NCT05569512

Title: A Phase 1/2 Trial of Uproleselan Combined with High Dose Busulfan Pre-Transplant Conditioning in Hematopoietic Stem Cell Transplantation for Patients with Chemotherapy Resistant Acute Myeloid Leukemia

Protocol Version: 2023NOV27

**DF/HCC Protocol #:** 22-063

**TITLE:** A Phase 1/2 Trial of Uproleselan Combined with High Dose Busulfan Pre-Transplant Conditioning in Hematopoietic Stem Cell Transplantation for Patients with Chemotherapy Resistant Acute Myeloid Leukemia


**Sponsor-Investigator:**

John Horan, MD, MPH
Boston Children's Hospital
Dana Farber Cancer Institute
john_horan@dfci.harvard.edu

**Study Co-Chairs:**

John Horan, MD, MPH
Boston Children's Hospital
Dana Farber Cancer Institute
john_horan@dfci.harvard.edu

Joseph Chewning, MD
University of Alabama Birmingham
Children's of Alabama
jchewning@peds.uab.edu

Malika Kapadia, MD
Boston Children's Hospital
Dana-Farber Cancer Institute
Malika_Kapadia@dfci.harvard.edu

**Study Committee Members:**

Edward Anders Kolb, MD
Alfred I du Pont Hospital for Children
eakolb@nemours.org

Amy Keating, MD
Co-chair, Oncology Strategy Group
Pediatric Transplant and Cellular
Therapy Consortium (PTCTC)
Dana Farber Cancer Institute
amy_keating@dfci.harvard.edu

Troy C. Quigg. DO
Co-chair, Oncology Strategy Group
Pediatric Transplant and Cellular
Therapy Consortium (PTCTC)
Helen DeVos Children's Hospital
Troy.Quigg@spectrumhealth.org

Maria Luisa Sulis, MD
Memorial Sloan Kettering Cancer Center
sulism@mskcc.org

William G. Woods, MD
Children's Healthcare of Atlanta
Emory University
William.Woods@choa.org

Muna Qayed, MD, MSCR
Children's Healthcare of Atlanta
Emory University
Muna.Qayed@choa.org

Matthew A. Kutny, MD
University of Alabama Birmingham
Children's of Alabama
mkutny@peds.uab.edu

Jaap Jan Boelens, MD, PhD
Memorial Sloan Kettering Cancer Center
boelensj@mskcc.org

Melinda Pauly, MD
Children's Healthcare of Atlanta
Emory University
Melinda.Pauly@choa.org

Michael R. Verneris, MD
Children's Hospital Colorado
michael.verneris@ucdenver.edu

Malika Kapadia, MD
Boston Children's Hospital
Dana-Farber Cancer Institute
Malika_Kapadia@dfci.harvard.edu

Jessica Pollard, MD
Boston Children's Hospital
Dana-Farber Cancer Institute
Jessica_Pollard@dfci.harvard.edu

Franziska Wachter, MD
Boston Children's Hospital
Dana-Farber Cancer Institute
Franziska_Wachter@dfci.harvard.edu

**Pediatric Pharmacologist:**
Janel Long-Boyle, PharmD, PhD
University of California San Francisco
janel.long-boyle@ucsf.edu

**Pharmacometrician:**
Joga Gobburu, PhD, MBA
University of Maryland
jgobburu@rx.umaryland.edu

**Statistician:**
Wendy B. London, PhD
Boston Children's Hospital
Dana Farber Cancer Institute
wendy.london@childrens.harvard.edu

**SOS Adjudication**:
Christine Duncan, MD
Boston Children's Hospital
Dana Farber Cancer Institute
Christine_Duncan@dfci.harvard.edu

**Biomarkers:**
**E-selectin Ligand Related Gene Expression:**
Soheil Meshinchi, MD, PhD
Seattle Children's Hospital
smeshinc@fhcrc.org

