Clinical Study Protocol: 15-007

Study Title:	A Phase 3, Randomized, Adaptive Study Comparing the Efficacy and Safety of Defibrotide vs Best Supportive Care in the Prevention of Hepatic Veno-Occlusive Disease in Adult and Pediatric Patients Undergoing Hematopoietic Stem Cell Transplant
Study Phase:	3
Product Name:	Defibrotide (defibrotide sodium)
IND Number:	62,118
EUDRACT Number:	2016-002004-10
Indication:	Prevention of Hepatic Veno-Occlusive Disease (VOD)
Investigators:	Multicenter
Sponsor:	Jazz Pharmaceuticals, Inc. 3180 Porter Drive Palo Alto, CA 94304
Medical Director:	PI Jazz Pharmaceuticals 3180 Porter Drive Palo Alto, CA 94304

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Amendment 03 v4.0	20 August 2018

This study will be conducted under Good Clinical Practice guidelines.

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SYNOPSIS

SPONSOR	Jazz Pharmaceuticals, Inc.
PRODUCT	Defibrotide
TITLE	A Phase 3, Randomized, Adaptive Study Comparing the Efficacy and Safety of Defibrotide vs Best Supportive Care in the Prevention of Hepatic Veno-Occlusive Disease in Adult and Pediatric Patients Undergoing Hematopoietic Stem Cell Transplant
STUDY NUMBER	15-007
STUDY PHASE	Phase 3
LOCATION	This study will be conducted at approximately 100 enrolling study sites in countries throughout the world, including the United States (US).
PRIMARY OBJECTIVE	The primary objective of the study is to compare the efficacy of defibrotide prophylaxis in addition to best supportive care (BSC) (DP arm) vs BSC alone (BSC arm) for the prevention of veno-occlusive disease (VOD) as measured by VOD-free survival by Day +30 post-hematopoietic stem cell transplant (HSCT) in patients who are at high risk or very high risk for developing VOD.
SECONDARY OBJECTIVES	The key secondary objective of the study is to compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of VOD as measured by VOD-free survival by Day +100 post-HSCT in patients who are at high risk or very high risk for developing VOD. Other secondary objectives of the study are as follows: • To further compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) on additional variables, as follows: – Incidence of VOD by Day +30 post-HSCT – VOD-free survival by Day +180 post-HSCT – Non-relapse mortality (NRM) by Day +100 and by Day +180 post-HSCT – Incidence of VOD-associated multi-organ dysfunction (MOD) (i.e., severe VOD) by Day +30 and by Day +100 post-HSCT (in those patients who develop VOD) – Proportion of patients who have resolution of VOD by Day +180 post-HSCT and time to resolution of VOD (in those patients who develop VOD) – Incidence of VOD after Day +30 post-HSCT, by Day +100, and by Day +180 post-HSCT • To compare the health-related quality of life using the following questionnaires: – 5-Level EuroQol-5D (EQ-5D-5L) (adults only) – EuroQol-5D for Youth (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) • To characterize the pharmacokinetics (PK) of defibrotide • To compare the overall safety of defibrotide in addition to BSC vs BSC alone, including adverse event (AE) profile, serious adverse event (SAE) profile, laboratory abnormalities, and vital signs (including peri-infusional vital signs for patients who receive defibrotide) • To compare the overall safety of defibrotide in addition to BSC vs BSC

	alone by comparing the incidence of grades 2, 3, and 4 acute graft-versus-host disease (GvHD) by Day +30, Day +100, and Day +180 post-HSCT, and the incidence of chronic GvHD at Day +180 post-HSCT • To compare graft failure and time to neutrophil and platelet engraftment
EXPLORATORY OBJECTIVES	The exploratory objectives of this study are as follows: • To compare the hospital resource utilization for defibrotide prophylaxis and BSC patients • To evaluate plasma concentration of potential predictive or prognostic VOD biomarkers (which may include but will not be limited to vascular cell adhesion molecule 1 [VCAM1], von Willebrand factor [vWF], L-ficolin, plasminogen activator inhibitor [PAI-1], thrombomodulin, C-reactive protein [CRP], angiopoietin 2 [ANG2]) and/or GvHD biomarkers (which may include but will not be limited to tumor necrosis factor receptor 1 [TNFR1], interleukin-1 receptor-like-1 [IL1RL1, also known as ST2], and regenerating islet-derived 3-alpha [REG3α]) • To evaluate immunogenicity of defibrotide in patients who receive defibrotide for treatment or prophylaxis.
DESIGN	This is a Phase 3, randomized, adaptive study comparing the efficacy and safety of defibrotide vs BSC in the prevention of hepatic VOD in adult and pediatric patients undergoing HSCT who are at high risk or very high risk of developing VOD, as diagnosed using the modified Seattle criteria. A total of 400 patients are planned for enrollment to ensure completion of approximately 360 patients. An interim analysis overseen by an independent Data Monitoring Committee (DMC) is planned when 70% of patients are evaluable for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post-HSCT), with pre-specified rules for efficacy stop, futility stop, and possible sample size re-estimation up to a maximum of 600 patients total. After informed consent or assent has been obtained from patients, or legal parent/guardians or representatives, as applicable, screening procedures will be performed within 14 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 BSC ("DP arm") or BSC alone ("BSC arm") in a 1:1 ratio. Randomization will be stratified according to risk of developing VOD (high-risk or very high-risk), age (>16 years or ≤16 years), and country using an interactive web response system (IWRS). Enrollment of those patients meeting high-risk criteria will be capped at 65% of the total enrollment using IWRS. All patients enrolled in the study will receive individualized standard of care therapy based on local institutional guidelines and patient need. This standard of care therapy or "best supportive care" (BSC) is intended to serve as a study control for comparison with those patients randomized to receive defibrotide prophylaxis. Patients randomized to the "best supportive care" arm (BSC arm) will receive standard of care therapy per institutional guidelines and patient need; patients randomized to the defibrotide prophylaxis arm (DP arm) will also receive standard of care therapy based on local institutional guidelines and patient need plus defibrotide prophylaxis. Prophylactic use of defibrotide added to "best supportive care" (DP arm) will be compared to "best supportive care" (BSC arm). Administration of defibrotide to patients in the DP arm will begin within 24 hours of the first dose of conditioning regimen (i.e., Study Day 1), and will continue (for those patients without a VOD diagnosis) for a recommended minimum of 21 days and end no later than Day +30 post-HSCT. (Note: 21 days of defibrotide prophylaxis corresponds to approximately Day +15 post-HSCT, assuming 5 days of conditioning therapy prior to HSCT). For patients in the BSC arm, administration of BSC according to institutional

	guidelines and patient need will begin on the first day of conditioning (i.e., Study Day 1) and continue until Day +30 post-HSCT or hospital discharge, whichever is sooner, or diagnosis of VOD, if applicable. Patients receiving BSC (i.e., randomized to the BSC arm and not being treated for VOD) should not receive defibrotide as part of their BSC regimen. If patients in either the DP or BSC arm develop VOD, per the modified Seattle criteria, they may receive rescue defibrotide treatment for VOD as prespecified in the informed consent and/or assent forms. For patients in either arm who develop VOD, defibrotide for treatment of VOD should be administered until resolution of VOD or hospital discharge. Patients will continue to be monitored for development of late-onset VOD through Day +180 post-HSCT. Patients who develop clinical signs and symptoms of VOD after hospital discharge/Day +30 post-HSCT will require more frequent monitoring (refer to specific instructions within schedule of assessments), and re-admission to the hospital will be at the investigator's discretion.
ESTIMATED DURATION OF STUDY	The study is expected to last approximately 5 years, with an estimated enrollment period of 4.5 years for the maximum of 600 patients and duration of participation for each patient lasting approximately 6 months.
STUDY POPULATION	A total of 400 patients are planned for enrollment to ensure completion of approximately 360 patients. If DMC recommends to increase the sample size after the interim analysis, an increase in sample size up to a maximum of 600 patients total as recommended by DMC will be implemented.
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION	Adult and pediatric patients scheduled to undergo HSCT who are at high risk or very high risk of developing VOD will be enrolled in the study. Each patient must meet the following criteria to be enrolled in this study. Study treatment is defined as defibrotide prophylaxis in addition to BSC or BSC alone. 1. Patient must be above the age of 1 month as of Study Day 1 (as defined in Section 3.1). 2. Patient must be scheduled to undergo allogeneic (adults or pediatric patients) or autologous HSCT (pediatric patients only) and be at high risk or very high risk of developing VOD. a. High-risk patients must meet both of the following criteria (i and ii): i. Patient must be scheduled to receive myeloablative conditioning, defined as either of the following: a. At least 2 alkylating agents (e.g., cyclophosphamide, busulfan, melphalan); the investigator must document in the medical chart that the conditioning regimen is considered to be myeloablative or b. Total body irradiation (TBI) (single dose of ≥5 Gy, or ≥8 Gy fractionated dose) and at least 1 alkylating agent, and ii. Patient must meet at least 1 of the following criteria (a or b): a. Has at least 1 hepatic-related risk factor, as defined by the European Society for Blood and Marrow Transplantation (EBMT) position statement (adapted and modified from Mohty et al. 2015), during screening as follows: • Transaminase level >2.5 times the upper limit of normal (ULN) during screening or within 14 days prior to screening on a non-screening test if the test was performed as part of patient's routine standard of care • Serum total bilirubin level >1.5 times the ULN during screening or within 14 days prior to screening on a

non-screening test if the test was performed as part of patient's routine standard of care
- Prior history of cirrhosis (with biopsy evidence)
- Prior history of hepatic fibrosis (by histology or other diagnostic scoring system per institutional guidelines)
- Prior history of viral hepatitis within 1 year before the start of study treatment, as indicated by an available positive test result(s) for any of the following:
  - Hepatitis A virus (HAV) immunoglobulin M (IgM) (anti-HAV IgM)
  - Hepatitis B virus (HBV) core immunoglobulin G (IgG) or IgM (anti-HBc IgG or anti-HBc IgM)
  - HBV surface antigen (HBsAg)
  - HBV deoxyribonucleic acid (DNA) by polymerase chain reaction (PCR) or nucleic acid amplification testing (NAAT)
  - Hepatitis C virus (HCV) antibody (anti-HCV) and HCV RNA by PCR or NAAT
- Any prior hepatic irradiation, including abdominal irradiation covering the hepatic area
- Documented diagnosis and confirmed evidence of iron overload in medical notes (repeated serum ferritin >2000 ng/mL and/or liver iron content ≥5.0 mg/gdw as estimated by magnetic resonance imaging T2*) within 3 months prior to screening (Armand et al. 2007, Armand et al. 2011)

or

b. Has advanced-stage neuroblastoma requiring myeloablative conditioning (defined in 2.a.i. above). Note: if the patient is scheduled to receive a tandem transplant, then enrollment may only occur following the first transplant and prior to second transplant.

b. Very high-risk patients must meet 1 of the following criteria:
   i. Osteopetrosis and patient must be scheduled to receive myeloablative conditioning, defined as either of the following:
      a. At least 2 alkylating agents (e.g., cyclophosphamide, busulfan, melphalan); the investigator must document in the medical chart that the conditioning regimen is considered to be myeloablative

      or

      b. TBI (single dose of ≥5 Gy, or ≥8 Gy fractionated dose) and at least 1 alkylating agent
   ii. Primary hemophagocytic lymphohistiocytosis (HLH), Griscelli II Chediak-Higashi syndrome, Hermansky-Pudiak II, X-linked lymphoproliferative disorders, X-linked severe combined immunodeficiency, X-linked hypogammaglobulinemia, or familial HLH 1-5 and undergoing myeloablative conditioning as defined in 2.b.i. above) (Weitzman 2011, Naithani et al. 2013).
   iii. Prior treatment with an ozogamicin-containing monoclonal antibody using the minimum dose and schedule, according to the patient prescribing information:
      - Gemtuzumab ozogamicin, at least 9 mg/m^2 total dose (Wadleigh et al. 2003)
      - Inotuzumab ozogamicin, at least 1.5 mg/m^2 over 28 days

	iv. Class III, high-risk thalassemia (i.e., patients who are ≥7 years old and have confirmed diagnosis of hepatomegaly (e.g., a liver size ≥5 cm below the costal margin on clinical examination) at the time of screening [Mathews et al. 2007]) 3. Female patients (and female partners of male patients) of childbearing potential who are sexually active 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 or partner include abstinence (when this is in line with the preferred and usual lifestyle of the patient [periodic abstinence, e.g., calendar, post-ovulation, symptothermal methods, and withdrawal are not acceptable methods]), combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (i.e., birth control pills, patches, vaginal ring), progestogen-only hormonal contraception associated with inhibition of ovulation (i.e., progestin implant or injection), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), surgical sterilization, and vasectomy (>6 months before Study Day 1). Post-menopausal women (i.e., women with >2 years of amenorrhea) do not need to use contraception. 4. Adult patients must be able to understand and sign a written informed consent. For minor 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. Patients who meet any of the following criteria will be excluded from the study. Study treatment is defined as defibrotide prophylaxis in addition to BSC or BSC alone. 1. Patient has hemodynamic instability within 24 hours before the start of study treatment. 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 (e.g., 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 (e.g., pulmonary hemorrhage or central nervous system 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 (tPA), 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 use will be allowed in both treatment arms (up to 100 U/kg/day). 4. Patient is using or plans to use an investigational agent for the prevention or treatment of VOD. 5. Patient, in the opinion of the investigator, may not be able to comply with

	the safety monitoring requirements of the study. 6. Patient or parent/legal guardian or representative has a psychiatric illness that would prevent the patient or parent/legal guardian or representative from giving informed consent and/or assent. 7. 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. 8. Patient is pregnant or lactating and does not agree to stop breastfeeding. 9. Patient has a known history of hypersensitivity to defibrotide or any of the excipients. 10. Patient or parent/legal guardian or representative lacks the full mental capacity to understand and sign a written informed consent. 11. Patient is receiving or plans to receive other investigational therapy during study.
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. Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a 2-hour window between the end of an infusion and the start of the next infusion. Individual doses of defibrotide are determined for individual patients by the study site pharmacist and are based on body weight at baseline (i.e., the day before conditioning begins for the DP arm, and on the day that conditioning begins for patients in the BSC arm who develop VOD and subsequently receive defibrotide). For the DP arm, 2-4 doses of defibrotide must be administered within 24 hours prior to conditioning. Each of the 4 divided doses per day will be rounded to the nearest 10 mg for adults (>35 kg) and the nearest 5 mg for children (≤35 kg) to facilitate efficient drug administration.
REFERENCE THERAPY	Best supportive care: BSC will be administered according to institutional standards and patient need.
DURATION OF TREATMENT	For patients randomized to receive defibrotide prophylaxis, defibrotide will be administered starting within 24 hours of the first dose of the conditioning regimen (i.e., Study Day 1) for a recommended minimum of 21 days and ending no later than Day +30 post-HSCT. Patients in this arm will also receive individualized standard of care therapy based on local institutional guidelines and patient need. Patients must undergo Day +15 post-HSCT assessments before hospital discharge; if a patient is discontinued early from study/study treatment (prior to Day +15 post-HSCT), the Day +15 post-HSCT assessments must be completed on the day of study/study treatment discontinuation (+1 day) Best supportive care for patients in the BSC arm will be administered beginning on the first day of conditioning (i.e., Study Day 1) and will continue until Day +30 post-HSCT or hospital discharge, whichever is sooner, or diagnosis of VOD, if applicable. For patients in either the DP or BSC arm who develop VOD per modified Seattle criteria, defibrotide will be administered until resolution of VOD or hospital discharge, whichever is sooner, and may continue beyond Day +30 post-HSCT. Patients with VOD undergoing rescue treatment with defibrotide will continue to receive individualized standard of care therapy based on local institutional guidelines and patient need excluding agents that increase the risk of bleeding.
EFFICACY	Efficacy will be assessed through monitoring of hepatic function for

ASSESSMENTS	development of VOD, relapse of disease, VOD-associated MOD (i.e., severe VOD), resolution of VOD, and survival. Other assessments include health-related quality of life, hospital resource utilization, and measurement of biomarkers in blood indicative of potential predictive or diagnostic VOD and/or GvHD biomarkers, and immunogenicity of defibrotide in patients who receive defibrotide for treatment or prophylaxis.
PHARMACOKINETIC ASSESSMENTS	Blood samples (3 mL) for measurement of defibrotide concentrations will be collected according to an intensive schedule from a subset of patients in the DP arm (approximately 25 patients weighing ≥30 kg) to characterize the pharmacokinetics of defibrotide for prophylaxis as follows: • On Day +1 and Day +7 post-HSCT at the first defibrotide infusion that is administered after 6:00 AM at the following time points: – Within 15 minutes before the start of the infusion – At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) – At the following times after the start of the infusion: 2.25 hours (±5 minutes), 2.5 hours (±10 minutes), 2.75 hours (±10 minutes), 3.0 hours (±10 minutes), 3.5 hours (±15 minutes), 4.0 hours (±15 minutes), and 5.0 hours (±15 minutes) • On Day +15 and Day +30 post-HSCT (for patients still receiving defibrotide) – At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) at the first defibrotide infusion of the day starting after 6:00 AM. The remainder of patients in the DP arm (weighing >15 kg) will follow a sparse schedule for collection of pharmacokinetic (PK) samples. Blood samples will be collected after the first defibrotide infusion that is administered after 6:00 AM, as follows: • On Day +7 post-HSCT at the following time points – At 2 hours after the start of the infusion (within ±15 minutes before the end of infusion) – At any time during the 2-hour window after the end of infusion (exact time relative to infusion must be recorded) • On Day +15 and Day +30 post-HSCT (for patients still receiving defibrotide) – At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) Patients in the DP arm and BSC arm, weighing ≥30 kg who develop VOD and receive defibrotide for rescue treatment of VOD will also have fewer PK samples collected (contingent schedule). All feasible efforts will be employed to collect blood samples as follows: • On Day 1 after the start of rescue defibrotide, 5 samples over the course of any single dosing interval at the following time points: – Within 15 minutes before the start of the infusion – At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) – At the following times after the start of the infusion: 3 hours (±10 minutes), 4 hours (±15 minutes), and 5 hours (±15 minutes) • On Day 7 after the start of rescue defibrotide treatment, for patients still receiving defibrotide – At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) at the first defibrotide infusion

	that is administered after 6:00 AM of the day.
SAFETY ASSESSMENTS	Safety will be assessed through monitoring of AEs, clinical laboratory tests, vital signs (including peri-infusional vital signs for patients who receive defibrotide), physical examinations, monitoring for GvHD, neutrophil and platelet engraftment and graft failure, and Karnofsky/Lansky performance scales.
STATISTICAL ANALYSIS	Based on published studies and results from a previously conducted prevention study (Study 2004-000592-33) with defibrotide (Corbacioglu et al. 2012), the proposed sample size for this study is 200 patients per treatment arm for a total sample size of 400 patients. Through simulations, it is shown that this sample size provides a 90% power to detect a hazard ratio (HR) of 0.46 for VOD-free survival by Day +30 post-HSCT in DP arm as compared with BSC arm, with an average of 68 events total. The HR of 0.46 is based on 86% and 72% VOD-free survival rates by Day +30 post-HSCT for DP arm and BSC arm, respectively, which translate to 14% and 28% as the incidence of VOD or death by Day+30 post-HSCT for the 2 arms, respectively. Due to uncertainties associated with the study design assumptions, specifically the background rate of events in the BSC arm and the size of the treatment effect, an interim analysis to be overseen by the DMC is planned when 70% of patients are evaluated for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post-HSCT), with specific rules for efficacy stop (i.e., 1-sided alpha of 0.0005), futility stop (i.e., conditional power <10%), and possible sample size re-estimation when the conditional power is in the promising zone. Details on the content of the interim analysis and adaptive design decision rules will be provided in an interim Statistical Analysis Plan (iSAP) and specified along with the responsibilities of the DMC as part of the DMC charter. The primary objective of the study is to compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of hepatic VOD as measured by VOD-free survival by Day +30 in patients undergoing HSCT who are at high risk or very high risk for developing VOD by Day +30 post-HSCT. The study is designed with both an interim and a final analysis. To maintain an overall significance level at 1-sided alpha of 0.025, the incremental alpha is specified at 1-sided 0.0005 for interim analysis and 1-sided 0.0245 for final analysis (the corresponding nominal alpha is 1-sided 0.0005 for interim analysis and 1-sided 0.02498 for final analysis). The key secondary objective of the study is to compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of VOD as measured by VOD-free survival by Day +100 post-HSCT in patients who are at high risk or very high risk for developing VOD. To control the study-wise type I error, a sequential testing strategy will begin with the test on primary efficacy endpoint. If the test is significant, the test on the key secondary efficacy endpoint will be conducted at 1-sided alpha = 0.025. This gate-keeping approach will keep the family-wise error rate at 1-sided 0.025 for the comparisons of the 2 treatment arms in the primary and the key secondary efficacy endpoints. The intent-to-treat (ITT) population will include all randomized patients. This will be the primary analysis population for the primary efficacy endpoint and all other efficacy endpoints. The modified intent-to-treat (mITT) population will include all randomized patients who proceed to HSCT after randomization. The mITT population will be used in the sensitivity analysis of the primary efficacy endpoint and the key secondary efficacy endpoint. The overall safety population will include all patients randomized to the DP arm who receive at least 1 dose of defibrotide and all patients randomized to

	the BSC arm. The PK population will include patients who have evaluable PK data for the PK analyses.
DATE OF PROTOCOL AMENDMENT (Version 4.0)	20 August 2018

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

ADA	Anti-drug antibody
AE	Adverse event
ALT	Alanine aminotransferase
ANG2	Angiopoietin 2
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATIII	Antithrombin III
BiPAP	Bilevel positive airway pressure
BMT	Bone marrow transplant
BSA	Body Surface Area
BSC	Best supportive care
BUN	Blood urea nitrogen
CEC	Central ethics committee
CFR	Code of Federal Regulations
cGMP	Current Good Manufacturing Practices
CI	Confidence interval
CMH	Cochran-Mantel-Haenszel
CrCl	Creatinine clearance
CRO	Clinical research organization
CTCAE	Common Terminology Criteria for Adverse Events
CRP	C-reactive protein
CT	Computed tomography
CVVH	Continuous veno-venous hemofiltration
D5W	5% Dextrose in water
DMC	Data Monitoring Committee
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
DP	Defibrotide prophylaxis

EBMT	European Society for Blood and Marrow Transplantation
eCRF	electronic Case Report Form
eCOA	Electronic clinical outcome assessment
EMA	European Medicines Agency
EPAC	Endpoint Adjudication Committee
EQ-5D	EuroQol-5D
EQ-5D-5L	5-Level EuroQol-5D health questionnaire
EQ-5D-Y	EuroQol-5D health questionnaire for Youth
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GvHD	Graft-versus-host disease
HAV	Hepatitis A virus
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HLA	Human leukocyte antigen
HLH	Hemophagocytic lymphohistiocytosis
HR	Hazard ratio
HSCT	Hematopoietic stem cell transplant
ICF	Informed Consent Form
ICH	International Council for Harmonisation
ICU	Intensive care unit
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system

IL1RL1	Interleukin-1 receptor-like-1, also known as ST2
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
iSAP	Interim Statistical Analysis Plan
ITT	Intent-to-treat
IWRS	Interactive web response system
MedDRA	Medical Dictionary for Regulatory Activities
MCV	Mean corpuscular volume
mITT	Modified intent-to-treat
MOD	Multi-organ dysfunction
NAAT	Nucleic acid amplification testing
NRM	Non-relapse mortality
PAI-1	Plasminogen activator inhibitor 1
PCR	Polymerase chain reaction
PGE1	Prostaglandin E1
PK	Pharmacokinetic
PS	Performance scale
REG3α	Regenerating islet-derived 3-alpha
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SOP	Standard operating procedure
SOS	Sinusoidal obstruction syndrome
SUSAR	Suspected serious unexpected adverse reaction
TEAEs	Treatment-emergent adverse events
TBI	Total body irradiation
TNFR1	Tumor necrosis factor receptor 1
t-PA	Tissue plasminogen activator (alteplase)
ULN	Upper limit of normal

US	United States
VCAM1	Vascular cell adhesion molecule 1
VOD	Hepatic veno-occlusive disease
vWF	von Willebrand factor
WBC	White blood cell
WHODRUG	World Health Organization Drug Dictionary
WHVPG	Wedged hepatic vein pressure gradient

1 INTRODUCTION

1.1 Background and Rationale

Defibrotide (defibrotide sodium) (brand name Defitelio®) has been approved in the United States (US) for the treatment of adults and pediatric patients with hepatic veno-occlusive disease (VOD), also known as sinusoidal obstruction syndrome (SOS), with evidence of renal or pulmonary dysfunction following hematopoietic stem cell transplantation (HSCT). Defibrotide is currently marketed in Europe, Israel, and South Korea with the brand name Defitelio for the treatment of severe hepatic VOD, also known as SOS, in HSCT therapy.

