogeneic transplant patients receiving the most cytotoxic conditioning regimens (myeloablative conditioning [MAC]) and stem cell grafts from donors most genetically unlike themselves (unrelated donors and/or patient-donor pairs who are mismatched at genetic loci, ie, human leukocyte antigen [HLA] mismatched) have the highest risk of developing GvHD. Acute GvHD usually occurs within the first 100 days following HSCT; involves potentially severe damage to the skin, liver, and/or gastrointestinal tract; increases the risk of developing chronic GvHD (cGvHD); and is associated with high rates of morbidity and mortality. As the leading cause of transplant-related morbidity and non-relapse mortality, GvHD limits the potential curative effect of HSCT on many hematologic malignancies. One in every 5 patients receiving a transplant from an unrelated donor dies from GvHD, and overall up to 1 in 10 transplant patients dies from GvHD complications (Pasquini and Zhu 2015).

Acute GvHD is recognized to be a consequence of donor T-cell activation and proliferation following recognition of host alloantigens, and multiple physiological events prior to and after HSCT contribute to local inflammation and target tissue destruction (Nomura et al 2017). The first step in the pathophysiology of aGvHD is postulated to be EC damage, which can be caused

by direct conditioning regimen toxicity, proinflammatory agents used during transplant, translocation of bacterial endotoxins across the damaged gastrointestinal tract, and subsequent donor cell engraftment (Palomo et al 2010). The damage is subsequently coupled with processes that amplify the original insult and promote further endothelial and tissue dysfunction. For example, conditioning regimen toxicity causes endothelial apoptosis and release of polynucleotides from dying cells (ie, damage-associated molecular patterns), activation of antigen-presenting cells, with release of proinflammatory cytokines, resulting in increased expression of adhesion molecule receptors at the EC surface (Mir et al 2017), while translocation of bacterial endotoxin may enhance these effects and increase antigenicity of ECs toward allogeneic CD8+ cytotoxic T-lymphocytes (Eissner et al 2002).

The potential for allogeneic HSCT to be curative depends on the complete destruction of malignant cells by 2 processes: the direct cytotoxic effects of pre-transplant conditioning; and the immunologically-mediated cytotoxic effects of the stem cell graft (GvT effect). This immunologically-mediated GvT effect (alloreactivity) is also the underlying basis of GvHD, when donor T-cells are stimulated to initiate variable degrees of acute and/or chronic host tissue inflammation, leading to aGvHD or cGvHD. Overall survival is dependent on the relative difference between the severity of aGvHD (which increases mortality mainly by direct effects on the gastrointestinal tract and from complications of immunosuppressive therapy) and the magnitude of the GvT effect (which decreases mortality by lowering relapse rates). Successful prevention of Grade B-D aGvHD, while preserving the GvT effect, would likely decrease morbidity and mortality by reducing the need for aggressive and prolonged immunosuppression, decreasing the symptoms of moderate and severe aGvHD, and maintaining the graft's positive effect on residual malignant cells after pre-transplant conditioning (decreased risk of relapse).

Most prophylactic treatments in use remain investigational in aGvHD, and progress in developing new treatments has been limited. The backbone of most T-cell replete conventional aGvHD prophylaxis regimens includes: 1) methotrexate (MTX) or mycophenylate mofetil (MMF) plus 2) a calcineurin inhibitor (cyclosporine A [CSA] or tacrolimus [TAC]). Of these, MMF is used more frequently in non-MAC and reduced intensity conditioning (RIC) regimens and umbilical cord blood transplants (Holtan et al 2014). Sirolimus is sometimes substituted for MTX, but VOD is a concern when used in combination with TAC, especially in busulfan-containing regimens. Steroids are typically not used as prophylaxis due to a lack of proven benefit (Holtan et al 2014). Anti-thymocyte globulin (ATG) has been shown to reduce the incidence of cGvHD and aGvHD (Finke et al 2009, Kroger et al 2016). However its adoption is not universal, and recent data showed that ATG is associated with increased disease relapse, reactivation of Epstein-Barr virus, delayed engraftment, and reduced overall survival (Walker et al 2016, Soiffer et al 2016). In summation, current therapies demonstrate variable efficacy and safety, which highlight the profound limitations in treatments to prevent this life-threatening disease.

Despite the use of various immunosuppressive regimens, aGVHD remains the most important and life-threatening complication after HSCT. Approximately 40-59% of adult and pediatric patients receiving allogeneic transplants from unrelated donors develop clinically significant Grade B-D aGvHD (Jagasia et al 2012, Jacobsohn 2008). First-line therapy consists of intravenous (IV) steroids, but nearly 40% of all patients with Grade B-D aGvHD are refractory to steroid-based therapy and further treatment options are limited. Second-line therapies are associated with significant toxicities, high failure rates, and patients with steroid refractory GvHD have 1-year overall survival rates of approximately 20-30% (Pavletic and Fowler 2012). In addition, approximately 8-30% of patients with unrelated donors will develop severe (Grade C-D) aGvHD (Jagasia et al 2012, Jacobsohn 2008), with estimated 5-year survival rates of 25% (Grade C) and 5% (Grade D) (Cahn et al 2005).

Patients developing aGvHD exhibit an enhanced degree of EC dysfunction compared with patients who do not develop this complication (Mir et al 2017). In patients with aGvHD, there is an increase in the biomarkers associated with EC damage (endothelial microparticles, E-selection and intercellular adhesion molecule-1 [ICAM-1], von Willebrand factor; Palomo et al 2010, Pihusch et al 2006, Matsuda et al 2001, Biedermann et al 2003). Furthermore, factors in serum from patients with aGvHD promote EC activation (Mir et al 2017) and there is an association between Grade C-D GvHD and increased angiopoietin-2 levels at baseline that suggests a connection to early damage to the endothelium (Dietrich et al 2013). Finally, circulating ECs in peripheral blood increase from engraftment to onset of GvHD (Almici et al 2014).

Ultimately, the goal of aGvHD prevention is to minimize the graft's negative effects on the patient while preserving its positive effects on both the underlying disease (GvT) and immune reconstitution. Current standard of care can limit the beneficial GvT effect and increase the risk of opportunistic infection and disease relapse. Given the high morbidity and mortality associated with GvHD and the limitations of current therapies, prevention of aGvHD remains an area with significant unmet need.

1.2. Nonclinical Experience

Nonclinical and clinical data suggest that the protective effect of defibrotide may be mediated via fibrinolytic, antithrombotic, anti-ischemic, anti-inflammatory, and anti-adhesive actions (reviewed in Pescador et al 2013). The mechanism of action of defibrotide is as a multi-target compound (Pescador et al 2013, Benimetskaya et al 2008, Echart et al 2009, Cella et al 2001, Coccheri et al 1988, Falanga et al 2003). Nonclinical studies have shown that defibrotide increases tissue plasminogen activator (t-PA) and decreases plasminogen activator inhibitor 1. In vitro studies showed that incubation of defibrotide with ECs upregulated thrombomodulin mRNA levels (Zhao et al 1994). In addition, defibrotide has been reported to induce the release of tissue factor pathway inhibitor from ECs and inhibit platelet aggregation by increasing the plasma concentration of prostacyclin and prostaglandin E2. Other experimental studies have shown that defibrotide can contribute to maintaining vascular integrity and reducing vascular permeability and inflammation. It has been shown to modulate the production of some inflammatory mediators such as interleukin 6, vascular endothelial growth factor, thromboxane

A2, leukotriene B4, and tumor necrosis factor. Defibrotide has been shown to down-regulate the gene expression, protein level, and activity of several inflammation-related biomarkers, including heparanase, inflammatory cytokines, and chemokines in activated ECs, reduce P-selectin and ICAM-1.

Defibrotide was shown to localize at the EC membranes and was subsequently internalized through micropinocytosis. However, defibrotide did not reach the cell nucleus even after 24 hours. The anti-inflammatory and antioxidant properties of defibrotide seem to be caused by the interaction of the drug with the cell membrane (Palomo et al 2016). These data suggest that the action of defibrotide is exerted through its interaction with the cell membrane, rather than acting through specific changes in expression of selected genes. Therefore, protection of the endothelium may prevent the development of vascular complications associated with GvHD that are caused by both the conditioning regimens and immunosuppressive agents.

The pharmacological activity of defibrotide in aGvHD in a mouse model of allogeneic stem cell transplantation is underway at Duke University in the laboratory of Nelson Chao. In this model, BALB/c mice are lethally irradiated (8.5 Gy) and transplanted with 1×10^7 T-cell depleted bone marrow (BM) cells together with 1×10^6 T-cells from C57BL/6 mice (Chen et al 2007). At the highest dose tested (800 mg/kg twice daily by intraperitoneal injection), defibrotide showed significant benefit in clinical aGvHD scores in mice and increased overall survival during a 30-day treatment period. Work is ongoing to elucidate relationships between dose schedules and benefit.

The nonclinical safety profile of defibrotide has been characterized in a comprehensive program in compliance with the ICH S6 (R1). The safety assessment program completed for defibrotide comprised the following: repeat-dose general toxicology (rat, rabbit, and dog), in vitro and in vivo mouse and rat genotoxicity, mouse and rat oral carcinogenicity, rat and rabbit developmental and reproductive toxicology, and allergenicity, antigenicity, and immunogenicity assessments. Segment II reproductive development studies were not warranted since the conditioning regimen precludes pregnancy, and long-term toxicology studies were not warranted given the duration of dosing (median 21 days).

Safety pharmacology evaluations were included in the repeat-dose toxicity studies in rat and dog to fulfill the regulatory requirement for central nervous system and cardiovascular evaluations. Local tolerance was reported as part of the general toxicology studies. In the 13-week dog toxicology study, defibrotide was well-tolerated, and the no observed effect level was 60 mg/kg/day in males and less than 60 mg/kg/day in females. Spleen, liver, and kidneys were considered the target organs with evidence of defibrotide uptake by Kupffer cells and kidney macrophages. Toxicokinetic studies showed that the systemic exposure in dogs after infusion was generally dose dependent, tended to plateau at about 1 hour after the start of infusion, and showed no accumulation over the study duration, which was consistent with a short half-life of approximately 11 minutes. Defibrotide administration did not demonstrate genotoxic potential or carcinogenic (oral) potential. No developmental or reproductive toxicity was observed after IV or IM bolus administration in rats; however, the dose levels were considered

low relative to the human equivalent. Defibrotide repeat-dose administration did not appear to cause immunogenicity, allergenicity, and immunotoxicity.

1.3. Overview of Defibrotide Clinical Development

The clinical program for defibrotide spans more than 20 years, with clinical studies evaluating the safety and efficacy of defibrotide for the treatment of adult and pediatric patients with hepatic VOD (beginning in April 2000) and for the prevention of hepatic VOD in high-risk pediatric patients (starting in January 2006). Additional clinical pharmacology studies have evaluated defibrotide in healthy and renally impaired subjects and have evaluated the effect of defibrotide on QTc intervals. Data on the effects of defibrotide in the prevention of aGvHD were generated as a pre-specified endpoint in a Phase 3 randomized study (Study 2004) as part of the overall clinical program. An ongoing Phase 3 randomized study (Study 15-007) is evaluating the efficacy and safety of defibrotide in the prevention of VOD in adult and pediatric patients undergoing HSCT. For additional information on studies in the defibrotide clinical development program, refer to the Investigator's Brochure (IB) for defibrotide.

1.4. Clinical Experience

The clinical evidence supporting defibrotide for the prevention of aGvHD comes mainly from the Phase 3 randomized trial Study 2004, with supportive evidence from published data and retrospective analyses done by Tekgündüz et al 2016 and Chalandon et al 2016. Additional supportive information has also been presented by Strouse et al 2016.

Study 2004 was a Phase 3, open-label, randomized, controlled trial that enrolled patients from 28 European university hospitals or academic medical centers. The trial included 356 patients of 18 years of age or younger who had undergone MAC prior to allogeneic or autologous HSCT and were at high risk of hepatic VOD. Among the 356 patients in the intent-to-treat (ITT) population, 180 patients received defibrotide prophylaxis and 176 were in the control arm. The mean age (±standard deviation [SD]) was 6.12±5.11 years and 6.28±5.32 years in the defibrotide and control arms respectively, with 26% and 25% being <2 years; and 61% and 57%, respectively, were males. The incidence and severity of aGvHD was a pre-specified endpoint in Study 2004 and was analysed in 241 allogeneic patients: 124 patients received defibrotide prophylaxis and 117 were in the control arm. Demographic and baseline characteristics were similar in the defibrotide and control arms of the allogeneic subset. Busulfan was used in conditioning in 62% and 64% of patients in the 2 arms, and melphalan in 60% and 53%, respectively. Grafts were from related donors in 40% and 30% of the defibrotide and control arms, respectively. Standard GvHD prophylaxis was allowed according to best practice and was generally comparable (CSA: 80% vs 89%, MTX: 45% vs 56%, in defibrotide and control arms, respectively). However, there was a difference in patients who received ATG in the defibrotide arm compared to controls (54% vs 70%, respectively). By Day +100, the incidence of aGvHD was 47% in the defibrotide prophylaxis arm compared with 67% in the control arm. The Grade B-D aGvHD by Day +100 equaled 22% in the defibrotide prophylaxis arm vs 37% in the control arm (p=0.015).

In Tekgündüz et al 2016, 195 adult patients at high risk for VOD who received an allogeneic HSCT were retrospectively evaluated in 3 groups to ascertain any differences in the incidence of aGvHD: no defibrotide, defibrotide post-HSCT for 14 days (Day +1 to Day +14), and defibrotide pre-HSCT (beginning on the first day of conditioning) for 14 days. The defibrotide groups received defibrotide 10 mg/kg/day for 14 days in addition to low-molecular weight heparin (enoxaparin, 0.4 mg), ursodeoxycholic acid (URSO), and N-acetylcystine (NAC), while the control group received the same prophylaxis treatments less defibrotide. High risk criteria for VOD were similar to those for Study 2004. The GvHD prophylaxis was per institutional guidelines, and included MTX/CSA, ATG-Fresenius (ATG-F), and post-transplant cyclophosphamide (PTCy). Of these, 85% of patients had a matched related donor (MRD) graft. All other patients were grouped as "Other" and included matched unrelated donors (MUDs), mismatched related/unrelated donors (MMRDs, MMURDs), and haploidentical donors. Treatment arms were comparable according to age, conditioning intensity, use of total-body irradiation (TBI)-based preparative regimen, female donor/male recipient combination, cytomegalovirus (CMV) seropositivity of recipients, and donor age. There was a trend in favor of defibrotide in decreasing cumulative incidence of severe forms (Grades C-D) of aGvHD at Day +180 (all grades aGvHD, 25.5% vs 40% vs 46.5%, defibrotide/pre-HSCT vs defibrotide/post-HSCT vs control, p=0.057). No defibrotide/pre-HSCT patient developed Grade C-D GvHD in the pre-HSCT group vs 15.5% in the control and 11.2% in the defibrotide/post-HSCT group. This trend was seen despite more patients in the defibrotide/pre-HSCT group having more "Other" donors than MRDs and more peripheral blood stem cell (PBSC) grafts than BM grafts (difference among no defibrotide vs defibrotide/post-HSCT vs defibrotide/pre-HSCT, $p<0.001$ and p=0.018, respectively), both of which are considered risk factors for aGvHD and would be expected to decrease the ability to show a treatment effect with defibrotide.

Chalandon and colleagues (2016) retrospectively analysed data from 248 patients who received defibrotide intravenously (800-2400 mg/day) in combination with heparin for VOD prophylaxis compared to patients not treated with defibrotide as controls. The Day +100 cumulative incidence of Grade B-D aGvHD was not significantly different between the 2 groups: 27% (95% confidence interval [CI] 22% to 33%) in the defibrotide group vs 29% (95% CI 24% to 35%) in the control group (p=0.707). The 1-year Grade B-D aGvHD cumulative incidence was significantly reduced in the defibrotide group (31% [95% CI 25% to 37%]) compared with the control group (42% [95% CI 36% to 48%]) (p=0.026). Multivariate analysis, performed taking into account clinical factors known to influence the risk of SOS, confirmed the favorable impact of defibrotide on 100 day VOD cumulative incidence [HR $7.5x10^{-7}$ (95%CI $1.8x10^{-7}$-$3.2x10^{-6}$), $p<0.00001$]; conversely, multivariate analysis failed to confirm the impact of defibrotide on 1 year event-free survival or the cumulative incidence of acute GvHD.

Strouse and colleagues (2016) reviewed Center for International Blood and Marrow Transplant Research (CIBMTR) data for 8341 patients who received HSCT between 2008-2011 for cases of VOD. Patients identified with severe VOD (VOD occurring in the setting of renal impairment requiring dialysis or any non-infectious pulmonary abnormality) were divided into 2 groups for analysis: those treated with defibrotide (n=41), and those not treated with defibrotide (n=55). The cumulative incidence of Grade B-D aGvHD at Day +100 post-HSCT was 23.1% (95% CI 11.4% to 37.4%) vs 37.7% (95% CI 25.3% to 51.0%) (defibrotide vs control, difference of -14.6% [95% CI -33.1% to 3.9%]). The cumulative incidence of Grade C-D aGvHD at Day +100 post-HSCT was 10.9% (95% CI 3.0% to 22.8%) vs 28.6% (95% CI 17.3% to 41.4%) (defibrotide vs control, difference of -17.7% [95% CI -33.6% to 2.0%]). Although the study was not in patients given defibrotide for prophylaxis of VOD or GvHD, the author concluded that the results supported the previously observed protective effect that defibrotide appeared to have on the incidence and severity of aGvHD. The author stated that the finding may be mechanistically related to down-regulation of heparanase or inhibition of T-cell activity, which preclinical and clinical studies have demonstrated with the use of defibrotide.

1.5. Justification for Dosage and Dosage Regimen

The dosage of defibrotide selected for this study is 25 mg/kg/day, administered intravenously as 4 2-hour infusions of 6.25 mg/kg every 6 hours beginning prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days and stopping no later than Day +30 post-HSCT. Using a recommended minimum duration of defibrotide prophylaxis provides a relatively consistent minimum duration of inpatient treatment to maximize the potential defibrotide treatment effect, while not imposing unnecessary inpatient hospitalization on patients who may be ready for hospital discharge prior to 21 days of study drug administration.

The safety of defibrotide 25 mg/kg/day as prophylaxis has been demonstrated in children at high risk of VOD who received MAC (Corbacioglu et al 2012). Incidences of adverse events (AEs), serious adverse events (SAEs), and events leading to discontinuation were similar between the defibrotide and control groups (207 SAEs in 108 patients in the defibrotide group and 231 SAEs in 103 patients in the control group). The most common AEs considered by the investigator to be related to defibrotide were hemorrhage-type events. During the prophylactic phase of the study, hemorrhage-type events were reported in 17.5% of patients in the defibrotide group vs 13.6% of patients in the control group. There was no notable difference between study arms in the incidence of any specific hemorrhage-type event. Overall, most fatal AEs were either neoplasms (12 [7%] patients in the defibrotide group and 14 [8%] patients in the control group) or infections (6 [3%] patients in the defibrotide group and 11 [6%] patients in the control group).

1.6. Justification for Choice of Primary Efficacy Endpoint

Acute graft-versus-host disease is a common complication of allogeneic HSCT that classically presents in the early post-transplantation period and has historically been measured at Day +100 post-HSCT. Although Grades C-D aGvHD are associated with the highest mortality, Grade B also represents a clinically meaningful point in the progression of aGvHD. Active physician engagement and concerted clinical efforts at (further) immunosuppression typically begin once the patient is diagnosed with Grade B aGvHD in order to 1) treat the Grade B symptoms and 2) prevent more severe and/or other organ involvement. Initial immunosuppression treatment with systemic steroids will ultimately fail in up to 40% of patients and is associated with multiple possible side effects, including an increased risk of systemic infection due to further immunosuppression and an increased probability of immunosuppressant drug toxicity (eg, renal toxicity) (Pavletic and Fowler 2012). Overall, these data suggest that a reduction in Grade B-D aGvHD at Day +100 post-HSCT would be clinically meaningful due to decreased patient morbidity and possibly decreased mortality.