**Flow cytometric measurement of E-selectin Ligand Expression:**
Michael R. Loken, PhD
Hematologics, Inc. Fred Hutchinson Cancer Research Center
mrloken@hematologics.com

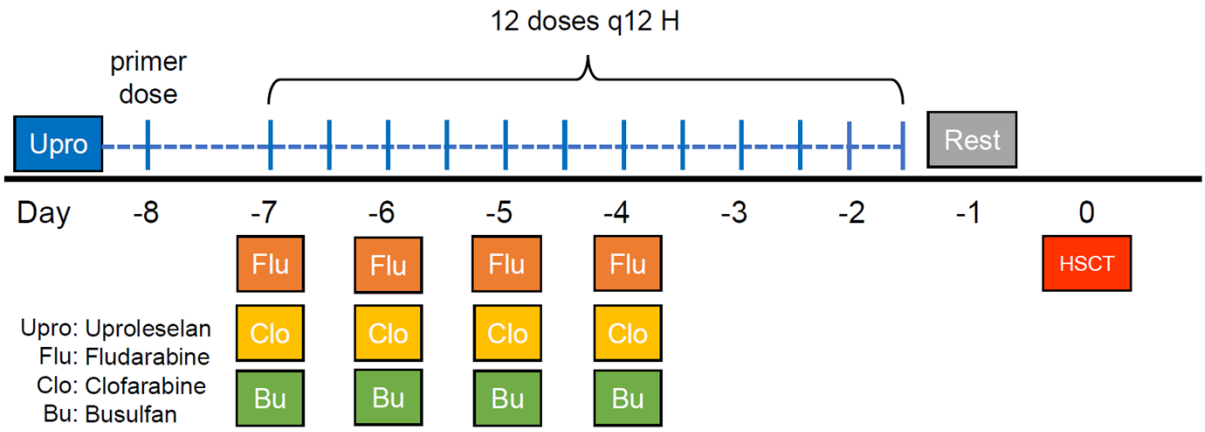
**Agent Supply:**
GlycoMimetics, Inc.: Uproleselan (investigational supply)

Commercial supply: busulfan, clofarabine, tacrolimus, methotrexate, mycophenolate mofetil

**IND #:** 155923
**IND Sponsor:** John Horan, MD, MPH
**PTCTC Study #:** ONC 2201

**SCHEMA**

Dosing schedule for uproleselan and standard conditioning



# TABLE OF CONTENTS

# OBJECTIVES

## 1.1 Hypothesis

The long range hypothesis of this project is that incorporating uproleselan into pre-transplant conditioning for acute myeloid leukemia will decrease post-transplant relapse and improve leukemia free survival.

## 1.2 Study Design

Single arm, multi-center, phase 1/2 trial.

## 1.3 Primary Objectives

Phase 1. To assess the safety and tolerability and identify the recommended phase 2 dose (RP2D) of uproleselan when combined with myeloablative, busulfan-based, pre-transplant conditioning regimens for treatment of acute myeloid leukemia.

Phase 2. To describe the safety of uproleselan at the RP2D when combined with myeloablative, busulfan-based, pre-transplant conditioning regimens for treatment of acute myeloid leukemia.

## 1.4 Secondary Objectives

1) To describe the pharmacokinetics of uproleselan in pediatric patients receiving myeloablative, busulfan based pre-transplant conditioning (this will encompass both phase 1 and phase 2).

2) To describe the preliminary efficacy of uproleselan by estimating the 12-month leukemia-free survival (LFS), overall survival (OS), and relapse in patients receiving uproleselan at the RP2D

3) To describe the maximal severity of oral and gastrointestinal mucositis in patients receiving uproleselan at the RP2D.

## 1.5 Exploratory Objectives

These preliminary assessments will be performed to prepare for more definitive assessments in a full scale clinical trial.

1) To obtain preliminary data on AML blast E-selectin ligand (EsL) expression by multi-dimensional flow cytometry as predictor of uproleselan response.