The pathophysiology of VOD is not well understood but is associated with numerous risk factors including the conditioning regimen that patients receive prior to HSCT. In particular, conditioning regimens that include high-dose alkylating chemotherapy agents or total body irradiation (TBI) can injure the sinusoidal and endothelial cells along with hepatocytes, causing clots at the injury sites and consequently, obstruction of the small veins in the liver. Later pathologic changes associated with VOD include deposition of collagen in the sinusoids, sclerosis of venular walls, and fibrosis of venular lumens (Shulman et al. 1994). Hepatocellular necrosis and vascular occlusion can lead to reversal of hepatic flow, hepatorenal pathophysiology, and ultimately, liver, pulmonary, and renal failure leading to death (Bearman et al. 1992, Bearman 1995, McDonald et al. 1993). Since conditioning regimen toxicity is considered to be the eliciting stimulus for endothelial cell damage and resultant VOD, prophylaxis of VOD requires initiation of therapy prior to this injury.

Currently, there are no approved therapies for the prophylaxis of VOD. Several experimental approaches for the prevention of VOD have not proven successful, leaving the prevention of VOD limited to supportive care and use of non-toxic agents with limited, if any, clinical benefit (Essell et al. 1998, Ohashi et al. 2000, Ruutu et al. 2002, Barkholt et al. 2008, Brown et al. 1998).

Prevention of hepatic VOD, therefore, represents an unmet medical need. Due to the lack of effective therapeutic measures, prevention of VOD would have substantial clinical impact. An optimal preventive approach would decrease the incidence of VOD itself, and the progression to VOD-associated complications (including renal and/or pulmonary failure, dialysis, encephalopathy, and admission to an intensive care setting), and increase VOD-free survival.

1.2 Clinical Experience

Defibrotide is a highly complex polydisperse collection of single-stranded polydeoxyribonucleotides prepared by controlled depolymerization of deoxyribonucleic acid (DNA) from porcine intestinal tissue that acts on the vascular endothelium to protect or repair damaged cells in the setting of VOD. Defibrotide has a complex mechanism of action with demonstrated endothelial protective properties, as well as profibrinolytic, antithrombotic, anti-ischemic, anti-inflammatory, and anti-adhesive activities, but no significant systemic anticoagulant effects (Palmer & Goa 1993, Falanga et al. 2003, Eissner et al. 2002). Nonclinical studies have revealed that defibrotide can protect human endothelial

cells against chemotherapy-induced cell death and activation, without compromising the antitumor effects of cytotoxic therapy (Eissner et al. 2002). This provides a local mechanism for maintenance of sinusoidal vascular integrity.

The acceptable tolerability of defibrotide and its biological activity as a protective effect on the endothelium suggest that defibrotide may provide a means of prophylaxis for VOD if administered near the time of vascular insult resulting from HSCT conditioning regimens. Furthermore, earlier initiation of defibrotide following a diagnosis of VOD has shown to be more successful than later initiation of treatment, as evidenced by improved Day +100 survival (Richardson et al. 2016). Defibrotide regimens for the prevention of VOD have consisted of doses ranging from approximately 10 to 40 mg/kg/day, administered 4 times daily from the start of conditioning until 28 to 30 days after HSCT. Data have been published from 10 investigators in 595 patients, including at least 124 children (Joshi et al. 2002, Chalandon et al. 2004, Versluys et al. 2004, Hasenkamp et al. 2005, Corbacioglu et al. 2006, Bonini et al. 2007, Dignan et al. 2007, Qureshi et al. 2008, Milone et al. 2008, Cappelli et al. 2009). The tolerability of the drug was consistently demonstrated in these studies. In uncontrolled studies, the development of VOD was lower with defibrotide compared to the usual risk in this patient population. In 3 controlled studies (Table 1), patients treated with defibrotide prophylaxis had a lower incidence of VOD than patients in control groups.

Table 1 Use of Defibrotide for the Prevention of Hepatic Veno-Occlusive Disease

Study	Study Design	Dose, Duration of treatment	Total Number of Patients		% of Patients who Developed VOD	
			Defibrotide	Control	Defibrotide	Control
Chalandon et al. 2004	Open-label, historically controlled	200-400 mg/day (10-25 mg/kg/day) in 4 divided doses + heparin from Day –7 to Day +20 post-HSCT	52 (age range, 5-60 years)	52 (age range, 4-60 years)	0	19%
Corbacioglu et al. 2006	Open-label, historically controlled	20-40 mg/kg/day in 4 divided doses with conditioning until Day +30 post-HSCT	9 (all <2 years)	11 (all <2 years)	11%	63%
Corbacioglu et al. 2012	Open-label vs best supportive care	25 mg/kg/day in 4 divided doses prior to conditioning until Day +30 post-HSCT	180 (all <18 years)	176 (all <18 years)	12%	20%

HSCT=hematopoietic stem cell transplant; VOD=veno-occlusive disease.

Chalandon et al. reported data on 52 successive patients transplanted for hematological malignancies between 1999 and 2002 (Chalandon et al. 2004). Prophylactic defibrotide (dose range, 10 to 25 mg/kg/day) was administered intravenously, starting before conditioning and continuing to Day +20 post-HSCT, in addition to heparin. None of the 52 patients developed VOD, and no side effects were attributed to defibrotide. These results were significantly

different (p=0.0012) to results from 52 successive patients who were not given defibrotide and who served as historical controls between 1997 and 1999. Of these 52 patients, 10 (19%) patients developed VOD, and 3 of these patients died of severe VOD. The prophylactic use of defibrotide did not adversely affect engraftment.

Corbacioglu et al. reported data from 9 children who underwent HSCT for osteopetrosis between 2001 and 2005 and were treated with defibrotide for prophylaxis of VOD (Corbacioglu et al. 2006). The incidence of VOD was retrospectively compared with a control group of 11 comparable patients who underwent HSCT for osteopetrosis between 1996 and 2000 and who did not receive defibrotide for prophylaxis. Hematopoietic stem cell transplant as treatment for this rare disease is associated with a high incidence of VOD. The overall incidence of VOD in the control group was 63.4% (7 of 11 patients) compared with 11.1% (1 of 9 patients) in the defibrotide prophylaxis group. Severe VOD occurred in 3 patients in the control group compared with no patients in the defibrotide prophylaxis group. The overall mortality rate was 18.2% (2 of 11 patients) in the control group and 11% (1 of 9 patients) in the defibrotide prophylaxis group, although only 1 of the 2 deaths in the control group was due to severe VOD. The other patient with VOD in the control group and the patient in the defibrotide prophylaxis group died from complications related to graft rejection. Defibrotide was well tolerated.

Finally, Corbacioglu et al. reported data from 356 children who were considered to be at high risk of VOD following myeloablative HSCT (Corbacioglu et al. 2012). Disease or transplant characteristics that placed a child at “high risk” of VOD included pre-existing liver disease (including any hepatic complications before transplantation, doubling of transaminase concentrations, and previous abdominal irradiation), second myeloablative HSCT, allogeneic HSCT for leukemia beyond the second relapse, conditioning with busulfan and melphalan, previous treatment with gemtuzumab ozogamicin, and diagnosis of inherited hemophagocytic lymphohistiocytosis (HLH), adrenoleukodystrophy, or osteopetrosis. Children were randomly assigned to treatment with defibrotide (25 mg/kg/day starting prior to conditioning through Day +30 post-HSCT) or best supportive care (BSC) between 2006 and 2009. Crossover of children randomly assigned to receive BSC was permitted upon a clinical diagnosis of VOD using modified Seattle criteria (see Section 1.4). Twenty-two (12%) of 180 patients randomly assigned to the defibrotide group developed VOD by Day +30 post-HSCT compared with 35 (20%) of 176 patients in the control group (risk difference -7.7%; 95% confidence interval (CI) –15.3 to –0.1; p=0.0488 [Z test for competing risk analysis]; p=0.0507 [log-rank test]). Of 177 patients in the defibrotide group, 154 (87%) had adverse events (AEs) by Day +180 post-HSCT compared with 155 (88%) of 176 patients in the control group.

The Sponsor will investigate the effectiveness and safety of prophylactic defibrotide in the current study, which will include both adult and pediatric patients at high risk or very high risk of developing VOD (see Section 1.5 and Section 4.1). Patients in both arms of the study will receive individualized standard of care therapy based on local institutional guidelines and patient need. This standard of care therapy or “best supportive care” (BSC) is intended to serve as a study control for comparison with those patients randomized to receive defibrotide prophylaxis. Patients randomized to the “best supportive care” arm (BSC arm) will receive

standard of care therapy per institutional guidelines and patient need; patients randomized to the defibrotide prophylaxis arm will also receive standard of care therapy based on local institutional guidelines and patient need plus defibrotide prophylaxis. Prophylactic use of defibrotide added to "best supportive care" (DP arm) will be compared to "best supportive care" alone (BSC arm). Any patient in either arm who develops VOD will be permitted to receive defibrotide.

1.3 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 the day before the first day of the conditioning regimen for a recommended minimum of 21 days and stopping no later than Day +30 post-HSCT. Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a 2-hour window between the end of an infusion and the start of the next infusion. Using a consistent minimum duration of inpatient treatment in the DP arm is seen as necessary for accurate assessment of a treatment effect. Although VOD is typically diagnosed before Day +21 post-HSCT (Bearman 1995, Richardson and Guinan 1999), 21 days of prophylactic treatment (corresponding to approximately Day +15 post-HSCT, assuming 5 days of conditioning therapy prior to HSCT) was selected in order to allow an optimal time for diagnosis of VOD while minimizing patients' exposure to the unnecessary risks of prolonged hospitalization should a patient be ready for early hospital discharge. Discharge of patients from the hospital before they have received the recommended minimum duration of treatment (i.e., 21 days) will not constitute a protocol violation as this does not impact efficacy or safety analyses.

The safety of defibrotide 25 mg/kg/day as prophylaxis has been demonstrated in children undergoing myeloablative conditioning considered to be at high risk of developing VOD (Corbacioglu et al. 2012). Incidences of 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). Although the most common AE considered by the investigator to be related to defibrotide was hemorrhage, the cumulative incidence of hemorrhage was similar between groups (39 [22%] of 177 patients in the defibrotide group vs 37 [21%] of 176 patients in the control group; p=0.8176). Overall, most fatal AEs were either neoplasms (12 [7%] of 177 patients in the defibrotide group and 14 [8%] of 176 patients in the control group) or infections (6 [3%] patients in the defibrotide group and 11 [6%] patients in the control group). In the intent-to-treat (ITT) population, patients who underwent allogeneic HSCT and who received defibrotide prophylaxis had a lower overall incidence of acute graft-versus-host disease (GvHD) (all grades) by Day +30 and +100 post-HSCT compared with those in the control group (Day +30 post-HSCT, 34% vs 53% p=0.004; Day +100 post-HSCT, 47% vs 67% p=0.002). A lower incidence of acute GvHD was also observed when grade 1 toxicity was excluded.

1.4 Justification for Choice of Primary Efficacy Endpoint

Veno-occlusive disease is considered to be a clinical diagnosis, using non-specific signs and symptoms, as histopathological evidence by biopsy is frequently too dangerous to obtain. Veno-occlusive disease is typically diagnosed within the first 21 days following HSCT, although infrequent cases can occur up to Day +65 post-HSCT (Bearman 1995, Richardson & Guinan 1999). Two classification systems are commonly employed for diagnosis of VOD, Baltimore criteria (Jones et al. 1987) and Seattle criteria (McDonald et al. 1984), the key difference being that Seattle criteria do not include a strict requirement for elevated total bilirubin for diagnosis of VOD:

- Baltimore criteria require elevated total bilirubin >2 mg/dL before Day +21 post-HSCT and 2 of the following criteria: hepatomegaly, ascites, or weight gain ≥5% from baseline
- Seattle Criteria and Modified Seattle criteria require at least 2 of the following 3 criteria before Day +20 post-HSCT: elevated total bilirubin >2 mg/dL; ascites and/or unexplained weight gain ≥5% from baseline; or hepatomegaly with or without right upper quadrant pain
  Note: These are "modified Seattle criteria;" the original definition of Seattle Criteria required weight gain ≥2% from baseline.

Note: Baseline values for weight and bilirubin level will be obtained within 24 hours before the first dose of conditioning regimen for the DP arm and on the day that conditioning begins for the BSC arm. Baseline values for hepatic size and presence of ascites will be obtained during ultrasound examination at the screening visit.

A literature review of previous clinical studies investigating drugs for prevention of VOD (Table 2) shows a consistent use of VOD as a landmark endpoint to assess the utility or lack of utility of a drug in the prevention of VOD occurrence. Although some clinical studies reported VOD by both Baltimore and modified Seattle/Seattle criteria, the most commonly used criteria were modified Seattle criteria. The most commonly used time point was Day +30 post-HSCT, although several studies used either an earlier time point (Day +20 or Day +21 post-HSCT) or a later time point (Day +40, Day +90, or Week +24 post-HSCT). In many clinical studies, VOD-free survival reported by Day +100 post-HSCT was also used as a key secondary endpoint.

Table 2 Previous Clinical Studies Investigating Drugs for Prevention of Veno-Occlusive Disease

Reference	Agent	Trial Design	VOD Efficacy Endpoint	VOD as Primary Efficacy Endpoint?	Patient Population (n)	VOD Incidence [Comments]
Defibrotide Study						
Corbacioglu et al. 2012	Defibrotide	Randomized, controlled (vs BSC)	VOD by D+30 by modified Seattle criteria	Yes	180 vs 176	12% vs 20%

Table 2 Previous Clinical Studies Investigating Drugs for Prevention of Veno-Occlusive Disease

Reference	Agent	Trial Design	VOD Efficacy Endpoint	VOD as Primary Efficacy Endpoint?	Patient Population (n)	VOD Incidence [Comments]
Non-defibrotide Studies						
Gluckman et al. 1990	PGE1	Randomized, controlled (vs BSC)	VOD (at any time or autopsy)	Yes	50 vs 59	12.2% vs 25.5%
Bearman et al. 1993	PGE1	4 dose levels	VOD by D+20	Yes	24	33% [Severe drug-related toxicity]
Song et al. 2006	Liposomal PGE1 (vs low dose heparin or BSC)	Retrospective comparator	VOD by D+20	Yes	40 vs 10 vs 35	35% PGE1 20% Heparin 26% BSC
Attal et al. 1993	Pentoxifylline + heparin vs heparin	Randomized prospective trial	VOD by Seattle criteria by D+35	No (regimen related toxicity)	70 vs 71	3/70 and 2/71 patients
Ferra et al. 1997	Pentoxifylline, ciprofloxacin, prednisolone	Parallel enrollment (not randomized)	VOD by Seattle Criteria	No (regimen related toxicity)	37 vs 16	8.1% vs 25.0% [but higher incidence of death by D+35: 11% vs 6%]
Essell et al. 1998	Ursodeoxycholic acid	Randomized placebo-controlled prospective trial	VOD by D+30 by modified Seattle criteria	Yes	32 vs 34	15.0% vs 40.0%
Ohashi et al. 2000	Ursodeoxycholic acid	Randomized open-label prospective trial	VOD by Week 24 by modified Seattle	Yes	67 vs 65	3.0% vs 18.5%
Ruutu et al. 2002	Ursodeoxycholic acid	Randomized open-label prospective trial	VOD by Baltimore and Seattle criteria	No (increased total bilirubin)	123 vs 119	2% vs 4% (Baltimore) 11% vs 12% (Seattle)
Attal et al. 1992	Heparin	Prospective comparison vs BSC	VOD by D+30	Yes	81 vs 80	2.5% vs 13.7%
Rosenthal et al. 1996	Heparin	Historically controlled trial	VOD by D+20 by Seattle criteria	Yes	50 vs 70	10.0% vs 25.7%
Marsa-Vila et al. 1991	Historical control Heparin vs BSC Non-randomized arm	Historically controlled trial Randomized prospective trial Non-randomized arm (high risk)	VOD by D+30 by Baltimore criteria	Yes	98 52 vs 46 38	3.1% 2.2% vs 7.7% 16.1%
Reiss et al. 2002	Heparin	Retrospective chart review vs matched controls	VOD by D+20 by Seattle criteria	Yes	175 vs 66	30% (heparin prophylaxis) vs 18% (without heparin prophylaxis)
Barkholt et al. 2008	N-acetyl cysteine	Randomized open-label prospective trial	VOD by D+21 by modified Seattle criteria	Yes	72 vs 88	2/72 and 3/88
Or et al. 1996	LMWH (enoxaparin)	Randomized open-label prospective trial	VOD by D+40 or hospital discharge by Seattle criteria	No (safety)	28 vs 33	"VOD parameters occurred less frequently" with LMWH

Table 2 Previous Clinical Studies Investigating Drugs for Prevention of Veno-Occlusive Disease

Reference	Agent	Trial Design	VOD Efficacy Endpoint	VOD as Primary Efficacy Endpoint?	Patient Population (n)	VOD Incidence [Comments]
Simon et al. 2001	Heparin Heparin + PGE1 Heparin LMWH BSC	Retrospective chart review	VOD by D+30	Yes	104 110 106 142	11% 12% 4% 22%
Haussmann et al. 2006	Antithrombin III vs HC	Sequential groups	VOD by D+30 by modified Seattle	Yes	91 vs 71	15% vs 18%

VOD=veno-occlusive disease; D=day; BSC=best supportive care; HC=historical control; LMWH=low molecular weight heparin; PGE1=prostaglandin E1

For the current study, the primary efficacy endpoint is a composite of survival status and VOD occurrence as determined by modified Seattle criteria measured as VOD-free survival by Day +30 post-HSCT. The VOD diagnosis will be adjudicated by a blinded Endpoint Adjudication Committee (EPAC).

VOD will be diagnosed using the modified Seattle criteria. Although other criteria are used for this clinical diagnosis, modified Seattle criteria are considered more sensitive, resulting in 2- to 3-fold higher rates of VOD diagnosis (for example, a recent series of 4171 patients [4290 HSCTs] demonstrated VOD occurrence of 11.0% using modified Seattle criteria compared to 3.9% using Baltimore criteria, although Baltimore criteria may better predict for higher VOD-associated mortality (Yakushijin et al. 2015). The lack of a mandatory elevation of total bilirubin (especially in the pediatric population in whom hyperbilirubinemia may be the last parameter to meet VOD criteria) permits earlier diagnosis of VOD, which may correlate with improved outcome.

The use of VOD-free survival by Day +30 post-HSCT as the primary endpoint should provide bone marrow transplant physicians with the most informative assessment of the treatment effect of defibrotide, taking into account both the potential observation of VOD by Day +30 post-HSCT as well as any potential for fatal toxicity with the examination of survival by this time point.

1.5 Justification for Selection of the Patient Population

In an unselected population, the incidence of VOD following HSCT has been estimated to be approximately 10% to 15% (Coppell et al. 2010). The incidence of VOD is estimated to be 20% to 25% in patients at high risk of VOD and 30% to 60% in patients at very high risk of VOD (Table 3). The assumed VOD-free survival at Day +30 post-HSCT considered in the design of this study takes into account a split between high risk and very high risk patients of 65% and 35%, respectively, which will be ensured at interim analyses, at the end of the study, and monitored throughout.

The all-cause mortality at this early time point (i.e., Day +30 post-HSCT) is not high; however, deaths do occur secondary to infection, GvHD, cardiopulmonary causes, relapse of underlying malignancy, and liver disease (primarily VOD). A recent publication noted a

decrease in all-cause mortality throughout the post-transplant course following myeloablative allogeneic HSCT; although Day +30 post-HSCT mortality was not separately reported, it would be estimated to be between 5% to 10% by Day +30 post-HSCT (Gooley et al. 2010). A separate study of intravenous versus oral busulfan for myeloablative HSCT reported 4% and 8% all-cause mortality by Day +30 post, respectively (Gupta et al. 2005). Given this, the incidence rate of VOD or death by Day +30 post-HSCT for this study (although it cannot be separately estimated by risk factor for VOD) would likely be 25% to 30% in patients at high risk of developing VOD and 33% to 63% in patients at very high risk of developing VOD.

The majority of patients in the high-risk population is predicted to be adult, whereas, patients in the very high-risk population are expected to be pediatric due to the presence of inherited diseases such as osteopetrosis and inherited hemophagocytic syndromes. There will however be adults in the very high-risk population (those receiving the ozogamicin antibody conjugates) and pediatric patients in the high-risk population (those with pre-existing liver disease and neuroblastoma).