The severity of aGvHD is determined by assessing the degree of involvement of the skin, liver, and gastrointestinal tract, and several systems for grading aGvHD have been developed. The most widely used grading scales in aGvHD prevention trials are the Consensus Criteria (Przepiorka et al 1995) and the International Bone Marrow Transplant Registry (IBMTR) Severity Index (Rowlings et al 1997). These 2 grading systems differ slightly in the staging criteria used to define each grade, but both have been shown to correlate well with treatment response and patient survival. In the proposed study, collection of data for the diagnosis and staging of aGvHD will be based on the recommendation of the Mount Sinai Acute GvHD International Consortium (MAGIC; Harris et al 2016; Appendix 2). Grading of aGvHD for assessment of the primary and applicable secondary efficacy endpoints will be based on the IBMTR Severity Index (Rowlings et al 1997; Appendix 3). As sensitivity analyses, all GvHD-related efficacy endpoints will also be analyzed using the modified Consensus Criteria detailed in the aGvHD grading system from MAGIC (Harris et al 2016; Appendix 4).

In the proposed study, the primary endpoint is the cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT. Given defibrotide's postulated mechanism of action in protection/stabilization of EC and the proposed treatment regimen for a recommended ≥21 days up to a maximum of Day +30 post-HSCT, defibrotide will be optimally placed to prevent aGvHD cases arising from transplant toxicity.

1.7. Justification for Selection of the Patient Population

Allogeneic transplant is offered to some patients with hematologic malignancies such as acute leukemia or myelodysplastic syndrome (MDS) as a potential curative therapy. Acute graft-versus-host disease is a common complication of allogeneic HSCT that classically presents prior to Day +100 post-HSCT despite intensive prophylaxis with immunosuppressive agents. Numerous risk factors are associated with the development of aGvHD. Two of the most important risk factors for developing Grade B-D aGvHD are the use of stem cell grafts from unrelated donors (Flowers et al 2011, Arora et al 2009) and the use of MAC regimens (Jagasia et al 2012).

Allogeneic transplant patients receiving stem cell grafts from donors most genetically unlike themselves (unrelated donors and/or patient-donor pairs who are mismatched at genetic loci [ie, HLA mismatched]) have the highest risk of developing aGvHD because of the increased degree of genetic variation between donor and patient. Approximately 40%-59% of adult and pediatric patients receiving allogeneic transplants from unrelated donors develop clinically significant moderate or severe (Grade B-D) aGvHD, despite the use of immunosuppressive aGvHD prophylaxis regimens (Jagasia et al 2012, Jacobsohn 2008). The prognosis for these patients is poor. First-line therapy consists of intravenous steroids, but nearly 40% of all patients with Grade B-D aGvHD are refractory to steroid-based therapy, and further treatment options are limited. Second-line therapies are associated with significant toxicities, high failure rates, and patients with steroid refractory GvHD have 1-year overall survival rates of approximately 20%-30% (Pavletic and Fowler 2012). In addition, approximately 8%-30% of patients with unrelated donors will develop severe (Grade C-D) aGvHD (Jagasia et al 2012, Jacobsohn 2008), with estimated 5-year survival rates of 25% (Grade C) and 5% (Grade D) (Cahn et al 2005).

Patients receiving the most cytotoxic conditioning regimens (ie, MAC) have a somewhat increased risk of developing aGvHD thought to be due to direct cellular toxicity and damage to the gastrointestinal tract. This promotes release of inflammatory cytokines and translocation of gut bacteria (Harris et al 2013), both of which stimulate and amplify the cytokine cascade that is central to the pathophysiology of aGvHD (Couriel et al 2004). Reduced intensity conditioning regimens have been used in older patients to avoid transplant-related morbidity and mortality, but while initial engraftment is often incomplete and mixed donor chimerism evolves over time to full donor chimerism, these regimens have not substantially truncated the risks of GvHD and the accompanying immunologically-based anti-tumor effects (Weisdorf 2017).

Patients with acute leukemia (CR1 or CR2) or MDS who have received MAC and undergo allogeneic HSCT with unrelated donor grafts represent 19% (20% adult and 17% pediatric) of allogeneic transplants in the European Union (EU) and 21% (23% adult and 17% pediatric) of allogeneic transplants in the US (Passweg et al 2016, Pasquini and Zhu 2015). This population excludes patients with advanced disease and represents a well-defined, high-risk group that is relatively homogeneous in its risk for developing aGvHD, while remaining broad enough to represent a significant proportion of the allogeneic HSCT population. In addition, the Sponsor has included both PBSC and BM as graft sources in this study. This allows for the inclusion of

both adult (who receive more PBSC grafts) and pediatric (who receive more BM grafts) patients. Allowing grafts from these sources is not likely to affect overall background risk, because results from a Phase 3 randomized trial demonstrated no difference in the incidence of Grade B-D aGvHD or survival between PBSC and BM grafts from unrelated donors in patients who received either MAC or RIC (Anasetti et al 2012). The reduction of aGvHD is expected to be similar in both adult and pediatric patients. With approximately 80% adult patients estimated to be in the study, the overall background incidence of Grade B-D aGvHD is estimated to be approximately 44%.

In summary, adult and pediatric patients with acute leukemia or MDS who undergo MAC or RIC prior to receiving PBSC or BM grafts from URDs are a patient population at high risk of aGvHD and have an unmet medical need for improved aGvHD prophylaxis therapies.

1.8. Potential Risks

The defibrotide clinical development program, spanning over 20 years, comprises 8 clinical studies, as well as expanded access/compassionate use programs, examining defibrotide in both the VOD treatment and prevention settings. The overall defibrotide safety database includes patient data from clinical trial and post-approval use. As of April 2017, over 6000 adult, adolescent, and pediatric patients have been exposed to defibrotide. Through clinical trial use, a total of 1690 patients have received defibrotide: approximately 1441 patients for treatment of VOD, 179 patients for prevention of VOD, 12 patients for severe or end-stage renal disease, and 58 healthy volunteers. Through post-approval exposure and use on a named patient basis, it is estimated that 4387 patients have received defibrotide. The safety profile is consistent across clinical trial and post-approval use. Safety data for prophylactic use of defibrotide is available from the randomized, prospective Study 2004-000592-33 (prevention of VOD in high risk pediatric patients post-HSCT).

In Study 2004-000592-33, defibrotide at a dose of 25 mg/kg/day administered as prophylaxis in pediatric patients undergoing HSCT at high risk for VOD was generally safe and well tolerated. In the prophylactic phase of the study, 353 patients were randomized and treated (n=177 defibrotide and n=176 controls). Between patients who received defibrotide prophylaxis and patients in the control arm who received no VOD prophylaxis (but received standard care post-transplant), respectively, there were similar incidences overall of AEs (79.7% vs 80.1%), AEs leading to death (14.1% vs 10.2%), severe AEs (32.2% vs 27.8%), and AEs leading to discontinuation (7.3% vs. 6.3%). The most commonly reported AEs during the prophylactic phase of the study were typical for a high-risk pediatric population undergoing HSCT and were generally balanced between the study arms. The overall incidence of AEs considered related to defibrotide was low (5.1%). Treatment-related AEs reported in more than 1 patient were gastrointestinal hemorrhage and epistaxis (reported in 2 patients each). During the prophylactic phase of the study, hemorrhage events were reported in 31 (17.5%) patients in the defibrotide prophylaxis arm compared with 24 (13.6%) patients in the control arm. There was no notable difference between study arms in the incidence of any specific hemorrhage event.

A review of the literature has revealed a similar safety profile. Defibrotide has been generally well tolerated, and the overall safety profile of defibrotide appears to be acceptable. The principal toxicity of concern continues to be the potential for increased risk of hemorrhage.

Hemorrhage is commonly observed in patients following HSCT. Clinically severe hemorrhage is reported in 12-27% of all post-HSCT patients, and in a study of over 1500 patients, the risk of post-HSCT clinically significant hemorrhage was independently associated with a diagnosis of VOD (odds ratio=2.2, 95% CI 1.4-3.6; Gerber et al 2008). Fatal hemorrhage has been reported to occur in 3-5% of all HSCT patients (Gerber et al 2008, Pihusch et al 2006, Nevo et al 1998). In this population, causality is difficult to determine given multiple comorbidities in VOD patients with associated organ dysfunction such as infection/sepsis, coagulopathy, thrombotic thrombocytopenic purpura, disseminated intravascular coagulation, GvHD, acute respiratory distress syndrome, and systemic inflammatory reaction.

Therefore, given the potential benefit to patients in decreasing the incidence of Grade B-D aGvHD (Section 1.4), and no appreciable safety concerns in data from the use of defibrotide in the treatment and prevention of VOD post-HSCT, the benefit-risk evaluation is considered to be positive for this study of defibrotide for the prevention of aGvHD.

Please refer to Section 6 of the defibrotide IB for additional details and guidance for the investigator.

1.9. Compliance Statement

This study will be conducted in compliance with this protocol, Good Clinical Practice (GCP), and applicable regulatory requirements.

Sponsor signatures indicating approval of this protocol are provided in Appendix 7.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of the study is to compare the efficacy of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone for the prevention of aGvHD as measured by the cumulative incidence of Grade B-D aGvHD by Day +100 post-allogeneic HSCT in adult and pediatric patients.

2.2. Secondary Objectives

The secondary objectives of the study are as follows:

- To compare the efficacy of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone on additional variables, as follows:
  - Grade B-D aGvHD-free survival by Days +100 and +180 post-HSCT
  - Cumulative incidence of Grade B-D aGvHD by Day +180 post-HSCT
  - Cumulative incidence of Grade C-D aGvHD by Days +100 and +180 post-HSCT
  - Cumulative incidence of relapse by Days +100 and +180 post-HSCT

    Note: Disease relapse per local institutional guidelines
- To evaluate steroid use in the treatment of aGvHD by Day +180 post-HSCT
- To compare the health-related quality of life (HRQoL) using the following questionnaires:
  - Functional Assessment of Cancer Therapy-Bone Marrow Transplant-Trial Outcomes Index (FACT-BMT-TOI) (adults only)
  - EuroQoL-5D (EQ-5D; version dependent on age group)
- To compare the safety of defibrotide added to standard of care immunoprophylaxis vs standard of care immunoprophylaxis alone, including AE profile, SAE profile, laboratory abnormalities, neutrophil and platelet engraftment, graft failure, and infectious disease occurrence

2.3. Exploratory Objectives

The exploratory objectives of the study are to evaluate the efficacy, hospital resource utilization and potential biomarkers associated with defibrotide treatment:

- GvHD-free, relapse-free survival (ie, patients alive without relapse, without Grade B-D aGvHD and without cGvHD) by Day +180 post-HSCT
- Grade C-D aGvHD-free survival by Days +100 and +180 post-HSCT
- Cumulative incidence of non-relapse mortality by Days +100 and +180 post-HSCT
- Cumulative incidence of cGvHD by Day +180 post-HSCT
- Overall survival by Day +180 post-HSCT
- Cumulative incidence of VOD with or without multi-organ dysfunction by Days +30 and +100 post-HSCT
- Cumulative incidence of transplant-associated thrombotic microangiopathy (TA-TMA) by Day +180 post-HSCT
- To compare the hospital resource utilization for defibrotide prophylaxis and standard of care patients
- To evaluate plasma concentrations of potential predictive and prognostic biomarkers of aGvHD and cGvHD

3. STUDY DESIGN

3.1. Overall Study Design and Plan

This is a Phase 2, prospective, randomized, open-label study comparing the efficacy and safety of defibrotide added to standard of care immunoprophylaxis (defibrotide prophylaxis arm) vs standard of care immunoprophylaxis alone (standard of care arm) for the prevention of aGvHD as measured by the cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT in adult and pediatric patients who are at high risk of developing aGvHD. Investigative site diagnosis of aGvHD and treatment decisions will be based on clinical judgment with guidance on clinical data collection and clinical staging from the recommendations of MAGIC (Harris et al 2016; Appendix 2). An overview of the study design is illustrated in Figure 1.

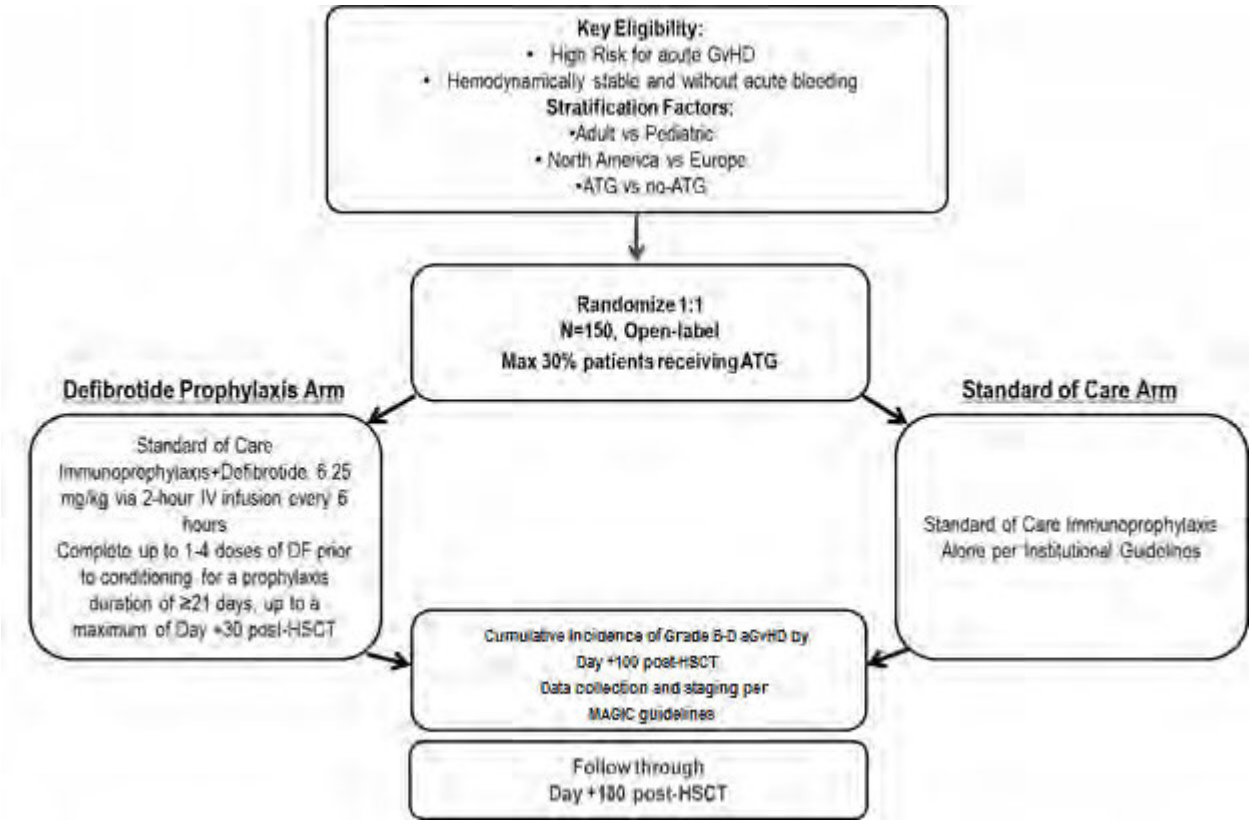
A total of 150 patients are planned for enrollment in the study. After informed consent and/or assent is obtained from patients, parents/legal guardians or representatives, as applicable, screening procedures will be performed within 28 days of the scheduled start of the patient's HSCT conditioning regimen. Eligible patients will be randomly assigned to receive defibrotide prophylaxis 25 mg/kg/day in addition to standard of care immunoprophylaxis ("defibrotide prophylaxis arm") or standard of care immunoprophylaxis alone ("standard of care arm") in a 1:1 ratio. Standard of care immunoprophylaxis will consist of MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG, with dosing and treatment schedules per local institutional guidelines, physician preference, and patient need. Randomization will be stratified by age at screening (<17 years vs ≥17 years), geographical region (North America vs Europe), and use of ATG (ATG vs no-ATG) using an interactive response technology (IRT). Use of ATG will be limited to 30% of patients.

All patients enrolled in the study (defibrotide prophylaxis and standard of care arms) should receive individualized standard of care immunoprophylaxis therapy based on local institutional guidelines, physician preference, and patient need. This standard of care therapy, intended to serve as a study control for comparison with those patients randomized to receiving defibrotide prophylaxis, will not be provided by the Sponsor. Patients randomized to the defibrotide prophylaxis arm should receive standard of care immunoprophylaxis therapy added to defibrotide prophylaxis. Prophylactic use of defibrotide added to standard of care immunoprophylaxis (defibrotide prophylaxis arm) will be compared to standard of care immunoprophylaxis alone (standard of care arm).

Patients randomized to the defibrotide prophylaxis arm will receive defibrotide prior to the start of conditioning therapy (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days (and stopping no later than Day +30 post-HSCT) at a dose of 25 mg/kg/day given as a 2-hour intravenous infusion every 6 hours.

For patients in the standard of care arm, standard of care should be administered according to local institutional guidelines, physician preference, and patient need, beginning on the first day of conditioning and continuing until hospital discharge. Patients randomized to the standard of care arm and who are receiving standard of care immunoprophylaxis should not receive defibrotide as part of their standard of care regimen. Patients will be monitored for the primary endpoint through Day +100 post-HSCT and secondary endpoints through Day +180 post-HSCT.

Figure 1: Study Diagram



ATG=anti-thymocyte globulin; DF=defibrotide; GvHD=graft-versus-host disease; HSCT=hematopoietic stem cell transplant; IV=intravenous; MAGIC=Mount Sinai Acute Graft-versus-Host Disease International Consortium.

Investigative site clinical diagnosis of aGvHD will be based on clinical, laboratory, and histological assessments on site. Collection of data for determination of clinical diagnosis of aGvHD and clinical staging of aGvHD will be based on the recommendations of MAGIC (Harris et al 2016; Appendix 2). Grading of aGvHD for assessment of the primary and applicable secondary efficacy endpoints will be based on the IBMTR Severity Index (Rowlings et al 1997; Appendix 3). As sensitivity analyses, all aGvHD-related efficacy endpoints will also be analyzed using the modified Consensus Criteria detailed in the aGvHD grading system from MAGIC (Harris et al 2016; Appendix 4). Grading of cGvHD will be based on the National Institutes of Health (NIH) criteria (Jagasia et al 2015; Appendix 5). Patients who develop clinical signs and

symptoms of aGvHD after hospital discharge or Day +30 post-HSCT may require more frequent monitoring, and re-admission to the hospital will be at the investigator's discretion.

For both treatment arms of the study, patients who develop aGvHD should be treated for aGvHD based on local institutional guidelines, physician preference, and patient need. Patients in the defibrotide prophylaxis arm diagnosed with aGvHD prior to Day +30 post-HSCT and receiving defibrotide prophylaxis at the time of diagnosis may continue to receive defibrotide at the discretion of their physician up to a maximum of Day +30 post-HSCT. Patients in the standard of care arm will not receive defibrotide for the treatment of aGvHD.

If a patient in either the defibrotide prophylaxis arm or standard of care arm develops VOD during the course of the study, the investigator may treat with Defitelio (commercially available defibrotide) if clinically indicated. Investigators should not use study drug for treatment of VOD. If patients in the defibrotide prophylaxis arm are diagnosed with VOD prior to Day +30 post-HSCT and are receiving defibrotide prophylaxis at the time of diagnosis, they may continue to receive defibrotide up to Day +30 post-HSCT and start Defitelio treatment thereafter. They will need to clearly demonstrate the diagnosis of VOD and the use of Defitelio should be clearly stated as for use in VOD. Details of VOD diagnosis will be captured as per the schedule of procedures and assessments.

Efficacy will also be assessed through monitoring for aGvHD-free survival, GvHD-free and relapse-free survival, relapse of disease, relapse-free survival, cGvHD, and overall survival.

Steroid use will be evaluated for the treatment of aGvHD by Day +180 post-HSCT.Other assessments include HRQoL, and hospital resource utilization. Measurement of biomarkers in blood for assessment of their potential as predictive or diagnostic biomarkers of GvHD and response to defibrotide prophylaxis will also be performed.