2) To obtain preliminary data on AML transcript expression of the EsL related glycan synthesis genes ST3GAL4 and FUT7.

# 2. BACKGROUND

## 2.1 IND Agent: Uproleselan

E-selectin is constitutively expressed on the sinusoidal endothelial cells of the marrow's vascular niche and plays a critical role in the regulation of hematopoietic stem cell quiescence, self-renewal, activation and homing. AML cells hijack the vascular niche through secretion of TNFα and other inflammatory mediators, increasing endothelial expression of E-selection, and through alterations in the E-selectin ligand, augmenting their E-selectin binding potential. E-selectin activates pro-survival signaling in AML cells through AKT/NF-kB pathways. This protects AML leukemia regenerating cells from cytotoxic chemotherapy. Uproleselan, a small molecule E-selectin mimetic, blocks this interaction, abrogating pro-survival signaling and, thereby, sensitizing AML cells to chemotherapy.[1]

A phase I/II trial combining uproleselan with conventional chemotherapy for treatment of adults with AML has been completed (ClinicalTrials.gov identifier: NCT02306291).[2] In the phase I portion, patients ≥18 years with relapsed or refractory disease were enrolled and received uproleselan combined with mitoxantrone, etoposide and cytarabine (MEC) as induction therapy. Three dose levels (5 mg/kg, 10 mg/kg, and 20 mg/kg bid IV) were assessed, and no dose limiting toxicities were identified. 10 mg/kg was selected as the dose for phase II assessment based on the pharmacokinetic and pharmacodynamic assessments. The phase II portion involved two strata: one for patients ≥18 years with relapsed or refractory disease who received uproleselan combined with MEC for induction (patients achieving remission could receive a second course as consolidation) ); and a second stratum for patients ≥60 years with newly diagnosed AML who received uproleselan combined with daunomycin and cytarabine ("7+3") as induction (patients achieving remission could receive a course of consolidation with uproleselan combined with idarubicin and cytarabine).

Severe (≥ grade 3) non-hematologic adverse events were uncommon; the incidences of oral mucositis, intestinal mucositis and blood stream infections were low and consistent with what would be expected for adult AML patients receiving intensive chemotherapy. The induction mortality rate was low for both the relapsed refractory and the newly diagnosed cohort. There were no DLT's attributed to uproleselan. For subjects who received uproleselan (any dose level) plus MEC, 23/66 (35%) experienced at least 1 CTCAE Grade 3 or 4 treatment emergent adverse event (TEAE) assessed to be related to uproleselan. A total of 8/25 subjects (32%) who received uproleselan 10 mg/kg plus 7+3 experienced at least 1 related CTCAE Grade 3 or 4 TEAE assessed to be related to uproleselan. The most frequently reported CTCAE Grade 3 or 4 TEAEs assessed to be related to uproleselan for subjects who received uproleselan (any dose level) plus MEC were febrile neutropenia (11/66 subjects; 17%), thrombocytopenia (7/66; 11%), anemia (5/66; 8%), neutropenia (3/66; 5%), sepsis (3/66; 5%), and platelet count decreased (4/66 subjects; 6%). The most frequently reported CTCAE Grade 3 or 4 TEAEs assessed to be related to uproleselan for subjects who received uproleselan 10 mg/kg plus 7+3 were febrile neutropenia (3/25 subjects; 12%), thrombocytopenia (3/25; 12%), anemia (2/25; 8%), and pneumonia (2/25 subjects; 8%). Durable high remission rates were achieved in both groups. The complete

remission/complete remission with incomplete hematologic recovery (CR/CRi) rate was 41% (27/66 subjects) for subjects who received uproleselan (any dose level) plus MEC and 43% (23/54 subjects) for subjects who received the RP2D uproleselan 10 mg/kg plus MEC. For patients receiving "7+3" the CR/CRi was 68% (17/25). Overall survival was also encouraging in both groups. The median OS was 8.8 months (range 0.9 – 28.8 months) for participants who received RP2D of uproleselan plus MEC and 8.8 months (range 0.9 – 28.8 months) for subjects who received the RP2D uproleselan 10 mg/kg plus MEC. The median OS was 12.6 months (range 0.1 – 22.0 months) for participants who received uproleselan 10 mg/kg plus 7+3. Correlative studies yielded two important findings: one, that E-selectin ligand is detectable on blasts of all patients; two, that the proportion of functional E-selectin binding by circulating blasts is associated with the achievement of remission and survival.