Table 3 Estimated Risk of Developing Veno-Occlusive Disease by Day +30 Following Hematopoietic Stem Cell Transplant in High-Risk and Very High-Risk Patient Populations

Patient Subset	Characteristics	Estimated Incidence of VOD by Day +30 post-HSCT	Reference
High-risk population	Myeloablative conditioning and:		
	• ≥1 hepatic-related risk factor	5-60%	Mohty et al. 2015
	• Neuroblastoma (high-risk)	24%	Horn et al. 2002
Very high-risk population	Osteopetrosis	67%	Corbacioglu et al. 2012
		19%	Orchard et al. 2015
	An inherited hemophagocytic syndrome, e.g., familial HLH, Griscelli syndrome, or Chediak-Higashi syndrome	40%	Corbacioglu et al. 2012
		38%	Naithani et al. 2013
	Ozogamicin-containing monoclonal antibody prior to HSCT:		
	• Gemtuzumab ozogamicin	11% - 64%	Wadleigh et al. 2003, Chevallier et al. 2010
	• Inotuzumab ozogamicin	17- 31%	Pfizer INO-VATE ALL Study 1022,[a] 2012
			Kantarjian et al. 2013

	Class III, high-risk thalassemia (i.e., patients who are ≥7 years old and have a liver size ≥5 cm at the time of screening)	40%	Mathews et al. 2007

HLH=hemophagocytic lymphohistiocytosis; HSCT=hematopoietic stem cell transplant; VOD=veno-occlusive disease.
[a] A Phase 3 Study of Inotuzumab Ozogamicin versus Investigator's Choice of Chemotherapy in Patients with Relapsed or Refractory Acute Lymphoblastic Leukemia.

1.6 Potential Risks

Potential risks associated with defibrotide administration include the risk of bleeding in patients with VOD after HSCT, hypersensitivity reactions, and risk to pregnancy.

1.6.1 Hemorrhage

Defibrotide increased the activity of fibrinolytic enzymes in vitro and may increase the risk of bleeding in patients with VOD after HSCT. Defibrotide should not be used in patients with active bleeding. If a patient on defibrotide develops bleeding, defibrotide should be discontinued. Additionally, temporary discontinuation of defibrotide is recommended for patients at significant risk of major bleeding who undergo surgery or invasive procedures.

Concomitant use of defibrotide and a systemic anticoagulant or fibrinolytic therapy may increase the risk of bleeding. Therefore, anticoagulants and fibrinolytic agents should be discontinued prior to defibrotide treatment and the start of defibrotide administration should be delayed until the effects of the anticoagulant have abated. However, in this study, anticoagulant fibrinolytic therapy for routine central venous line management and intermittent dialysis or ultrafiltration is permitted, fibrinolytic instillation for central venous line occlusion is also permitted, and heparin use is allowed in both treatment arms (up to a maximum of 100 U/kg/day).

1.6.2 Hypersensitivity

Hypersensitivity reactions have occurred in less than 2% of patients treated with defibrotide; 1 case of anaphylactic reaction was reported in a patient who had previously received defibrotide (see Investigator's Brochure [IB] for Defibrotide). Patients should be monitored for hypersensitivity reactions, especially if there is a history of previous exposure to defibrotide. If a severe hypersensitivity reaction occurs, defibrotide should be discontinued and the hypersensitivity treated according to standard of care, and monitored until symptoms resolve. Patients with a known hypersensitivity to defibrotide or its excipients will be excluded from the study.

1.6.3 Pregnancy

There are no available data on the use of defibrotide in pregnant women. However, due to a possible increased risk of hemorrhage, which may increase the risk of miscarriage, defibrotide should not be used during pregnancy.

1.6.4 Other Risks

Bolus administration of defibrotide may cause flushing or a sensation of "generalized heat." For other potential risks, refer to the IB for Defibrotide

1.7 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 prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of VOD as measured by VOD-free survival by Day +30 post-HSCT in patients who are at high risk or very high risk for developing VOD.

2.2 Secondary Objectives

The key secondary objective of the study is to compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of VOD as measured by VOD-free survival by Day +100 post-HSCT in patients who are at high risk or very high risk for developing VOD.

Other secondary objectives of the study are as follows:

- To further compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) on additional variables, as follows:
  - Incidence of VOD by Day +30 post-HSCT
  - VOD-free survival by Day +180 post-HSCT
  - Non-relapse mortality (NRM) by Day +100 and by Day +180 post-HSCT
  - Incidence of VOD-associated multi-organ dysfunction (MOD) (i.e., severe VOD) by Day +30 and by Day +100 post-HSCT (in those patients who develop VOD)
  - Proportion of patients who have resolution of VOD by Day +180 post-HSCT and time to resolution of VOD (in those patients who develop VOD)
  - Incidence of VOD after Day +30 post-HSCT, by Day +100, and by Day +180 post-HSCT
- To compare the health-related quality of life using the following questionnaires:
  - 5-Level EuroQol-5D (EQ-5D-5L) (adults only)
  - EuroQol-5D for Youth (EQ-5D-Y), proxy version 1 (pediatric patients 4 to 7 years of age)
  - EQ-5D-Y, self-report version 1 (pediatric patients 8 to <16 years of age)
- To characterize the pharmacokinetics of defibrotide
- To compare the overall safety of defibrotide in addition to BSC vs BSC alone, including AE profile, SAE profile, laboratory abnormalities, and vital signs (including peri-infusional vital signs for patients who receive defibrotide)
- To compare the overall safety of defibrotide in addition to BSC vs BSC alone by comparing the incidence of grades 2, 3, and 4 acute GvHD by Day +30, Day +100, and Day +180 post-HSCT, and the incidence of chronic GvHD at Day +180 post-HSCT
- To compare graft failure and time to neutrophil and platelet engraftment

2.3 Exploratory Objectives

The exploratory objectives of this study are as follows:

- To compare the hospital resource utilization for defibrotide prophylaxis and BSC patients
- To evaluate plasma concentration of potential predictive or prognostic VOD biomarkers (which may include but will not be limited to vascular cell adhesion molecule 1 [VCAM1], von Willebrand factor [vWF], L-ficolin, plasminogen activator inhibitor [PAI-1], thrombomodulin, C-reactive protein [CRP], angiopoietin 2 [ANG2]) and/or GvHD biomarkers (which may include but will not be limited to tumor necrosis factor receptor 1 [TNFR1], interleukin-1 receptor-like-1 [IL1RL1, also known as ST2], and regenerating islet-derived 3-alpha [REG3α])
- To evaluate immunogenicity of defibrotide in patients who receive defibrotide for treatment or prophylaxis

3 STUDY DESIGN

3.1 Overall Study Design and Plan

This is a Phase 3, randomized, adaptive study comparing the efficacy and safety of defibrotide vs BSC in the prevention of hepatic VOD in adult and pediatric patients undergoing HSCT who are at high risk or very high risk of developing VOD, as diagnosed using the modified Seattle criteria.

A total of 400 patients are planned for enrollment to ensure completion of approximately 360 patients. An interim analysis overseen by an independent Data Monitoring Committee (DMC) is planned when 70% of patients are evaluable for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post-HSCT), with pre-specified rules for efficacy stop, futility stop, and possible sample size re-estimation of up to 600 patients total (see Section 9.3).

After informed consent or assent has been obtained from patients, or parent/legal guardians or representatives, as applicable, screening procedures will be performed within 14 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 BSC ("DP arm") or BSC without defibrotide prophylaxis ("BSC arm") in a 1:1 ratio. Randomization will be stratified according to risk of developing VOD (high-risk or very high-risk, as defined in Section 4.1), age (>16 years or ≤16 years), and country using an interactive web response system (IWRS). Enrollment of those patients meeting high-risk criteria will be capped at 65% of the total enrollment using IWRS.

All patients enrolled in the study (DP and BSC arms) will receive individualized standard of care therapy based on local institutional guidelines and patient need. This standard of care therapy or "best supportive care" (BSC) is intended to serve as a study control for comparison with those patients randomized to receive defibrotide prophylaxis. Patients randomized to the "best supportive care" arm (BSC arm) will receive standard of care therapy per institutional guidelines and patient need; patients randomized to the defibrotide

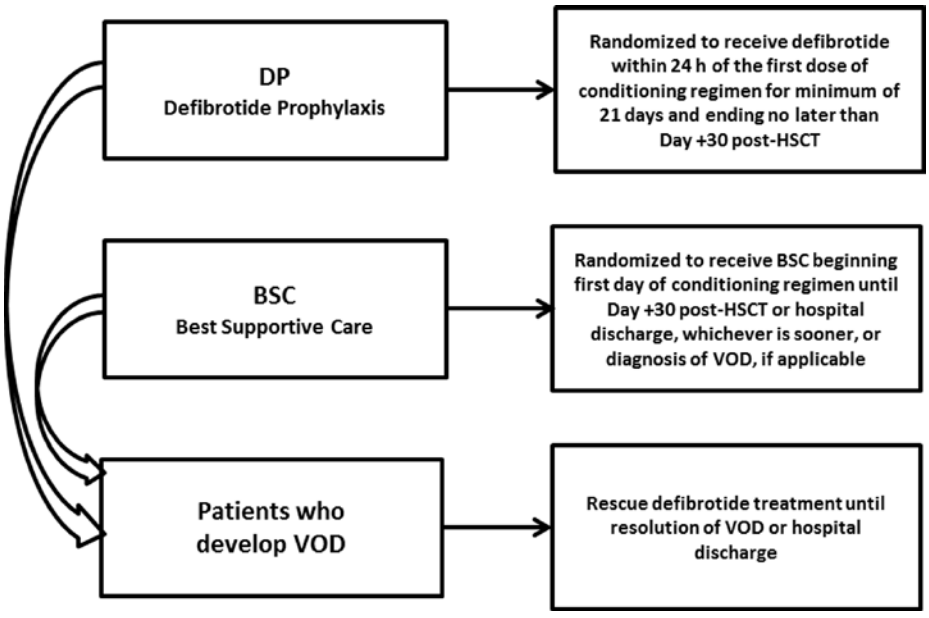
prophylaxis arm (DP arm) will also receive standard of care therapy based on local institutional guidelines and patient need plus defibrotide prophylaxis. Prophylactic use of defibrotide added to "best supportive care" (DP arm) will be compared to "best supportive care" (BSC arm).

Administration of defibrotide to patients in the DP arm will begin within 24 hours of the first dose of conditioning regimen (i.e., Study Day 1), and will continue (for those patients without a VOD diagnosis) for a recommended minimum of 21 days and end no later than Day +30 post-HSCT.

For patients in the BSC arm, administration of BSC according to institutional guidelines and patient need will begin on the first day of conditioning (i.e., Study Day 1) and continue until Day +30 post-HSCT or hospital discharge, whichever is sooner, or diagnosis of VOD, if applicable. Patients receiving BSC (i.e., randomized to the BSC arm and not being treated for VOD as rescue treatment) should not receive defibrotide as part of their BSC regimen.

If patients in either the DP or BSC arm develop VOD, per the modified Seattle criteria, they may receive rescue defibrotide treatment for VOD as prespecified in the informed consent and/or assent forms. For patients in either arm who develop VOD, defibrotide for rescue treatment of VOD should be administered until resolution of VOD or hospital discharge (see Figure 1 and Appendix 2 for details).

Figure 1. Study Diagram



HSCT=hematopoietic stem cell transplant; VOD=veno-occlusive disease.

Patients will continue to be monitored for development of late-onset VOD through Day +180 post-HSCT. Patients who develop clinical signs and symptoms of VOD after hospital discharge/Day +30 post-HSCT will require more frequent monitoring, and re-admission to

the hospital will be at the investigator's discretion. For all patients, confirmation (i.e., yes/no) that the modified Seattle criteria for diagnosis of VOD (e.g., total bilirubin level, hepatomegaly, weight gain, and/or ascites) have been met must be entered into the IWRS to allow dispensation of defibrotide for rescue treatment of VOD.

To reduce potential bias considering the open-label design, an independent EPAC that is blinded to study treatment assignment will be established to confirm retrospectively whether a patient met the criteria for VOD by Day +30 post-HSCT or by Day +100 post-HSCT, using the modified Seattle criteria. The EPAC will review relevant electronic case report forms (eCRFs), results of diagnostic procedures, and ultrasound results; alternate etiology (e.g., viral hepatitis, acute GvHD) will also be considered during review of patient data.

Efficacy will also be assessed through monitoring of hepatic function for development of VOD, relapse of disease, VOD-associated MOD, resolution of VOD, and survival.

Other assessments include health-related quality of life, and hospitalization resource utilization. Measurement of biomarkers in blood indicative of potential predictive or diagnostic VOD and/or GvHD biomarkers will also be performed. Blood samples for measurement of defibrotide concentrations will be collected according to an intensive schedule from a subset of patients in the DP arm to characterize the pharmacokinetics of defibrotide for prophylaxis. The remainder of patients in the DP arm will follow a sparse schedule for collection of pharmacokinetic (PK) samples; and all patients who develop VOD and receive defibrotide for rescue treatment of VOD will also have fewer PK samples collected. Serum samples to assess the immunogenicity of defibrotide will also be collected in any patients receiving defibrotide either as prophylaxis or rescue treatment (irrespective of treatment assignment).

Safety will be assessed through monitoring of AEs, clinical laboratory tests, vital signs (including peri-infusional vital signs for patients who receive defibrotide), physical examinations, monitoring for graft-versus-host disease, neutrophil and platelet engraftment and graft failure, and Karnofsky/Lansky performance scales.

Schedules of procedures and assessments are provided in Appendix 1 (Schedule of Procedures and Assessments for Patients Receiving Defibrotide Prophylaxis or BSC) and Appendix 2 (Schedule of Procedures and Assessments for Patients who Develop VOD and Receive Rescue Defibrotide).

3.2 Rationale for Study Design and Control Arm

The control for this study, BSC as reference therapy, was selected because no effective prophylactic treatment for VOD is currently available. Use of a placebo control was considered unethical due to increased risk of fluid overload and infection with intravenous administration.

The patient population selected for this study will be patients undergoing HSCT who are at high risk or very high risk of developing VOD. It is hypothesized that defibrotide prophylaxis will reduce the incidence of VOD in a high-risk population compared with BSC.

The clinical benefit, if confirmed, would be significant considering that the mortality of VOD with associated MOD has been estimated to be more than 80% by Day +100 post-HSCT (Coppell et al. 2010).

Throughout the trial, the Sponsor will monitor the defibrotide rescue treatment as well as investigator adherence to the pre-defined schedule of assessments. The importance of an accurate local diagnosis of VOD will be addressed through training at the investigator meetings and site initiation visits and periodically will be reemphasized through site communication (e.g., newsletters).

3.3 Study Duration and Dates

The study is expected to last approximately 5 years, with an estimated enrollment period of 4.5 years for the maximum of 600 patients and duration of participation for each patient lasting approximately 6 months.

The study will consist of a screening period lasting up to 14 days, a treatment period of variable time (dependent on length of conditioning and time on defibrotide/observation), and a follow-up period lasting 6 months following HSCT.

4 SELECTION OF STUDY POPULATION

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

A total of 400 patients from approximately 100 enrolling study sites in countries throughout the world, including the US, are planned. If DMC recommends an increase in sample size after the interim analysis, an increase in sample size up to a maximum of 600 patients total will be implemented.

4.1 Inclusion Criteria

Each patient must meet the following criteria to be enrolled in this study. Study treatment is defined as defibrotide prophylaxis in addition to BSC or BSC alone.

1. Patient must be above the age of 1 month as of Study Day 1 (as defined in Section 3.1).
2. Patient must be scheduled to undergo allogeneic (adults or pediatric patients) or autologous HSCT (pediatric patients only) and be at high risk or very high risk of developing VOD.
   a. High-risk patients must meet both of the following criteria (i and ii):
      i. Patient must be scheduled to receive myeloablative conditioning, defined as either of the following:
         a. At least 2 alkylating agents (e.g., cyclophosphamide, busulfan, melphalan); the investigator must document in the medical chart that the conditioning regimen is considered to be myeloablative
            or
         b. TBI (single dose of ≥5 Gy, or ≥8 Gy fractionated dose) and at least 1 alkylating agent, and
      ii. Patient must meet at least 1 of the following criteria (a or b):
         a. Has at least 1 hepatic-related risk factor, as defined by the European Society for Blood and Marrow Transplantation (EBMT) position statement (adapted and modified from Mohty et al. 2015), during screening as follows:
- Transaminase level >2.5 times the upper limit of normal (ULN) during screening or within 14 days prior to screening on a non-screening test if the test was performed as part of patient's routine standard of care
- Serum total bilirubin level >1.5 times the ULN during screening or within 14 days prior to screening on a non-screening test if the test was performed as part of patient's routine standard of care
- Prior history of cirrhosis (with biopsy evidence)
- Prior history of hepatic fibrosis (by histology or other diagnostic scoring system per institutional guidelines)
- Prior history of viral hepatitis within 1 year before the start of study treatment as indicated by a positive test for any of the following:
  – hepatitis A virus (HAV) immunoglobulin M (IgM) (anti-HAV IgM)

– hepatitis B virus (HBV) core immunoglobulin G (IgG) or IgM (anti-HBc IgG or anti-HBc IgM)

– HBV surface antigen (HBsAg)

– HBV DNA by polymerase chain reaction (PCR) or nucleic acid amplification testing (NAAT)

– Hepatitis C virus (HCV) antibody (anti-HCV) and HCV RNA by PCR or NAAT

- Any prior hepatic irradiation, including abdominal irradiation covering the hepatic area
- Documented diagnosis and confirmed evidence of iron overload in medical notes (repeated serum ferritin >2000 ng/mL and/or liver iron content ≥5.0 mg/gdw as estimated by magnetic resonance imaging T2*) within 3 months prior to screening (Armand et al. 2007; Armand et al. 2011)

or

b. Has advanced-stage neuroblastoma requiring myeloablative conditioning (defined in 2.a.i. above). Note: if the patient is scheduled to receive a tandem transplant, then enrollment may only occur following the first transplant and prior to second transplant.

b. Very high-risk patients must meet 1 of the following criteria:

i. Osteopetrosis and patient must be scheduled to receive myeloablative conditioning, defined as either of the following:

a. At least 2 alkylating agents (e.g., cyclophosphamide, busulfan, melphalan); the investigator must document in the medical chart that the conditioning regimen is considered to be myeloablative

or

b. TBI (single dose of ≥5 Gy, or ≥8 Gy fractionated dose) and at least 1 alkylating agent

ii. Primary HLH, Griscelli II Chediak-Higashi syndrome, Hermansky-Pudiak II, X-linked lymphoproliferative disorders, X-linked severe combined immunodeficiency, X-linked hypogammaglobulinemia, or familial HLH 1-5 and undergoing myeloablative conditioning (as defined in 2.b.i. above) (Weitzman 2011, Naithani et al. 2013)

iii. Prior treatment with an ozogamicin-containing monoclonal antibody using the minimum dose and schedule, according to the patient prescribing information:

- Gemtuzumab ozogamicin, at least 9 mg/m^2 total dose (Wadleigh et al. 2003)
- Inotuzumab ozogamicin, at least 1.5 mg/m^2 over 28 days

iv. Class III, high-risk thalassemia (i.e., patients who are ≥7 years old and have a confirmed diagnosis of hepatomegaly (e.g., a liver size ≥5 cm below the costal margin on clinical examination) at the time of screening [Mathews et al. 2007])

3. Female patients (and female partners of male patients) of childbearing potential who are sexually active 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 or partner

include abstinence (when this is in line with the preferred and usual lifestyle of the patient [periodic abstinence, e.g., calendar, post-ovulation, symptothermal methods, and withdrawal are not acceptable methods]), combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation (i.e., birth control pills, patches, vaginal ring), progestogen-only hormonal contraception associated with inhibition of ovulation (i.e., progestin implant or injection), intrauterine device (IUD), intrauterine hormone-releasing system (IUS), surgical sterilization, and vasectomy (>6 months before Study Day 1). Post-menopausal women (i.e., women with >2 years of amenorrhea) do not need to use contraception.

4. Adult patients must be able to understand and sign a written informed consent. For minor 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. Study treatment is defined as defibrotide prophylaxis in addition to BSC or BSC alone.

1. Patient has hemodynamic instability within 24 hours before the start of study treatment.
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 (e.g., 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/24 hours) to replace blood loss, or
   b. bleeding from a site which, in the investigator's opinion, constitutes a potential life-threatening source (e.g., pulmonary hemorrhage or central nervous system 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 (tPA), streptokinase, urokinase, antithrombin III (ATIII), and 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 use will be allowed in both treatment arms (up to a maximum of 100 U/kg/day).
4. Patient is using or plans to use an investigational agent for the prevention or treatment of VOD.
5. Patient, in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.
6. Patient or parent/legal guardian or representative has a psychiatric illness that would prevent the patient or parent/legal guardian or representative from giving informed consent and/or assent.
7. 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.

8. Patient is pregnant or lactating and does not agree to stop breastfeeding.
9. Patient has a known history of hypersensitivity to defibrotide or any of the excipients.
10. Patient or parent/legal guardian or representative lacks the full mental capacity to understand and sign a written informed consent.
11. Patient is receiving or plans to receive other investigational therapy during study.

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 BSC according to institutional guidelines and patient need, excluding defibrotide (see Section 5.7).

5.2 Treatments Administered

Patients will be randomly assigned in a 1:1 ratio to receive defibrotide prophylaxis 25 mg/kg/day or BSC.

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. Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a 2-hour window between the end of an infusion and the start of the next infusion. Individual doses of defibrotide are determined for individual patients based on body weight at baseline (i.e., the day before conditioning begins for the DP arm, and on the day that conditioning begins for patients in the BSC arm who develop VOD and subsequently receive defibrotide). For the DP arm, 2-4 doses of defibrotide must be administered within 24 hours prior to conditioning. Each of the 4 divided doses per day will be rounded to the nearest 10 mg for adults (>35 kg) and the nearest 5 mg for children (≤35 kg) to facilitate efficient drug administration.

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

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 alter substantially.

5.2.1.1 Defibrotide Prophylaxis

For patients randomized to receive defibrotide prophylaxis, 2-4 doses of defibrotide should be administered within 24 hours before the first dose of conditioning regimen. Defibrotide administration is recommended for a minimum of 21 days and ending no later than Day +30 post-HSCT. Patients in this arm of the study will also receive standard of care therapy based on institutional guidelines and patient need (i.e., BSC). Patients must undergo Day +15 post-HSCT assessments before hospital discharge. If a patient is discontinued early from study/study treatment (prior to Day +15 post-HSCT), the Day +15 post-HSCT assessments must be completed on the day of study/study treatment discontinuation (+1 day).