Safety will be assessed through monitoring of AEs, SAEs, vital signs, physical examinations, clinical laboratory tests, and Karnofsky/Lansky performance scales (PSs). In addition, the following parameters will be assessed for safety:

- Time to neutrophil and platelet engraftment and cumulative incidence of graft failure by Day +100 post-HSCT
- Incidence of infectious disease by Days +100 and +180 post-HSCT (see Section 6.8.7).

Schedule of procedures and assessments is provided in Appendix 1.

3.2. Rationale for Study Design and Control Arm

Standard of care immunoprophylaxis was selected as the control for this study, and the options available for use as aGvHD immune-prophylaxis are the most common regimens offered to patients with URDs receiving either MAC or RIC. Use of placebo control was considered unethical due to increased risk of infection, bleeding, possible fluid overload, and patient discomfort should additional venous access need to be obtained in order to blind study staff to treatment arm assignment. Criteria for diagnosis of aGvHD and staging of aGvHD organ involvement in both arms of the study using the MAGIC guidelines is an effort to minimize the subjectivity in aGvHD diagnosis and staging and distribute this risk equally between study arms in this open-label trial. While comprehensive, the MAGIC guidelines do not cover every conceivable scenario; thus Medical Monitor adjudication may be required. Additional information (eg., related labs, adverse events, concomitant medications, etc.) will be recorded for adjudication purposes.

3.3. Study Duration and Dates

The study is expected to last approximately 24 months, with an estimated enrollment period of 18 months for the planned total of 150 patients. Duration of participation for each patient is expected to last approximately 6 months.

The study will consist of a screening period lasting up to 28 days, a treatment period of variable time (dependent on length of conditioning and time on defibrotide/observation), and a follow-up period up to 180 days post-HSCT (the last visit in the study for each patient). The end of trial is the date when the last patient is assessed or receives an intervention for evaluation in the study, at the follow-up of Day +180 post-HSCT, early termination visit, or last visit at which the patient is assessed or receives an intervention as part of the study, if the patient does not complete the Day +180 post-HSCT/early termination visit.

4. SELECTION OF STUDY POPULATION

Adult and pediatric patients scheduled to undergo HSCT who are at high risk of developing aGvHD will be enrolled in the study.

A total of 150 patients from approximately 60 enrolling study sites in North America and Europe are planned.

4.1. Inclusion Criteria

Each patient must meet the following criteria to be enrolled in this study:

1. Patient must be ≥1 year of age at screening and undergoing allogeneic HSCT.
2. Patient must be diagnosed with acute leukemia in morphologic complete remission (CR1 or CR2) or with MDS with no circulating blasts and with less than 5% blasts in the BM.
3. Patient must have planned to receive either MAC or RIC regimen (patients who receive non-myeloablative regimens are not eligible) and have an URD who is HLA matched or single-allele mismatched (7/8 or 8/8 match at HLA-A, -B, -C, -DRB; or 9/10 or 10/10 match at HLA-A, -B, -C, -DRB, and -DQB1 at high resolution using DNA-based typing).
4. Patient must receive the following medical regimen as part of standard of care immunoprophylaxis for GvHD in either study arm at doses and regimen determined by local institutional guidelines, physician preference, and patient need:

   MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (ATG use is limited to 30% of patients).
5. Graft must be a CD3+ T-cell replete PBSC graft or non-manipulated BM graft.
6. Patient has total bilirubin <2x the upper limit of normal (ULN; unless elevated bilirubin is attributed to Gilbert's Syndrome) and both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) <3x ULN.
7. Female patients of childbearing potential who are sexually active and male patients who are sexually acitive and have female partners must agree to use a highly effective method of contraception with their partners during exposure to defibrotide and for 1 week after the last dose of defibrotide. Highly effective methods of contraception that may be used by the patient include abstinence (when this is in line with the preferred and usual lifestyle of the patient [periodic abstinence, eg, calendar, post-ovulation, symptothermal methods, and withdrawal are not acceptable methods]), combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (ie, birth control pills, patches, vaginal ring), progestogen-only hormonal contraception associated with inhibition of ovulation (ie, progestin implant or injection), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), surgical sterilization, and vasectomy (>6 months before study Day 1). Surgically sterile woman and men and post-menopausal women (ie, women with >2 years of amenorrhea) do not need to use contraception.

8. Negative serum pregnancy tests (for female patients) (performed locally) at Screening prior to treatment with study drug, and as required by local guidelines.
9. Adult patients must be able to understand and sign a written informed consent. For pediatric patients, the parent/legal guardian or representative must be able to understand and sign a written informed consent. Assent, when appropriate, will be obtained according to institutional guidelines.

4.2. Exclusion Criteria

Patients who meet any of the following criteria will be excluded from the study:

1. Patient has had a prior autologous or allogeneic HSCT.
2. Patient has acute bleeding that is clinically significant within 24 hours before the start of study treatment, defined as either of the following (a or b):
   a. Hemorrhage requiring >15 cc/kg of packed red blood cells (eg, pediatric patient weighing 20 kg and requiring 300 cc packed red blood cells/24 hours, or an adult weighing >70 kg and requiring 3 units of packed red blood cells/24hours) to replace blood loss, or
   b. Bleeding from a site which, in the investigator's opinion, constitutes a potential life-threatening source (eg, pulmonary hemorrhage or CNS bleeding), irrespective of amount of blood loss.
3. Patient used any medication that increases the risk of bleeding within 24 hours before the start of study treatment, including, but not limited to, systemic heparin, low molecular weight heparin, heparin analogs, alteplase, streptokinase, urokinase, antithrombin III (ATIII), oral anticoagulants including warfarin, and other agents that increase the risk of bleeding. Patients may receive heparin or other anticoagulants for routine central venous line management and intermittent dialysis or ultrafiltration. Fibrinolytic instillation for central venous line occlusion is also permitted.
   Note: Heparin used to keep catheters open will be allowed (up to 100 U/kg/day).
4. Patient is using or plans to use an investigational agent for the prevention of GvHD.
5. Patient is receiving or plans to receive other investigational therapy.
6. Patient, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.
7. Patient has a psychiatric illness that would prevent the patient or legal guardian or representative from giving informed consent and/or assent.
8. Patient has a serious active disease or co-morbid medical condition, as judged by the investigator, which would interfere with the conduct of this study.
9. Patient is pregnant or lactating and does not agree to stop breastfeeding.
10. Any other condition that would cause a risk to the patient if he/she participated in the trial.

11. Patient has a known history of hypersensitivity to defibrotide or any of its excipients. Patient has a known hypersensitivity to agent(s) (or excipients of agent[s]) within the immunoprophylaxis regimens allowed by the protocol and also to be part of the patient's planned immunoprophylaxis regimen (refer to the approved product label and reference therapy section for additional details).
12. Patient has any contraindications to agent(s) (or excipients of agent[s]) within the immunoprophylaxis regimens allowed by the protocol and also to be part of the patient's planned immunoprophylaxis regimen (refer to approved product label and reference therapy section for additional details).

5. STUDY TREATMENT

5.1. Description of Treatment

5.1.1. Study Drug

Defibrotide (defibrotide sodium) is a clear light yellow to brown solution supplied as 200 mg/ 2.5 mL (concentration of 80 mg/mL) in single-patient-use clear, glass vials. After dilution with 0.9% sodium chloride or 5% dextrose in water (D5W), the final solution should be free of particulates and turbidity.

Excipients include sodium citrate dihydrate, hydrochloric acid and sodium hydroxide (for pH adjustment), and water for injection.

5.1.2. Reference Therapy

The reference therapy in this study is standard of care according to local institutional guidelines, physician preference, and patient need, excluding defibrotide and agents that increase the risk of bleeding (see Section 5.7). Reference therapy will not be provided by the Sponsor. Please refer to SmPC or approved label for warnings and precautions associated with reference therapy.

5.2. Treatments Administered

Patients will be randomly assigned in a 1:1 ratio to receive defibrotide 25 mg/kg/day added to standard of care immunoprophylaxis or standard of care immunoprophylaxis alone.

5.2.1. Defibrotide Administration

Defibrotide solution is administered intravenously by study site personnel at a dose of 25 mg/kg/day, divided into 4 equal doses of 6.25 mg/kg/dose given as 2-hour infusions every 6 hours. Defibrotide may be held for surgical procedures or to accommodate other urgent medication delivery without necessitating dose modification. For surgical procedures, it is recommended that defibrotide administration be completed more than 2 hours prior to intervention. Scheduling may also be adjusted to accommodate other medications and interventions, such as dialysis. While the intent is to treat every 6 hours, the schedule may be adjusted without rendering the patient ineligible or causing protocol violation as long as the daily dose does not substantially alter. A dosing window of ±30 minutes is allowed with a minimum of 2 hours separating the end of 1 infusion and the beginning of another. Individual doses of defibrotide are determined for individual patients based on body weight at baseline. Baseline is defined as the day defibrotide starts for the defibrotide prophylaxis arm or conditioning therapy starts for the standard of care arm. To facilitate efficient drug administration, each of the 4 divided doses per day will be rounded to the nearest 10 mg for patients weighing >35 kg and the nearest 5 mg for patients weighing ≤35 kg.

After dilution, with D5W or 0.9% sodium chloride, the final concentration of defibrotide for administration should be in the range of 4 mg/mL to 20 mg/mL, as appropriate for infusion over 2 hours. Detailed procedures for preparation of study drug will be provided separately.

If a patient on defibrotide develops bleeding or hypersensitivity, defibrotide should be discontinued. Additionally, temporary discontinuation of defibrotide is recommended for patients at significant risk of major bleeding who are receiving defibrotide and undergo surgery or invasive procedures (see Section 1.8).

5.2.1.1. Defibrotide Prophylaxis

For patients randomized to receive defibrotide prophylaxis, defibrotide will be administered prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended prophylaxis duration of ≥21 days and ending no later than Day +30 post-HSCT. Patients in this arm of the study should also receive standard of care immunoprophylaxis using the agents listed in Section 5.1.2, with dosing and duration of therapy based on local institutional guidelines, physician preference, and patient need. Patients must undergo Day +14 post-HSCT assessments before hospital discharge. If a patient is discontinued from study/study drug prior to Day +14 post-HSCT, the Day +14 post-HSCT assessments must be completed on the day of study/study drug discontinuation (±2 days).

5.2.1.2. Extended Defibrotide Administration in the Defibrotide Prophylaxis Arm for Patients who Develop aGvHD

Patients in the defibrotide prophylaxis arm diagnosed with aGvHD prior to Day +30 post-HSCT and receiving defibrotide prophylaxis at the time of diagnosis may continue to receive defibrotide at the discretion of their physician up to Day +30 post-HSCT.

Note: There are no known benefits in the use of defibrotide as treatment for aGvHD. Patients in the defibrotide prophylaxis arm who have discontinued defibrotide prophylaxis and are subsequently diagnosed with aGvHD prior to Day+30 may not re-start defibrotide as treatment for aGvHD.

5.2.1.3. Defitelio Rescue Treatment for Patients who Develop VOD

If a patient in either the defibrotide prophylaxis arm or standard of care arm develops VOD during the course of the study, the investigator may treat with Defitelio (commercially available defibrotide) if clinically indicated. Investigators should not use study drug for the treatment of VOD. If patients in the defibrotide prophylaxis arm are diagnosed with VOD prior to Day +30 post-HSCT and are receiving defibrotide prophylaxis at the time of diagnosis, they may continue to receive defibrotide up to Day +30 post-HSCT and start Defitelio treatment thereafter. The patient must have a documented diagnosis of VOD and the use of Defitelio as treatment for VOD must be clearly indicated on the appropriate case report form. Details of the criteria used to diagnose VOD must be captured on the appropriate case report form. Defitelio may be administered as treatment for VOD until resolution of VOD or hospital discharge, whichever is sooner, and may continue beyond Day +30 post-HSCT. Patients must be hemodynamically

stable and not at risk of bleeding, as listed in Section 4.2, to receive Defitelio as treatment for VOD. For additional information regarding the use of Defitelio to treat VOD, please refer to the package insert included with the prescribed supply of Defitelio.

5.2.2. Reference Therapy

Patients randomized to the standard of care arm should receive standard of care immunoprophylaxis therapy according to local institutional guidelines, physician preference, and patient need. The standard of care options available are intended to serve as study control for comparison with those patients randomized to receive defibrotide prophylaxis in addition to standard of care immunoprophylaxis. Administration of standard of care immunoprophylaxis should be based on local institutional guidelines, physician preference, and patient need regarding dosing and duration of each immunosuppressive agent. Please refer to SmPC or approved label for warnings and precautions associated with reference therapy.

5.3. Selection and Timing of Defibrotide Dosing for Each Patient

All patients who receive defibrotide will receive the same dosage (25 mg/kg/day divided into 4 equal doses of 6.25 mg/kg/dose) and will follow the same regimen (2-hour infusions every 6 hours daily). To facilitate efficient drug administration, each of the 4 divided doses per day will be rounded to the nearest 10 mg for patients weighing >35 kg and the nearest 5 mg for patients weighing ≤35 kg.

5.4. Method of Assigning Patients to Treatment Arms

Patients will be randomly assigned to receive defibrotide prophylaxis in addition to standard of care immunoprophylaxis or standard of care immunoprophylaxis alone in a 1:1 ratio in an open-label fashion after they qualify for participation in the study. The investigator or designee will access IRT to obtain treatment assignments for patients eligible to participate in the study.

5.5. Randomization

A copy of the master randomization code will be provided to the head of the Sponsor's Quality Department or a designee in a sealed envelope, and the Sponsor will be blinded to the master randomization code.

Randomization will be stratified by age at screening (<17 years vs ≥17 years), geographical region (North America vs Europe), and use of ATG (ATG vs no-ATG) using an IRT. Use of ATG will be limited to 30% of patients. A permuted block design with above stratification factors will be used.

5.6. Blinding

Blinding is not applicable in this open-label study.

5.7. Prior and Concomitant Therapy

5.7.1. Prior Medications

All medications and therapies current at the time of screening through baseline, and all prior therapies for the malignant disease will be recorded as prior medications.

5.7.2. Medications for Conditioning Prior to HSCT

All medications and therapies (including radiation therapy) planned to be administered as part of the conditioning regimen (also known as the preparative regimen) will be recorded. In addition, actual doses and administration will be recorded. Patients who do not proceed to MAC or RIC will be identified.

5.7.3. Concomitant Medications and Concomitant Medications of Special Interest

All medications and therapies taken between baseline and Day +63 post-HSCT will be recorded as concomitant medications. Medications and therapies administered as standard of care (for both defibrotide prophylaxis and standard of care arms) will also be recorded.

All special interest medications and therapies taken between baseline and Day +180 post-HSCT (end of study or early termination) will be recorded as concomitant medications of special interest. Concomitant medications of special interest in either study arm include: steroid medication and therapies, treatment and prophylaxis for GvHD, and Defitelio used to treat VOD. Please see Appendix 2 for further details on the MAGIC guidelines for steroid treatment guidance.

5.7.4. aGvHD Prophylaxis or Treatment Medications

Graft-versus-host disease prophylaxis should be administered according to local institutional guidelines, physician preference, and patient need, and should include the following:

- MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (rabbit-derived ATG-Fresenius or Thymoglobulin); ATG use is limited to 30% of patients.

Acute GvHD medications will be recorded as follows:

- Medications used for the prevention of aGvHD: prophylaxis
- Medications used for the treatment of aGvHD: treatment

5.7.5. Prohibited Medications

Medications that increase the risk of hemorrhage are prohibited at the time of HSCT or within 24 hours of the first dose of study drug (defibrotide prophylaxis) and throughout the study. These include, but are not limited to, systemic heparin, low molecular weight heparin, heparin analogs, alteplase (t-PA), streptokinase, urokinase, ATIII, and oral anticoagulants, including warfarin, and other agents that increase the risk of bleeding. Note: Patients may receive heparin or other anticoagulants for routine central venous line management, and intermittent dialysis or

ultrafiltration. Fibrinolytic instillation for central venous line occlusion is also permitted. Heparin use is allowed throughout the study for patients in both treatment arms (up to a maximum of 100 U/kg/day).

If patients are randomized to the standard of care arm, investigators should not prescribe Defitelio as part of their standard of care regimen. Defitelio is not prohibited, however, for treatment of VOD.

The Sponsor must be notified of any instances in which excluded therapies are administered.

5.8. Treatment Compliance

Defibrotide will be administered by study site personnel, and all administrations will be recorded in the electronic case report form (eCRF). Treatment compliance will be monitored throughout the study.

5.9. Packaging and Labeling

Defibrotide will be supplied to the study sites by the Sponsor in vials containing 200 mg defibrotide at a concentration of 80 mg/mL.

All packaging and labeling operations will be performed according to current Good Manufacturing Practices, GCP, and local requirements.

5.10. Storage and Accountability

Defibrotide will be stored, inventoried, reconciled, and retained or destroyed according to applicable state and federal regulations and instructions from the Sponsor.

Defibrotide solution does not contain preservatives. Unopened vials of defibrotide are to be stored at room temperature according to the carton/vial label. Diluted defibrotide solution must be used within 4 hours if stored at room temperature, or within 24 hours if stored under refrigeration (2°C to 8°C), and then subsequently discarded. Use of diluted defibrotide is not permitted outside of these time ranges. Partially used vials should also be discarded, and must not be used across patients.

The investigator or pharmacist will maintain accurate records of receipt of all defibrotide, including dates of receipt. Defibrotide must be kept in a secure area. Unused (or partially used) supplies must be accounted for on the drug inventory record. The receipt and dispensing of all defibrotide must be documented throughout the study and reconciled at study completion.

After the study has been completed and all drug accountability records have been completed and reviewed, all unused clinical supplies are to be disposed of per instructions from the Sponsor. The investigator must provide a written explanation for any missing study drug. One copy of the drug inventory record will be retained at the study site and the other will be retained by the Sponsor.

6. STUDY PROCEDURES

6.1. Informed Consent/Assent

All patients will provide their written informed consent and/or assent, as applicable, before any study-related procedures are performed. Parents/legal guardians or representatives of minor patients will also provide written informed consent in accordance with local Institutional Review Board (IRB)/Independent Ethics Committee (IEC) requirements.

Each patient's chart will have his or her signed informed consent form (ICF) and/or assent form for study participation attached to it. When the study treatment is completed and the eCRF has been monitored, the ICF will be kept in the investigator's central study file. Regulatory authorities may check the existence of the signed ICF in this central study folder. All patients will be given a copy of their signed ICF.

6.2. Medical History, Information Pertaining to Underlying Disease/ Any Prior HSCTs, and Current HSCT

6.2.1. Medical History

A complete medical history including standard body systems and any ongoing infections will be collected.

6.2.2. Information Pertaining to Underlying Disease

Information pertaining to the underlying disease, including date of initial diagnosis, date of recurrent disease, if applicable, prior treatment for diagnosis (eg, surgery, radiation, chemotherapy), if applicable, will be collected.

6.2.3. Information Pertaining to Current HSCT

Information pertaining to the current HSCT, including type of graft, source of graft, degree of HLA matching, and any other pertinent information will be collected.

6.3. Efficacy Assessments

Efficacy will be assessed through monitoring of patient symptoms, physical examinations, laboratory testing, imaging studies as needed, biopsy/pathology for development of aGvHD, disease relapse, and survival.

6.3.1. Primary Efficacy Assessment for Cumulative Incidence of Grade B-D aGvHD by Day +100 Post-HSCT

6.3.1.1. Diagnosis of aGvHD

The clinical diagnosis of aGvHD will be based on clinical judgment guided by absolute quantification of symptoms (eg, extent of rash, total bilirubin level, volume of diarrhea) according to the guidance detailed by MAGIC, regardless of suspected/proven etiology, and by

the results of any laboratory, imaging, biopsy and/or other patient evaluations deemed necessary by the treating physician per local guidelines and standard of care. However, per the MAGIC guidelines, aGvHD will be considered present and counted as an event in the evaluation of all aGvHD endpoints if the confidence level for the diagnosis is either "Confirmed" or "Probable" per criteria detailed in Table 1. Confidence levels for attribution of symptoms to a diagnosis of aGvHD are based on treatment decisions made by the clinician. When symptoms develop that are concerning for aGvHD, an overall confidence level is assigned to the diagnosis of aGvHD based on the highest organ-specific confidence level assigned as supported by categorized facts (eg, biopsy results and infectious studies) and by clinical action (Table 1). Thus, each target organ is assigned a confidence level and the highest confidence level in any organ becomes the overall confidence level for the diagnosis of aGvHD ("Confirmed" is the highest confidence level). Please see Appendix 2 for further details on the MAGIC guidelines for aGvHD data collection, staging and grading.