This trial's encouraging results paved the way for two full scale clinical trials: a phase II/III RCT of daunomycin and cytarabine induction +/- uproleselan in older adults with newly diagnosed AML being conducted by the NCI and led by the Alliance (ClinicalTrials.gov identifier: NCT03701308); and a phase III RCT of MEC or fludarabine, cytarabine and idarubicin (FAI) +/- uproleselan in adults with relapsed or refractory AML being conducted by GlycoMimetics, Inc. (ClinicalTrials.gov identifier: NCT03616470). In these trials, a fixed dose of 800 mg of uproleselan is being used to provide an exposure approximating the weight-based dose of 10 mg/kg. In the Phase I/II study, the RP2D dose was 10 mg/kg, and subjects had a median weight of 81.3 kg. The results of the population PK analysis suggest that clearance does not vary with body size. As a result, a fixed dose, rather than a weight-adjusted dose is likely to minimize between-subject variability. Combining the RP2D and median weight with the lack of body size as a covariate, suggests that a fixed dose of 800 mg would provide equivalent exposure as the RP2D (10 mg/kg) (Helen Thackray, GlycoMimetics, Inc.-personal communication, January, 2020).

2.1.1 Uproleselan's potential to decrease transplant-related mortality and morbidity by reducing mucositis.

As noted above, in the phase 1/2 trial of uproleselan combined with intensive AML chemotherapy, the incidences of severe oral and intestinal mucositis were low. Uproleselan has been shown to diminish oral and intestinal mucosal barrier injury following high dose chemotherapy or total body irradiation in mice. Mechanistic studies indicate that this is due to inhibition of E-selectin mediated migration of macrophages to sites of cytotoxic mucosal injury, thereby, abrogating mucosal inflammation.[3,4] Protection against mucosal barrier injury could yield two additional benefits in the setting of allogeneic HSCT. First, it could mitigate bacterial translocation, decreasing the risk for blood stream infections.[5] Notably, the incidence of bacteremia in the phase 1/2 trial was low. Second, protection against mucosal barrier injury could lessen the risk for acute graft-versus-host disease (GVHD), since gastrointestinal mucosal injury plays a central role in the pathogenesis of acute GVHD.[6]

2.1.2 Protection of normal hematopoietic stem cells from the cytotoxic effects of chemotherapy by uproleselan

In murine models, the effects of uproleselan on normal hematopoietic stem cells and leukemia cells are divergent. Rather than sensitizing normal hematopoietic stem cells to the cytotoxic effects of chemotherapy, it protects them. With conventional chemotherapy treatment, uproleselan accelerates neutrophil recovery, providing another mechanism by which uproleselan could prevent mucositis and blood stream infections.[4] In the setting of allogeneic HSCT, uproleselan's protection of normal hematopoietic stem cells could be disadvantageous. By protecting host hematopoietic stem cells from the effects of conditioning, uproleselan could hinder donor hematopoietic stem cell engraftment. However. according to recent experiments conducted by GlycoMimetics, Inc. the protective effect of uproleselan on normal hematopoietic stem cells may not be sufficient to impede donor engraftment. In a murine congenic transplant model, uproleselan administered prior to myeloablative total body irradiation did not alter engraftment.[7]

2.1.3 Justification for dose levels and pharmacokinetic assessment

Previous trials in adult patients have provided a robust pharmacokinetic (PK) dataset for use in population PK modeling, which allows simulation of dose levels and subsequent exposure across different weight and age groups. Doses from 2 to 40 mg/kg were assessed in adults, in both healthy volunteer and patient trials. Minimal accumulation of uproleselan at any dose level was observed, as expected from its short half-life. The PK optimal model was a three-compartment model that included a time-lagged input and the effect of renal function (as estimated by creatinine clearance, using the Cockcroft-Gault equation) on clearance. Clearance of uproleselan varies with renal function and does not vary with body size or any other covariate evaluated in the adult population (50-150kg).