If a patient on defibrotide develops bleeding, defibrotide should be discontinued. Additionally, temporary discontinuation of defibrotide is recommended for patients at significant risk of major bleeding who are receiving defibrotide (prophylactically or as rescue medication) and who undergo surgery or invasive procedures (see Section 1.6.1).

5.2.1.2 Defibrotide Rescue Treatment for Patients who Develop VOD

For patients who develop VOD per modified Seattle criteria in either the DP or BSC arms, defibrotide will 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 defined in the first 3 exclusion criteria (#1, #2, #3) listed in Section 4.2, to receive defibrotide as rescue treatment for VOD. Patients with VOD undergoing rescue treatment with defibrotide will continue to receive individualized standard of care therapy (BSC) based on local institutional guidelines and patient need. Medications that increase risk of bleeding must be stopped 24 hours prior to the start of defibrotide rescue treatment (see Section 5.7.6).

Defibrotide administration must be stopped if a patient has clinically significant bleeding. Defibrotide may be re-initiated if the bleeding is controlled and is of severity $\leq$ grade 1.

The daily dose of defibrotide in patients who receive rescue treatment with defibrotide after being randomized to the DP or BSC arm will be based on body weight at baseline (i.e., within 24 hours before conditioning begins for the DP arm, and on the day that conditioning begins for patients in the BSC arm who develop VOD and subsequently receive defibrotide).

5.2.2 Reference Therapy

Patients randomized to the BSC arm will receive standard of care therapy according to institutional guidelines and patient need. Best supportive care is intended to serve as study control for comparison with those patients randomized to receive defibrotide prophylaxis. Administration of BSC will begin on the first day of conditioning and continue until Day +30 post-HSCT or hospital discharge, whichever is sooner, or diagnosis of VOD, if applicable (see Section 5.7.5 and Section 5.7.6).

5.3 Selection and Timing of Defibrotide Dosing for Each Patient

All patients randomly assigned to receive defibrotide prophylaxis and all patients who receive defibrotide subsequent to diagnosis of VOD 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). Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a 2-hour window between the end of an infusion and the start of the next infusion. Each of the 4 divided doses per day will be rounded to the nearest 10 mg for adults (>35 kg) and the nearest 5 mg for children (≤35 kg) to facilitate efficient drug administration.

5.4 Method of Assigning Patients to Treatment Arms

Patients will be randomly assigned to receive defibrotide prophylaxis or BSC in a 1:1 ratio in an open-label fashion after they qualify for participation in the study. The investigator or designee will access IWRS 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 according to risk of developing VOD (high risk or very high risk, as defined in Section 4.1), age (>16 years or ≤16 years), and country. If a patient meets both the high-risk and the very high–risk criteria, the patient will be classified as very high risk.

5.6 Blinding

This is an open-label study. To minimize potential for bias, the following blinding measures will be employed:

- The central reviewer of imaging studies will be blinded to study treatment assignment and all non-radiologic patient data (see Section 6.3.1.2).
- Members of the EPAC will be blinded to study treatment assignment, will not be employees of the Sponsor, and will not be otherwise involved in the study (Section 6.3.1.3 and Section 11.9.2).

Details of Sponsor blinding will be provided in a separate document.

5.7 Prior and Concomitant Therapy

5.7.1 Prior Medications

All medications and therapies taken from the time of screening through baseline will be recorded as prior medications. All previous anticancer treatments will also be recorded.

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 myeloablative conditioning (inclusion criterion #2(i) in Section 4.1) will be identified.

5.7.3 Concomitant Medications

All medications and therapies taken between baseline and Day +60 post-HSCT will be recorded as concomitant medications. Medications and therapies administered as BSC (for both DP and BSC arms) will also be recorded.

5.7.4 Graft-versus-Host-Disease Medications

Graft-versus-host disease prophylaxis should be administered according to institutional guidelines.

For patients who develop VOD per modified Seattle criteria as assessed by the investigator, use of sirolimus (also known as rapamycin [Rapamune©]) must be discontinued, and substituted with alternate GvHD prophylaxis.

GvHD medications will be recorded as follows:

- Medications used for the prevention of GvHD: prophylaxis
- Medications used after the initial clinical or pathological diagnosis of GvHD: treatment

5.7.5 Permitted Medications

Best supportive care includes all medications per institutional guidelines and patient need. For medications prohibited for use in the study, see Section 5.7.6.

5.7.6 Prohibited Medications

Medications that increase the risk of bleeding are prohibited within 24 hours of the first dose of study treatment (defibrotide prophylaxis or as rescue treatment for VOD) and throughout the duration of defibrotide administration. Medications that increase risk of bleeding may be administered in the BSC alone arm as per institutional guidelines and patient need but not

concomitantly with defibrotide while they are taking rescue treatment for VOD. 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).

Patients receiving BSC (i.e., randomized to the BSC arm and not being treated for VOD) should not receive defibrotide as part of their BSC regimen.

All investigational therapies are prohibited from use in this study, with the exception of defibrotide 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 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 (cGMP), 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.

Unopened vials of defibrotide are to be stored according to the carton/vial label. Use the diluted defibrotide solution within 4 hours if stored at room temperature or within 24 hours if stored under refrigeration (2°C to 8°C). Partially used vials should be discarded.

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 off per instructions from the Sponsor. The investigator must provide a written explanation for any missing study

treatment. 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 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 (e.g., surgery, radiation, chemotherapy), if applicable, will be collected. Information pertaining to any prior HSCTs, including type of graft, source of graft, degree of human leukocyte antigen (HLA) matching, and number of prior HSCTs, if any, will be collected.

Information will also be collected regarding previous history of liver disease (results of liver biopsies, previous hepatitis serology testing).

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, number of prior HSCTs, if any, will be collected.

6.3 Efficacy Assessments

Efficacy will be assessed through monitoring of hepatic function and signs and symptoms for development of VOD, VOD-associated MOD, resolution of VOD, and survival.

6.3.1 Primary Efficacy Assessment for Veno-Occlusive Disease-Free Survival by Day +30 Post-Hematopoietic Stem Cell Transplant

6.3.1.1 Development of Veno-Occlusive Disease

Diagnosis of VOD per the modified Seattle criteria requires at least 2 of the following criteria:

- Total bilirubin >2 mg/dL or 34 µmol
- Hepatomegaly post-HSCT (with or without right upper quadrant pain) or an increase from baseline in hepatomegaly. Hepatomegaly (both adults and pediatrics) is defined as a >15% increase in liver size and an absolute increase of at least 1 cm in length in the mid-clavicular line compared with baseline as determined by ultrasonography
- Unexplained weight gain of ≥5% above baseline or ascites

Note: Ascites "Yes" on EPAC or clinical diagnosis of VOD per the modified Seattle criteria is defined as the presence of any amount of free fluid greater than trace fluid in the abdomen as determined by ultrasonography in a patient with " ascites absent" at baseline (see Table 4 for details and definitions).

Table 4 Ascites Assessment

Ascites Assessment by Ultrasound		Ascites Assessment for EPAC or Clinical Diagnosis of VOD[a]
Ascites at Baseline	**Ascites Post-baseline**	
Absent[b]	Absent	No
Absent	Present	Yes
Present[c]	Absent	Not Evaluable[d]
Present	Present	Not Evaluable
Not Evaluable[e]	Absent or Present or Not Evaluable	Not Evaluable
Absent or Present	Not Evaluable	Not Evaluable

a For diagnosis of VOD using the modified Seattle criteria.
b Ascites "absent" at baseline or any post-baseline ultrasound examination is defined as trace ascites or no ascites as determined by ultrasonography on the specified ultrasound examination.
c Ascites "present" at baseline or any post-baseline ultrasound examination is defined as the presence of any amount of free fluid greater than trace as determined by ultrasonography on the specified examination.
d Ascites "Not evaluable" in the context of ascites assessment for EPAC or clinical diagnosis VOD per modified Seattle criteria means that ascites cannot be used when determining whether or not VOD is present.
e Ascites "Not evaluable" at baseline or any post-baseline ultrasound examination means the measurement was not obtainable for any reason on the specified ultrasound examination
EPAC= Endpoint Adjudication Committee; VOD=veno-occlusive disease.

Note: Liver size and ascites must be confirmed by ultrasound as physical examination is insufficient to document these parameters. All imaging should be made available to the central imaging facility.

Note: Baseline values for weight and bilirubin level will be obtained within 24 hours before the first dose of conditioning regimen for the patients in the DP arm and on the day that conditioning begins for patients in the BSC arm. Baseline values for hepatic size and presence of ascites will be obtained during ultrasound examination at the screening visit.

Histopathological evidence of VOD by liver biopsy is sufficient to confirm diagnosis of VOD with or without clinical characteristics of the disease; liver biopsy is not mandatory and should only be obtained if this can be safely performed. A standardized protocol for the histopathological analysis of liver biopsy specimens is provided in Appendix 3. When liver biopsy is indicated, it is recommended that the wedged hepatic vein pressure gradient (WHVPG) also be measured, if this can be safely performed.

Total bilirubin values will be measured and physical examinations will be performed during screening. An abdominal ultrasound to assess liver and ascites will be performed to evaluate the status of VOD according to a standardized protocol provided by the central imaging laboratory (see Appendix 4). If assessment of Doppler flow pattern is otherwise performed, these results will be captured in the eCRF. Ultrasounds may be performed more frequently than at the time points specified in Appendix 1 and Appendix 2, as clinically indicated. Clinical signs or symptoms that may require more frequent ultrasound assessments for VOD diagnosis include the following:

- Evidence by physical examination of hepatomegaly (or worsening from baseline) or ascites (with or without right upper quadrant pain)
- Unexplained weight gain of ≥5% from the baseline value
- Total bilirubin >2 mg/dL or 34 µmol

6.3.1.2 Central Review of Imaging Studies

All imaging studies will be reviewed at a central imaging facility by personnel who will be blinded to study treatment and all non-radiologic data.

The central imaging facility will address the following:

- Liver size (change from baseline, hepatic length in the mid-clavicular line, associated date)
- Ascites ("absent/present/not evaluable", description, associated date);
  "Ascites present" at baseline or any post-baseline ultrasound examination is defined as the presence of any amount of free fluid greater than trace as determined by ultrasonography on the specified examination. If there is trace ascites or no ascites on a specific ultrasound examination, this is considered "ascites absent." If the presence of ascites is not obtainable for any reason during an ultrasound examination, then the ultrasound finding for ascites will be considered "not evaluable" (see Table 4 for details).

6.3.1.3 Endpoint Adjudication Committee

Development of VOD for individual patients will be adjudicated by members of the EPAC, who will be blinded to study treatment. The EPAC will receive weight data, clinical laboratory test results, imaging reports from the central imaging facility, biopsy data (any organ, when available), and additional data as clinically indicated. The EPAC will base its adjudication decision on review of the following as outlined in the central imaging charter:

- Weight gain ≥5% from baseline (yes/no, date of assessment)
- Total bilirubin >2 mg/dL (yes/no, date of assessment)
- Imaging reports from the central imaging facility providing data regarding liver size and/or ascites
- Physical exam data (assessment of right upper quadrant pain)
- Additional data as clinically indicated
- Biopsy data (any organ)
- For hyperbilirubinemia/liver dysfunction (such as GvHD, intravascular hemolysis, viral infection(s), prior liver disease)
- For ascites and/or weight gain (fluid overload, cardiac failure, capillary leak syndrome)

6.3.2 Key Secondary Efficacy Assessment

Assessment of the key secondary efficacy variable consists of monitoring VOD-free survival by Day +100 post-HSCT. Diagnosis of VOD will be made as described for the primary efficacy assessment (see Section 6.3.1).

6.3.3 Other Secondary Efficacy Assessments

Other secondary efficacy assessments include monitoring for incidence of VOD by Day +30, Day +100, and Day +180 post-HSCT, VOD-free survival by Day +180 post-HSCT, relapse of disease, VOD-associated MOD, and resolution of VOD.

6.3.3.1 Veno-Occlusive Disease-Free Survival by Day +180 Post-Hematopoietic Stem Cell Transplant

For monitoring of VOD-free survival through Day +180 post-HSCT, the diagnosis of VOD by Day +100 post-HSCT will be made by EPAC as described for the primary efficacy assessment (see Section 6.3.1), and the diagnosis of VOD after Day +100 post-HSCT will be based on investigator assessment.

6.3.3.2 Relapse of Malignant Disease

Monitoring for relapse of malignant disease will vary as clinically indicated, and will be diagnosed according to institutional standards. Relapse of disease, if applicable, will be documented and recorded on the eCRF.

6.3.3.3 Veno-Occlusive Disease-Associated Multi-Organ Dysfunction

Veno-occlusive disease-associated MOD is defined as renal or pulmonary dysfunction in the presence of VOD. Renal dysfunction will be diagnosed if any of the following criteria are met by Day +30, Day +100, or Day +180 post-HSCT:

- Serum creatinine ≥3 times the value at baseline or the lowest value during HSCT conditioning
- Creatinine clearance (CrCl) ≤40% of the value at baseline or the greatest value during HSCT conditioning, calculated using Cockcroft-Gault for adults and Schwartz formula for pediatric patients, or the glomerular filtration rate (GFR) (calculated if cystatin C is measured), ≤40% of the value at baseline or the greatest value during HSCT conditioning, (Grubb et al. 2005; Larsson et al. 2004).
- Dialysis dependence
  Note: Renal dysfunction must be attributable to fluid overload or mechanical impingement from abdominal distention or hepatic enlargement and not due to nephrotoxic drugs (e.g., cyclosporine, amphotericin B).

Pulmonary dysfunction will be diagnosed if the patient requires oxygen supplementation using any of the following by Day +30, Day +100, or Day +180 post-HSCT:

- Face mask for at least 24 hours
- Bilevel positive airway pressure (BiPAP) device or
- Ventilator support
  Note: Oxygen supplementation by nasal cannula is not a criterion for pulmonary dysfunction. Pulmonary dysfunction must be attributable to fluid overload or mechanical impingement from abdominal distention or hepatic enlargement and not due to infection (e.g., pneumonia).

6.3.3.4 Resolution of Investigator-Diagnosed Veno-Occlusive Disease and Veno-Occlusive Disease-Associated Multi-Organ Dysfunction

Investigators will determine whether patients have resolution of VOD which requires that patients who develop VOD (according to investigator diagnosis) have not only resolution of their VOD, but also resolution of any VOD-associated MOD, if applicable and as defined below by Day +30, Day +100, or Day +180 post-HSCT:

- Resolution of VOD will be defined as follows:
  - For patients who had a total bilirubin value >2 mg/dL attributed to VOD at any point while participating in the study, these patients must have:
    1. Total bilirubin <2.0 mg/dL for 3 consecutive days, and
    2. Resolution of VOD-associated MOD, if applicable

Note: Resolution of abnormal total bilirubin level as a parameter for resolution will be omitted if there is documented alternate cause for additional hepatic injury (e.g., GvHD of the liver).

- For patients who never had a total bilirubin value >2 mg/dL, or total bilirubin was >2 mg/dL and not attributed to VOD, these patients must have:
  1. Resolution of ascites or return of weight to baseline (for patients >20 kg at randomization) or weight adjusted for growth (for patients <20 kg), and
  2. Clinically significant decrease (return to baseline measurement) of liver size confirmed by ultrasound, and
  3. Resolution of VOD-associated MOD, if applicable, and
  4. Normalization of the portal flow (if present at VOD diagnosis) and documented by ultrasound

- Resolution of VOD-associated MOD will be defined as follows:
  - Renal MOD
    1. Serum creatinine level <1.5 times baseline or the lowest value during HSCT conditioning, or CrCl (or optional GFR calculated if cystatin C is measured) >80% at baseline or the greatest value during HSCT conditioning, and
    2. No need for dialysis (intermittent or continuous veno-venous hemofiltration [CVVH])
  - Pulmonary MOD
    1. No need for supplemental oxygen by either face mask or BiPAP device for 3 consecutive days, and
    2. No need for ventilator support

The dates when resolution of VOD and resolution of VOD-associated MOD are met will be recorded.

6.4 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 health-related quality of life questionnaires will be administered to adults, and children and their parents, as applicable (Table 5).

Table 5 Health-Related Quality of Life Questionnaires

Questionnaire		Adults	Pediatric		Recall period
			Self-report	Parent report	
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-5L=5-Level EuroQol-5D health questionnaire; EQ-5D-Y=EuroQol-5D health questionnaire for Youth.

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

6.5 Health Economics

6.5.1 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.

6.5.2 Number of Days Spent in the Intensive Care Unit

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.

6.5.3 Inpatient Resource Use

Inpatient resource use and/or use of diagnostic tests during the initial hospital stay or during any readmissions will be documented. Information will be recorded for blood product transfusions, coagulation factors administered, ventilator use, dialysis, biopsies, ultrasounds, chest images, head images, bone marrow biopsies, abdominal or thoracic drain placement, and abdominal or pelvic CT scans.

6.6 Biomarker and Pharmacokinetic Assessments

6.6.1 Biomarker Assessments

For all patients weighing >15 kg, blood samples (3 mL for pediatric patients; 7 mL for adult patients) to evaluate plasma concentration of potential VOD biomarkers (which may include but will not be limited to VCAM1, vWF, L- ficolin, PAI-1, thrombomodulin, CRP, ANG2) and/or GvHD biomarkers (which may include but will not be limited to TNFR1, IL1RL1 [also known as ST2], and REG3α) will obtained from all patients at the following time points:

- At baseline/Study Day 1, before the start of study treatment
- On Day +7 post-HSCT
- On Day +15 post-HSCT

If hospital discharge occurs prior to Day +15 post-HSCT, then the Day +15 biomarker sample should be obtained either on the day of discharge or at 1 of the twice weekly visits occurring between Early Hospital Discharge and Day +30 post HSCT (see Appendix 1, Schedule of Procedures and Assessments).

In addition, for all patients (including those who were randomly assigned to the BSC arm) who develop VOD and receive rescue treatment with defibrotide, blood samples (3 mL for pediatric patients; 7 mL for adult patients) will be collected for biomarker analyses at the following time points (see Appendix 2):

- Upon local diagnosis of VOD (pre-dose)
- On Days 7 and 14 after the start of rescue defibrotide
- Upon resolution of VOD, as applicable

- On Day +180 post-HSCT

For those patients whose VOD has not resolved within 21 days of local diagnosis, one additional sample should be drawn on approximately day 21 after diagnosis.

For all patients at any point in the study, blood samples for biomarker analysis will also be collected

- Upon diagnosis of acute GvHD
- 14 days following diagnosis of acute GvHD

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

6.6.2 Pharmacokinetic Assessments in Any Patient who Receives Defibrotide

Three PK populations and schedules are planned for this study. The intensive PK population will consist of approximately 25 patients (weighing ≥30 kg) who were randomly assigned to receive defibrotide (DP arm) at select study centers. The remainder of patients in the DP arm (weighing >15 kg) will follow a sparse schedule for collection of PK samples. The sparse PK population will include those patients who were randomly assigned to receive defibrotide (DP arm) and were not included in the intensive PK population. The contingent PK population will include all patients (including those randomized to the BSC arm) who receive defibrotide as rescue treatment subsequent to a diagnosis of VOD.

6.6.2.1 Intensive Pharmacokinetic Sampling Schedule

For the intensive PK analysis, blood samples for measurement of defibrotide concentrations will be collected from approximately 25 patients weighing ≥30 kg who were randomly assigned to receive defibrotide (DP arm), including approximately 10 children (≤16 years old) to characterize the PK profile of defibrotide. An attempt will be made to recruit 3 to 5 patients receiving continuous veno-venous hemodialysis (CVVH).

Blood samples (3 mL) will be collected as follows:

- Day +1 post-HSCT and Day +7 post-HSCT at the first defibrotide infusion of the day starting after 6:00 AM at the following time points:
  - Within 15 minutes before the start of the infusion
  - At 2 hours after the start of infusion (within ±15 minutes before the end of infusion)
  - At the following times after the start of the infusion: 2.25 hours (±5 minutes), 2.5 hours (±10 minutes), 2.75 hours (±10 minutes), 3.0 hours (±10 minutes), 3.5 hours (±15 minutes), 4.0 hours (±15 minutes), and 5.0 hours (±15 minutes)
- At Day +15 post-HSCT*, and at Day +30 post-HSCT (for patients still receiving defibrotide):
  - At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) at the first defibrotide infusion of the day starting after 6:00 AM

*NOTE: If hospital discharge occurs prior to Day +15 post-HSCT, the Day +15 post-HSCT PK samples do not need to be collected.

Patients should be carefully monitored so as not to exceed the blood draw maximums detailed in Appendix 6; PK sampling can be eliminated or discontinued if investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (see Section 7 for details).

6.6.2.2 Sparse Pharmacokinetic Sampling Schedule

For the sparse PK analysis, patients weighing >15 kg who were randomly assigned to receive defibrotide (DP arm) and who were not included in the intensive PK analysis will have blood samples (3 mL) collected after the first defibrotide infusion of the day starting after approximately 6:00 AM, as follows:

- At Day +7 post-HSCT
  - At 2 hours after the start of the infusion (within ±15 minutes before the end of infusion)
  - At any time during the 2-hour window after the end of infusion (exact time relative to infusion must be recorded)
- At Day +15 post-HSCT*, and at Day +30 post-HSCT (for patients still receiving defibrotide)
  - At 2 hours after the start of infusion (within ±15 minutes before the end of infusion)

*NOTE: If hospital discharge occurs prior to Day +15 post-HSCT, the Day +15 post-HSCT PK samples do not need to be collected.

Patients should be carefully monitored so as not to exceed the blood draw maximums detailed in Appendix 6; PK sampling can be eliminated or discontinued if investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (see Section 7 for details).

6.6.2.3 Contingent Pharmacokinetic Sampling Schedule

For the contingent PK analysis in all patients weighing ≥30 kg (including those who were randomly assigned to the BSC arm) who receive defibrotide as treatment subsequent to a diagnosis of VOD, per modified Seattle criteria and as determined by the investigator, all feasible efforts will be employed to collect blood samples (3 mL), as follows:

- Day 1 after the start of rescue defibrotide, 5 samples over the course of any single dosing interval as follows:
  - Within 15 minutes before the start of the infusion
  - At 2 hours after the start of infusion (within ±15 minutes before the end of infusion)
  - At the following times after the start of infusion: 3 hours (±10 minutes), 4 hours (±15 minutes), and 5 hours (±15 minutes)
- Day 7 after the start of rescue defibrotide, for patients still receiving defibrotide
  - At 2 hours after the start of infusion (within ±15 minutes before the end of infusion) at the first defibrotide infusion of the day starting after 6:00 AM

Patients should be carefully monitored so as not to exceed the blood draw maximums detailed in Appendix 6; PK sampling can be eliminated or discontinued if investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (see Section 7 for details).