Table 1: Confidence Level Criteria for Diagnosis of aGvHD

Confidence level	Pathologic evidence	Clinician assessment	Treatment for aGVHD	Comments
Confirmed (GvHD diagnosis proven by biopsy)	Unequivocal pathologic evidence of GvHD	GvHD is the etiology for symptoms	Yes	GvHD is clearly present even if other etiologies may co-exist simultaneously
Probable (Clinician assessed as GvHD diagnosis and/or treatment started)	Not required	GvHD most likely etiology for symptoms	Yes	GvHD is most likely present but other etiologies may also explain the symptoms and there is insufficient evidence to make a confirmed diagnosis
Possible (aGvHD in differential)	Not required	GvHD in differential diagnosis	No	GvHD may be present, but other etiologies are favored to the degree that GvHD treatment is not initiated
Negative (only 1 required)	Unequivocal evidence of a diagnosis other than GvHD (eg, drug rash)	GvHD is not considered as an explanation for the symptoms	No **and** the symptoms resolve without GvHD treatment[a]	A "negative" biopsy (eg, normal skin) is not unequivocal evidence of a diagnosis other than GvHD

Note: Because aGvHD rarely occurs prior to Day +14 post-HSCT and symptoms such as rash, nausea, and diarrhea are often present, a confidence level of "Possible" is not entertained in this early time period. However, when aGvHD is diagnosed and treated, a confidence level of "Probable" or Confirmed" can be assigned during the first 2 weeks post-HSCT depending on whether aGvHD is biopsy-proven.

[a] If GvHD in 1 organ (eg, gastrointestinal tract) is being treated and another symptomatic organ does not have GvHD (eg, liver biopsy confirms VOD and absence of GVHD), then the symptom in the non-GvHD organ does not need to resolve without treatment for the organ to be considered uninvolved (and negative confidence level).

aGvHD=acute graft-versus-host disease.

The aGvHD diagnosis date is the earlier of the following:

- Date physician clearly determines, or confirms in response to a query, a diagnosis of aGvHD (**Probable** confidence level [CL]), or
- Date systemic treatment is started for a presumed diagnosis of aGvHD as the most likely etiology for symptoms (**Probable** CL), or
- Date a biopsy was performed that demonstrates the presence of aGvHD (**Confirmed** CL)

Biopsy reports will be assessed locally. Pathology results will be used to assess aGvHD target organ confidence levels per MAGIC (Appendix 2).

The primary endpoint is the cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT and assigned an overall confidence level for aGvHD diagnosis of either "Confirmed" or "Probable."

6.3.1.2. Staging of aGvHD

Staging of aGvHD will be based on target organ criteria as described per MAGIC (Appendix 2).

6.3.1.3. Grading of aGvHD

Grading of aGvHD for assessment of the primary and applicable secondary efficacy endpoints will be based on the IBMTR Severity Index (Rowlings et al 1997; Appendix 3). As sensitivity analyses, all GvHD-related efficacy endpoints will also be analyzed using the modified Consensus Criteria detailed in the aGvHD grading system from MAGIC (Harris et al 2016; Appendix 4). Refer to Section 9.8 for additional details.

6.3.2. Secondary Efficacy Assessments

Secondary efficacy assessments include Grade B-D aGvHD-free survival by Days +100 and +180 post-HSCT; cumulative incidence of Grade B-D aGvHD by Day +180 post-HSCT; cumulative incidence of Grade C-D aGvHD by Days +100 and +180 post-HSCT; and cumulative incidence of relapse by Days +100 and +180 post-HSCT.

6.3.2.1. Cumulative Incidence of Grade B-D aGvHD by Day +180 Post-HSCT

Cumulative incidence of Grade B-D aGvHD by Day +180 post-HSCT will be assessed based on criteria for aGvHD assessment in Section 6.3.1.3.

6.3.2.2. Cumulative Incidence of Grade C-D aGvHD by Days +100 and +180 Post-HSCT

Cumulative incidence of Grade C-D aGvHD by Days +100 and +180 post-HSCT will be assessed based on criteria for aGvHD assessment in Section 6.3.1.3.

6.4. Evaluation of Steroid Use in the Treatment of aGvHD

The incidence of systemic steroid use in the treatment of aGvHD will be evaluated by Day +180 post-HSCT.

6.5. Health-Related Quality of Life

Patient-reported outcomes, including multi-dimensional questionnaires to measure quality of life and functional status, are a powerful method used to assess a patient's experience with an experimental therapeutic regimen. Different HRQoL questionnaires will be administered to adults, and children and their parents, as applicable (Table 2).

Table 2: Health-Related Quality of Life Questionnaires

Questionnaire			Adults	Pediatric		Recall period
				Self-report	Parent report	
FACT-BMT-TOI			≥16 years			Past 7 days
EQ-5D	EQ-5D-5L		≥16 years			Current health
	EQ-5D-Y	Proxy version 1			4 to 7 years	Current health
		Self-report version		8 to <16 years		Current health

EQ-5D=EuroQoL-5D health questionnaire; EQ-5D-5L=5-Level EuroQoL-5D health questionnaire; EQ-5D-Y=EuroQoL-5D health questionnaire for Youth; FACT-BMT-TOI=Functional Assessment of Cancer Therapy-Bone Marrow Transplant-Trial Outcomes Index.

The EQ-5D will be used to calculate utility values for the different health states experienced by patients in the study.

6.6. Health Economics (Duration of Hospital Stay)

The duration of hospital stay (including any re-admissions) will be recorded in the eCRF as the date and time of admission and the date and time of discharge. As a subset of hospitalization days, the duration of days spent in the intensive care unit (ICU) will be recorded in the eCRF as the date and time of admission to the ICU and the date and time of discharge from ICU.

6.7. Biomarker Assessments

Blood samples (3 mL for pediatric patients; 7 mL for adult patients) to evaluate plasma concentration of potential GvHD biomarkers will be obtained from all patients (weighing >15 kg) who have provided consent or assent, at the following time points:

- At baseline (prior to conditioning)
- On Day +7 post-HSCT

- On Day +14 post-HSCT
- On last day of treatment with study drug
- On Day +100 post-HSCT
- On Day +180 post-HSCT
- Upon diagnosis of aGvHD
- 14 days following diagnosis of aGvHD

If hospital discharge occurs prior to Day +14 post-HSCT, then the Day +14 biomarker sample should be obtained on the day of discharge (see Appendix 1).

To ensure patient safety, biomarker sampling will not be done in any patient predicted to exceed the blood draw maximums detailed in Appendix 6.

6.8. Safety Assessments

Safety will be assessed through monitoring of AEs, SAEs, vital signs, physical examinations, clinical laboratory tests, neutrophil and platelet engraftment and graft failure, Karnofsky/Lansky PSs, and infectious disease.

6.8.1. Adverse Events

6.8.1.1. Reporting of Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered related to study drug or procedure.

Adverse events include, but are not limited to, the following: (1) a worsening or change in nature, severity, or frequency of conditions present at the start of the study; (2) patient deterioration due to primary illness; (3) intercurrent illness; (4) drug interaction; and/or (5) laboratory values assessed as clinically significant by the investigator.

All AEs, whether observed by the investigator, reported by the patient, determined from laboratory findings, vital signs, physical examinations, or other means, must be recorded.

Patients should be questioned in a general way, without asking about the occurrence of any specific symptom. The investigator should attempt to establish a diagnosis (including syndromes) based on signs, symptoms, and/or other clinical information. When a diagnosis or syndrome is established or confirmed, the diagnosis or syndrome, not the individual signs/symptoms, should be documented as the AE.

Following questioning and evaluation, all AEs, whether believed by the investigator to be related or unrelated to the study drug or procedure, must be documented in the patient's medical records, in accordance with the investigator's normal clinical practice. Each AE is to be evaluated for duration, severity, seriousness, outcome, action taken with study drug, and causal relationship to the study drug or procedure.

6.8.1.2. Severity Assessment

Adverse events will be classified by the investigator using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. All appropriate treatment areas should have access to a copy of the CTCAE v4.03. A copy of the CTCAE v4.03 can be downloaded from the Cancer Therapy Evaluation Program website (http://ctep.cancer.gov).

If the CTCAE grade is not specified for a particular event or if the event term does not appear in the CTCAE, general guidelines for grading severity of AEs are provided in Table 3.

When the severity of an AE changes over time, an increase in the severity will be recorded as a new AE, and the original AE will stop when the new AE starts.

Table 3: National Cancer Institute Common Terminology Criteria for Adverse Events Severity Grades General Guidelines

Severity Grade	Terminology Criteria
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[a]
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting age-appropriate self-care ADL[b]
Grade 4	Life-threatening consequences; urgent intervention indicated
Grade 5	Death related to AE

[a] Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[b] Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden
ADL=activities of daily living; AE=adverse event; CTCAE=Common Terminology Criteria for Adverse Events.
Source: CTCAE v4.03 Accessed at: https://evs.nci nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

6.8.1.3. Serious Adverse Events

An SAE is an AE that fulfills any of the following criteria, as per International Council for Harmonisation (ICH) E2A.II.B:

- Is fatal (results in death)
- Is life-threatening (Note: The term "life-threatening" refers to an event in which the patient was at immediate risk of death at the time of the event; it does not refer to an event that could hypothetically have caused death had it been more severe. Grade 4 laboratory values secondary to HSCT are not necessarily serious unless the patient was at immediate risk of death.)
- Requires inpatient hospitalization or prolongs existing hospitalization
- Results in persistent or significant incapacity or disability, defined as substantial disruption of the ability to conduct normal life functions
- Results in a congenital anomaly/birth defect
- Is an important medical event

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above in the definition of an SAE.

Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, and the development of drug dependency or drug abuse.

Suspected transmission of an infectious agent via a medicinal product is considered an important medical event.

An AE should be recorded as an SAE when it meets at least one of the criteria for seriousness. A patient's underlying disease that results in the initial hospitalization for HSCT is not considered an SAE. The following reasons for hospitalization are also NOT considered SAEs:

- Procedures that were planned prior to the patient entering the study
- Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Procedures that are elective in nature and not related to worsening of an underlying condition

Complications that occur during hospitalizations are AEs. If a complication prolongs the hospitalization, it is an SAE.

"Inpatient hospitalization" means the patient has been formally admitted to a hospital for medical reasons, for any length of time. Emergency room care without admission to a hospital is considered outpatient care.

Overdose, medication errors, and drug misuse of the study drug are considered reportable experiences and should be reported by study site personnel on an Other Reportable Experience Form. The form and contact information for submission of the form will be provided to the study sites separately.

6.8.1.4. Causal Relationship to Study Drug or Procedure

The investigator's assessment of the relationship of AE to study drug and to study procedures is required. The relationship or association of the study drug or procedure in causing or contributing to the AE will be characterized using the following classification and criteria:

Related to Study Drug or Procedure	***There is a reasonable possibility that the study drug or procedure caused the event, ie, there is evidence to suggest a causal relationship.*** Some temporal relationship exists between the event and the administration of the study drug or procedure and the event is unlikely to be explained by the patient's medical condition, other therapies, or accident.
Not Related to Study Drug or Procedure	***There is not a reasonable possibility or clinical evidence that the study drug or procedure caused the event.*** The event can be readily explained by other factors such as the patient's underlying medical conditions, concomitant therapy, or accident; or there is no temporal relationship between study drug or procedure and the event.

6.8.1.5. Adverse Event Recording and Reporting

For patients in either the defibrotide prophylaxis or standard of care arm, the investigator must record all AEs and SAEs that occur from the time written informed consent is obtained until Day +63 post-HSCT (or screen failure), regardless of their relationship to study drug or procedure.

For patients who do not receive HSCT, AEs will be collected up to 70 days after baseline.

In addition, any SAE assessed as related to study drug or study procedures by the investigator after Day +63 post-HSCT, must be reported as described below.

SAEs must be reported to the Sponsor or its designee using the SAE Reporting Form within 24 hours of first knowledge of the event by study site personnel. The SAE Reporting Form and contact information for submission of the form, will be provided to the study sites separately.

The SAE Reporting Form must be completed as thoroughly as possible before transmittal to the contact provided on the form. The investigator must provide his/her assessment of causality to the study drug and the study procedure at the time of the initial report.

6.8.1.6. Follow-up of Adverse Events and Serious Adverse Events

All AEs and SAEs assessed as not related to study drug or procedure, including clinically significant laboratory tests, or physical examination findings, must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the final study visit occurs, whichever comes first.

AEs and SAEs assessed as related to study drug or procedure will be followed for as long as necessary to adequately evaluate the patient's safety, or until the event stabilizes, or the patient is lost to follow up. If resolved, a resolution date should be recorded.

AEs and SAEs resulting in termination will be followed to the satisfactory resolution and determination of outcome as ascertained by the investigator (and/or the Sponsor or its designee).

The investigator is responsible for ensuring that follow-up includes any supplemental investigations indicated to elucidate the nature and/or causality of the event. This may include additional clinical laboratory testing or investigations, examinations, histopathological examinations, or consultation with other health care professionals as is practical, according to the Sponsor's requests.

The investigator should provide follow-up SAE information for any updates to information previously provided to the Sponsor or as requested by the Sponsor.

6.8.1.7. Pregnancy

Patients' HSCT preparative regimens commonly include agents which may cause infertility and are known to have teratogenic effects. As such, there is a low likelihood that patients may become pregnant. However, due to the teratogenic effects of the preparative regimens, and the unknown effects of defibrotide use during pregnancy, highly effective methods of contraception are required (see Section 4.1, inclusion criterion #7).

If a female patient (or female partner of a male patient) of childbearing potential becomes pregnant at any time after the first dose of study drug and up to Day +63 post-HSCT, it must be reported to the Sponsor or its designee using the Pregnancy Report Form within 24 hours of first knowledge of the event by study site personnel. Study drug must be stopped for any pregnant patient (see Section 6.9.1).

The pregnancy of a female patient (or female partner of a male patient) must be followed until the outcome of the pregnancy is known, and in the case of a live birth, for 6 months following the birth of the child. The Infant Follow-up Form should be used to report information regarding the status of the infant.

The Pregnancy Report Form, Infant Follow-up Form, and contact information for submission of the form, will be provided to the study sites separately.

6.8.1.8. Regulatory Reporting

The Sponsor or its designee is responsible for reporting to the relevant regulatory authorities, central ethics committees (CECs), and participating investigators, and will report in accordance with ICH guidelines, the US Code of Federal Regulations (CFR), the EU Clinical Trial Directive, and local regulatory requirements.

The reference safety information to determine expectedness of SAEs is specified in the IB for defibrotide.

All suspected unexpected serious adverse reactions (SUSARs) will be reported to the relevant regulatory authorities, CECs, and all participating investigators no later than 15 days after first knowledge of the event.

SUSARs that are fatal or life-threatening will be reported to the relevant regulatory authorities, CECs, and participating investigators no later than 7 days after knowledge of such a case, and relevant follow-up information provided within an additional 8 days.

Once a year throughout the clinical study, a report listing of all SUSARs (and SAEs if required by local regulation) that have occurred during this period and a report of the patient's safety will be submitted to the applicable authorities, and as otherwise required by local laws.

Reporting of SAEs by the investigator to his/her local IRB/IEC will be done in accordance with the standard operating procedures and policies of the IRB/IEC. Adequate documentation must be maintained showing that the IRB/IEC was properly notified.

6.8.2. Clinical Laboratory Tests

Clinical laboratory tests will be performed at local laboratories. It is anticipated that patients will undergo laboratory testing both as an inpatient and an outpatient. The investigator will provide to the Sponsor or its designee the current licensure and laboratory reference ranges for all laboratories used during the study.

Refer to Appendix 1 for the individual laboratory tests to be performed during the study. If a patient is at risk for exceeding maximal allowable blood draw limits (per Seattle Children's Hospital Guideline for Maximum Blood Volumes, Appendix 6), the blood draw schedules for laboratory assessments may be adjusted per local physician's practice to ensure patient safety and to remain below the blood draw maximums.

Laboratory values assessed as clinically significant by the investigator are AEs and must be recorded as AEs (see Sections 6.8.1.1 and Section 6.8.1.5).

6.8.3. Vital Signs

Vital signs will be measured through Day +63 only.

Adverse events determined from vital sign measurements must be recorded (see Section 6.8.1.5).

6.8.4. Physical Examination

Complete physical examination, including weight and assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen, lymph nodes, extremities, and a general assessment (including edema) will be performed. Height will be measured at baseline only.

Adverse events determined from physicial examinations must be recorded (see Section 6.8.1.5).

6.8.5. Neutrophil and Platelet Engraftment

Patients will be monitored for engraftment per the Schedule of Procedures and Assessments (Appendix 1). If a patient is discontinued early from the study, engraftment status can be reported at the early termination visit.

The investigator will determine the dates for neutrophil engraftment and platelet engraftment based on an algorithm, as described in the subsequent sentences. The dates for neutrophil and platelet engraftment will be recorded separately. The date of neutrophil engraftment is defined as the first date after HSCT of an absolute neutrophil count >0.5 x 10^9/L that is maintained for 3 consecutive days. The definition of "absolute neutrophil count" includes both segmented neutrophils and "bands", immature neutrophils. The date of platelet engraftment is defined as the first date after HSCT of a platelet count >20 x 10^9/L without a platelet transfusion in the preceding 7 days.

6.8.6. Karnofsky/Lansky Performance Scores

Functional impairment will be assessed using the Karnofsky PS for patients ≥16 years of age and the Lansky PS for patients <16 years (Table 4).

Table 4: Karnofsky and Lansky Performance Scores

Percentage	Karnofsky Scale (Patients ≥16 years of age)	Lansky Scale (Patients <16 years of age)
100%	Normal, no complaints, no evidence of disease	Fully active
90%	Able to carry on normal activity, minor signs or symptoms of disease	Minor restriction in physically strenuous play
80%	Normal activity with effort, some signs or symptoms of disease	Restricted in strenuous play, tires more easily, otherwise active
70%	Cares for self, unable to carry on normal activity or to do active work	Both greater restriction of and less time spent in active play
60%	Requires occasional assistance from others but able to care for most needs	Ambulatory up to 50% of time, limited active play with assistance/supervision
50%	Requires considerable assistance from others and frequent medical care	Considerable assistance required for any active play, fully able to engage in quiet play
40%	Disabled, requires special care and assistance	Able to initiate quiet activities
30%	Severely disabled, hospitalization indicated, death not imminent	Needs considerable assistance in quiet activities
20%	Very sick, hospitalization necessary, active support, treatment necessary	Limited to very passive activity initiated by others
10%	Moribund, fatal process progressing rapidly	Completely disabled, not even passive play

Source: Karnofsky et al 1948; Lansky et al 1987.

6.8.7. Infectious Diseases

Infectious disease markers will be assessed per institutional practice to include at minimum: CMV, Hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab) during screening, and CMV at Days +100 and +180 post-HSCT.

6.9. Removal of Patients from the Study or Study Drug

6.9.1. Handling of Early Terminations

All patients are free to withdraw from participation in this study at any time, for any reason, and without prejudice. The investigator must withdraw any patient from the study if the patient states that he/she wants to stop participating in the study. In addition, the investigator, the Sponsor, or its designee, may remove a patient from the study treatment or the study at any time and for any reason, for example, in the case of disease relapse, non-compliance with study drug, or protocol deviation.

However, patients must early terminate/discontinue study drug or study if they have any of the following events:

Reasons to early terminate/discontinue study drug	Reasons to early terminate/discontinue study
• Withdrawal of consent by patient or patient's parent/legal guardian or representative • Adverse event which in the opinion of the investigator precludes continuation of study drug for patient safety • Investigator considers it not in the patient's best interest to continue • Death • Pregnancy • Sponsor (or its designee) decision to terminate study	• Withdrawal of consent by patient or patient's parent/legal guardian or representative • Death • Lost to follow-up • Sponsor (or its designee) decision to terminate study

The specific reason for the discontinuation from study or study drug should be documented on the corresponding eCRFs. If a patient or patient's parent/legal guardian or representative withdraws informed consent, the specific reason for withdrawing the informed consent should be stated. It is vital to obtain follow-up data on any patient who discontinued study drug because of an AE.