Since this trial is the first in children and the first in hematopoietic stem cell transplantation (HSCT), dosing will start at 10 mg/kg/dose q12H and will not be escalated. It includes a provision for de-escalation to 5 mg/kg/dose q 12 H, if the starting dose is not well tolerated. PK data obtained from this trial will be assessed using a population PK model. to determine actual exposure and coefficient of variation in children and adolescents . These results will inform uproleselan in future pediatric trials.

## 2.2 Relapse after HSCT for AML

Recurrence of disease represents the most important cause of treatment failure after allogeneic hematopoietic stem cell transplantation (HSCT) for patients with acute myeloid leukemia (AML).[8-12] Most relapses occur in the first year after transplant and durable remissions after relapse are rare.[13] Hence, prevention of relapse represents a major focus of research. In the Second Annual International Cancer Institute's Workshop on the Biology, Prevention and Treatment of Relapse after HSCT, the incorporation of novel agents into pre-transplant conditioning regimens to enhance their anti-leukemic effect was proposed as one of the key strategies to be explored for relapse prevention.[14]

## 2.3 Incorporating Uproleselan into Busulfan Conditioning to Enhance its Anti-leukemic Effect

In this trial, uproleselan will be combined with the busulfan, fludarabine and clofarabine regimen, a regimen developed from the standard busulfan and fludarabine regimen commonly employed for both adults and children with AML.[15-19] Building on pre-clinical research demonstrating that the newer purine analogue clofarabine possesses greater activity against AML than fludarabine, and that clofarabine's activity can be enhanced by low doses of fludarabine, investigators at MD Anderson have conducted two randomized controlled trials to assess the effects of combining fludarabine and clofarabine with busulfan. In the first, an early phase trial in adults with AML or MDS, they examined varying dose combinations of the two purine analogues and identified 10 mg/m2/dose of fludarabine and 30 mg/m2/dose of clofarabine as the optimal combination.[20,21] In the second, a recently completed phase 3 trial, 250 (median age =51 years, range =3 to 70 years) patients with AML (n=181) or MDS (n=69) receiving HLA matched related or unrelated donor transplants were randomized to receive busulfan with fludarabine (40 mg/m2/dose) or busulfan with fludarabine (10 mg/m2/dose) and clofarabine (30 mg/m2/dose). Preliminary trial results indicate that busulfan-fludarabine-clofarabine's (BFC) is associated with a lower risk of relapse, especially in patients whose disease is not in remission at the time of

admission, but is also associated with a higher risk of non-relapse mortality, especially in patients with multiple pre-existing co-morbidities. As a result for the group as a whole, there were no differences in progression-free or overall survival. In a sub-group analysis of the patients without significant co-morbidity (co-comorbidity index=0-2) and not remission, however, overall survival was significantly higher patients receiving BFC[22].

Importantly, BFC has been extended to a large group of children and adolescents at the Leiden University Medical Center and University Medical Center Utrecht transplant centers in the Netherlands. 155 children (median age 9.7 years) with hematologic malignancies were enrolled, including 69 with AML (28 in CR1, 40 in CR2, 1 with active disease). 119 patients received transplants from unrelated donor. Most patient received either bone marrow (n=79) or cord blood (n=66) grafts. There were only 2 cases of non-engraftment. Non-relapse mortality at 3 years was 10.3%. No veno-occlusive disease of the liver was observed. The cumulative incidence of relapse in AML was 22.5% and event free survival for patients transplanted in AML in 1st CR and 2nd CR was 64% and 65%, respectively. These results demonstrate that in pediatric patients BFC is safe, well suited for cord blood transplantation and effective for AML[23].