6.6.2.4 Sample Collection, Storage, and Shipping

For PK samples, blood samples (3 mL) will be collected from a venous cannula different from the site of drug infusion into K2-ethylenediaminetetraaceticacid (K2EDTA) vacutainers at specified time points. For biomarker samples, 3 mL blood for pediatric patients and 7 mL blood for adult patients will be obtained for processing. If a peripheral venous cannula is not available, an indwelling central venous double-lumen catheter can be used (defibrotide infusion will be via the distal port and blood sampling will be from the proximal port).

Details for PK and biomarker sample collection, processing, storage, and shipping instructions are provided in a separate laboratory manual.

6.6.2.5 Pharmacokinetic Parameters

Depending on the number of samples obtained, the following PK parameters for a given patient may be calculated using noncompartmental methods:

- C_{max}: maximum concentration
- T_{max}: time of maximum concentration
- $T_{1/2}$: elimination half-life
- AUC_t: area under the plasma concentration-time curve to the last quantifiable concentration
- $AUC_{0-\infty}$: area under the plasma concentration-time curve extrapolated to infinity
- CL: clearance
- V: volume of distribution

Clearance and volume of distribution will also be estimated by population PK analysis using all PK samples collected during the study

6.7 Immunogenicity Assessment in Patients who Receive Defibrotide

Immunogenicity of defibrotide in patients who received defibrotide will be assessed using a validated method.

For all patients weighing ≥15 kg in the DP and BSC arms, blood samples (10 mL) will be obtained at baseline.

For patients weighing ≥15 kg who receive defibrotide prophylaxis (DP arm), blood samples (5 mL) will be collected within 15 minutes before the start of infusion at the first infusion of the day (where applicable) that is done after approximately 6:00 AM on the following days:

- Day +15 post-HSCT*
- Day +30 post-HSCT
- Day +100 post-HSCT
- Day +180 post-HSCT

*NOTE: If hospital discharge occurs prior to Day +15 post-HSCT, then the Day +15 immunogenicity sample should be obtained either on the day of discharge or at 1 of the twice weekly visits occurring between Early Hospital Discharge and Day +30 post HSCT (see Appendix 1, Schedule of Procedures and Assessments).

For all patients weighing ≥15 kg (including those randomly assigned to the BSC arm) who receive defibrotide as treatment subsequent to a diagnosis of VOD, per modified Seattle criteria and as determined by the investigator, all feasible efforts will be employed to collect blood samples (5 mL) within 15 minutes before the start of infusion at the first infusion of the day (where applicable) after approximately 6:00 AM, on the following days:

- Day 14 after the start of rescue defibrotide
- Day 30 after the start of rescue defibrotide
- Day +100 post-HSCT
- Day +180 post-HSCT

Patients weighing <15 kg may have immunogenicity samples collected if it is determined by the investigator that the collection would not pose safety concerns or cause a need for transfusion. In these patients, blood samples (5 mL) will be obtained at baseline and at 1 or more of the sampling times specified above for patients weighing ≥15 kg. Anti-drug antibody assessment will be conducted with stored serum samples from patients treated with defibrotide in this study to enable simultaneous assessment of multiple time points from the same patient.

Patients should be carefully monitored so as not to exceed the blood draw maximums detailed in Appendix 6.

Immunogenicity of defibrotide will be assessed by evaluating the ADA and neutralizing antibody for ADA-positive patients. In case of failure to develop a validated method for ADA or neutralization antibody evaluations after reasonable amount of efforts, alternative appropriate assays may be utilized to evaluate the immunogenicity of defibrotide in patients receiving defibrotide treatment.

6.7.1 Sample Collection, Storage, and Shipping

Blood for immunogenicity samples will be collected from a peripheral venous cannula opposite to the site of drug infusion into serum collection tubes at specified time points. If a peripheral venous cannula is not available, an indwelling central venous double-lumen catheter can be used (defibrotide infusion will be via the distal port and blood sampling will be from the proximal port).

Details for immunogenicity sample collection, processing, storage, and shipping instructions are provided in a separate laboratory manual.

6.8 Safety Assessments

Safety will be assessed through monitoring of AEs, clinical laboratory tests, vital signs (including peri-infusional vital signs for patients who receive defibrotide), physical examinations, monitoring for GvHD, neutrophil and platelet engraftment and graft failure, and Karnofsky/Lansky performance scales.

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) clinically significant laboratory values.

All AEs, whether observed by the investigator, reported by the patient, determined from laboratory findings, or other means, will 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 of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis, 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 6. 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 6 CTCAE Adverse Event Severity Grades General Guidelines

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: http://evsnci.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 adverse event 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, i.e., 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

At a minimum, for patients in either the DP or BSC arm, the investigator must record all AEs and SAEs that occur from the time written informed consent is obtained until Day +60 post-HSCT (or screen failure), regardless of their relationship to study drug or procedure.

Even if the minimum of Day +60 post-HSCT is met, the investigator must continue to record all AEs and SAEs that occur within 30 days after the last dose of defibrotide, regardless of their relationship to study drug or procedure.

For patients who do not receive HSCT, AEs must be collected for 70 days after baseline or early termination, whichever occurs first. For patients randomized but not treated, AEs will be collected for 70 days after randomization or early termination, whichever occurs first.

Any SAE assessed as related to defibrotide or study procedures by the investigator, regardless of time after study termination, must also be recorded.

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. Serious adverse event Reporting Forms and contact information 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. If the investigator does not provide a causality assessment of the SAE at the time of the initial report, the event by default will be presumed to be “related”. Follow-up SAE information must be provided to the Sponsor if there are any updates to SAE information previously provided.

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 provided.

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.

6.8.1.7 Pregnancy

Pregnancy of a patient or a male patient's partner must be reported within 24 hours of first knowledge of the event by study site personnel.

If a patient or a male patient's partner becomes pregnant any time after the first dose of study drug until 30 days after the last dose of study drug, the pregnancy form should be used to report the pregnancy to the Sponsor or its designee, and study medication must be stopped for any pregnant patient. The pregnancy of a patient or a male patient's partner 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.

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 European Union (EU) Clinical Trial Directive, and local regulatory requirements.

The reference safety information to determine expectedness of SAEs is specified in the Investigator's Brochure for defibrotide.

Suspected serious unexpected adverse reactions (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.

All other SUSARs (i.e., non-fatal or life-threatening) will be reported to the relevant regulatory authorities, CECs, and all participating investigators no later than 15 days after first knowledge of the event.

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 IEC will be done in accordance with the standard operating procedures and policies of the IEC. Adequate documentation must be maintained showing that the 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.

6.8.2.1 Laboratory Parameters

Clinical laboratory tests will include serum chemistry, hematology, and coagulation (Table 7). Collection frequency is outlined in the Schedule of Procedures and Assessments in Appendix 1 and Appendix 2. 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 within the guidelines outlined in Section 7 to ensure patient safety.

Clinical laboratory tests can be measured as part of the screening visit or within 14 days prior to the start of the screening window on a non-screening test if the test was performed as part of patient's routine standard of care. Therefore, if a patient's normal SOC labs include all labs required as screening labs for the protocol and are done within 28 days prior to baseline, these labs can be considered to serve as the patient's protocol-mandated screening labs, and elevated AST/ALT (> 2.5 times the upper ULN) or bilirubin (> 1.5 times the ULN) values therein can be considered as liver risk factors in the inclusion criteria for the study.

During the study, clinically significant changes in routine laboratory tests will be considered AEs (Section 6.8.1.1).

Table 7 Clinical Laboratory Tests

Serum Chemistry	**Hematology**
Alanine aminotransferase (ALT)	Hemoglobin
Albumin	Hematocrit
Alkaline phosphatase	Mean corpuscular volume (MCV)
Aspartate aminotransferase (AST)	White blood cell count (WBC) with differential
Blood urea nitrogen (BUN)	Platelet count
Calcium	
Chloride	
Creatinine	**Coagulation**
Creatinine clearance (CrCl)[a]	Activated partial thromboplastin time (aPTT)
Cystatin C (optional)[b]	International normalized ratio (INR)
Glomerular filtration rate (GFR; optional)[b]	
Glucose	
Magnesium	
Phosphorus	
Potassium	

Table 7 Clinical Laboratory Tests

Serum Chemistry	Hematology
Sodium Total bilirubin, direct bilirubin, and indirect bilirubin Total protein	

[a] Creatinine clearance will be calculated using Cockcroft-Gault formula for adults and Schwartz formula for pediatric patients.
[b] If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
GFR= Glomerular filtration rate

Clinical laboratory parameters listed in the inclusion/exclusion criteria must be reviewed (see Section 4.1 and Section 4.2). The investigator will provide an explanation of all clinically significant laboratory observations, and these findings will be recorded as AEs.

6.8.2.2 Other Laboratory Tests

Screening laboratory tests include a serum or urine pregnancy test for women of child-bearing potential. A serum or urine pregnancy test will also be performed at Day +30 post-HSCT and Day +60 post-HSCT. For women of child-bearing potential who develop VOD and receive defibrotide, a serum or urine pregnancy test will be performed 30 days after the first dose of defibrotide and 30 days after the last dose of defibrotide.

6.8.3 Vital Signs

Vital signs will include blood pressure, pulse, respiratory rate, and body temperature. The method for measuring body temperature (e.g., oral, tympanic, rectal, or axillary) is to be recorded. For all patients, vital signs will be recorded during screening, once daily from baseline through Day+30 post-HSCT or hospital discharge, whichever is sooner; twice weekly after early hospital discharge until Day +30 post-HSCT (if applicable); weekly between Days +31 and +60 post-HSCT; and at the Days +100 and +180 post-HSCT visits. For patients who develop VOD during the study and are administered rescue defibrotide, vital signs will be recorded daily from diagnosis of VOD through hospital discharge, twice weekly through resolution of VOD, 30 days after the last dose of rescue defibrotide, and at the Days +100 and +180 post-HSCT visits.

More intensive vital signs monitoring will be conducted on the first day of defibrotide dosing, which occurs on:

- the day before the first day of the conditioning regimen for patients in the DP arm who receive defibrotide prophylaxis and
- on the first day of defibrotide administration for patients in the BSC arm who receive defibrotide treatment for VOD

For this intensive monitoring, vital signs will be recorded at pre- dose, at 15, 30, 60, and 120 minutes (±5 minutes) following start of infusion and at 15 minutes (±5 minutes) following the end of the first and second infusions.

If patients in the DP arm who receive defibrotide prophylaxis receive rescue defibrotide for the treatment of VOD, intensive vital signs monitoring will be conducted only on the first day of defibrotide prophylaxis regardless of when they move into the rescue arm.

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 (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Height will be measured during screening only. Whenever possible, assessment of right upper quadrant pain will be performed.

6.8.5 Graft-versus-Host Disease

Diagnosis of GvHD will be made through monitoring of the skin (i.e., rash), total bilirubin level (i.e., liver), stool output (i.e., gut), and by biopsy, as clinically indicated.

Acute GvHD (defined separately for patients ≥45 kg <45 kg) is defined using stages and grades according to Jacobsohn and Vogelsang (Jacobsohn and Vogelsang 2007), as shown in Table 8.

Table 8 Staging and Grading of Acute Graft versus Host Disease

	Skin	Liver Total bilirubin (mg/dL)	Gut Stool output (mL/day)
Stage			
0	No GvHD rash	<2	<500 or persistent nausea
1	Maculopapular rash <25% BSA	2-3	500-999 or 10-20 mL/kg/day for patients <45 kg
2	Maculopapular rash 25%-50% BSA	3.1-6	1000-1500 or >20-35 mL/kg/day for patients <45 kg
3	Maculopapular rash >50% BSA	6.1-15	>1500 or >35 mL/kg/day for patients <45 kg
4	Generalized erythroderma plus bullous formation	≥15	Severe abdominal pain with or without ileus
Grade	**Skin**	**Liver**	**Gut**
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	—	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	—

GvHD=graft-versus-host disease; BSA=body surface area.
Source: Jacobsohn andVogelsang 2007

Assessment for chronic GvHD will be performed using the Seattle Cancer Care scoring form (see Appendix 5). Assessment includes examination of rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated.

6.8.6 Neutrophil and Platelet Engraftment

Patients will be monitored for engraftment per the Schedules of Procedures and Assessment (Appendix 1), i.e., weekly during hospitalization up to Day +60 post-HSCT (including on Day +30 post-HSCT), and at the Day +100 post-HSCT and Day +180 post-HSCT, study completion, or early termination visits.

For patients who develop VOD and are administered rescue defibrotide, engraftment will be monitored weekly after administration of the first dose of defibrotide through resolution of VOD; at 30 days after the last dose of defibrotide; and at the Day +100 post-HSCT and Day +180 post-HSCT/ study completion/early termination visits (Appendix 2).The investigator will determine the dates for neutrophil engraftment and platelet engraftment based on the algorithm below. 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.

6.8.7 Karnofsky/Lansky Performance Scores

Functional impairment will be assessed using the Karnofsky Performance Scale (PS) for patients ≥16 years of age and the Lansky PS for patients <16 years (Table 9).

Table 9 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.

6.9 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 at any time and for any reason. Reasons for early termination from the study or study drug may include the following:

- Withdrawal of consent by patient or patient's parent/legal guardian or representative
- Adverse event which in the opinion of the investigator contraindicates continuation in the study
- Investigator considers it not in the patient's best interest to continue in the study
- Death
- Disease relapse
- Non-compliance with study drug
- Pregnancy
- Sponsor (or its designee) decision to terminate study

The specific reason for the discontinuation from study or study drug should be documented on the termination eCRF. 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
- Termination based on recommendation of DMC at interim analysis

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 3 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 DP and BSC arms is provided in Appendix 1. A schedule of study procedures and assessments for patients who develop VOD during the study and are administered rescue defibrotide is provided in Appendix 2.

Throughout the study, if a patient in either arm, and during either phase of the study (prophylaxis phase or VOD treatment phase), is at risk for exceeding maximal allowable blood draw limits (per Seattle Children's Hospital Guideline for Maximum Blood Volumes [in the opinion of the investigator], Appendix 6), the blood draw schedules may be adjusted or eliminated within the following guidelines to ensure patient safety. The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests:

- Investigators can eliminate or discontinue PK sampling and/or biomarker sampling as a first option.
- If elimination or discontinuation of sampling for PK (first) and biomarker (second) assessments still leaves a patient at risk of exceeding blood draw maximums as per Appendix 6, investigators can opt for a less rigorous schedule of laboratory assessments (serum chemistry, hematology, and coagulation testing). Under these circumstances, the

sampling schedule for laboratory assessments, to be based on local institutional practice, must include all of the following (at a minimum), during all time periods when daily or twice weekly laboratory assessments are otherwise required:

- Sampling for bilirubin testing to be performed daily (up to Day +30 post HSCT if hospitalized)
- Sampling for serum chemistry and hematology testing to be performed at least 3 times weekly
- Sampling for coagulation testing to be performed at least once weekly

7.1 Pre-Treatment Period

7.1.1 Screening Period (Study Day –14 to –1)

The following procedures will be performed or information will be collected during the screening visit:

- Administration of informed consent/assent
- Review of inclusion/exclusion criteria
- Demographics
- Medical history
- Prior medications (i.e., current at the time of screening), all prior therapies for the underlying disease, and conditioning regimen
- Physical examination, including height, assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Serum/urine pregnancy test, for females of childbearing potential only
- Clinical laboratory tests (serum chemistry, hematology, and coagulation). NOTE: Clinical laboratory tests can be measured as part of the screening visit or within 14 days prior to the start of the screening window on a non-screening test if the test was performed as part of patient's routine standard of care. Therefore, if a patient's normal SOC labs include all labs required as screening labs for the protocol and are done within 28 days prior to baseline, these labs can be considered to serve as the patient's protocol-mandated screening labs, and elevated AST/ALT (> 2.5 times the upper ULN) or bilirubin (> 1.5 times the ULN) values therein can be considered as liver risk factors in the inclusion criteria for the study.
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.

- Abdominal ultrasound (to assess liver and ascites). If possible, the spot at which the mid-clavicular line intersects with the lower costal margin should be marked with a surgical marker pen. NOTE: Abdominal ultrasound during the screening visit can be completed after randomization (if required) but must be done prior to the baseline visit.

7.1.2 Randomization

Eligible patients will be randomly assigned to study treatment in IWRS on or before the baseline visit. Baseline/Study Day 1 is defined as day the before conditioning begins for patients randomized to the DP arm and the day that conditioning begins for patients randomized to the BSC arm.

For patients whose HSCT/conditioning is postponed after the patient has been screened and randomized but before starting in the study/study treatment:

- If the date of the next planned transplant is >30 days from last screening test, clinical laboratory tests (including for bilirubin values), and ultrasound need to be repeated, and patient eligibility for inclusion in the study needs to be re-assessed before the next planned date for HSCT. Please see additional guidance below:
    - If the patient is no longer eligible then the patient needs to be removed from the study via early termination and the reason for the early termination needs to be clearly documented.

7.2 Treatment Period (DP and BSC Arms); Baseline/Study Day 1 Through Day +30 Post-HSCT

The treatment period for the study is from Study Day 1/baseline through Study Day +30 post-HSCT. Baseline/Study Day 1 is defined as the day before conditioning begins for patients randomized to the DP arm and the day that conditioning begins for patients randomized to the BSC arm.

Best supportive care will be administered to all patients enrolled in the study, beginning on the day prior to conditioning for patients in the DP arm and on the day of conditioning for patients in the BSC arm.

For patients randomized to DP arm, defibrotide prophylaxis will be administered in addition to BSC beginning the day before the first day of the conditioning regimen (Study Day 1) for a recommended minimum of 21 days (corresponding to approximately Day +15 post HSCT) and will end no later than Day +30 post-HSCT.

For patients randomized to the BSC arm, BSC will be administered starting on the day of conditioning (Study Day 1) and continue until Day +30 post HSCT or hospital discharge, whichever occurs earlier.

7.2.1 Assessments During Hospitalization

Hospitalization for patients in both the DP and BSC arms is from Study Day 1/baseline through hospital discharge. Patients must undergo Day +15 post-HSCT assessments before hospital discharge. If discharge occurs earlier (before Day +15 post-HSCT), patients must undergo the Day +15 post-HSCT assessments as close to the day of discharge as possible. If a patient is discontinued early from study/study drug (prior to Day +15 post-HSCT), the Day +15 post HSCT assessments must be completed on the day of study/study drug discontinuation (+1 day).

7.2.1.1 Baseline/Study Day 1

The following procedures will be performed or information will be collected at baseline.

PRE-DOSE (BEFORE THE START OF STUDY TREATMENT):

These assessments should be performed pre-dose for patients in the DP arm and before the start of conditioning for patients in the BSC arm.

- Updates to prior medications (i.e., from the time of screening and all prior therapies for underlying disease)
- Review of planned conditioning regimen and GvHD prophylaxis
- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight. NOTE: Weight can be measured up to 3 days prior to baseline visit. However, this will need to be re-measured at baseline visit in order to record baseline weight.
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adult patients only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- *Biomarker blood sample (see Section 6.6.1 for details)
- Immunogenicity sample (DP and BSC arms, see Section 6.7.1 for details)

*Note: Biomarker sampling can be eliminated if the investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (Appendix 6).

START OF STUDY TREATMENT:

- Administration of defibrotide 25 mg/kg/day, given as 4 2-hour infusions of 6.25 mg/kg every 6 hours. Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a 2-hour window between the end of an infusion and the start of the next infusion (if randomized to DP arm) or administration of BSC medication(s) (if randomized to BSC arm)
- Patients randomized to DP arm only: Vital signs (blood pressure, pulse, respiratory rate, and body temperature), and intensive monitoring of vital signs only during the first 2 infusions of defibrotide prophylaxis, as follows: 15, 30, 60, and 120 minutes (± 5minutes) following start of infusion and at 15 minutes (± 5minutes) following the end of the first and second infusions

POST DOSE (AFTER THE START OF STUDY TREATMENT):

- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Concomitant medications (including actual conditioning and GvHD prophylaxis)
- Monitoring of AEs
- Survival status

7.2.1.2 Assessments Beginning on Study Day 2, After the Start of Study Treatment

DAILY DURING HOSPITALIZATION

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Clinical laboratory tests (serum chemistry, hematology, and coagulation). Note: bilirubin must be assessed daily during hospitalization; other serum chemistry and hematology tests may be performed a minimum of 3 times weekly and coagulation parameters may be assessed a minimum of once weekly at the discretion of the investigator, to ensure patient safety (see Appendix 6 and Section 7 for details).
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Administration of defibrotide 25 mg/kg/day, given as 4 2-hour infusions of 6.25 mg/kg every 6 hours. Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a

2-hour window between the end of an infusion and the start of the next infusion (if randomized to DP arm) or administration of BSC medication(s) (if randomized to BSC arm)
- Concomitant medications (including actual conditioning and GvHD prophylaxis)
- Monitoring of AEs
- Survival status

TWICE WEEKLY DURING HOSPITALIZATION

- After HSCT only: Acute GvHD (i.e., rash, total bilirubin, stool output)
- Monitoring for VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)

WEEKLY DURING HOSPITALIZATION

- After HSCT only: Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- After HSCT only: Relapse of malignant disease

DAY +1, DAY +7, DAY +15, DAY +22 POST-HSCT

- Karnofsky PS (≥16 years of age); on Days +1, +7 and +15 post-HSCT
- Lansky PS (<16 years of age); on Days +1, +7 and +15 post-HSCT
- EQ-5D-5L (adult patients only); on Days +7 and +15 post-HSCT
- EQ-5D-Y (pediatric patients and parents [proxy version 1]); on Days +7 and +15 post-HSCT
- Abdominal ultrasound (to assess liver and ascites); on Days +7, +15, and +22 post-HSCT
- *PK samples (patients randomized to DP arm only); on Days +1, +7, and +15 post-HSCT (see Sections 6.6.2.1 and 6.6.2.2 for PK populations and exact time points)
- **Biomarker samples; on Days +7 and +15 post-HSCT (see Section 6.6.1 for exact time points)
- Immunogenicity sample; on Day +15; patients randomized to DP arm only (see Section 6.7.1 for exact time points)

Note: Abdominal ultrasounds can be performed more frequently if VOD is suspected.

*PK sampling on Day +15 post-HSCT is for patients still receiving defibrotide; PK sample will not be collected if discharge occurs prior to Day +15 post HSCT.