Adverse events resulting in termination will be followed to the satisfactory resolution and determination of outcome as ascertained by the investigator (and/or the Sponsor or its designee). The data will be recorded on the appropriate eCRF.

6.9.2. Sponsor's Termination of Study

The Sponsor reserves the right to terminate the study at any time for clinical or administrative reasons, including but not limited to, the following:

- Low enrollment
- Concern for patient safety

Upon notification by the Sponsor, such a termination must be implemented promptly by the investigator, if instructed to do so by the Sponsor, in a timeframe that is compatible with the patients' well-being.

6.10. Appropriateness of Measurements

The safety assessments used in this study are typical for a Phase 2 study and are based on the safety profile of defibrotide, as characterized in several clinical studies and post-marketing experience in another indication.

7. STUDY ACTIVITIES

A schedule of study procedures and assessments for patients in the defibrotide prophylaxis and standard of care arms is provided in Appendix 1. It should be noted that HSCT Day (Day 0) in the study is defined as the day of transplant.

7.1. Pre-HSCT Evaluations

The following evaluations should be completed during screening (≤28 days prior to baseline), unless otherwise indicated:

- Informed consent.
- Inclusion/exclusion criteria (eligibility must be confirmed before randomization; review of planned conditioning regimen for GvHD prophylaxis is required to ensure that the eligibility criteria will continue to be met prior to randomization).
- Demographics.
- Medical history: Medical history, information pertaining to underlying disease, any prior HSCTs, and current HSCT, should be collected.
- Prior medications: Include those current at the time of screening and all prior therapies for the malignant disease.
- Physical examination and weight.
- Complete blood count (CBC) with WBC differential and platelet count, and blood chemistries (serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT). Performed locally.
- Infectious disease markers per institutional practice to include at minimum: CMV antibody and Hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab). Performed locally.
- Disease evaluation for acute leukemia and MDS (performed locally): Bone marrow aspirate and/or biopsy for pathology and cytogenetics. Cytogenetics, molecular, or other institutional assessment to determine Minimal Residual Disease will be recorded at screening to document patient's pre-transplant Minimal Residual Disease status. Bone marrow aspirate and biopsy are required for assessment of disease relapse. If disease relapse assessment can be made using only an aspirate sample, results from assessment of a core biopsy are not required. Results of a biopsy and aspirate performed prior to HSCT as part of the standard of care will be recorded during the screening period. This bone marrow biopsy and aspirate is not required to be performed between Day -28 and baseline.

Disease relapse is defined by either morphological evidence of acute leukemia or MDS consistent with pre-transplant features, documented or not by biopsy. The event is defined as an increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy. Disease relapse will be diagnosed when there is morphological or clinical evidence of:

- Reappearance of leukemia blast cells in the peripheral blood, or
- >5% blasts in the BM, not attributable to another cause (eg, BM regeneration), or
- The appearance of previous or new dysplastic changes (MES specific) within the BM, with or without falling donor chimerism, or
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid, or
- Institution of therapy to treat relapsed disease, including donor lymphocyte infusion

- Serum pregnancy test (performed locally): Must be performed ≤30 days before the start of the transplant conditioning regimen. Pregnancy test is required for females of child-bearing potential (ie, not postmenopausal or surgically sterile), and may be performed per institutional practices.
- Adverse event assessments.
- Randomization (this should be the last screening activity to be completed).

The following evaluations should be completed at baseline (baseline is defined as the day defibrotide prophylaxis starts for the defibrotide prophylaxis arm or conditioning therapy starts for the standard of care arm):

- Prior medications: Include those current at the time of screening through baseline, and all prior therapies for the malignant disease.
- Vital signs
- Physical examination, weight, and height.
- CBC with WBC differential and platelet count, and blood chemistries (serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT). Performed locally.
- Karnofsky/Lansky PS: Functional impairment will be assessed using the Karnofsky PS for patients ≥16 years of age and the Lansky PS for patients <16 years.
- Hospitalization data: Includes dates of hospitalization, dates in ICU, and dates of readmissions.
- Health-related quality of life assessments include the following patient questionnaires: FACT-BMT-TOI and EQ-5D (version dependent on age group; see Section 6.5 for additional details).

- Biomarkers: Blood samples to evaluate plasma concentration of potential GvHD biomarkers will be collected with an assessment window of -3 days.
- Concomitant medications: Include conditioning regimen for HSCT, and medications and therapies administered as standard of care (for both defibrotide prophylaxis and standard of care arms).
- Concomitant medications of special interest in either study arm: Include all steroid medications and therapies, all treatment and prophylaxis for GvHD, and Defitelio used to treat VOD.
- Adverse event assessments.
- Study drug administration:
  - Defibrotide prophylaxis: Administered intravenously as 4 2-hour infusions of 6.25 mg/kg every 6 hours beginning prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days of treatment and ending no later than Day +30 post-HSCT.
  - Standard of care prophylaxis: Administered according to local institutional guidelines, physician preference, and patient need, and include MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (rabbit-derived ATG-Fresenius or Thymoglobulin; ATG use is limited to 30% of patients).
- Survival status.

7.2. HSCT Day (Day 0)

The following evaluations should be completed according to Appendix 1:

- Vital signs.
- Physical examination and weight
- HLA typing (recipient and donor; performed locally): HLA typing information should be available prior to HSCT Day.
- CBC with WBC differential and platelet count, and blood chemistries (serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT). Performed locally.
- Hospitalization data: Includes dates of hospitalization, dates in ICU, and dates of readmissions.
- Concomitant medications: Include conditioning regimen for HSCT, and medications and therapies administered as standard of care (for both defibrotide prophylaxis and standard of care arms).
- Concomitant medications of special interest: Include all steroid medications and therapies, all treatment and prophylaxis for GvHD, and Defitelio used to treat VOD in either study arm.

- Adverse event assessments.
- Study drug administration:
  - Defibrotide prophylaxis: Administered intravenously as 4 2-hour infusions of 6.25 mg/kg every 6 hours beginning prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days of treatment and ending no later than Day +30 post-HSCT.
  - Standard of care prophylaxis: Administered according to local institutional guidelines, physician preference, and patient need, and include MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (rabbit-derived ATG-Fresenius or Thymoglobulin; ATG use is limited to 30% of patients).
- Survival status.

7.3. Post-HSCT Evaluations

The following evaluations should be completed according to Appendix 1:

- Vital signs completed weekly through Day +63 post-HSCT.
- Physical examination and weight completed weekly through Day +91 post-HSCT, then at Days +100 and +180 post-HSCT (end of study or early termination).
- CBC and manual WBC differential performed at least 2 times weekly from day of transplant (ie, HSCT Day [Day 0]) until ANC >0.5 x 10^9/L for 3 days and platelet count >20 x 10^9/L without a platelet transfusion in the preceding 7 days. CBC then performed weekly through Day +100 post-HSCT and at Day +180 post-HSCT (end of study or early termination). Performed locally.
- Blood chemistries (serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT) performed at least 2 times weekly until hospital discharge. Blood chemistries performed weekly after hospital discharge until Day +100 post-HSCT, then at Day +180 post-HSCT (end of study or early termination). If a patient is at risk for exceeding maximal allowable blood draw limits (per Seattle Children's Hospital Guideline for Maximum Blood Volumes, Appendix 6), the blood draw schedules for laboratory assessments may be adjusted per local physician's practice to ensure patient safety and to remain below the blood draw maximums. Performed locally.
- Infectious disease markers per institutional practice to include at minimum: CMV monitoring at Days +100 and +180 post-HSCT. Performed locally.

- Disease evaluation for acute leukemia and MDS (performed locally): Bone marrow aspirate and/or biopsy for pathology and cytogenetics at Day +100 post-HSCT and Day +180 post-HSCT (end of study or early termination). Bone marrow aspirate and biopsy are required for assessment of disease relapse. If disease relapse assessment can be made using only an aspirate sample, results from assessment of a core biopsy are not required. Disease relapse is defined by either morphological evidence of acute leukemia or MDS consistent with pre-transplant features, documented or not by biopsy. The event is defined as an increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy. Disease relapse will be diagnosed when there is morphological or clinical evidence of:
  - Reappearance of leukemia blast cells in the peripheral blood, or
  - >5% blasts in the BM, not attributable to another cause (eg, BM regeneration), or
  - The appearance of previous or new dysplastic changes (MES specific) within the BM, with or without falling donor chimerism, or
  - The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid, or
  - Institution of therapy to treat relapsed disease, including donor lymphocyte infusion
- Pregnancy test (performed locally): Performed per institutional practices prior to treatment with study drug, at Day +28 post-HSCT, at Day +63 post-HSCT, and 1 week after the last dose of defibrotide.
- Neutrophil and platelet engraftment/graft failure performed weekly through Day +91 post-HSCT, then at Days +100 and +180 post-HSCT (end of study or early termination). Performed locally.
- GvHD assessments: MAGIC staging/assessment should be completed at least a) weekly through Week 13, at Day +100 post-HSCT and at Day +180 post-HSCT (aGvHD present or not) and b) at GvHD diagnosis and/or GvHD treatment and for 4 weeks of follow-up for both events (to obtain 4 weeks response data). Example: If GvHD is diagnosed at Day +100 post-HSCT, MAGIC staging/assessments are required at GvHD diagnosis (Day +100 post-HSCT) and for 4 weeks of follow-up (with assessment periods ending on Days +107, +114, +121, and +128 post-HSCT). The last visit of the study is the Day +180 post-HSCT visit, thus follow-up visits are not required to continue beyond the Day +180 post-HSCT visit.

Each assessment period corresponds to a 7 day interval from the first day of stem cell infusion (ie, ends on 7, 14, 21, etc, days from the first day of transplant). Patient GvHD staging is **reported** once per assessment period except for weeks in which GvHD is first diagnosed or treated, in which case the GvHD staging should be completed on those days as well.

Guidance: Patients may be **assessed** for GvHD more than once per week (eg, inpatient where daily assessments are often performed) but only 1 assessment should be **reported**. The GvHD staging(s) and corresponding date that should be reported for an assessment period should be:

- If GvHD staging was performed only once during an assessment period, report the staging and date of the clinical assessment.
- If GvHD staging was performed on multiple days during an assessment period, **and** the staging did not change during the assessment period, report the staging and date closest to the end of the assessment period.
- If GvHD staging was performed on multiple days during an assessment period, report the staging for the day corresponding to the **peak staging** for that period. If the peak stage occurred on more than 1 day, report the day closest to the end of the assessment period.
- If either GvHD diagnosis or treatment develops during an assessment period, report the staging on the day of GvHD diagnosis and GvHD treatment on the *GvHD Diagnosis or Treatment form* **and** report the peak staging from the assessment period on the *Weekly form*. These *might* be the same date but require separate forms (Weekly Assessment and GvHD Diagnosis/Treatment forms).
- For assessment periods where no staging is available, report that the patient was not assessed. Unless patient status is "Off Study", GvHD weekly assessment forms need to be completed for all 13 weeks, at Day +100 post-HSCT and at Day +180 post-HSCT. If the patient was not assessed in Week 13, continue to report Weekly forms, regardless of whether GvHD is active, until the final reporting week is one in which patient was assessed.

- VOD assessments completed up to Day +30 post-HSCT and at Day +100 post-HSCT.
- Hospitalization data collected weekly through Day +91 post-HSCT, then at Days +100 and +180 post-HSCT (end of study or early termination).
- Quality of life assessments completed weekly through Day +28 post-HSCT, then at Days +100 and +180 post-HSCT (end of study or early termination). The assessments include the following patient questionnaires: FACT-BMT-TOI and EQ-5D (version dependent on age group; see Section 6.5 for additional details).

- Biomarkers: Blood samples to evaluate plasma concentration of potential GvHD biomarkers will be collected on Days +7 and +14 post-HSCT, on last day of treatment with study drug, on Days +100 and +180 post-HSCT, upon diagnosis of aGvHD, and 14 days following diagnosis of aGvHD. If hospital discharge occurs prior to Day +14 post-HSCT, then the Day +14 biomarker sample should be obtained on the day of discharge.
- Concomitant medications: Include conditioning regimen for HSCT, and medications and therapies administered as standard of care (for both defibrotide prophylaxis and standard of care arms). Concomitant medications will be assessed weekly through Day +63 post-HSCT.
- Concomitant medications of special interest in either study arm: Include all steroid medications and therapies, all treatment and prophylaxis for GvHD, and Defitelio used to treat VOD. Concomitant medications of special interest will be assessed weekly through Day +180 post-HSCT (end of study or early termination).
- Adverse event assessments: Investigator must record all AEs and SAEs that occur from the time written informed consent is obtained up to Day +63 post-HSCT, regardless of their relationship to study drug or procedure. Only SAEs considered by the investigator to be related to study drug or study procedures should be reported more than Day +63 post-HSCT.
- Study drug administration:
  - Defibrotide prophylaxis: Administered intravenously as 4 2-hour infusions of 6.25 mg/kg every 6 hours beginning prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days of treatment and ending no later than Day +30 post-HSCT.
  - Standard of care prophylaxis: Administered according to local institutional guidelines, physician preference, and patient need, and include MTX or MMF + calcineurin inhibitor (CSA or TAC) +/- ATG (rabbit-derived ATG-Fresenius or Thymoglobulin; ATG use is limited to 30% of patients).
  - If a patient is discontinued from study/study drug prior to Day +14 post-HSCT, the Day +14 post-HSCT assessments must be completed on the day of study/study drug discontinuation (+2 days).
- Survival status collected weekly through Day +91 post-HSCT, then at Days +100 and +180 post-HSCT (end of study or early termination).

8. QUALITY CONTROL AND ASSURANCE

The study will be conducted according to applicable GCP regulations and guidelines. Quality assurance audits may be performed at the discretion of the Sponsor.

9. PLANNED STATISTICAL METHODS

9.1. General Considerations

All study data will be summarized by treatment group using descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) for continuous variables (eg, age, weight) and by the number and percentage of patients for categorical variables (eg, gender, race). All summaries, statistical analyses, and individual patient data listings described below will be completed using Version 9.3 or later of the Statistical Analysis System (SAS Institute, Inc Cary, NC).

9.2. Tests of Hypotheses and Significance Levels

The primary objective of the study is to compare the efficacy of defibrotide added to standard of care immunoprophylaxis (defibrotide prophylaxis arm) vs standard of care immunoprophylaxis alone (standard of care arm) for the prevention of aGvHD as measured by the cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT in adult and pediatric patients. The trial is hypothesis generating and is not powered to detect minimal clinically meaningful differences between treatment groups at a significant (type I) error level of 5%. The trial is designed to obtain meaningful estimates of the treatment difference of the cumulative incidence rates between the 2 treatment arms.

9.3. Determination of Sample Size

The sample size of 150 patients (75 patients per treatment arm) will provide a 90% CI of (-0.28, -0.03) for the treatment difference of the primary endpoint (ie, cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT), assuming the cumulative incidence of 28.6% and 44% for the defibrotide prophylaxis arm and standard of care arm, respectively. The calculation of the CI is based on the large sample normal approximation. The 44% cumulative incidence for the standard of care arm is based on published studies and results from a previously conducted study (Study 2004-000592-33). The 28.6% cumulative incidence for the defibrotide prophylaxis arm is projected based on a relative 35% improvement from the standard of care arm.

9.4. Analysis Sets

The ITT Analysis Set will include all randomized patients. Patients will be analyzed according to the treatment to which they were randomized. This is the primary analysis set for efficacy analysis.

The Safety Analysis Set will include all patients randomized to the defibrotide prophylaxis arm who receive at least 1 dose of defibrotide and all patients randomized to the standard of care arm. Patients will be analyzed according to the treatment they actually received. This is the primary analysis set for safety analysis.

9.5. Handling of Dropouts and Missing Data

Every effort will be made to minimize missing data. For efficacy analysis with time-to-event methodology, data for patients who discontinue from the study early will be included in the analysis of efficacy endpoints up to their censoring time.

9.6. Pooling of Investigation Centers

Data from all study centers will be pooled. Data may also be pooled by region or country for exploratory or sensitivity analyses, as appropriate.

9.7. Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized by treatment group. The summaries of demographics and baseline characteristics will be provided for the Safety Analysis Set and the ITT Analysis Set.

Relevant medical history findings and prior medications will be summarized by system organ class and anatomical therapeutic chemical codes, respectively, using descriptive statistics.

9.8. Efficacy Endpoints and Analyses

Grading of aGvHD for assessment of the primary and applicable secondary efficacy endpoints will be based on the IBMTR Severity Index (Rowlings et al 1997). As sensitivity analyses, all GvHD-related efficacy endpoints will also be analyzed using the modified Consensus Criteria detailed in the aGvHD grading system from MAGIC (Harris et al 2016).

9.8.1. Primary Efficacy Endpoint and Analysis

The primary efficacy analysis will be performed using the ITT Analysis Set.

The primary efficacy endpoint is cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT. It will be estimated by the cumulative incidence competing risk estimator, as described by Marubini and Valsecchi (1995). Death prior to Grade B-D aGvHD by Day +100 post-HSCT will be considered as the competing risk. The difference in the cumulative incidence between the 2 treatment arms will be estimated with 2-sided 90% CI (Zhang and Fine 2008) and the treatment comparison will be based on the Gray's test (Gray 1988).

As a sensitivity analysis, the primary efficacy analysis will be repeated by censoring patients in the standard of care arm at the time of rescue Defitelio initiation if patients in the standard of care arm received rescue Defitelio treatment for VOD.

The timing variable for the primary efficacy endpoint will be anchored at time of HSCT (time=0). It is anticipated that fewer than 2% of patients will not undergo HSCT in the ITT Analysis Set. For those patients, time 0 is counted at randomization. The timing variable is defined as the number of days from time 0 to the onset of aGvHD or death by Day +100 post-HSCT. If a patient does not have aGvHD and is not followed for 100 days, the censoring for that endpoint will be defined at the time of last available evaluation of aGvHD.

9.8.2. Secondary Efficacy Endpoints and Analyses

Secondary efficacy endpoints include the following:

- Grade B-D aGvHD-free survival by Days +100 and +180 post-HSCT
- Cumulative incidence of Grade B-D aGvHD by Day +180 post-HSCT
- Cumulative incidence of Grade C-D aGvHD by Days +100 and +180 post-HSCT
- Cumulative incidence of relapse by Days +100 and +180 post-HSCT

The treatment differences along with the 90% CI will be presented for the secondary efficacy endpoint. These secondary efficacy endpoints will be tested for difference between treatment groups without multiplicity adjustments, and nominal p-values will be reported.

9.8.2.1. Grade B-D aGvHD-free Survival by Days +100 and +180 Post-HSCT

Kaplan-Meier estimates of Grade B-D aGvHD-free survival by Day +100 and Day +180 post-HSCT will be presented for the 2 treatment arms. The grade B-D aGvHD or death will be considered an event in the analyses. Patients will be censored at the last available evaluation of aGvHD if no event occurs. The hazard ratio (HR) of defibrotide prophylaxis arm compared with standartd of care arm from the Cox proportional hazard model will be presented along with the 90% CI. The log rank test will be performed to test the treatment difference.

9.8.2.2. Cumulative Incidence of Grade B-D aGvHD by Day +180 Post-HSCT and Cumulative Incidence of Grade C-D aGvHD by Days +100 and +180 Post-HSCT

In addition to cumulative incidence of Grade B-D aGvHD by Day +100 post-HSCT, cumulative incidence of Grade B-D aGvHD by Day +180 post-HSCT and cumulative incidence of Grade C-D aGvHD by Days +100 and +180 post-HSCT will be analyzed similarly to the primary efficacy endpoint.

9.8.2.3. Cumulative Incidence of Relapse by Days +100 and +180 Post-HSCT

Cumulative incidence of relapse by Days +100 and +180 post-HSCT will be analyzed similarly to the primary efficacy endpoint of treating death prior to disease relapse as a competing event.