BFC was chosen for use as the conditioning backbone in this trial because it may be more effective in patients with chemotherapy-resistant AML, because it is safe in pediatric patients and because it is immunosuppressive enough to produce robust engraftment in cord blood transplantation.

The current trial builds on other recent attempts to add novel chemotherapy agents to BF or BFC conditioning in adults with AML. The list of agents that have been tested or are currently being tested in early phase trials includes plerixafor[24], CPX-35121 and vorinostat[25] (NCT02083250).

## 2.4 Rationale

This phase 1/2 trial will allow us to prepare for a full-scale clinical trial, open to patients with chemo-sensitive as well as chemo-resistant AML and to patients receiving a wider variety of donor/graft types and forms of conditioning and GVHD prophylaxis. We anticipate that in the full-scale clinical trial, patients with chemo-sensitive disease will be assigned to a randomized stratum (uproleselan vs. placebo), while patients with chemo-resistant disease will be non-randomly assigned to treatment with uproleselan.

Eligibility: Eligibility for the trial will be limited to those patients at highest risk for relapse: those transplanted with refractory disease (not in remission) or transplanted in a complete remission, but with measurable residual disease on marrow testing by multidimensional flow cytometry (MRD positive). The risk of relapse associated with pre-transplant MRD approaches that associated with refractory disease. A large analysis of children and adults transplanted for AML in first or second complete remission (CR1 or CR2) showed that patients with any level of MRD pre-transplant had a nearly five-fold higher risk of relapse at 3 years compared to those who were MRD negative. Importantly, pre-transplant MRD obviated the significance of traditional risk factors, including cytogenetic risk and remission number.[26] Similar results were shown in a pediatric only cohort in the Pediatric Blood and Marrow Transplant Consortium study ONC001 for patients receiving allo-HSCT in CR1 or CR2. The 2-year cumulative incidence of

relapse was 32% for the 113 patients who were MRD negative prior to HSCT as compared to 70% for the 10 patients who were MRD pos. (p=0.01).[27] In both of these studies, most of the relapses in patients who had pre-HSCT MRD occurred by 6 months. In both studies, relapse-free survival for MRD positive patients was approximately 40% at 6 months.

## 2.5 Correlative Studies Background

In this trial, we will explore E-selectin related biomarkers. We will assess the proportion of baseline bone marrow blasts with detectable E-selectin ligand (EsL), which could be an important determinant of benefit from uproleselan. The proportion of blast cells expressing EsL could thereby inform eligibility for future trials of uproleselan in HSCT.

In patients not receiving uproleselan, EsL expression has been shown to be associated with poorer prognosis. Investigators from the Children's Oncology Group (COG) examined the prognostic importance of EsL expression in 1074 children, adolescents and young adults with newly diagnosed AML enrolled on the phase 3 AAML1031 trial. They assessed expression with transcriptome analysis of 24 genes involved in glycosylation of EsL. In their transcriptome analysis, they identified 7 genes where expression was associated with increased risk of treatment failure. Of these, they selected two for further analysis, ST3GAL4 and FUT7, as they directly synthesize sLex. Overall survival ranged from 45.8% in patients with high expression (highest quartile) of both genes (SFhigh), to 55.5% in patients with high expression of one gene (SFinter), to 71.0% in patients without high expression of either (SFlow, $p<0.0001$). These investigators also observed variation in expression of these genes across patient sub-groups. 71.5% of infants <1 year were SFlow, and only 4.66% SFhigh. 97% of patients with core binding factor AML were SFlow and 0% SFhigh. These investigators also performed multidimensional flow cytometry (MDF) to detect cell surface EsL expression and demonstrated that SF gene expression was strongly correlated with EsL surface expression[7].