**If hospital discharge occurs prior to Day +15 post-HSCT, then the Day +15 biomarker and ADA samples should be obtained either on the day of discharge or at 1 of the twice weekly visits occurring between early hospital discharge and Day +30 post-HSCT (see Appendix 1).

Additionally, biomarker and/or PK sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6) with the reason for omitting samples clearly documented.

RELATIVE TO DIAGNOSIS OF ACUTE GVHD

- Biomarker sample (see Section 6.6.1 for exact time points)
  - Upon diagnosis of acute GvHD
  - 14 days after diagnosis of acute GvHD

7.2.2 After Early Discharge From Hospital until Day +30 Post-Hematopoietic Stem Cell Transplant

Patients who are discharged from the hospital before Day +30 post-HSCT must be monitored frequently on an outpatient basis until Day +30 post-HSCT, as follows:

TWICE WEEKLY (±1 DAY)

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Clinical laboratory tests (serum chemistry, hematology, and coagulation). Note: bilirubin must be assessed twice-weekly; other serum chemistry and hematology tests may be performed at the discretion of the investigator (see Section 7 for details) to ensure recommended blood draw limits (Appendix 6) are not exceeded.
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Monitoring for VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Concomitant medications
- Monitoring of AEs
- Survival status

WEEKLY (±1 DAY)

- Abdominal ultrasound (to assess liver and ascites)
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Relapse of malignant disease

If patients who are discharged before Day +30 post-HSCT have clinical signs or symptoms of VOD before Day +30 post-HSCT, they must be re-admitted to the hospital and will follow the schedule of assessments described in Section 7.2.1 during hospitalization. If these

patients are diagnosed with VOD per the modified Seattle criteria, they will begin treatment for VOD with defibrotide and follow the schedule of procedures and assessments in Section 7.4.

7.2.3 Day+30 Post-HSCT Visit

The daily efficacy assessments (including physical exam, weight, and total bilirubin; see Section 7.2.1.2 and Appendix 1) performed on Day +30 post-HSCT support the evaluation of the primary efficacy endpoint for this study. Patients should have this assessment performed on Day +30 post-HSCT (-2 to 0 days) wherever possible. The following procedures will also be performed on Day +30 post-HSCT (-2 to 0 days) (if the twice weekly visit falls within the Day +30 post-HSCT window of -2 to 0 days, the Day +30 post HSCT assessments should be performed at that visit).

- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Abdominal ultrasound (to assess liver and ascites)
- Serum/urine pregnancy test, for females of childbearing potential only
- Acute GvHD
- Relapse of malignant disease
- Monitoring for VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- *PK sample (for patients still receiving defibrotide; see Section 6.6.2.1 and Section 6.6.2.2 for PK populations and exact time points)
- Immunogenicity sample (patients randomized to DP arm; see Section 6.7.1)

*Note: PK sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6) with the reason for omitting samples clearly documented.

7.2.4 At Hospital Discharge

- Abdominal ultrasound (liver size and ascites)j
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])

7.3 Follow-up Period (DP and BSC Arms)

The follow-up period begins after hospital discharge or on Day +31 post-HSCT and continues through Day +180 post-HSCT, study completion, or early termination. Patients

will be monitored weekly through Day +60 post-HSCT, on Day +100 post-HSCT, and on Day +180 post-HSCT as detailed below.

Patients who develop signs and symptoms of VOD after discharge from the hospital must be monitored twice weekly instead of weekly through Day +60 post-HSCT. If these patients are diagnosed with VOD per the modified Seattle criteria, they will be re-admitted to the hospital, begin defibrotide for treatment of VOD, and follow the schedule of procedures and assessments outlined in Section 7.4 and Appendix 2.

If late-onset VOD is diagnosed (after Day +30 post-HSCT), patients will be eligible to receive defibrotide rescue treatment (see Section 7.4.1).

7.3.1 From Hospital Discharge/Day +31 through Day +60 Post-Hematopoietic Stem Cell Transplant (DP and BSC Arms)

The following procedures and assessments are to be performed or information will be collected after hospital discharge or Day +31 post-HSCT through Day +60 post-HSCT:

WEEKLY (±3 DAYS) THROUGH DAY +60 POST-HSCT

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Monitoring for VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])
- Relapse of malignant disease
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Concomitant medications
- Monitoring of AEs
- Survival status

An abdominal ultrasound may also be performed to assess liver and ascites, if clinically indicated.

AT DAY +60 (±3 DAYS) POST-HSCT

- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Serum/urine pregnancy test for females of childbearing potential only
- Abdominal ultrasound (to assess liver and ascites)

7.3.2 Day +100 Post-Hematopoietic Stem Cell Transplant (-7 to 0 days) (DP and BSC Arms)

The following procedures and assessments are to be performed or information will be collected at Day +100 post-HSCT (-7 to 0 days):

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Abdominal ultrasound (to assess liver and ascites)
- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Chronic GvHD (i.e., rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated)
- Monitoring for VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])
- Relapse of malignant disease
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Immunogenicity sample (patients randomized to DP arm; see Section 6.7.1)
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Monitoring of SAEs considered to be related to study drug

- Survival status

7.3.3 Day +180 Post-Hematopoietic Stem Cell Transplant, Study Completion, or Early Termination (DP and BSC Arms)

The following procedures and assessments are to be performed or information will be collected at Day +180 post-HSCT, study completion, or early termination (±30 days):

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Abdominal ultrasound (to be performed only if the patient's total bilirubin is >2.0 mg/dL or the patient has signs/symptoms of VOD)
- Acute GvHD (i.e., rash, bilirubin, stool output)
- Chronic GvHD (i.e., rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated)
- Monitoring for VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])
- Relapse of malignant disease
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Immunogenicity sample (patients randomized to DP arm; see Section 6.7.1)
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Monitoring of SAEs considered to be related to study drug
- Survival status

7.4 Treatment of Patients Diagnosed with Veno-Occlusive Disease

A schedule of study procedures and assessments for patients who develop VOD and receive rescue treatment with defibrotide is provided in Appendix 2.

Administration of defibrotide for treatment of VOD should begin as soon as possible after the diagnosis of VOD and continue until hospital discharge or resolution of VOD, whichever occurs sooner (treatment may go beyond Day +30 post-HSCT). The Day +30 post-HSCT, Day +100 post-HSCT, and Day +180 post-HSCT assessments should be performed whether the patient is hospitalized or not or whether the patient's VOD has resolved or not (Sections 7.4.2.3, 7.4.2.4, and 7.4.2.5, respectively, and Appendix 2).

7.4.1 Treatment Period for Veno-Occlusive Disease

7.4.1.1 Assessments at Time of Diagnosis of Veno-Occlusive Disease

The following procedures and assessments are to be performed at the time of diagnosis of VOD:

- IWRS registration
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- *Biomarker sample (see Section 6.6.1)

*Note: Biomarker sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6) with the reason for omitting samples clearly documented.

7.4.1.2 Assessments During Hospitalization

DAILY DURING HOSPITALIZATION

The following procedures and assessments are to be performed daily during hospitalization for treatment of VOD, unless otherwise specified:

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature), and intensive monitoring of vital signs only during the first 2 infusions of defibrotide treatment, as follows: pre-dose, and 15, 30, 60, and 120 minutes (±5 minutes) following start of infusion and at 15 minutes (±5 minutes) following the end of the first and second infusions
- Clinical laboratory tests (serum chemistry, hematology, and coagulation). Note: bilirubin must be assessed daily during hospitalization; other serum chemistry and hematology tests may be performed a minimum of 3-times weekly and coagulation parameters may be assessed a minimum of once weekly at the discretion of the investigator (see Section 7 for details) to ensure patient safety (Appendix 6).

- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Administration of defibrotide 25 mg/kg/day, given as 4 2-hour infusions of 6.25 mg/kg every 6 hours. Each dose (infused over a 2 hour ±15 min infusion period) may be administered within ±1 hour of the scheduled dosing time provided that there is at least a 2-hour window between the end of an infusion and the start of the next infusion (all patients); and intensive monitoring of vital signs only during the first 2 infusions of defibrotide treatment, as follows: pre-dose, and 15, 30, 60, and 120 minutes (± 5 minutes) following start of infusion and at 15 minutes (±5 minutes) following the end of the first and second infusions
- Concomitant medications
- Monitoring of AEs
- Survival status

TWICE WEEKLY (±1 DAY) DURING HOSPITALIZATION

- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Resolution of VOD (i.e., total bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)

WEEKLY (±1 DAY) DURING HOSPITALIZATION

- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Abdominal ultrasound (to assess liver and ascites)
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Relapse of malignant disease

DAY 7 AFTER THE START OF RESCUE DEFIBROTIDE

- *Biomarker blood sample (see Section 6.6.1)

*Note: Biomarker sampling can be eliminated if investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (Appendix 6).

DAY 14 AFTER THE START OF RESCUE DEFIBROTIDE

- *Biomarker blood sample (see Section 6.6.1)
- *PK samples (see Section 6.6.2.3 for population and time points)
- Immunogenicity sample (see Section 6.7.1)

*Note: Biomarker and PK sampling can be eliminated if investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (Appendix 6).

DAY 30 (AFTER THE START OF RESCUE DEFIBROTIDE)

- *PK samples (see Section 6.6.2.3 for PK population and time points)
- Immunogenicity sample (see Section 6.7.1)
- Serum/urine pregnancy test, for females of childbearing potential only

*Note: PK sampling can be eliminated if investigator believes such sampling will compromise patient safety by exceeding daily or monthly blood draw maximums or cause need for transfusion (Appendix 6).

RELATIVE TO VOD RESOLUTION AND ACUTE GVHD DIAGNOSIS

- Biomarker samples (see Section 6.6.1)
  - Upon resolution of VOD
    Note: For patients whose VOD has not resolved by 21 days after local diagnosis, an additional biomarker sample will be collected on approximately day 21 after diagnosis.
  - Upon diagnosis of acute GvHD
  - 14 days after diagnosis of acute GvHD

AT HOSPITAL DISCHARGE

- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])

7.4.2 Follow-up Period for VOD

The follow-up period begins after discharge from the hospital and continues through Day +180 post-HSCT, study completion, or early termination.

7.4.2.1 Through Resolution of Veno-Occlusive Disease

Patients who had a diagnosis of VOD and who are discharged from the hospital before resolution of VOD will be monitored until resolution of VOD, as follows.

TWICE WEEKLY (±1 DAY)

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.

- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Clinical laboratory tests (serum chemistry, hematology, and coagulation). Note: bilirubin must be assessed twice weekly; other serum chemistry and hematology tests may be performed at the discretion of the investigator (see Section 7 for details) to ensure patient safety (Appendix 6) with the reason for omitting samples clearly documented.
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Resolution of VOD (i.e., total bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Monitoring of AEs
- Survival status

WEEKLY (±1 DAY)

- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Abdominal ultrasound (assess liver and ascites)
- Relapse of malignant disease

7.4.2.2 30 Days (+7 days) After the Last Dose of Defibrotide

The following procedures and assessments are to be performed 30 days (+7 days) after the last dose of rescue defibrotide:

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Urine/serum pregnancy test for females of childbearing potential only
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)

- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Relapse of malignant disease
- Resolution of VOD (i.e., total bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Monitoring of AEs
- Survival status

This visit may be omitted if a weekly scheduled visit between Day +100 and +180 post-HSCT occurs within 7 days after this visit would be scheduled.

7.4.2.3 Day+30 Post-Hematopoietic Stem Cell Transplant (-2 to 0 days) Visit

Patients who develop VOD and receive and receive defibrotide for treatment of VOD should have the Day +30 post-HSCT (-2 to 0 days) assessments performed wherever possible. If the twice weekly visit falls within the Day +30 post-HSCT window of -2 to 0 days, the Day +30 post HSCT assessments should be performed at that visit. The following assessments will be performed:

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Abdominal ultrasound (to assess liver and ascites)
- Serum/urine pregnancy test, for females of childbearing potential only
- Acute GvHD

- Relapse of malignant disease
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Resolution of VOD (i.e., bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)
- Immunogenicity sample (patients randomized to DP arm; see Section 6.7.1)
- Hospitalization data (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Administration of defibrotide (if applicable)
- Concomitant medications
- Adverse events
- Survival status

7.4.2.4 Day +100 Post-Hematopoietic Stem Cell Transplant

The following procedures and assessments are to be performed or information will be collected at Day +100 post-HSCT (-7 to 0 days):

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Abdominal ultrasound (to assess liver and ascites)
- Acute GvHD (i.e., rash, total bilirubin, stool output)
- Chronic GvHD (i.e., rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated)
- Relapse of malignant disease
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Resolution of VOD (i.e., bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)
- Immunogenicity sample (see Section 6.7.1)

- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Administration of defibrotide (if applicable)
- Monitoring of SAEs considered related to study drug
- Survival status

7.4.2.5 Day +180 Post-Hematopoietic Stem Cell Transplant, Study Completion, or Early Termination (±30 Days)

The following procedures and assessments are to be performed or information will be collected at Day +180 post-HSCT, study completion, or early termination (±30 days):

- Physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver; and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.
- Weight
- Vital signs (blood pressure, pulse, respiratory rate, and body temperature)
- Karnofsky PS (≥16 years of age)
- Lansky PS (<16 years of age)
- EQ-5D-5L (adults only)
- EQ-5D-Y (pediatric patients and parents [proxy version 1])
- Clinical laboratory tests (serum chemistry, hematology, and coagulation)
- Calculation of CrCl (using Cockcroft-Gault for adults and Schwartz formula for pediatric patients). If cystatin C is measured, GFR will be calculated using either the Grubb or Larsson formula.
- Neutrophil and platelet engraftment/graft failure (see Section 6.8.6)
- Acute GvHD (i.e., rash, bilirubin, stool output)
- Chronic GvHD (i.e., rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated)
- Abdominal ultrasound (to be performed only if the patient's total bilirubin is >2.0 mg/dL or the patient has signs/symptoms of VOD)
- Relapse of malignant disease
- Monitoring for VOD-associated MOD (i.e., serum creatinine/CrCl or GFR [optional]/dialysis and supplemental oxygen/ventilator support)
- Resolution of VOD (i.e., bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)
- *Biomarker sample (see Section 6.6.1)
- Immunogenicity sample (see Section 6.7.1)
- Hospitalization information (i.e., dates of hospitalization, dates in ICU, dates of readmission) and inpatient resource use
- Administration of defibrotide (if applicable)
- Monitoring of SAEs considered to be related to study drug

- Survival status

*Note: Biomarker sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6) with the reason for omitting samples clearly documented.

8 QUALITY CONTROL AND ASSURANCE

The study will be conducted according to GCP guidelines and according to national law. 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. Categorical variables will be reported using frequency and percentage (e.g., gender, race). Continuous variables will be reported using number of patients, mean, standard deviation, median, minimum, and maximum (e.g., age, weight). 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 prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of hepatic VOD as measured by VOD-free survival by Day +30 in patients undergoing HSCT who are at high risk or very high risk for developing VOD by Day +30 post-HSCT. The study is designed with both an interim and a final analysis. To maintain an overall significance level at 1-sided alpha of 0.025, the incremental alpha is specified at 1-sided 0.0005 for interim analysis and 1-sided 0.0245 for final analysis (the corresponding nominal alpha is 1-sided 0.0005 for interim analysis and 1-sided 0.02498 for final analysis).

The key secondary objective of the study is to compare the efficacy of defibrotide prophylaxis in addition to BSC (DP arm) vs BSC alone (BSC arm) for the prevention of VOD as measured by VOD-free survival by Day +100 post-HSCT in patients who are at high risk or very high risk for developing VOD. To control the study-wise type I error, a sequential testing strategy will begin with the test on primary efficacy endpoint. If the test is significant, the test on the key secondary efficacy endpoint will be conducted at 1-sided alpha = 0.025. This gate-keeping approach will keep the family-wise error rate at 1-sided 0.025 for the comparisons of the 2 treatment arm in the primary and the key secondary efficacy endpoints.

9.3 Determination of Sample Size

On the basis of literature and results from a previously conducted prevention study (Study 2004-000592-33) with defibrotide (Corbacioglu et al. 2012; see Section 1.5), the proposed sample size for this study is 200 patients per treatment arm for a total sample size of 400 patients. Through simulations, it is shown that this sample size provides a 90% power

to detect a hazard ratio (HR) of 0.46 for VOD-free survival by Day +30 post-HSCT in DP arm as compared with BSC arm, with an average of 68 events total. The HR of 0.46 is based on 86% and 72% VOD-free survival rates by Day +30 post-HSCT for DP arm and BSC arm, respectively, which translate to 14% and 28% as the incidence of VOD or death by Day+30 post-HSCT for the 2 arms, respectively. The assumptions for the simulations to calculate the sample size also include: (1) a 2-look group sequential design at 1-sided significance level of 0.025 (overall) with 1 interim analysis for efficacy stopping (1-sided significance level of 0.0005) or non-binding futility stopping at ≤10% conditional power; and (2) 10% dropout rate. Due to uncertainties associated with the study design assumptions, specifically the background rate of events in the BSC treatment arm and the size of the treatment effect, an interim analysis to be overseen by the DMC is planned when 70% of patients are evaluated for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post-HSCT), with specific rules for efficacy stop (i.e., 1-sided alpha of 0.0005), futility stop (i.e., conditional power <10%), and possible sample size re-estimation up to a maximum of 600 patients total when the conditional power is in the promising zone. Details on the content of the interim analysis and adaptive design decision rules will be provided in an interim Statistical Analysis Plan (iSAP) and specified along with the responsibilities of the DMC as part of the DMC charter.

9.4 Analysis Populations

The ITT population will include all randomized patients. This will be the primary analysis population for the primary efficacy endpoint and all other efficacy endpoints. The modified intent-to-treat (mITT) population will include all randomized patients who proceed to HSCT after randomization. The mITT population will be used in the sensitivity analysis of the primary efficacy endpoint and the key secondary efficacy endpoint.

The overall safety population will include all patients randomized to the DP arm who receive at least 1 dose of defibrotide and all patients randomized to the BSC arm.

The PK population will include patients who have evaluable PK data for the PK analyses.

9.5 Handling of Dropouts and Missing Data

Every effort will be made to minimize missing data. The primary and key secondary efficacy analysis use time-to-event methodology so patients who discontinue from the study early will be included in the analysis of efficacy endpoint up to their censoring time. Although a very small proportion of missing data is expected, the details of missing data handling and potential need for windowing in the sensitivity analyses that consider binary outcomes will be discussed in the Statistical Analysis Plan (SAP).

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 using descriptive statistics for the safety population and the ITT Population.

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

9.8 Efficacy Endpoints and Analyses

Although the randomization will be stratified by 3 variables, only 2 stratification variables for randomization will be used in the stratified tests as described in this section (e.g., stratified log-rank test and Cochran-Mantel-Haenszel [CMH] test) for the primary efficacy endpoint and the secondary efficacy endpoints, as appropriate - very high risk/high risk and pediatric (≤16 years)/adults (>16 years). These 2 stratification variables yield 4 distinct strata corresponding to unique levels of randomization strata. If there are less than 20 patients in at least 1 of the 4 distinct strata, only the very high risk/high risk strata will be used in the stratified tests. Although the randomization will be stratified by country as well to ensure balance of treatment, due to the expected low enrollment within some countries, the stratified efficacy analyses will not be stratified by country.

9.8.1 Primary Efficacy Endpoint and Analysis

The primary efficacy analysis will be performed using the ITT population.

The primary efficacy endpoint is the VOD-free survival rate by Day +30 post-HSCT, as adjudicated by the independent EPAC. Kaplan-Meier estimates of the VOD-free survival rate by Day +30 post-HSCT will be presented for the 2 treatment arms and a stratified log rank test will be performed as the primary efficacy endpoint analysis to establish the treatment difference.

In addition, as a sensitivity analysis, the CMH test will be used to compare a composite binary endpoint of VOD free + alive at Day +30 post-HSCT between the 2 treatment arms, where the composite binary endpoint is defined by the proportion of patients who are VOD free and alive at Day +30 post-HSCT.

Additional sensitivity analyses for the primary efficacy endpoint will include but not limited to: the unstratified log rank test for the ITT population and the stratified log rank test using the mITT population.

Both the stratified log rank test and the CMH test will be performed by the 2 stratification variables for randomization - very high risk/high risk and pediatric (≤16 years)/adults (>16 years).

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 population. For those patients, time 0 is counted at randomization. The timing variable is

defined as the number of days from time 0 to the earlier of VOD or death by Day +30 post-HSCT. If a patient is not followed for 30 days, the censoring for that endpoint will be defined at the time of last available evaluation of VOD by EPAC. If a patient in BSC arm receives rescue treatment with defibrotide but VOD is not confirmed by EPAC then the patient will be censored at the time of rescue defibrotide initiation. Additional analysis based on other timing and censoring strategies will be considered and detailed in the SAP.

Patients assessed for the primary efficacy endpoint before the interim analysis make up the stage 1 sample, and those patients assessed after the interim analysis make up the stage 2 sample. At interim analysis, the stage 1 sample will be used to construct the treatment difference of the primary efficacy endpoint and the stratified log rank test of efficacy. For the final analysis, the method of Cui, Hung, and Wang (Cui et al. 1999) will be used to combine the independent stratified log rank test statistics from stage 1 and stage 2.

To maintain an overall significance level at 1-sided alpha=0.025, the incremental alpha is specified at 1-sided 0.0005 for interim analysis and 1-sided 0.0245 for final analysis (the corresponding nominal 1-sided alpha is 0.0005 for interim analysis and 1-sided 0.02498 for final analysis).

9.8.2 Key Secondary Efficacy Endpoint and Analysis

The key secondary efficacy endpoint is VOD-free survival by Day +100 post-HSCT and will be analyzed similarly to the primary efficacy endpoint.

9.8.3 Other Secondary Efficacy Endpoints and Analyses

Other secondary efficacy endpoints include the following:

- Incidence of VOD by Day +30 post-HSCT
- VOD-free survival by Day +180 post-HSCT
- NRM by Day +100 and by Day +180 post-HSCT
- Incidence of VOD-associated MOD (i.e., severe VOD) by Day +30 and by Day +100 post-HSCT (in those patients who develop VOD)
- Proportion of patients who have resolution of VOD by Day +180 post-HSCT and time to resolution of VOD
- Incidence of VOD after Day +30 post-HSCT, by Day +100, and by Day +180 post-HSCT

These secondary efficacy endpoints will be tested without multiplicity adjustments, and nominal p-values will be reported.