9.9. Steroid Use in the Treatment of aGvHD by Day +180 Post-HSCT

The cumulative incidence of systemic steroid use in the treatment of aGvHD by Day +180 post-HSCT will be analyzed similarly to the primary efficacy endpoint using a competing risk analysis.

9.10. Health-Related Quality of Life

For the FACT-BMT, the TOI which consists of the physical well-being scale, the functional well-being scale, and the BMT specific items of the FACT-BMT will be analyzed. Descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) will be provided by time point and treatment arm. Actual values and changes over time in scores will also be summarized. The EQ-5D will also be scored and descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be summarized by time point, treatment group and age category. Actual values and changes over time in scores will also also be summarized. Different versions of the EQ-5D will be used in each of 3 age categories:

- EQ-5D-5L (adults only)
- EQ-5D-Y, proxy version 1 (pediatric patients 4 to 7 years of age)
- EQ-5D-Y, self-report version (pediatric patients 8 to <16 years of age)

9.11. Biomarkers

Summaries for biomarkers (ie, exploratory endpoint) will be provided by treatment arm and collection time. Additional exploratory analyses may be performed and will be specified in the Statistical Analysis Plan, as appropriate.

9.12. Safety Endpoints and Analyses

Safety analyses will be conducted using the Safety Analysis Set.

9.12.1. Adverse Events

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) to classify events under primary system organ class and preferred term.

The number and percentage of patients who experienced treatment-emergent adverse events (TEAEs), serious TEAEs, TEAEs leading to discontinuation of study drug, Grade 3 and 4 TEAEs, and deaths will be summarized by treatment group using the Safety Analysis Set. Results will be presented by system organ class and preferred term. The overview will also report TEAEs by maximum severity.

The number and percentage of patients with treatment-related TEAEs, serious TEAEs, TEAE leading to discontinuation of study drug, Grade 3 and 4 TEAEs, and patients who have died will also be summarized by treatment group.

For all AE summaries, if a patient has more than 1 AE within a preferred term, the patient is counted only once at the maximum severity and with the closest relationship to study drug. If a patient has more than 1 AE within a system organ class, the patient is similarly counted once when reporting results for that system organ class.

All AE data will be listed. The information presented will include patient number, treatment, primary system organ class and preferred term, date of onset, severity, relationship to study drug, action taken, and stop date (if available).

9.12.2. Clinical Laboratory Results

Laboratory parameters will be summarized (eg, total bilirubin, serum creatinine). Descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) as well as changes from baseline (baseline is defined as the day defibrotide prophylaxis starts for the defibrotide prophylaxis arm or conditioning therapy starts for the standard of care arm) will be summarized by treatment group using the Safety Analysis Set. The number and percentage of patients with abnormal values at post baseline will be presented by treatment group.

9.12.3. Graft Failure and Time to Neutrophil and Platelet Engraftment

Time to neutrophil and platelet engraftment will be defined as the time from the date of HSCT to the date that the criterion for neutrophil or platelet engraftment, respectively, is met (see Section 6.8.5). Kaplan-Meier curves and summary statistics (including the percent of patients achieving engraftment up to Day +100 post-HSCT) will be presented by treatment arm.

The proportion of patients with graft failure will be summarized by treatment group using the Safety Analysis Set.

9.12.4. Karnofsky and Lansky Performance Scores

For Karnofsky and Lansky PS, descriptive statistics (sample size, mean, standard deviation, median, minimum, and maximum) as well as changes from baseline (unless otherwise specified) will be summarized by treatment group using the Safety Analysis Set.

9.12.5. Infectious Diseases

The incidence of infectious disease by Days +100 Day and +180 post-HSCT will be summarized by treatment group using the Safety Analysis Set (see Section 6.8.7).

9.12.6. Concomitant Medications

Concomitant medications will be coded using the World Health Organization Drug Dictionary (WHODrug) and will be summarized separately by treatment group using descriptive statistics.

9.13. Subgroup Analyses

Exploratory analyses of the primary efficacy, secondary efficacy, and safety endpoints may be conducted for the following subgroups of interest, but not limited to:

- Pediatric population (<17 years old)
- Patients with ATG use

10. DATA QUALITY ASSURANCE

Steps to assure the accuracy and reliability of data include the selection of qualified investigators and appropriate study sites, review of protocol procedures with the investigator and associated personnel prior to the study, and periodic monitoring visits by Jazz Pharmaceuticals or its designee. Data are reviewed throughout the study through programmed checks, reports, and manual review. Any discrepancies will be resolved with the investigator or designees as appropriate.

10.1. Clinical Data Management

The standard procedures for handling and processing clinical data will be followed in compliance with 21 CFR Part 11, Food and Drug Administration (FDA) and ICH Regulations and Guidelines, Good Clinical Practices, and the Standard Operating Procedures (SOPs) of Jazz Pharmaceuticals or the contract research organization (CRO). A comprehensive Data Management Plan (DMP) will be developed to document data sources, systems, and handling.

The lack of standardized approaches toward collection and evaluation of staging data for aGvHD diagnosis likely contributes to why promising GvHD treatments reported from single centers have failed to show benefit in randomized multicenter clinical trials (Harris et al 2016). Collection of data pertaining to aGvHD diagnosis and clinical staging will be done according to MAGIC guidelines (Appendix 2), which have been developed through international expert consensus opinion to standardize the diagnosis and clinical staging of GvHD.

10.2. Electronic Case Report Forms

All patient data required by the protocol to be reported to the sponsor on each trial patient will be recorded by clinical site staff in eCRFs developed by Jazz Pharmaceuticals or its designee, unless such data are transmitted to the sponsor or designee electronically (eg, central laboratory data, data from an IRT, etc). Electronic data sources will be identified in the DMP. The Principal Investigator must review the eCRFs and provide his/her signature certifying that he/she has reviewed the data and considers them complete and accurate to the best of his/her knowledge. Regardless of who signs or completes the forms, it is the Principal Investigator's responsibility to ensure their completeness and accuracy.

10.3. Retention of Data

The investigator/institution should maintain the study documents as specified in Essential Documents for the Conduct of a Trial (ICH E6 Good Clinical Practice) and as required by the applicable regulatory requirement(s). The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the responsibility of the Sponsor to inform the investigator/institution when these documents no longer need to be retained.

11. ADMINISTRATIVE CONSIDERATIONS

11.1. Investigators and Study Administrative Structure

Parties (eg, Sponsor, CROs, and vendors) responsible for the various functions in this study will be listed in a separate document and filed in the Trial Master File.

11.2. Institutional Review Board or Independent Ethics Committee Approval

This study will be conducted in accordance with IRB regulations (eg, US 21 CFR 56.103) or IEC regulations. The final approved protocol and the ICF will be reviewed by the IRB/IEC. In addition, the IRB/IEC will review any other written information to be provided to the patient, advertisements for patient recruitment (if used), and patient compensation (if any). The committee's decision concerning conduct of the study will be sent in writing to the investigator and a copy will be forwarded to the Sponsor. The investigator will agree to make any required progress reports, as well as reports of SAEs, life-threatening problems, death, or any significant protocol deviations, as required by the IRB/IEC.

A list of the IRB/IEC members who actually participated in the review, their respective titles (occupational identification), and institutional affiliations or an IRB/IEC assurance number must be provided to the Sponsor. The approval letter or notice must be provided on IRB/IEC letterhead and contain the date of the meeting and sufficient information to identify the version of the protocol unambiguously (by name and number) and state that the ICF was also reviewed.

A clinical study may not be initiated before the proposed protocol and ICF have been reviewed and unconditionally approved by an IRB/IEC. The clinical study remains subject to continuing review by the IRB/IEC at least annually. The Sponsor or its designee will supply all necessary data for the investigator to submit to the IRB/IEC. The Sponsor will not ship clinical supplies to an investigational site until written signed approval from the site's IRB/IEC has been received by the Sponsor.

The investigator is responsible for ensuring initial and continued review and approval of the clinical study by the IRB/IEC at his/her site. The investigator must also ensure that he/she will promptly report to the IRB/IEC and the Sponsor all changes in the research activity and all unanticipated problems involving risk to human patients or others, and that he/she will not make any changes in the research without IRB/IEC approval, except where necessary to eliminate apparent hazards to human patients. If the study remains in progress for more than 1 year, documentation of annual review by the IRB/IEC must be maintained.

11.3. Ethical Conduct of the Study

The study will be conducted in accordance with applicable local regulations relating GCP and with the SOPs of the CRO or the Sponsor, as applicable.

Endorsement of the ethical principles embedded in the above guidances and regulations ensures that the rights, safety, and well-being of study patients are protected and are consistent with the principles that have their origin in the Declaration of Helsinki, World Medical Association – "Ethical Principles for Medical Research Involving Human Subjects."

11.4. Patient Information and Consent

All patients or their parents/legally authorized representatives will provide their written informed consent before the performance of any study-related procedures.

Written informed consent is to be obtained from each patient prior to enrollment into the study, and/or from the patient's legally authorized representative. If the patient is under the legal age of consent, the consent must be signed by the legally authorized representative in accordance with the relevant country and local regulatory requirements.

Written parental/legal guardian informed consent in addition to a separate written minor consent and/or assent (in accordance with any applicable state and local laws) are required for each minor patient prior to any study related procedures in the study. Each patient's chart will have his/her signed ICF for study participation attached to it. When the study treatment is completed and the eCRF has been monitored, the ICF will be kept in the investigator's central study file. Regulatory authorities may check the existence of the signed ICF in this central study folder if not having done so during the performance of the trial.

11.5. Patient Confidentiality

All reports and communications relating to the patients in the study will identify each patient only by the patient's study number. These documents will be treated with strict adherence to professional standards of confidentiality and will be filed at the study site under adequate security and restricted access.

Portions of the patient's medical records pertinent to the study may be reviewed by the Sponsor or its designee, the governing IRB/IEC, and governmental agency to ensure accuracy and completeness of the source documents and data in the eCRFs.

11.6. Protocol Adherence and Protocol Amendments

The protocol must be read thoroughly and the instructions must be followed exactly.

The investigator must not implement any deviation from the protocol. Any changes in the protocol will require a formal amendment. The IRB/IEC will be notified of all amendments to the protocol. Amendments to the protocol will not be implemented until written IRB/IEC approval has been received.

11.7. Required Documents

The investigator must provide the Sponsor or its designee with the applicable regulatory documents before the enrollment of any patient (copies should be kept by the investigator in the investigator's regulatory document binder).

11.8. Study Monitoring

Throughout the course of the study, the study monitor will make frequent contacts with the investigator. This will include telephone calls and onsite visits. During the onsite visits, the eCRFs will be reviewed for completeness and adherence to the protocol. As part of the data verification, source documents will be made available for review by the site. The study monitor will also perform drug accountability checks and will periodically request review of the investigator study file to assure completeness of documentation in all respects of clinical study conduct.

Upon completion of the study, the study monitor will arrange for a final review of the study files after which the files should be secured for the appropriate time period. The investigator or appointed delegate will receive the study monitor during these onsite visits and will cooperate in providing the documents for review and respond to inquiries. In addition, the investigator will permit inspection of the study files by authorized representatives of the regulatory agencies.

11.9. Protocol Violations/Deviations

All major protocol violations must be reported to the IRB/IEC in an expedited fashion. Reports of protocol violations should be submitted to the Sponsor continuously.

11.10. Access to Source Documentation

The Sponsor (or its designee) will be responsible for monitoring this clinical study. The Sponsor will monitor the study conduct, proper eCRF entry, source documentation completion and retention, and accuracy of study drug accountability. To this end, a monitor will visit the study site at suitable intervals and be in frequent contact with the site through verbal and written communication. It is essential that the monitor have access to all documents (related to the study and the individual patients) at any time they are requested. In turn, the monitor will adhere to all requirements for patient confidentiality as outlined in the ICF. The investigator and his/her staff

will be expected to cooperate with the monitor, to be available during the monitoring visit to answer questions, and to provide relevant information.

In addition, representatives of the Quality Assurance Department at the Sponsor (or equivalent), or appointed monitoring organization(s), and representatives of the FDA or other regulatory agencies may request to inspect the study documents (eg, study protocol, eCRFs, study drug accountability records, original medical records/files). All patient data will be treated confidentially.

11.11. Data Generation and Analysis

Information regarding data management and data collection is provided in Sections 10.1 and 10.2, respectively. Information on planned data analyses is provided in Section 9.

11.12. Publication and Disclosure Policy

Please refer to individual site contracts for specific contractual obligations and requirements.

All information concerning defibrotide, operations at Jazz Pharmaceuticals, patent applications, formulas, manufacturing processes, basic scientific data, and formulation information supplied by Jazz Pharmaceuticals to the investigator and not previously published, are considered confidential and remain the sole property of Jazz Pharmaceuticals. Electronic CRFs also remain the property of Jazz Pharmaceuticals. The investigator agrees to use this information only to complete this study and will not use it for other purposes without written consent of Jazz Pharmaceuticals as further detailed in the Clinical Study Agreement signed by the investigator and/or institution.

It is understood by the investigator that Jazz Pharmaceuticals will use the information obtained in this clinical study in connection with the study of defibrotide, and therefore may disclose this information as required to other Jazz Pharmaceuticals investigators; appropriate international regulatory agencies; or others. In agreeing to participate in this study, the investigator understands that he/she has an obligation to provide complete test results and all data developed during this study to Jazz Pharmaceuticals. Jazz Pharmaceuticals requires that permission to publish details of this study must be obtained in writing as further detailed in the Clinical Study Agreement signed by the investigator and/or institution. It is intended that the results of this study may be published in scientific literature. In addition, results will be provided for Applicable Clinical Trials on ClinicalTrials.gov. The conditions noted here are intended to protect commercial confidential materials (patents, etc) and not to restrict publication.

12. REFERENCE LIST

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Appendix 1 Schedule of Procedures and Assessments

	Pre-HSCT		HSCT Day	Days Post-HSCT														
Evaluation	**Screening**	**Baseline[a]**	**0**	**+7**	**+14[b]**	**+21**	**+28**	**+35**	**+42**	**+49**	**+56**	**+63**	**+70**	**+77**	**+84**	**+91**	**+100**	**+180/ EOS or ET**
Window (days)	*≤28 days prior to baseline*	*NA*	*NA*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*-4 to 0*	*±5*
Informed consent	X																	
Inclusion/exclusion criteria[c]	X																	
Randomization[d]	X																	
Demographics	X																	
Medical history[e]	X																	
Prior medications[f]	X	X																
Vital signs		X	X	X	X	X	X	X	X	X	X	X						
Physical exam, weight, and height[g]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HLA typing (recipient and donor)[h]			X															
CBC[i], differential, platelet count, and blood chemistries[j]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Infectious disease markers[k]	X																X	X
Disease evaluation[l]	X[m]																X	X
Pregnancy test[n]	X						X					X						
Karnofsky or Lansky performance scale[o]		X																

Appendix 1 Schedule of Procedures and Assessments

	Pre-HSCT		HSCT Day	Days Post-HSCT													
Evaluation	**Screening**	**Baseline[a]**	**0**	**+7**	**+14[b]**	**+21**	**+28**	**+35**	**+42**	**+49**	**+56**	**+63**	**+70**	**+77**	**+84**	**+91**	**+100**	**+180/ EOS or ET**
Window (days)	*≤28 days prior to baseline*	*NA*	*NA*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*-4 to 0*	*±5*
Neutrophil and platelet engraftment/graft failure				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
GvHD assessments[p,q]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VOD assessments				X	X	X	X	X[r]									X	
Hospitalization data[s]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Quality of life assessments[t]		X		X	X	X	X										X	X
Biomarkers[u]		X[v]		X	X												X	X
Concomitant medications		X	X	X	X	X	X	X	X	X	X	X						
Concomitant medications of special interest		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events assessments	X	X	X	X	X	X	X	X	X	X	X	X[w,x]					X[x]	X[x]
Study drug administration[y]		X	X	X	X	X	X	X[r]										
Survival status		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Note: HSCT Day (Day 0) in the study is defined as the day of transplant.

a. Baseline is defined as the day defibrotide prophylaxis starts for the defibrotide prophylaxis arm or conditioning therapy starts for the standard of care arm.

b. Patients randomized to receive defibrotide prophylaxis must undergo Day +14 post-HSCT assessments before hospital discharge. If a patient is discontinued from study/study drug prior to Day +14 post-HSCT, the Day +14 post-HSCT assessments must be completed on the day of study/study drug discontinuation (±2 days).

c. Eligibility must be confirmed before randomization. Review of planned conditioning regimen for GvHD prophylaxis is required to ensure that the eligibility criteria will continue to be met prior to randomization.

d. Randomization of patients should be the last screening activity performed.

e. Medical history, information pertaining to underlying disease, any prior HSCTs, and current HSCT, should be collected.

f. Prior medications include those current at the time of screening through baseline, and all prior therapies for the malignant disease.

g. Height is only required at the baseline visit.

h. HLA typing information should be available prior to HSCT Day.

i. CBC and WBC differential performed at least 2 times weekly from day of transplant (ie, HSCT Day [Day 0]) until ANC >0.5 x 10^9/L for 3 days and platelet count >20 x 10^9/L without a platelet transfusion in the preceding 7 days. CBC then performed weekly through Day +100 post-HSCT and at Day +180 post-HSCT.

j. Blood chemistries include: serum creatinine, bilirubin, alkaline phosphatase, AST, and ALT. Blood chemistries performed at least 2 times weekly until hospital discharge. Blood chemistries performed weekly after hospital discharge until Day +100 post-HSCT, then at Day +180 post-HSCT. If a patient is at risk for exceeding maximal allowable blood draw limits (per Seattle Children's Hospital Guideline for Maximum Blood Volumes, Appendix 6), the blood draw schedules for laboratory assessments may be adjusted per local physician's practice to ensure patient safety and to remain below the blood draw maximums.

k. Infectious disease markers will be assessed per institutional practice to include at minimum: CMV, Hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab) during screening, and CMV at Days +100 and +180 post-HSCT.

l. Disease evaluation for acute leukemia and MDS (performed locally) includes a BM aspirate and/or biopsy for pathology and cytogenetics. Cytogenetics, molecular, or other institutional assessment to determine Minimal Residual Disease will be recorded at screening to document patient's pre-transplant Minimal Residual Disease status. Bone marrow aspirate and biopsy are required for assessment of disease relapse. If disease relapse assessment can be made using only an aspirate sample, results from assessment of a core biopsy are not required. Disease relapse is defined by either morphological evidence of acute leukemia or MDS consistent with pre-transplant features, documented or not by biopsy. The event is defined as an increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy. Disease relapse will be diagnosed when there is morphological or clinical evidence of i) reappearance of leukemia blast cells in the peripheral blood, or ii) >5% blasts in the BM, not attributable to another cause (eg, BM regeneration), or iii) the appearance of previous or new dysplastic changes (MES specific) within the BM, with or without falling donor chimerism, or iv) the development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid, or v) institution of therapy to treat relapsed disease, including donor lymphocyte infusion.

m. Results of a biopsy and aspirate performed prior to HSCT as part of the standard of care will be recorded during the screening period. This bone marrow biopsy and aspirate is not required to be performed between D-28 and baseline.

n. Serum pregnancy test must be performed ≤30 days before the start of the transplant conditioning regimen. In addition, pregnancy test must be performed prior to treatment with study drug, at Day +28 post-HSCT, at Day +63 post-HSCT, 1 week after the last dose of defibrotide, and as required by local guidelines. Pregnancy test is required for females of child-bearing potential (ie, not postmenopausal or surgically sterile), and may be performed per institutional practices.

o. Functional impairment will be assessed using the Karnofsky PS for patients ≥16 years of age and the Lansky PS for patients <16 years.

p. MAGIC staging/assessment should be completed at least 1) weekly through Week 13, at Day +100 post-HSCT and at Day +180 post-HSCT (aGvHD present or not) and 2) at GvHD diagnosis and/or GvHD treatment and for 4 weeks of follow-up for both events (to obtain 4 weeks response data). Example: If GvHD is diagnosed at Day +100 post-HSCT, MAGIC staging/assessments are required at GvHD diagnosis (Day +100 post-HSCT) and for 4 weeks of follow-up (with assessment periods ending on Days +107, +114, +121, and +128 post-HSCT). The last visit of the study is the Day +180 post-HSCT visit, thus follow-up visits are not required to continue beyond the Day +180 post-HSCT visit.