9.8.3.1 Incidence of VOD by Day +30 Post- Hematopoietic Stem Cell Transplant

Incidence of VOD by Day +30 post-HSCT will be analyzed as a binary endpoint using the CMH test stratified by 2 stratification variables for randomization - very high risk/high risk and pediatric (≤16 years)/adults (>16 years).

9.8.3.2 Veno-Occlusive Disease-Free Survival by Day +180 Post-Hematopoietic Stem Cell Transplant

In addition to VOD-free survival by Day +100 post-HSCT, VOD-free survival by Day +180 post-HSCT will be analyzed similarly to the primary efficacy endpoint.

9.8.3.3 Non-Relapse Mortality

Non-relapse mortality is defined for patients with a diagnosis of malignancy as death from HSCT without evidence of relapse of malignant disease.

Non-relapse mortality rate at Day +100 and Day +180 post-HSCT will be evaluated by Kaplan-Meier estimates and analyzed by stratified log rank test between the 2 treatment arms using 2 stratification variables for randomization - very high risk/high risk and pediatric (≤16 years)/adults (>16 years).

9.8.3.4 Incidence of Multi-Organ Dysfunction

The proportion of patients who develop VOD-associated MOD (i.e., severe VOD), as defined in Section 6.3.3.2, by Day +30 and Day +100 post-HSCT will be summarized and compared between the 2 treatment arms using a chi-square test.

9.8.3.5 Resolution of Veno-Occlusive Disease and Time to Resolution of Veno-Occlusive Disease

Of those patients who were diagnosed with VOD by Day +30 post-HSCT by the investigator, the proportion of patients who have resolution of VOD by Day +180 post-HSCT will be summarized by treatment arm.

The time to resolution of VOD will be defined as the date of VOD diagnosis to the date that the last criterion for resolution of VOD (see Section 6.3.3.4) was met. Time to resolution of VOD will be summarized and compared between the 2 treatment arms using a log-rank test.

9.8.3.6 Development of Veno-Occlusive Disease after Day +30 and By Day +180 Post-Hematopoietic Stem Cell Transplant

The incidence of VOD after Day +30 post-HSCT will also be summarized and compared between the 2 treatment arms using a chi-square test as appropriate.

9.9 Health-Related Quality of Life

For health-related quality of life questionnaires, descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be summarized by time point and treatment arm. Actual values and changes over time in scores will also be summarized.

- To compare the health-related quality of life using the following questionnaires:
  - 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)

9.10 Biomarkers

Summaries for biomarkers (i.e., exploratory endpoint) will be provided by treatment arm and collection time. Additional exploratory analyses may be performed and will be specified in the SAP, as appropriate.

9.11 Pharmacokinetic Endpoints and Analyses

Depending on the number of samples obtained, noncompartmental methods may be used to calculate PK parameters (see Section 6.6.2.5) for patients in the intensive and contingent sampling populations.

Pharmacokinetic parameters will be summarized separately for the different PK sampling populations using descriptive statistics.

Additionally, a population PK analysis will be performed using all PK samples collected in the study to further characterize the pharmacokinetics of defibrotide, to examine potential sources of PK variability, and to explore the relationship between exposure and response.

9.12 Immunogenicity Testing

A descriptive analysis of immunogenicity of defibrotide results will be performed.

9.13 Health Economics/Outcomes Research

Summaries for hospitalization resource utilization (i.e., exploratory endpoints) will be provided by treatment arm. Additional exploratory analyses may be performed and will be specified in the SAP, as appropriate.

Duration of hospital stay is defined as the total number of days, including partial days, from the date of admission for administration of the HSCT conditioning regimen through the date of hospital discharge.

The total number of days, including partial days and not necessarily contiguous, spent in the ICU from the date of admission for administration of the HSCT conditioning regimen through hospital discharge will be recorded.

Inpatient resource use and/or use of diagnostic tests during the initial hospital stay or during any readmissions will be documented. Information regarding the following resources will be collected:

- Number of blood product transfusions
- Duration of ventilator use
- Duration of dialysis
- Number of biopsies
- Number of bone marrow biopsies

The number of hospital readmissions after the initial hospital discharge following HSCT during the 6-month follow-up period will be recorded. The duration of hospital stay, number of days spent in the ICU, and inpatient resource use will also be recorded for each readmission as described in Sections 6.5.1, 6.5.2, and 6.5.3, respectively.

Analyses that will be performed for safety or concomitant medications will not be repeated but will be used to evaluate the health economic impact. Additional exploratory analyses may be performed and will be specified in the SAP, as appropriate.

9.14 Safety Endpoints and Analyses

Safety analyses will be conducted using the safety population.

In this study, the occurrence of VOD and the administration of rescue defibrotide represent a clinical landmark time point for safety. Therefore, for the safety analyses, 2 study phases are defined with respect to the administration of rescue defibrotide, as follows:

- Prophylaxis Phase – For the overall safety population, this phase is defined as the period between baseline and start date of rescue defibrotide, if applicable, or the period between baseline and Day +180 post-HSCT if no VOD occurred. (End date of Prophylaxis Phase=start date of rescue defibrotide – 1, or Day +180 post-HSCT if no VOD occurred)
- Treatment Phase – For the subset of patients in the safety population who developed VOD and received rescue defibrotide, this phase is defined as the period between start date of rescue defibrotide and Day +180 post-HSCT. (Start date of Treatment Phase=start date of rescue defibrotide)

Patients will be analyzed by study phase and by treatment received during the Prophylaxis Phase.

9.14.1 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 study phase and treatment received during the Prophylaxis Phase. 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, Grade 3 and 4 TEAEs, and patients who have died will also be summarized by study phase and treatment received during the Prophylaxis Phase.

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).

9.14.2 Clinical Laboratory Results

Laboratory parameters will be summarized (e.g., total bilirubin, serum creatinine). Descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) as well as changes from baseline (i.e., within 24 hours before conditioning begins for the DP arm and on the day conditioning begins for the BSC arm) will be summarized by study phase and treatment received during the Prophylaxis Phase. The number and percentage of patients with abnormal values postbaseline and other summary statistics (i.e., mean, minimum, maximum, standard deviation, and number of patients) will be presented by study phase and treatment received during the Prophylaxis Phase for each laboratory parameter as per protocol schedule.

9.14.3 Vital Signs Results

For vital signs, descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) will be summarized by study phase and treatment received during the Prophylaxis Phase. Changes from baseline to maximum decrease in systolic and diastolic blood pressure will also be summarized. Abnormal vital signs (to be defined in the SAP), the number and percentage of patients with any postbaseline vital sign results above and/or below specified levels, and summary statistics (i.e., mean, median, minimum, maximum, standard deviation, and number of patients) will be presented by study phase and treatment received during the Prophylaxis Phase for each vital sign as per protocol schedule.

9.14.4 Physical Examinations

A finding identified by the investigator as abnormal on the physical examination at the screening visit will be recorded on the Medical History eCRF. A clinically significant adverse change (i.e., worsening) of a physical examination finding after screening will be recorded as an AE.

9.14.5 Graft-versus-Host Disease

9.14.5.1 Incidence of Acute Graft versus Host Disease

The proportion of patients of grade 2, 3, or 4 acute GvHD, as defined in Section 6.8.5 by Day +30, Day +100 and Day +180 post-HSCT will be summarized by study phase and treatment received during the Prophylaxis Phase.

9.14.5.2 Incidence of Chronic Graft-versus-Host Disease

The proportion of patients who develop chronic GvHD, as defined in Appendix 5, will be summarized by study phase and treatment received during the Prophylaxis Phase.

9.14.6 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 6.8.6). 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 study phase and treatment received during the Prophylaxis Phase.

9.14.7 Karnofsky and Lansky Performance Scores

For Karnofsky and Lansky PS, descriptive statistics (n, mean, standard deviation, median, minimum and maximum) as well as changes from baseline (unless otherwise specified) will be summarized by study phase and treatment received during the Prophylaxis Phase.

9.14.8 Concomitant Medications

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

9.15 Subgroup Analyses

Exploratory analyses of the primary efficacy, key secondary efficacy, and safety endpoints may be conducted for the following subgroups of interest:

- Patients at very high risk of developing VOD (see Section 4.1)
- Patients with prior liver disease (see Section 4.1 criterion 2.a.ii.a)
- Pediatric population (≤16 years)

9.16 Interim Analysis and Data Monitoring

Due to uncertainties associated with the study design assumptions, specifically the background rate of events in the BSC arm and the size of the treatment effect, an interim analysis to be overseen by the DMC is planned when 70% of patients are evaluated for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post HSCT), with specific rules for efficacy stop (i.e., 1-sided alpha=0.0005), futility stop (i.e., conditional power <10%), and possible sample size re-estimation up to a maximum of 600 patients total when the conditional power is in the promising zone. Details on the content of the interim analysis and adaptive design decision rules will be provided in an interim Statistical Analysis Plan (iSAP) and specified along with the responsibilities of the DMC as part of the DMC charter.

It is expected that the prevalence of the very high risk patients and the high risk patients will be approximately 35% and 65%, respectively, at the interim analysis. The enrollment split between high risk and very high risk patients will be monitored and managed to ensure that the minimum number of patients in the very high risk category is enrolled.

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 records 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.

10.2 Electronic Case Report Forms

All subject data required by the protocol to be reported to the sponsor on each trial subject 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 (e.g., central laboratory data, data from an IWRS, electronic Clinical Outcome Assessment [eCOA] data, 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 (e.g., 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 (IRB) or Independent Ethics Committee (IEC) Approval

The final approved protocol and the informed consent form 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 agrees 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 informed consent form was also reviewed.

A clinical study may not be initiated before the proposed protocol and informed consent form have been reviewed and unconditionally approved by an IRB meeting federal regulations. The clinical study remains subject to continuing review by the IRB. 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 renewal must be submitted to the Sponsor or its designee. Within 3 months of study completion or termination, a final report must be provided to the IRB/IEC by the clinical site.

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. These standards respect the following guidelines or laws:

- Guideline for Good Clinical Practice E6 (R1): ICH, May 1996).
- US Code of Federal Regulations (CFR) pertaining to conduct and reporting of clinical studies (Title 21 CFR Parts 11, 50, 54, 56, 312, and 314).
- Clinical Trials Directive (European Medicines Agency [EMA]) Directive 2001/20/EC

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/legal guardians or representatives will provide their written informed consent before the performance of any study-related procedures. Study sites will follow local guidelines regarding assent of minor children.

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 will be reviewed by Sponsor personnel or its designee and possibly by governmental agency personnel to ensure adequate source documentation, accuracy, and completeness of the eCRFs. The IRB has the authority to review patient records.

11.6 Protocol Adherence – Amendments

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

Any changes in the protocol will require a formal amendment. Such amendments will be agreed upon and approved in writing by the investigator and the Sponsor designees. 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.

To minimize introduction of bias into the trial we are proposing to capture via IWRS the objective measures for VOD diagnosis, which will be verified during on-site monitoring of the study. Of the 4 parameters for VOD diagnosis 2 are clinically objective (weight gain and bilirubin). For hepatomegaly and ascites, we will attempt to provide within the protocol a numerical method to quantify a diagnosis and provide guidance and education for assessment of these parameters. We will monitor the rescue treatment throughout the trial as well as investigator adherence to the pre-defined schedule of assessments. VOD cases in the BSC arm which are not confirmed by the blinded endpoint committee will be specifically reviewed for consideration of any critical protocol violations. The specific concern of patients in the BSC arm receiving defibrotide prior to a diagnosis of VOD will be addressed through training at the investigator meetings, site initiation visits and periodically reemphasized through site communication (e.g., newsletters).

11.9 Oversight Committees

11.9.1 Data Monitoring Committee

An independent DMC will oversee periodic safety analyses and one interim analysis that is part of the adaptive design of this study. Periodic safety analyses will begin after 50 patients have been randomly assigned to study treatment and monitored for a minimum of 100 days after HSCT, and will occur approximately every 6 months thereafter. The interim analysis is planned when 70% of patients are evaluable for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post-HSCT). Specific rules for efficacy stop, futility stop, and possible sample size re-estimation are outlined in the iSAP and DMC charter.

Sponsor personnel will attend and prepare presentations for the Open Sessions of the DMC. Sponsor personnel will not be involved in the statistical function for DMC assessments in this study, and data will be delivered to the DMC in a restricted manner.

The members and functions of the DMC will be detailed in the DMC Charter.

11.9.2 Endpoint Adjudication Committee

An independent EPAC that is blinded to study treatment assignment will be established to determine whether a patient meets the criteria for the primary efficacy endpoint (i.e., VOD-free survival by Day +30 post HSCT), the key secondary efficacy endpoint (i.e., VOD-free survival at Day +100 post-HSCT), using the modified Seattle criteria for diagnosis of VOD. The EPAC will review all available data, including relevant eCRFs, results of diagnostic procedures, and ultrasound results; alternate etiology (see Section 6.3.1.3) will also be considered during review of patient data.

The members and functions of the EPAC will be detailed in the EPAC Charter.

11.9.3 Steering Committee

A Steering Committee will be formed, consisting of international physicians knowledgeable of the therapeutic area, clinical trial methodology, and transplantation. The role of the Steering Committee is to provide oversight on study design, monitor patient enrollment, review DMC recommendations, and provide recommendations to the Sponsor regarding any suggested changes in study conduct.

11.10 Protocol Violations/Deviations

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

11.11 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 and source documentation completion and retention, and accurate 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 participants) at any time they are requested. In turn, the monitor will adhere to all requirements for patient confidentiality as outlined in the informed consent form. The investigator and his/her staff will be expected to cooperate with the monitor, to be available during a portion of the monitoring visit to answer questions, and to provide any missing information.

In addition, representatives of the Quality 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 (e.g., study protocol, eCRFs, study drug, original medical records/files). All patient data will be treated confidentially.

11.12 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.

11.13 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. The conditions noted here are intended to protect commercial confidential materials (patents, etc.) and not to restrict publication.

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Appendix 1 Schedule of Procedures and Assessments for Patients Receiving Defibrotide Prophylaxis or BSC

Evaluation	Pre-treatment Period	Treatment Period Through Day +30 post-HSCT					Follow-Up Period From Hospital Discharge Through Completion		
	Screening	During Hospitalization: Conditioning/HSCT			Early Hospital Discharge	Day +30 post-HSCT	Day +31 to Day +60 post-HSCT	Day +100 post-HSCT	Day +180 post-HSCT/Study Completion or Early Termination.
		Baseline/ Study Day 1[a]	Daily	Other	Twice Weekly or as indicated[b]	NA	Other[c]		
Window (days)	*-14 to -1*	*NA*	*NA*	*±1*	*±1*	*-2 to 0*[d]	*±3*	*-7 to 0*	*±30*
Informed consent	X								
Inclusion/exclusion criteria	X[e]								
Randomization[f]	X								
Demographics	X								
Medical history	X								
Prior medications[g]	X	X							
Physical examination[h]	X	X	X		X	X	Weekly	X	X
Weight	X	X	X		X	X	Weekly	X	X
Vital signs[i]	X	X	X		X	X	Weekly	X	X
Karnofsky PS (≥16 years of age)		X		Days +1,+7,+15 post-HSCT/ At discharge		X	Day +60 post-HSCT	X	X
Lansky PS (<16 years of age)		X		Days +1,+7,+15 post-HSCT/ At discharge		X	Day +60 post-HSCT	X	X
EQ-5D-5L (adults only)		X		Days +7,+15 post-HSCT/ At discharge		X	Day +60 post-HSCT	X	X
EQ-5D-Y (pediatric patients and parents [proxy version 1])		X		Days +7,+15 post-HSCT/ At discharge		X	Day +60 post-HSCT	X	X

Appendix 1 Schedule of Procedures and Assessments for Patients Receiving Defibrotide Prophylaxis or BSC

Evaluation	Pre-treatment Period	Treatment Period Through Day +30 post-HSCT					Follow-Up Period From Hospital Discharge Through Completion		
	Screening	During Hospitalization: Conditioning/HSCT			Early Hospital Discharge	Day +30 post-HSCT	Day +31 to Day +60 post-HSCT	Day +100 post-HSCT	Day +180 post-HSCT/Study Completion or Early Termination.
		Baseline/ Study Day 1[a]	Daily	Other	Twice Weekly or as indicated[b]	NA	Other[c]		
Window (days)	*-14 to -1*	*NA*	*NA*	*±1*	*±1*	*-2 to 0*[d]	*±3*	*-7 to 0*	*±30*
Serum/urine pregnancy test (females of childbearing potential)	X					X	Day +60 post-HSCT		
Clinical laboratory tests (including CrCl or GFR [optional])[j]	X	X	X[k]		X[l]	X	Weekly	X	X
Neutrophil and platelet engraftment/graft failure				Weekly[m]	Weekly[n]	X[n]	Weekly	X	X
Abdominal ultrasound[o]	X			Days +7,+15 post-HSCT /At discharge, Day +22 post-HSCT	Weekly	X	Day +60 post-HSCT	X	X[o]
Chronic GvHD[p]								X	X
Acute GvHD (i.e., rash, total bilirubin, stool output)				Twice weekly[m]	X	X	Weekly	X	X
VOD (i.e., total bilirubin, hepatomegaly, ascites, weight gain, biopsy [as applicable])				Twice weekly	X	X	Weekly	X	X
Relapse of malignant disease				Weekly	Weekly	X	Weekly	X	X
VOD-associated MOD (i.e., serum creatinine/CrCl/GFR [optional]/dialysis and supplemental O_2/ventilator support)				Twice weekly	X	X	Weekly	X	X

Appendix 1 Schedule of Procedures and Assessments for Patients Receiving Defibrotide Prophylaxis or BSC

Evaluation	Pre-treatment Period	Treatment Period Through Day +30 post-HSCT					Follow-Up Period From Hospital Discharge Through Completion		
	Screening	During Hospitalization: Conditioning/HSCT			Early Hospital Discharge	Day +30 post-HSCT	Day +31 to Day +60 post-HSCT	Day +100 post-HSCT	Day +180 post-HSCT/Study Completion or Early Termination.
		Baseline/ Study Day 1[a]	Daily	Other	Twice Weekly or as indicated[b]	NA	Other[c]		
Window (days)	*-14 to -1*	*NA*	*NA*	*±1*	*±1*	*-2 to 0*[d]	*±3*	*-7 to 0*	*±30*
PK samples[q]				Days +1,+7,+15 post-HSCT		X			
Biomarker blood samples (patients weighing >15 kg)[r]		X		Days +7,+15 post-HSCT/At discharge	X[s]				
Immunogenicity samples[t]		X		Day+15 post-HSCT/At discharge[u]	X[s]	X[u]		X	X
Hospitalization data[v]		X	X			X	Weekly	X	X
Study drug administration: DP (2-4 doses administered within 24 hours prior to conditioning) or BSC (beginning on the first day of conditioning)		X	X			X (if applicable)			
Concomitant medications[w]		X	X		X	X	Weekly		
Adverse event assessment		X	X		X	X	Weekly	X[x]	X[x]
Survival status		X	X		X	X	Weekly	X	X

a. Baseline is defined as day the before conditioning begins for patients randomized to the DP arm and the day that conditioning begins for patients randomized to the BSC arm. Baseline assessments (with the exception of hospitalization data, study drug administration, concomitant medications, AE assessments, and survival status) are to be performed pre-dose for patients in the DP arm and before conditioning begins for patients in the BSC arm.

b. If patients who are discharged from the hospital before Day +30 post-HSCT develop clinical signs of VOD before Day +30 post-HSCT, they must be re-admitted to the hospital and will follow the schedule of events for hospitalization during the treatment period. If these patients are diagnosed with VOD per the modified Seattle criteria, they will begin treatment for VOD and follow the schedule of events in Appendix 2.

c. Patients who develop clinical signs and symptoms of VOD after discharge from the hospital must be monitored twice weekly instead of weekly through Day +60 post-HSCT. If these patients are diagnosed with VOD per the modified Seattle criteria, they will begin treatment for VOD and follow the schedule of events in Appendix 2.

d. If the twice weekly visit falls within the window of the Day +30 post-HSCT visit, the Day +30 post-HSCT assessments should be performed at that visit.

e. Eligibility must be confirmed before randomization.

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

g. Prior medications include those current at the time of screening through baseline, all prior therapies for the malignant disease, conditioning regimen for HSCT, and GvHD prophylaxis. Review of planned conditioning regimen for GvHD prophylaxis to ensure that the eligibility criteria will continue to be met.

h. Complete physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver and with measurement of abdominal girth for pediatric patients), lymph nodes, extremities, and a general assessment (including edema). Height will be measured during screening only. Whenever possible, assessment of right upper quadrant pain will be performed.

i. Vital signs include blood pressure, pulse, respiratory rate, and body temperature. For the DP arm, intensive monitoring of vital signs will be recorded during the first and second infusion of defibrotide on the first day of defibrotide dosing (within 24 hours before the first dose of the conditioning regimen). For this intensive monitoring, vital signs will be recorded at pre-dose, and 15, 30, 60, and 120 minutes (±5 minutes) following start of infusion and at 15 minutes (±5 minutes) following the end of the first and second infusions.

j. Clinical laboratory tests include serum chemistry (ALT, albumin, alkaline phosphatase, AST, BUN, calcium, chloride, creatinine, glucose, magnesium, phosphorus, potassium, sodium, total bilirubin [direct and indirect], total protein, CrCl [using the Cockcroft-Gault formula for adults and Schwartz formula for pediatric patients] and if cystatin C is measured, then GFR [using the Grubb or Larsson formula]); hematology (hemoglobin, hematocrit, MCV, WBC count with differential, platelet count); and coagulation (aPTT, INR).

k. Bilirubin must be assessed daily during hospitalization; other serum chemistry and hematology assessments may be performed a minimum of 3 times weekly and coagulation parameters may be assessed a minimum of once weekly at the discretion of the investigator (see Section 7 for details) to ensure patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

l. Bilirubin must be assessed twice weekly; other serum chemistry, hematology, and coagulation parameters may be assessed at the discretion of the investigator (see Section 7 for details) to ensure patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

m. These assessments are to be performed post-HSCT only.

n. If a patient is discontinued early from the study, engraftment status can be reported at the Day+30 post-HSCT visit.

o. Abdominal ultrasounds (see Appendix 4) will be performed to assess liver and ascites and may be performed more frequently than at the protocol-specified time points, as clinically indicated (e.g., if VOD is diagnosed). If possible, the spot at which the mid-clavicular line intersects with the lower costal margin should be marked with a surgical pen. The ultrasound during the screening visit can be completed after randomization (if required) but prior to baseline. The ultrasound at Day +180 post-HSCT is to be performed only if the patient's total bilirubin is >2.0 mg/dL or the patient has signs/symptoms of VOD.

p. Assessment of chronic GvHD includes examination of rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated.

q. Intensive PK analysis: For patients (approximately 25) in the DP arm weighing ≥30 kg, blood samples will be collected as follows: on Days +1 and +7 post-HSCT at the first defibrotide infusion of the day starting after 6:00 AM, within 15 minutes before the start of the infusion; at 2 hours after the start of infusion (within ±15 minutes before the end of infusion); and at 2.25 hours (±5 minutes), 2.5 hours (±10 minutes), 2.75 hours (±10 minutes), 3 hours (± 10 minutes), 3.5 hours (±10 minutes), 4 hours (± 10 minutes), and 5 hours (± 10 minutes) after the start of infusion. On Days +15 and +30 post-HSCT (for patients still receiving defibrotide) at 2 hours after start of infusion (within ±15 minutes before the end of infusion) at the first defibrotide infusion of the day starting after 6:00 AM.

Sparse PK analysis: For patients in the DP arm (weighing >15 kg) who are not included in the intensive PK population, blood samples will be collected as follows: on Day +7 post-HSCT, 1 sample at 2 hours after the start of the infusion (within ±15 minutes before the end of infusion) and 1 sample during the 2-hour window after the end of infusion (exact time relative to infusion must be recorded); on Days +15 and +30 post-HSCT (for patients still receiving defibrotide) at 2 hours after the start of infusion (within ±15 minutes before the end of infusion).
Note: If early hospital discharge occurs before Day +15 post-HSCT, the PK sample does not need to be obtained. Furthermore, PK sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

r. In addition to baseline, Day +7, and Day+15 post-HSCT, biomarker plasma samples to evaluate plasma concentration of potential VOD biomarkers (which may include but will not be limited to VCAM1, vWF, L-ficolin, PAI-1, CRP, ANG2, thrombomodulin) and/or GvHD biomarkers (which may include but will not be limited to TNFR1, IL1RL1 [also known as ST2], and REG3α) will be collected upon diagnosis of acute GvHD and 14 days following diagnosis of acute GvHD, as applicable. Note: If hospital discharge occurs before the Day +15 post-HSCT visit, the biomarker sample may be obtained on the day of early discharge or at 1 of the twice weekly visits between early discharge and the Day +30 post-HSCT visit. Biomarker sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

s. If discharged prior to Day +15 post-HSCT and not obtained on the day of discharge.

t. Patients <15 kg may have immunogenicity samples collected if it is determined by the investigator that the collection would not pose safety concerns or cause a need for transfusion.

u. On Day +15 post-HSCT and on Day +30 post-HSCT, blood samples for immunogenicity analysis are to be collected from patients randomized to the DP arm only within 15 minutes before the start of infusion at the first infusion of the day after 6:00 AM. Note: If hospital discharge occurs prior to Day +15 post-HSCT, then the Day +15 immunogenicity sample should be obtained either on the day of discharge or at 1 of the twice weekly visits occurring between Early Hospital Discharge and Day +30 post HSCT visit.

v. Hospitalization data includes dates of hospitalization, dates in ICU, dates of readmissions, and inpatient resource use (i.e., number of blood product transfusions, amount of coagulation factors administered, frequency and duration of ventilator use and dialysis, number of liver biopsies, ultrasounds, chest images, head images, bone marrow biopsies, abdominal or thoracic drains placed, abdominal or pelvic CT scans, and use of concomitant medications.

w. Concomitant medications will be recorded up to Day +60 post-HSCT.

x. Only SAEs considered by the investigator to be possibly related to study drug should be reported more than 30 days after the last dose of study drug.

ADA=antidrug antibodies; AE=adverse event; ALT=alanine aminotransferase; ANG2=angiopoietin 2; aPTT=activated partial thromboplastin time; AST=aspartate aminotransferase; BSC=best supportive care; BUN=blood urea nitrogen; CrCl=creatinine clearance; CRP=C-reactive protein; CT=computed tomography; DP=defibrotide prophylaxis; EQ-5D-5L=5-Level EuroQuol-5D health questionnaire; EQ-5D-Y=EuroQol-5D health questionnaire for Youth; GFR=glomerular filtration rate; GvHD=graft-versus host disease; HSCT=hematopoietic stem cell transplant; ICU=intensive care unit; IN1RL1=interleukin-1 receptor-like-1; INR=International Normalized Ratio; MCV=mean corpuscular volume; MOD=multi-organ dysfunction; NA=not applicable; PAI-1=plasminogen activator inhibitor 1; PS=performance scales; PK=pharmacokinetic; REG3α=regenerating islet-derived 3-alpha; SAE=serious adverse event; TNFR1= tumor necrosis factor receptor 1; VCAM1=vascular cellular adhesion molecule 1; VOD=hepatic veno-occlusive disease; vWF=von Willebrand factor; WBC=white blood cell.

Appendix 2 Schedule of Procedures and Assessments for Patients who Develop VOD and Receive Rescue Defibrotide

Evaluation	Treatment Period				Follow-Up Period From Hospital Discharge Through Completion[a]			Post-HSCT Assessments (Performed During Treatment or Follow-Up Period)[a]		
	Diagnosis of VOD Through Hospital Discharge				Through resolution of VOD[b]		30 days after last dose of defibrotide[c]	Day +30 post-HSCT	Day +100 post-HSCT[c]	Day +180 post-HSCT/ Study Completion or Early Termination
	At VOD Diagnosis	Daily	Other time points	After First Dose of Defibrotide	Twice weekly	Weekly				
Window (days)	*NA*	*NA*	*±1*	*NA*	*±1*	*±1*	*+7*	*-2 to 0*[d]	*-7 to 0*	*±30*
IWRS registration for rescue patients	X									
Physical examination[e]		X			X		X	X	X	X
Weight		X			X		X	X	X	X
Vital signs[f]		X			X		X	X	X	X
Karnofsky PS (≥16 years of age)	X							X	X	X
Lansky PS (<16 years of age)	X							X	X	X
EQ-5D-5L (adults only)			Weekly, At discharge			X	X	X	X	X
EQ-5D-Y (pediatric patients and parents [proxy version 1])			Weekly, At discharge			X	X	X	X	X
Serum/urine pregnancy test (females of childbearing potential)				Day 30			X	X		
Clinical laboratory tests (including CrCl or GFR [optional])[g]		X[h]			X[i]		X	X	X	X
Neutrophil and platelet engraftment/graft failure			Weekly			X	X	X	X	X
Acute GvHD (i.e., rash, total bilirubin, stool output)			Twice weekly		X		X	X	X	X
Chronic GvHD[j]									X	X
Abdominal ultrasound[k]			Weekly			X		X	X	X[k]
Relapse of malignant disease			Weekly			X	X	X	X	X
VOD-associated MOD (i.e., serum			Twice weekly		X		X	X	X	X

Appendix 2 Schedule of Procedures and Assessments for Patients who Develop VOD and Receive Rescue Defibrotide

Evaluation	Treatment Period				Follow-Up Period From Hospital Discharge Through Completion[a]			Post-HSCT Assessments (Performed During Treatment or Follow-Up Period)[a]		
	Diagnosis of VOD Through Hospital Discharge				Through resolution of VOD[b]		30 days after last dose of defibrotide[c]	Day +30 post-HSCT	Day +100 post-HSCT[c]	Day +180 post-HSCT/ Study Completion or Early Termination
	At VOD Diagnosis	Daily	Other time points	After First Dose of Defibrotide	Twice weekly	Weekly				
Window (days)	*NA*	*NA*	*±1*	*NA*	*±1*	*±1*	*+7*	*-2 to 0*[d]	*-7 to 0*	*±30*
creatinine/CrCl/GFR [optional]/dialysis and supplemental O_2/ventilator support)										
Resolution of VOD (i.e., bilirubin, ascites, weight, supplemental oxygen/ventilator support, creatinine)			Twice weekly		X		X	X	X	X
PK samples[l]				Days 1, 7						
Biomarker blood samples (patients weiging >15 kg)	X		X[m]	Days 7, 14						X
Immunogenicity samples[n]				Days 14[o], 30[o]				X	X	X
Hospitalization data[p]		X			X		X	X	X	X
Defibrotide administration		X						X (if applicable)	X (if applicable	X (if applicable)
Concomitant medications[q]		X						X		
Adverse event assessment		X			X		X	X	X[r]	X[r]
Survival status		X			X		X	X	X	X

a. The Day +30 post-HSCT, Day +100 post-HSCT, and Day +180 post-HSCT follow-up assessments should be performed whether the treatment-phase patient is hospitalized or not, or whether the patient's VOD has resolved or not

b. Patients who are discharged from the hospital before resolution of VOD will be monitored twice weekly until resolution of VOD.

c. This visit at 30 days after last dose of defibrotide may be omitted if a weekly scheduled visit between Day +100 and +180 post-HSCT occurs within 7 days after this visit would be scheduled. Safety assessments performed at the visit that occurs 30 days after the last dose of defibrotide need not be repeated at the Day +100 post-HSCT visit if these visits occur within 2 weeks of each other.

d. If the twice weekly visit falls within the window of the Day +30 post-HSCT visit, the Day +30 post-HSCT assessments should be performed at that visit

e. Complete physical examination, including assessments of the skin, head, eyes, ears, nose, throat, neck, thyroid, lungs, heart, abdomen (with palpation of the liver and with measurement of abdominal girth for pediatric patients only), lymph nodes, extremities, and a general assessment (including edema). Whenever possible, assessment of right upper quadrant pain will be performed.

f. Vital signs include blood pressure, pulse, respiratory rate, and body temperature. Intensive monitoring of vital signs will be recorded during the first and second infusion of defibrotide on the first day of defibrotide dosing. For this intensive monitoring, vital signs will be recorded at pre- dose, and 15, 30, 60, and 120 minutes (±5 minutes) following start of infusion and at 15 minutes (±5 minutes) following the end of the first and second infusions.

g. Clinical laboratory tests include serum chemistry (ALT, albumin, alkaline phosphatase, AST, BUN, calcium, chloride, creatinine, glucose, magnesium, phosphorus, potassium, sodium, total bilirubin [direct and indirect], total protein, CrCl [using the Cockcroft-Gault formula for adults and Schwartz formula for pediatric patients] and if cystatin C is measured, then GFR [using the Grubb or Larsson formula]); hematology (hemoglobin, hematocrit, MCV, WBC count with differential, platelet count); and coagulation (aPTT, INR).

h. Bilirubin must be assessed daily during hospitalization; other serum chemistry and hematology tests may be performed a minimum of 3-times weekly and coagulation parameters may be assessed a minimum of once weekly at the discretion of the investigator (see Section 7 for details) to ensure patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

i. Bilirubin must be assessed twice weekly; other serum chemistry, hematology, and coagulation parameters may be assessed at the discretion of the investigator (see Section 7 for details) to ensure patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

j. Assessment of chronic GvHD includes examination of rash, mouth, eyes, gastrointestinal tract, liver function tests, lungs, joints and fascia, genital tract, and other symptoms, as clinically indicated.

k. Abdominal ultrasounds (see Appendix 4) will assess liver and ascites, and may be performed more frequently than at the protocol-specified time points, as clinically indicated. If possible, the spot at which the mid-clavicular line intersects with the lower costal margin should be marked with a surgical pen. The ultrasound at Day +180 post-HSCT is to be performed only if the patient's total bilirubin is >2.0 mg/dL or the patient has signs/symptoms of VOD.

l. Contingent PK analysis applies to patients weighing ≥30 kg (including those who were randomly assigned to receive BSC) who receive defibrotide as treatment subsequent to a diagnosis of VOD. All feasible efforts will be made to collect blood samples on Day 1 after the start of rescue defibrotide, 5 samples over the course of any single dosing interval will be collected as follows: within 15 minutes before the start of the infusion, at 2 hours after the start of infusion (within ±15 minutes before end of infusion), and at 3, 4, and 5 hours (±10 minutes) after the start of infusion; on Day 7 after the start of rescue defibrotide, for patients still receiving defibrotide, samples will be collected at 2 hours after the start of infusion (within ±15 minutes before the end of infusion) at the first defibrotide infusion of the day starting after 6:00 AM. Note: PK sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

m. Biomarker plasma samples to evaluate plasma concentration of VOD biomarkers (which may include but will not be limited to VCAM1, vWF, L-ficolin, PAI-1, CRP, ANG2, thrombomodulin) and/or GvHD biomarkers (which may include but will not be limited to TNFR1, IL1RL1 [also known as ST2], and REG3α) will also be collected upon resolution of VOD (if applicable); at diagnosis of acute GvHD; and 14 days after diagnosis of acute GvHD. For those patients whose VOD has not resolved by 21 days after local diagnosis, an additional biomarker sample will be collected at approximately 21 days after diagnosis. Note: Biomarker sampling can be eliminated if investigator believes such sampling will compromise patient safety (Appendix 6). The investigator must document in medical records the rationale for eliminating any protocol-mandated blood tests.

n. Patients <15 kg may have immunogenicity samples collected if it is determined by the investigator that the collection would not pose safety concerns or cause a need for transfusion.

o. Blood samples for immunogenicity analysis are to be collected within 15 minutes before the start of the infusion at the first infusion of the day after approximately 6:00 AM.

p. Hospitalization data includes dates of hospitalization, dates in ICU, dates of readmissions, and inpatient resource use (i.e., number of blood product transfusions, amount of coagulation factors administered, frequency and duration of ventilator use and dialysis, number of liver biopsies, ultrasounds, chest images, head images, bone marrow biopsies, abdominal or thoracic drains placed, abdominal or pelvic CT scans, and use of concomitant medications.

q. Concomitant medications will be recorded up to Day +60 post-HSCT.

r. Only SAEs considered by the investigator to be possibly related to study drug should be reported more than 30 days after the last dose of study drug.

ADA=antidrug antibodies; ALT=alanine aminotransferase; ANG2=angiopoietin 2; aPTT=activated partial thromboplastin time; AST=aspartate aminotransferase; BSC=best supportive care; BUN=blood urea nitrogen; CrCl=creatinine clearance; CRP=C-reactive protein; CT=computed tomography; EQ-5D-5L=5-Level EuroQuol-5D health questionnaire; EQ-5D-Y=EuroQol-5D health questionnaire for Youth; GvHD=graft-versus host disease; GFR=glomerular filtration rate; HSCT=hematopoietic stem cell transplant; ICU=intensive care unit; IN1RL1=interleukin-1 receptor-like-1; INR=International Normalized Ratio; IWRS=interactive web response system; MCV=mean corpuscular volume; MOD=multi-organ dysfunction; NA=not applicable; PAI-1=plasminogen activator inhibitor 1; PK=pharmacokinetic; PS=performance scale; REG3α=regenerating islet-derived 3-alpha; SAE=serious adverse event; TNFR1= tumor necrosis factor receptor 1; VACM1=vascular cell adhesion molecule 1; VOD=hepatic veno-occlusive disease; vWF=von Willebrand factor; WBC=white blood cell.

Appendix 3 Diagnosis of Veno-Occlusive Disease by Liver Biopsy

Introduction

Confirmation of the clinical diagnosis of veno-occlusive disease (VOD) relies on histological findings including occlusion of terminal hepatic venules and sublobular veins (Shulman et al. 1980), sinusoidal fibrosis (Jones et al. 1987), and hepatic necrosis (Shulman et al. 1980). Using necropsy data in a prospective study of 255 bone marrow transplant (BMT) recipients between 1978 and 1980, McDonald et al. found the positive predictive value of a "certain" clinical diagnosis of VOD to be 88% compared with histological confirmation of venular occlusion in the cohort of 64 patients upon autopsy (1984). Similar results were obtained from an analysis of 235 BMT recipients at Johns Hopkins in whom a cohort of 48 autopsies was evaluable for VOD (Jones et al. 1987). The positive predictive value for a certain clinical diagnosis of VOD was 95%. Of note, the negative predictive value for a certain clinical diagnosis of "no VOD" was 96% and 93% in these 2 series, respectively.

Although the expectation is that the clinical diagnosis of VOD will be the primary information provided to the Endpoint Adjudication Committee (EPAC), it is possible that liver biopsy data will be presented in a subset of patients. This appendix provides a standardized protocol for tissue preparation and analysis of key histopathological VOD features.

Tissue Preparation

Representative liver tissue will be fixed in formalin per local procedures. From each block, serial 4- to 6-pm sections will be cut and stained with hematoxylin and eosin, Gomori's trichrome or Wilder's reticulin stain. Sections from a formalin-fixed liver block from each case are stained with an antibody directed against α1-antitrypsin (Dako, Carpinteria, CA) and stained by means of an avidin-biotin peroxidase technique so to enhance features of nodular regenerative hyperplasia or nodular transformation (Nakhleh et al. 1988; Wanless 1990)

Histological Analysis

The following histological features should be examined and provided in a report (to be sent to the EPAC):

1. Hepatic venules
    a. Occluded hepatic venules Yes/No (defined as concentric subendothelial narrowing of the internal diameter of terminal hepatic and small sublobular venules; use trichrome-stained and reticulin-stained sections)
    b. Occluded hepatic venules high grade Yes/No ("high grade" is defined as an average percentage of luminal narrowing >33%)
    c. Venules with eccentric luminal narrowing Yes/No (defined as localized (nonconcentric) widening of the subendothelial zone within hepatic venules and small sublobular veins)

d. Venules with phlebosclerosis (defined as specimens with fibrotic and unusual concentric thickening of the adventitial layer of the venule wall, often blending with sinusoidal fibrosis)

2. Sinusoidal fibrosis
   a. Sinusoidal fibrosis in zone 3, scored 0 to 3 (higher grades of sinusoidal fibrosis are associated with widening of the sinusoids by an obvious increase of connective tissue and some atrophy of intervening hepatocyte cords; the increase in sinusoidal connective tissue should be based on trichrome or reticulin stains)
3. Hepatocytes
   a. Hepatocyte necrosis in zone 3 (scored 0 to 3)
   b. Nodular regenerative hyperplasia Yes/No (this finding is best seen on reticulin staining and on immunostaining for α1-antitrypsin, which is expressed in the enlarged regenerating hepatocytes)

In addition, the pathologist should comment on graft-versus-host disease (GvHD) in the differential diagnosis:

- GvHD is defined histologically as degenerative and destructive changes of the small interlobular or marginal bile ducts.
- Changes of GvHD can be accompanied by a mixed chronic inflammatory infiltrate in the portal space, unrest of the hepatocyte cords and variable degrees of hepatocellular cholestasis in zone 3 and proliferation of dilated periportal cholangioles containing inspissated bile.

Jones RJ, Lee KSK, Beschorner WE, et al. Veno-occlusive disease of the liver following bone marrow transplantation. Transplantation 1987; 44: 778-83.

McDonald GB, Sharma P, Matthews DE, et al. Veno-occlusive disease of the liver after bone marrow transplantation: diagnosis, incidence and predisposing factors. Hepatology 1984; 4: 116-22.

Nakhleh RE and Snover DC. Use of alpha-1-antitrypsin staining in the diagnosis of nodular regenerative hyperplasia of the liver. Hum Pathol 1988; 19: 1048-52.

Shulman HM, McDonald GB, Matthews D, et al. An analysis of hepatic veno-occlusive disease and centrilobular hepatic degeneration following bone marrow transplantation. Gastroenterology 1980; 79: 1178-91.

Wanless IR. Micronodular transformation (nodular regenerative hyperplasia) of the liver: a report of 64 cases among 2,500 autopsies and a new classification of benign hepatocellular nodules. Hepatology 1990; 11: 787-97.

Appendix 4 Standardized Protocol for Abdominal Ultrasounds

A standardized protocol will be developed for all sites to follow regarding the minimal acceptable ultrasonography procedures to be performed. Please refer to the Imaging Manual.

Appendix 5 Graft versus Host Disease Morbidity Assessment

CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) ASSESSMENT AND SCORING FORM Page 1 of 2

Name:_____ **Date of birth:**_____ **Assessment date:**_____

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA involved** ☐ Rodnan score: ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No Symptoms/ Manifestation	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH *Diagnostic/Distinctive features* ☐ Present ☐ Absent ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No symptoms	☐ Symptoms such as nausea, vomiting,anorexia,dysphagia, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation

Name:

(continued) **CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) ASSESSMENT AND SCORING FORM** **Page 2 of 2**

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN
☐ *Abnormality present but NOT thought to represent GVHD*				
LUNGS ‡ ☐ PFTs not done **FEV1** ☐ **DLCO** ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
☐ *Abnormality present but NOT thought to represent GVHD*				
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but NOT thought to represent GVHD*				
GENITAL TRACT *Diagnostic/ Distinctive features* ☐ Present ☐ Absent ☐ Not examined	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum
☐ *Abnormality present but NOT thought to represent GVHD*				

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply):

☐ Weight loss	☐ Bronchiolitis obliterans	☐ Bronchiolitis obliterans with organizing pneumonia	
☐ Esophageal stricture or web	☐ Pericardial Effusion	☐ Pleural Effusion(s)	☐ Ascites (serositis)
☐ Nephrotic syndrome	☐ Peripheral Neuropathy	☐ Myasthenia Gravis	☐ Polymyositis
☐ Malabsorption	☐ Cardiac conduction defects	☐ Coronary artery involvement	☐ Cardiomyopathy
☐ Eosinophilia >500/microliter	☐ Other: _____		☐ **None**

Biopsy obtained: ☐**Yes** ☐**No** **Organ system(s) biopsied:**_____ **GVHD confirmed by histology:** ☐**Yes** ☐**No**

OVERALL severity of GVHD: ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Change from previous evaluation: ☐ **No prior or current GVHD** ☐ **Improved** ☐ **Stable** ☐ **Worse** ☐ **N/A (baseline)**

Completed by:_____ **Date form completed:**_____

‡ Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO (carbon monoxide diffusion capacity corrected for hemoglobin) is not available, grading using FEV1 (forced expiratory volume) should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established.[28] The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: > 80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; < 40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); AST (aspartate aminotransferase); ULN (upper limit of normal); LFS (Lung Function Score); N/A (not applicable).

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Source: https://www.fredhutch.org/content/dam/public/Treatment-Suport/Long-Term-Follow-Up/GVHD-Morbidity-Assessment.pdf

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, Ph.D. 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 3, Randomized, Adaptive Study Comparing the Efficacy and Safety of Defibrotide vs Best Supportive Care in the Prevention of Hepatic Veno-Occlusive Disease in Adult and Pediatric Patients Undergoing Hematopoietic Stem Cell Transplant
Study Number:	15-007
Original Protocol v1.0:	09 May 2016
Amendment 01 v2.0:	19 January 2017
Amendment 02 v3.0	24 February 2017
Amendment 03 v4.0	20 August 2018

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

PI

Jazz Pharmaceuticals

PI

Jazz Pharmaceuticals

PI

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