Each assessment period corresponds to a 7 day interval from the first day of stem cell infusion (ie, ends on 7, 14, 21, etc, days from the first day of transplant). Patient GvHD staging is **reported** once per assessment period except for weeks in which GvHD is first diagnosed or treated, in which case the GvHD staging should be completed on those days as well.

Guidance: Patients may be **assessed** for GvHD more than once per week (eg, inpatient where daily assessments are often performed) but only 1 assessment should be **reported**. The GvHD staging(s) and corresponding date that should be reported for an assessment period should be:

- If GvHD staging was performed only once during an assessment period, report the staging and date of the clinical assessment.
- If GvHD staging was performed on multiple days during an assessment period, **and** the staging did not change during the assessment period, report the staging and date closest to the end of the assessment period.
- If GvHD staging was performed on multiple days during an assessment period, report the staging for the day corresponding to the **peak staging** for that period. If the peak stage occurred on more than 1 day, report the day closest to the end of the assessment period.
- If either GvHD diagnosis or treatment develops during an assessment period, report the staging on the day of GvHD diagnosis and GvHD treatment on the *GvHD Diagnosis or Treatment form* **and** report the peak staging from the assessment period on the *Weekly form*. These *might* be the same date but require separate forms (Weekly Assessment and GvHD Diagnosis/Treatment forms).
- For assessment periods where no staging is available, report that the patient was not assessed. Unless patient status is "Off Study", GvHD weekly assessment forms need to be completed for all 13 weeks, at Day +100 post-HSCT and at Day +180 post-HSCT. If the patient was not assessed in Week 13, continue to report Weekly forms, regardless of whether GvHD is active, until the final reporting week is one in which patient was assessed.

q. GvHD assessment questions regarding individual case scenarios may require Medical Monitor adjudication. Additional information (eg, related labs, AEs, concomitant medications, etc.) will be recorded for adjudication purposes.

r. Up to Day +30 post-HSCT.

s. Hospitalization data includes dates of hospitalization, dates in ICU, and dates of readmissions.

t. Quality of life assessments include the following patient questionnaires: FACT-BMT-TOI and EQ-5D (version dependent on age group; see Section 6.5 for additional details.

u. Blood samples to evaluate plasma concentration of potential GvHD biomarkers will be collected at baseline, on Days +7 and +14 post-HSCT, on last day of treatment with study drug, on Days +100 and +180 post-HSCT, upon diagnosis of aGvHD, and 14 days following diagnosis of aGvHD. If hospital discharge occurs prior to Day +14 post-HSCT, then the Day +14 biomarker sample should be obtained on the day of discharge.

v. Blood samples to evaluate plasma concentration of potential GvHD biomarkers will be collected with an assessment window of -3 days.

w. Investigator must record all AEs and SAEs that occur from the time written informed consent is obtained up to Day +63 post-HSCT, regardless of their relationship to study drug or procedure.

x. Only SAEs considered by the investigator to be related to study drug or study procedures should be reported more than Day +63 post-HSCT.

y. Administered intravenously as 4 2-hour infusions of 6.25 mg/kg every 6 hours beginning prior to the start of the conditioning regimen (may have completed 1-4 doses of defibrotide prior to conditioning) for a recommended duration of ≥21 days of treatment and ending no later than Day +30 post-HSCT.

AE=adverse event; ALT=alanine aminotransferase; ANC=absolute neutrophil count; AST=aspartate aminotransferase; BM=bone marrow; CBC=complete blood count; CMV=cytomegalovirus; EOS=end of study; ET=early termination; EQ-5D=EuroQoL-5D health questionnaire; FACT-BMT-TOI=Functional Assessment of Cancer Therapy-Bone Marrow Transplant-Trial Outcomes Index; GvHD=graft-versus host disease; HepA=hepatitis A; HepB=hepatitis B; HepC=hepatitis C; HLA=human leukocyte antigen; HSCT=hematopoietic stem cell transplant; ICU=intensive care unit; LVEF=left ventricular ejection fraction; MAGIC=Mount Sinai Acute GvHD International Consortium; MDS=myelodysplastic syndrome; NA=not applicable; PS=Performance Scale; SAE=serious adverse event; VOD=veno-occlusive disease; WBC=white blood cell.

Appendix 2 MAGIC GvHD Guidance Manual (adapted for use by the Sponsor for this study)

PURPOSE: To provide guidance for uniform GVHD target organ staging and data collection

While comprehensive, this guidance manual does not cover every conceivable scenario and questions regarding individual case scenarios can be directed to the Medical Monitor for adjudication.

aGVHD Onset note:

Historically, the term "Onset" was used to describe both the event when GVHD was diagnosed and not systemically treated and the event when GVHD was diagnosed and also systemically treated. These could occur at different times. The term *GVHD Onset* will not be used in this guidance, and the terms GVHD Diagnosis and GVHD Treatment will be used instead.

The definition of each is as follows –

The aGVHD Diagnosis date is the earlier of the following:

- Date physician clearly determines, or confirms in response to a query, a diagnosis of acute GVHD. (**Probable** confidence level [CL])
- Date a biopsy was performed that demonstrates the presence of GVHD (**Confirmed** CL)

The aGVHD Treatment date is:

- Date systemic treatment was started for GVHD (**Probable** CL)

Guidance: GVHD Diagnosis and GVHD Treatment may be reported on the same date for a patient. If GVHD Treatment occurs within 3 days of GVHD Diagnosis, then only one set of GVHD data and samples is needed for the patient. If GVHD Treatment occurs greater than 3 days from GVHD Diagnosis, a new event has occurred and separate data forms and samples will be collected.

ASSESSMENT PERIODS

MAGIC Staging/Assessment should be completed at least **(a) weekly through Day 98/Week 14 (aGVHD present or not) and (b) at GVHD Diagnosis and/or GVHD Treatment and for four weeks of follow-up for both events (to obtain 4 week response data).**

MAGIC Assessment period: One week. Each assessment period corresponds to a 7 day interval from the first day of stem cell infusion (ie, ends on 7, 14, 21, etc, days from the first day of transplant). Patient GVHD staging is **reported** once per assessment period **except** for weeks in which GVHD is first diagnosed or treated, in which case the GVHD staging should be completed on those days as well.

Guidance: Patients may be **assessed** for GVHD more than once per week (eg, inpatient where daily assessments are often performed) but only one assessment should be **reported**. The GVHD staging(s) and corresponding date that should be reported for an assessment period should be:

- If GVHD staging was performed only once during an assessment period, report the staging and date of the clinical assessment.
- If GVHD staging was performed on multiple days during an assessment period, **and** the staging did not change during the assessment period, report the staging and date closest to the end of the assessment period.
- If GVHD staging was performed on multiple days during an assessment period, report the staging for the day corresponding to the **peak staging** for that period. If the peak stage occurred on more than one day, report the day closest to the end of the assessment period.
- If either GVHD Diagnosis or Treatment develops during an assessment period, report the staging on the day of GVHD Diagnosis and GVHD Treatment on the *GVHD Diagnosis or Treatment form* **and** report the peak staging from the assessment period on the *Weekly form*. These *might* be the same date but require separate forms (Weekly Assessment and GVHD Dx/Tx forms).
- For assessment periods where no staging is available, report that the patient was not assessed. Unless patient status is "Off Study", GVHD weekly assessment forms need to be completed for all 14 weeks. If the patient was not assessed in Week 14, continue to report Weekly forms, regardless of whether GVHD is active, until the final reporting week is one in which patient was assessed. Typically this will require reporting only until Week 15.

GVHD STAGING once treatment begins: During the initial treatment response period (four weeks), the GVHD staging should be reported for the date CLOSEST to the seven day interval from the start of treatment. For example, week 4 corresponds to days 22-28 post transplant. If a patient begins systemic steroid treatment for GVHD on day 24, the assessment reported for week 5 should be the one that occurred closest to day 31 (i.e, at a seven day interval). This practice allows for the GVHD staging at start of treatment and day 28 post-treatment to be used to determine day 28 treatment response.

Guidance 1: Steroid therapy alone cannot determine the date of GVHD treatment because patients may be receiving steroids for other indications. The date of GVHD treatment is the first date that systemic therapy is given for GVHD (as indicated on the GVHD treatment form).

Guidance 2: Patients may be assessed for GVHD more than once per week (eg, inpatient where daily assessments are often performed). The GVHD staging(s) and corresponding date that should be reported for an assessment period during treatment should be the date closest to the multiple of seven days calculated from the start of treatment.

SPURIOUS GVHD

Spurious GVHD Diagnosis and/or Spurious GVHD Treatment can occur multiple times per patient. Occasionally, GVHD is diagnosed, but further data review (ie, biopsy results) contradict the diagnosis. For example, a skin rash thought to be GVHD is proven to be infectious. In such cases, the diagnosis/treatment and weekly forms should be revised to indicate no GVHD, confidence level changed to negative, and comment field used to indicate spurious diagnosis or treatment. See also *Target Organ guidance (symptoms present, steroids given for non-GVHD cause).*

BIOPSY INFORMATION

Biopsies: Biopsy reports help determine the date of GVHD Diagnosis.

Guidance: Biopsies sometimes take place before the medical record indicates that GVHD is present.

- Clinical judgment determines the presence of GVHD even if biopsy results do not agree. For example, if a patient is staged and treated, then GVHD is considered present even if a biopsy indicates absence of GVHD (ie, early GVHD skin rash may be reported as normal skin by pathologist).
- In order to change a diagnosis from acute to chronic GVHD, clinical features of chronic GVHD **must** be present (eg, sclerodermatous or lichenoid skin changes) and acute GVHD features **must** be resolved. Histopathology is not required and could reflect treatment induced changes, especially when intensive therapy was given over a prolonged period. Acute GVHD staging and grading should continue in scenarios such as this.

Biopsy Result Definitions**:**

The pathologist interpretation of a biopsy does not always clearly resolve whether GVHD is present or not. The below guidance is to assist in reporting the biopsy findings, but should not replace transplant physician involvement in data reporting.

- **Positive:** GVHD is clearly identified as present on the biopsy. **Note** – multiple coexisting processes may be present on a biopsy along with GVHD. If other etiologies are noted, they should be reported but will not change staging. (example: CMV colitis and GI GVHD both present on the same biopsy).
- **Equivocal:** GVHD and/or other possible etiologies are identified on the biopsy and the pathologist cannot definitively state whether or not GVHD is present. (a common scenario)
- **Non-Diagnostic:** Either no abnormalities are identified, the abnormalities are insufficient to identify a potential etiology, or there is insufficient tissue for interpretation.
- **Non-GVHD Etiology:** One or more etiologies are identified on a biopsy, none of which are GVHD.
- **Unknown**: The pathological results are not available by the end of the assessment period.

Biopsy results are not changed for a completed assessment period unless the original report was incorrect based on the information available *at the time*.

Multiple biopsies from the same organ: All biopsies obtained in a given assessment period are reported. If a target organ biopsy is positive for GVHD, and a later biopsy for the same organ in the same or different assessment period is negative, the diagnosis of GVHD is unchanged (subsequent biopsies that do not show GVHD may reflect treatment effect).

- Clinician can determine if a patient has GVHD despite an unknown, non-diagnostic or equivocal biopsy result, or the absence of biopsy altogether.
  - If the patient has started treatment for GVHD then the confidence that GVHD is present is **Probable.**
  - If the patient has not started treatment for GVHD then the confidence that GVHD is present is **Possible**.
- When a positive biopsy result is obtained and clinician determines GVHD is not present, the case is referred for adjudication.

Pathology Results	Target Organ Confidence Level		
	Treated as GVHD	**Not treated but GVHD in differential diagnosis**	**Not treated and GVHD not in differential diagnosis**
Positive	**Confirmed**		
Equivocal	**Probable**	**Possible**	
Non-Diagnostic	**Probable**	**Possible**	**Negative**
Non-GVHD Etiology	**Probable**	**Negative**	
Unknown	Not changed from previous assessment period		

GVHD confirmed in a biopsied target organ raises the confidence level from possible to probable for other target organs where GVHD is suspected, even in the absence of treatment.

TARGET ORGAN CONFIDENCE LEVELS

Guidance: The level of confidence that symptoms are aGVHD (**Confirmed, Probable, Possible, and Negative**) is reported at each assessment period for each target organ.

Confidence levels may change over time as new information becomes available – these changes are reported for the assessment period during which they occur. Prior confidence levels are not changed as these reflect the real-time assessment. When new information and discussion retrospectively alters a previously reported confidence level, an **adjudication level**, will also be recorded for the assessment period. For example, a skin rash treated for GVHD is recorded in real time as probable GVHD. If later information revealed that the rash was likely due to a diagnosis other than GVHD, the adjudication level will be recorded as possible GVHD. This strategy allows both the real-time and adjudicated confidence levels to be recorded when there is a disagreement. Target organ specific rules are provided below to assist in establishing the confidence level.

Symptomatic GVHD Confidence Levels

	Pathologic evidence	Clinician assessment	Treatment for acute GVHD	Comments
Confirmed **(GVHD Diagnosis proven by bx)**	Unequivocal pathologic evidence of GVHD	GVHD is the etiology for symptoms	Yes	GVHD is clearly present even if other etiologies may co-exist simultaneously
Probable **(Clinician Assessed as GVHD Diagnosis and/or Treatment started)**	Not required	GVHD most likely etiology for symptoms	Yes	GVHD is most likely present but other etiologies may also explain the symptoms and there is insufficient evidence to make a confirmed diagnosis
Possible **(aGVHD in differential)**	Not required	GVHD in differential diagnosis	No	GVHD may be present, but other etiologies are favored to the degree that GVHD treatment is not initiated
Negative **(only 1 required)**	Unequivocal evidence of a diagnosis other than GVHD (eg, drug rash)	GVHD is not considered as an explanation for the symptoms	No **and** the symptoms resolve without GVHD treatment*	A "negative" biopsy (eg, normal skin) is not unequivocal evidence of a diagnosis other than GVHD

*If GVHD in one organ (eg, GI tract) is being treated and another symptomatic organ does not have GVHD (eg, liver biopsy confirms VOD and absence of GVHD), then the symptom in the non-GVHD organ does not need to resolve without treatment for the organ to be considered uninvolved (and negative CL).

Additional Guidance and Common Scenarios:

- **Symptoms are present, GVHD treatment started, but other etiologies are also present**. Example: A patient with diarrhea thought to be from GVHD is treated with high dose steroids, but C. difficile toxin is also present (a GI biopsy is not available). This is considered **Probable** GVHD and staging should be reported.
- **Symptoms are present but GVHD treatment is not given**: (1) A patient has a rash which might be GVHD, but no treatment is given and no biopsy results are available. (2) A patient has diarrhea, but no etiology is identified and no GVHD treatment is given. These situations are considered **Possible** GVHD if GVHD is a potential etiology. The extent of rash or diarrhea volume is reported, the GVHD symptoms are staged but this is not considered a diagnosis of GVHD. Note:
  - If a biopsy is subsequently reported/obtained that confirms the presence of GVHD, the confidence level changes to **Confirmed** GVHD as of the date of the positive biopsy, which then becomes the diagnosis of GVHD date.
  - If GVHD treatment is later initiated for persistent symptoms, the confidence level changes to **Probable** GVHD as of the date treatment is begun.
  - **Exception**: GVHD skin rash ≤50% may not be treated but is diagnosed as GVHD on clinical grounds. This case is considered **Probable** GVHD. The date of clinical staging is the diagnosis of GVHD date.
- **Prior to day 14 exception**. [GVHD rarely develops before day 14, but symptoms are often present - eg, nausea, vomiting, diarrhea]. A confidence level of Possible GVHD can only be assigned prior to day 14 as the result of adjudication. Otherwise, Possible GVHD is **not** an option prior to day 14.
  - Report Confirmed when biopsy confirmation of GVHD is available
  - Report Probable when start of treatment has begun for GVHD target organ symptoms but biopsy confirmation of GVHD is not yet available
  - Report Negative when target organ symptoms were present but no GVHD treatment was started and there is no confirmatory biopsy
  - If symptoms persist past Day 14, the confidence level stays the same until new evidence develops, such as change in symptom severity or physician judgment that GVHD should be included in the differential diagnosis(es).
- **Target organs symptoms are present, a non-GVHD etiology is diagnosed (ie, engraftment syndrome) but treatment similar to that used for GVHD (ie, steroids) is given**. This case is considered **Negative** GVHD.
  - If symptoms persist and the rationale for treatment is changed to GVHD treatment (eg, steroid dose is increased to manage presumed GVHD), the confidence level changes to **Probable** GVHD at the date of treatment change.
  - If a biopsy is subsequently reported/obtained that confirms the presence of GVHD, the confidence level changes to **Confirmed** GVHD as of the date of the positive biopsy, which then becomes the diagnosis of GVHD date.

- **Exception:** Steroid Guidance for potential GVHD – When steroids or other immunosuppression are given for treatment of unconfirmed aGVHD and then stopped within one week due to new information (ie, alternative non-GVHD diagnoses), the organ Confidence Levels can be downgraded by the Treating Center without central adjudication (Probable to Possible or, if aGVHD is no longer considered an etiology of symptoms, to Negative) retroactively. Furthermore, GVHD will no longer be considered present.
  - Steroid indication will be updated from an indication for aGVHD to an indication for Non-GVHD: Other: Adjudicated as not GVHD.

Confidence levels when symptoms are present in multiple target organs:

- If GVHD treatment is not being given, **Possible** or **Negative** GVHD confidence levels will be reported for each organ as defined in the table above.
- If **Probable** or **Confirmed** GVHD is present in one target organ (and treated), symptoms (simultaneous or that develop while still on treatment) in other target organs that are not biopsied at the same time are reported as **Probable** GVHD. This action is taken because it is not possible to exclude GVHD on clinical grounds in other symptomatic target organs when treatment is being given. For example, if biopsy proven skin GVHD is present and treated with systemic steroids and diarrhea develops, then the lower GI tract will be staged for GVHD and the confidence level will be **Probable** (even if an earlier GI biopsy was negative and no other etiology diagnosed).
  - **Exception:** If only topical therapy is being given for GVHD (eg, steroid creams for skin GVHD), other target organs with symptoms (eg, diarrhea) will be reported as **Possible** GVHD in the absence of systemic GVHD treatment.
  - **Exception:** If other symptomatic organs do not meet the minimum requirements for stage 1, then these should be reported as **Possible** (eg, diarrhea volume <500 ml/day)**.**
  - **Exception:** Jaundice attributable to another etiology (eg, SOS) that is present prior to GVHD Diagnosis /Treatment in another organ will not be considered GVHD unless confirmed by biopsy (see Liver GVHD Guidance section below).
  - **Confirmed** GVHD is reported for additional target organs ONLY when GVHD is confirmed by biopsy in that particular organ
  - **Upper GI rule:** When GVHD is present (probable or confirmed) in another target organ, suspected in the upper GI tract, but untreated, the UGI should be staged as 1 and confidence level = possible.

Guidance for Complicated Scenarios:

- Scenarios not addressed above should be referred for **adjudication**.

Adjudication Levels:

- When adjudication determines a different confidence level than the real time assessment, the Adjudication Confidence Level will be recorded as well.

ORGAN STAGING AND SYMPTOM INFORMATION

STAGING GUIDANCE:

- Staging is retrospectively modified during adjudication to take into account all information, including subsequent biopsy results, in order to be accurate. Confidence levels are not modified and should reflect the status at the time of reporting according to Table 1.
- There is no adjustment of GVHD staging when, along with confirmed/probable/possible GVHD, non-GVHD etiologies are also present.
  - For example, when a patient with hyperbilirubinemia from pre-existing veno-occlusive disease/sinusoidal obstruction syndrome develops **Confirmed/Probable** GVHD in another target organ and starts systemic GVHD therapy, liver GVHD staging will be based upon total bilirubin and not adjusted down because of the patient's other known cause for hyperbilirubinemia.
  - **Exception**: GVHD skin rash ≤50% may not be treated but is diagnosed as GVHD on clinical grounds. This case is considered **Probable** GVHD. The date of clinical staging is the date of GVHD diagnosis.

SKIN GVHD GUIDANCE:

- Only active rash (eg, erythema, maculopapular changes) is considered in body surface area (BSA) calculations. Hyper- or hypopigmentation or inactive areas (eg, brown/tanned rash, dry skin) representing post-inflammatory changes are not included in the BSA assessment.
- Always report BSA involvement (if available), use the Rule of 9's when necessary
- Always report if bullae and/or desquamation are present
  - Desquamation from severe skin GVHD is categorized by the loss of all skin layers.
  - These symptoms must be caused by GVHD and not directly related to a healing process.
- Peeling of the skin in the setting of quiescent GVHD is not stage 4 GVHD as this can be seen as a normal process in healing skin.
- **Working Definition of Engraftment Syndrome:**
  - No Acute GVHD
  - Some combination of the following symptoms around the time of WBC engraftment:
    - Fevers without infection
    - Rash
    - Jaundice
    - Nausea
    - Vomiting
    - Diarrhea
  - **Explanation: The presence of symptoms is not sufficient to document engraftment syndrome, rather it is a diagnosis by the treating physician when some of the above criteria are present and specifically attributed to engraftment syndrome.**

Skin GVHD staging rules:

- Stage 0: No GVHD rash
- Stage 1: Maculopapular rash < 25% BSA
- Stage 2: Maculopapular rash 25 – 50% BSA
- Stage 3: Maculopapular rash > 50% BSA
- Stage 4: Generalized erythroderma (>50% BSA) **plus** bullous formation and/or desquamation >5% BSA

Other common etiologies for skin symptoms: Drug rash, conditioning regimen toxicity, infection, cytokine storm/engraftment syndrome.

LIVER GVHD GUIDANCE:

- Use total (not direct/conjugated) bilirubin for staging.
- If jaundice is present before GVHD is diagnosed in another target organ (skin or GI), and GVHD is not the only diagnosis in the differential (whether in assessment or by history), liver GVHD will NOT be diagnosed.
- If jaundice develops at the same time or after GVHD in another target organ, liver GVHD is presumed to be present.
- Isolated liver GVHD can be diagnosed based on clinical judgment when GVHD is the only or most plausible etiology (GVHD treatment should be given to diagnose isolated liver GVHD).
- If Bilirubin is not above 2.0 but other liver enzymes are significantly elevated from the normal, use comment field to note elevated values but do not stage Liver as GVHD.

Liver GVHD staging:

- Stage 0: < 2 mg/dl
- Stage 1: 2-3 mg/dl
- Stage 2: 3.1-6 mg/dl
- Stage 3: 6.1-15 mg/dl
- Stage 4: >15 mg/dl

Other common etiologies for liver symptoms: Drug toxicity, conditioning regimen toxicity, VOD, TPN cholestasis, infection.

UPPER GI GVHD GUIDANCE:

- The determination that nausea, vomiting, and/or anorexia are persistent (one of the criteria for upper GI GVHD) is based on clinical judgment. As a general rule, nausea lasting fewer than 3 days, fewer than two vomiting episodes per day for at least two days, and anorexia without weight loss should not be considered persistent.

Upper GI GVHD staging rules:

- Stage 0: No persistent nausea or vomiting
- Stage 1: Persistent nausea, vomiting or anorexia

Other common etiologies for upper GI symptoms: Drug toxicity, conditioning regimen toxicity, infection, TPN.

LOWER GI GVHD GUIDANCE:

- For diarrhea volumes, report liquid stool volume in the following order; (1) Average of 3 consecutive days if available, (2) Otherwise the 3 days closest to each other, (3) Otherwise average of 2 consecutive days, (4) Otherwise the maximum volume from the isolated day(s) available. Formed or mostly formed stools should not be quantified or counted in the estimation of liquid stool volume.
- **When diarrhea is reported as episodes instead of individual volumes:**
  Episodes of non-quantified liquid stool will be counted as **200 cc** each for children ≥50 kg and adults, **4 ml/kg** for children up to 50 kg.
- Quantified episodes of mixed urine/stool: use 50% total volume
- Positive lower GI biopsy with stool <500cc is reported as stage 0

Lower GI GVHD staging (use adult staging for children ≥50kg):

- Stage 0: Adult: < 500 ml/day; Child: < 10 ml/kg/day
- Stage 1: Adult: 500–999 ml/day; Child: 10 -19.9 ml/kg/day
- Stage 2: Adult: 1000-1500 ml/day; Child: 20 – 30 ml/kg/day
- Stage 3: Adult: >1500 ml/day; Child: > 30 ml/kg/day
- Stage 4: Severe abdominal pain with or without ileus, or grossly bloody stool (regardless of stool volume).
  - For stage 4 GI: the term "severe abdominal pain" is defined as:
    (a) Pain that requires start of narcotic use, or a substantial increase in on-going narcotic use, PLUS
    (b) Pain that significantly impacts performance status, as determined by the treating physician.
  - When gross blood is used for staging: Bloody diarrhea is staged as 4, even if low volume. Blood streaked low volume (<500 mL) stools (as can be seen w/ bleeding hemorrhoids for example) are staged as 0.

Other common etiologies for lower GI symptoms: Drug toxicity, conditioning regimen toxicity, infection.

SYSTEMIC STEROIDS AND IMMUNOSUPPRESSIVE TREATMENT/PROPHYLAXIS

GVHD prophylaxis Guidance:

- Report actual, not planned, GVHD prophylaxis given. For example, if CSA is planned, but tacrolimus is actually given, report tacrolimus. If methotrexate is planned and at least one dose is given, even if one or more doses is not, report methotrexate as a prophylaxis.
- If prophylaxis changes prior to, or after, Diagnosis of GVHD/Start of Treatment for GVHD [eg, an intolerance to one agent leads to replacement with another agent(s)], report the replacement agent(s) and report this as a change in prophylaxis
- Report all GVHD prophylaxis agent(s) given during the assessment period, indication will be GVHD

General guidelines:

- Each weekly assessment period should report all GVHD treatments given (immunosuppressive or otherwise), regardless of whether GVHD is present,
- Systemic steroids administered as "one-time" doses, or given for a 3-day burst for reasons other than GVHD treatment (eg, mucositis, nausea/vomiting, premedication for transfusion, etc) will not be collected.
- Admission body weight is used for conversion of steroid dose to mg/kg; unless the clinician specifies a different body weight to use (eg, admission weight is inaccurate due to fluid overload). Use outpatient clinic visit weight if treatment for GVHD is started during an outpatient visit. The weight recorded at the start of steroids should be used on all subsequent forms and for calculations for steroid dosing.
- Steroid dose and form (prednisone, methylprednisolone, etc) should be reported corresponding to the date of GVHD assessment for that week. The dose being given on the date of GVHD assessment should be the one reported, even if the dose is changed one or more times during the assessment period.
  - **Exception:** When steroid therapy begins during the assessment period, report the starting dose.
- Indication for steroid therapy should be reported.
- If the patient develops GVHD while on steroid therapy for another indication, and the dose of steroids is changed, report the new dose of steroids as the treatment dose given for GVHD, the new indication for steroid therapy, and the date of the dose change.
- Other agents: list all other agents being given for GVHD treatment and/or prophylaxis.
- If a patient is receiving extracorporeal photopheresis (ECP), report the number of ECP treatments given during the assessment period.
- If the patient is on a study where the treatment assignment is not known (blinded), report this as experimental treatment and report which potential agents the patient may be receiving (ie, report both the study drug AND placebo).
- Topical therapies will not be collected.

Common indications for steroid treatment: GVHD treatment, GVHD prophylaxis, Cytokine storm/Engraftment syndrome, IPS

aGVHD Data Collection:
At each time period report if aGVHD is present, resolved or was not diagnosed since the last reporting period. If aGVHD has resolved, provide a resolution date. Report patient data starting from the last Weekly form. If aGVHD is present at Day +180, indicate each organ staging and Confidence Level.

Report "N/A" only if patient has not been evaluated since last report.

cGVHD Data Collection:
Follow the NIH criteria for assessing if cGVHD is present (see link in Appendix 5).
At each time period report if cGVHD is present and if on systemic treatment, as applicable. If cGVHD was diagnosed in a reporting period, provide a diagnosis date.

If cGVHD has previously been reported, check "Previously Reported".
Report "N/A" only if patient has not been evaluated since last report.

Donor Cellular Infusion (DCI) Data Collection:
Report any DCIs occurring since the last reporting period. If multiple infusions have occurred then report up to 3. DCI's can be multiple different cell types and are given for multiple indications.

The most common reason for DCI is to treat relapse (instead of a subsequent txp), and may be preceded by chemotherapy.

If DCI has been given, keep collecting GVHD Weekly data and Survival data. Patients do not come Off Study for DCI.
Report "N/A" only if patient has not been seen since last report.

Report the type of cell used for the infusion:
Lymphocytes, Peripheral Blood Mononuclear cells, Dendritic cells from the original donor, Mesenchymal cells, Other (specify)
Report the reason why the DCI was given:
Planned, Treat disease, Treat PTLD or EBV-Lymphoma, Treat Viral infection, Treat GVHD, Mixed Chimerism, Graft Failure, Other (specify)

3rd Party Infusion Data Collection:
Report any 3rd party infusions given since the last reporting period. If multiple infusions have occurred then report up to 3. Third party infusions are from donors other than the original donor or recipient.

If 3rd party infusion has been given, keep collecting GVHD Weekly data and Survival data. Patients do not come Off Study for 3rd party infusion.
Report "N/A" only if patient has not been evaluated since last report.

Report the date of infusion.

Relapse Data Collection:
Report if the patient has Relapsed since the last reporting period.

If "Yes", please indicate date relapse occurred. If relapse has previously been reported at any time, click "Previously Reported".
Report "N/A" only if patient has not been evaluated since last report.
If Relapse has occurred keep collecting GVHD Weekly data and Survival data.

Report the date of relapse.

Subsequent Transplant Data Collection:
Report if the patient has had a Subsequent Transplant since the last reporting period.

If "Yes", please indicate date occurred. If this has previously been reported at any time, click "Previously Reported".
Report "N/A" only if patient has not been seen since last report.
If Subsequent Transplant is Yes, Weekly GVHD data collection ends, but survival data will still be collected.

Report the date of the subsequent BMT

Patient Survival Data Collection:
Indicate if patient is Alive, Dead or Lost to Follow.
If patient has not been seen/assessed since last follow up, indicate Lost to Follow up.
If the patient is Alive, report the last date the patient was seen or known to be alive.
If the patient has Died, report the date of death. Primary and contributing cause of death will also be reported.

Cause of Death Hierarchy:
Cause of death is attributed to the key initiating event in the chain of events that culminate in death. It is inaccurate to report the proximate cause of death (eg, liver failure) when the liver failure is a result of severe liver GVHD. In that example, the cause of death should be recorded as GVHD. Cause of death may be difficult to ascertain and often requires adjudication. In some cases, no obvious cause of death can be determined, in which case the adjudication represents a best medical opinion. The following hierarchy will be followed for assigning cause of death:

Relapse
Recorded at any point prior to death, supersedes all other causes of death, including GVHD and Infection, when any other competing etiology is also present at time of death.
EXCEPTION: In most cases, COD can be determined using the above rule. In circumstances where the treating physician does not believe that relapse is the primary cause of death, an exception can be made with documentation emailed to the DCC and/or a comment entered into the Cause of Death comment box. All such cases will be adjudicated.

GVHD (Acute and Chronic)
GVHD supersedes all other causes of death except relapse. Relapse supersedes GVHD because relapse generally prompts measures that increase likelihood of GVHD (eg, abrupt cessation of immunosuppression or donor leukocyte infusion).

Deaths occurring with active GVHD symptoms are attributed to GVHD.
Deaths (usually incorrectly reported as Infection) are attributed to GVHD when symptoms are absent if the patient had been previously treated or remains on immunosuppression treatment for GVHD. The effect of GVHD treatment on the immune system persists beyond the last treatment dose and therefore guidance is provided for the time off immunosuppression to consider when assigning GVHD as cause of death.

Deaths are considered due to GVHD even when symptoms are absent (ie, complete response to treatment) if any of the following conditions are met:

- Patient was on 10 mg prednisone (or equivalent dose of alternative steroid) within two weeks of death
- Patient was on 20 mg prednisone (or equivalent dose of alternative steroid) within four weeks of death
- Patient on immunosuppressive agents (eg, ATG, Campath, etc) other than those used for prophylaxis
- In the event of missing or incomplete data, patients reported to have Grade 4 GVHD within two months of death, then COD is GVHD

Infection (specify infection)
Infection supersedes other causes of death except Relapse and GVHD. Infections are considered confirmed if a Positive culture or a Confirmatory Pathology report was obtained

Note: Please allow at least 5 days for pending culture reports to be finalized after death.
Provide the organism identified in the Specify field
If infection led to Sepsis, please document the organism identified as well as sepsis in the Specify field
When applicable, Infection should be listed as a secondary COD when GVHD is the Primary COD

Other causes of death:
Rejection/Poor Graft Function
Diffuse Alveolar Hemorrhage (DAH)
Idiopathic pneumonia syndrome (IPS)
Veno-occlusive Disease (VOD) / Sinusoidal obstruction syndrome (SOS)
New Malignancy, excluding PTLD
Post-transplant lymphoproliferative disease (PTLD)
Cardiac Event
Examples include: Arrhythmia, Sudden Death, Coronary Artery Disease / Myocardial Infarction (MI)
Pulmonary Event
Examples include: ARDS, Aspiration pneumonia, pulmonary embolism), excludes pneumonia or other infectious diseases (which should be reported under Infections)
Thrombotic Microangiopathy (TMA)
Other – HSCT related (specify)
Events not already described above but related to the transplant should be specified here. (ie, Immunosuppressant overdose)
Other – Non-HSCT related (specify)
Events not related to the transplant itself should be specified here. Example include motor vehicle accident, homicide, and accidental death
Unknown
If no information is known around the time of death, and none of the above definitions are applicable, report Unknown COD

Form Comment can be used to provide any further details about any major event, further clarification on the events surrounding a death, if COD has been reviewed with physician at site, if patient transferred care to another hospital, the general condition of the patient at this time, etc.

Appendix 3 IBMTR Severity Index for Grading of aGvHD (Rowlings et al 1997)

	Skin Involvement			Liver Involvement			GI Involvement	
Index[a]	Stage (max.)	Extent of rash		Stage (max.)	Total bilirubin (µmol/l)		Stage (max.)	Volume of diarrhea (ml/d)
A	1	<25%		0	<34		0	<500
B	2	25-50%	or	1-2	34-102	or	1-2	550-1500
C	3	>50%	or	3	103-255	or	3	>1500
D	4	Bullae	or	4	>255	or	4	Severe pain and ileus

[a] Assign Index based on maximum involvement in an individual organ system.

GI=gastrointestinal; max.=maximum.
Source: Rowlings PA, Przepiorka D, Klein JP, et al. IBMTR Severity Index for grading acute graft-versus-host disease: retrospective comparison with Glucksberg grade. Br J Haematol. 1997;97(4):855-864.

Appendix 4 Modified Consensus Criteria (by MAGIC) for Grading of aGvHD (Harris et al 2016)

GvHD Target Organ Staging

Stage	Skin (Active Erythema Only)	Liver (Bilirubin)	Upper GI	Lower GI (Stool Output/Day)
0	No active (erythematous) GvHD ash	<2 mg/dL	No or intermittent nausea, vomiting, or anorexia	Adult: <500 mL/day or <3 episodes/day Child: <10 mL/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2-3 mg/dL	Persistent nausea, vomiting or anorexia	Adult: 500-999 mL/day or 3-4 episodes/day Child: 10-19.9 mL/kg/day or 4-6 episodes/day
2	Maculopapular rash 25-50% BSA	3.1-6 mg/dL		Adult: 1000-1500 mL/day or 5-7 episodes/day Child: 20-30 mL/kg/day or 7-10 episodes/day
3	Maculopapular rash >50% BSA	6.1-15 mg/dL		Adult: >1500 mL/day or >7 episodes/day Child: >30 mL/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dL		Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

Abbreviations: BSA=body surface area; GI=gastrointestinal; GvHD=graft-versus-host disease.
Overall clinical grade (based on most severe target organ involvement):
Grade 0: No stage 1-4 of any organ.
Grade I: Stage 1-2 skin without liver, upper GI, or lower GI involvement.
Grade II: Stage 3 rash and/or stage 1 liver and/or stage 1 upper GI and/or stage 1 lower GI.
Grade III: Stage 2-3 liver and/or stage 2-3 lower GI, with stage 0-3 skin and/or stage 0-1 upper GI.
Grade IV: Stage 4 skin, liver, or lower GI involvement, with stage 0-1 upper GI.

Source: Harris AC, Young R, Devine S, et al. International, Multicenter, Standardization of Acute Graft-versus-Host Disease Clinical Data Collection: A Report from the Mount Sinai Acute GVHD International Consortium. Biol Blood Marrow Transplant. 2016;22(1):4-10.

Appendix 5 NIH Criteria for Grading of cGvHD (Jagasia et al 2015)

Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group Report. Biol Blood Marrow Transplant. 2015;21(3):389-401.

Appendix 6 Seattle Children's Hospital Guideline for Maximum Blood Volumes

Maximum Allowable Blood Draw Volumes:

PATIENT'S WEIGHT		TOTAL VOLUME	MAXIMUM mL IN ONE BLOOD DRAW	MAXIMUM mL IN A 30-DAY PERIOD
Kg	**lbs**	**mL**	**2.5% of total blood vol**	**5% of total blood vol**
1	2.2	100	2.5	5
2	4.4	200	5	10
3	6.6	240	6	12
4	8.8	320	8	16
5	11	400	10	20
6	13.2	480	12	24
7	15.4	560	14	28
8	17.6	640	16	32
9	19.8	720	18	36
10	22	800	20	40
11 thru 15	24 thru 33	880-1200	22-30	44-60
16 thru 20	35 thru 44	1280-1600	32-40	64-80
21 thru 25	46 thru 55	1680-2000	42-50	64-100
26 thru 30	57 thru 66	2080-2400	52-60	104-120
31 thru 35	68 thru 77	2480-2800	62-70	124-140
36 thru 40	79 thru 88	2880-3200	72-80	144-160
41 thru 45	90 thru 99	3280-3600	82-90	164-180
46 thru 50	101 thru 110	3680-4000	92-100	184-200
51 thru 55	112 thru 121	4080-4400	102-110	204-220
56 thru 60	123 thru 132	4480-4800	112-120	224-240
61 thru 65	134 thru 143	4880-5200	122-130	244-260
66 thru 70	145 thru 154	5280-5600	132-140	264-280
71 thru 75	156 thru 165	5680-6000	142-150	284-300
76 thru 80	167 thru 176	6080-6400	152-160	304-360
81 thru 85	178 thru 187	6480-6800	162-170	324-340
86 thru 90	189 thru 198	6880-7200	172-180	344-360
91 thru 95	200 thru 209	7280-7600	182-190	364-380
96 thru 100	211 thru 220	7680-8000	192-200	384-400

Based on blood volume of:

1 to 2 kg	100 mL/kg	(pre-term infant)
>2 kg	80 mL/kg	(term infant - adult)

This information is similar to that used by the Committee on Clinical Investigations at
Children's Hospital in Los Angeles, and at Baylor College of Medicine in Dallas, TX.
Adapted by Rhona Jack, PhD August 2001
Children's Hospital and Regional Medical Center Laboratory
Seattle, WA
Source: www.seattlechildrens.org/pdf/blood-volume-chart.pdf

Appendix 7 Signatures of Agreement for Protocol

Study Title: A Phase 2, Prospective, Randomized, Open-label Study on the Efficacy of Defibrotide Added to Standard of Care Immunoprophylaxis for the Prevention of Acute Graft-versus-Host-Disease in Adult and Pediatric Patients After Allogeneic Hematopoietic Stem Cell Transplant

Study Number: JZP963-201

Original Protocol: 19 September 2017

Amendment DE1: 07 March 2018

Amendment 1: 19 June 2018

This clinical study protocol was subject to critical review and has been approved by Jazz Pharmaceuticals.

[redacted]

Jazz Pharmaceuticals
Innovation that performs

Signature Manifestation

[redacted]