A cross-sectional study in adults with AML demonstrated much higher EsL expression by MDF in patients with relapsed/refractory AML than in patients with newly diagnosed AML. Similarly, EsL expression was much higher in patients with unfavorable cytogenetic/molecular abnormalities than those with favorable/intermediate risk markers.[28]

The adverse impact of EsL on prognosis may be negated by uproleselan. In fact, preliminary data from the phase 1/2 trial in adults discussed above suggests that degree of EsL expression may influence response to uproleselan. Data on EsL expression measured by MDF was available at study entry in 36 of 66 patients enrolled. Though EsL was detectable on blasts in all patients, there was great variation. Degree of expression on leukemia stem cells was dichotomized. In 22 patients (61%), the level was ≥ 10%. In the remaining 14 patients it was < 10 %. In the high expression group, the CR/Cri rate was 45% and median overall survival was 12.7 months. In the low expression group, the response rate was 29% and median survival 5.4 months28. More data is needed to determine if EsL expression can be used to identify patients likely to benefit from uproleselan. In the current trial, pre-transplant marrow samples will be evaluated for EsL expression by gene expression and MDF.

# 3. PARTICIPANT SELECTION

Once informed consent has been obtained, the investigator may begin screening studies to confirm eligibility. Once all eligibility criteria have been satisfied, the investigator may enroll the participant according to Section 4. All clinical and laboratory studies to determine eligibility must be performed within 28 days prior to enrollment.

**Exceptions:**

- Disease staging bone marrow testing, CSF assessment and assessment of other extramedullary disease must be performed **within 14 days** prior to enrollment.

Start of conditioning must be within 7 days of enrollment.

## 3.1 Eligibility Criteria

3.1.1 Age ≥12 months and ≤ 39 years

The minimum and maximum number of subjects enrolled on the study are 20 and 28, respectively. In order to ensure at least 70% of the population are under the age of 18 years of age, the number of subjects >=18 years old will be limited as follows:

- At least 7 of the first 10 subjects must be under 18 years old
- At least 7 of the second 10 subjects must be under 18 years old
- At least 6 of the last 8 subjects enrolled must be under 18 years old

3.1.2 Lansky/Karnofsky performance status ≥70% (see Appendix A)

3.1.3 Weight ≥10 kg

3.1.4 Acute myeloid leukemia that arises *de novo* or is secondary to:

- cytotoxic chemoradiotherapy
- myelodysplastic syndrome
- a leukemia predisposition syndrome or inherited marrow failure syndrome other than ones associated with transplant-related morbidity and mortality. A predisposition resulting from a germline RUNX1 mutation is example of an eligible disorder. Fanconi Anemia and Dyskeratosis Congenita are examples of ineligible disorders.

3.1.5 **Disease status:**

*Multidimensional flow cytometry (MDF) to assess disease status for eligibility will be performed centrally by Hematologics.*

- In a first or second complete remission (defined as marrow with ≤1% leukemic blasts by MDF and no evidence of extramedullary disease) with minimal residual disease (MRD, defined as marrow with ≥0.05% leukemic blasts by MDF) after at least 2 cycles of induction/re-induction chemotherapy.

- Have newly diagnosed disease or disease in first relapse that is refractory (defined as marrow with >1% leukemic blasts by MDF or persistence of extramedullary disease) to at least 2 cycles of induction/re-induction chemotherapy.

***This sample will be used for eligbility as well as correlative biomarkers. Please see section 9.2 for details regarding collection, processing, and shipping of the sample.***

3.1.6 **Graft and Donor Types:**

Patients must be receiving bone marrow or peripheral blood stem cells from a HLA identical related or HLA matched unrelated (allele level matched at A, B, C and DRB1 loci) donor.

Eligibility of prospective donors should be determined in compliance with requirements of 21 CFR Part 1271. This should include donor screening for COVID-19 exposure or infection. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/updated-information-human-cell-tissue-or-cellular-or-tissue-based-product-hctp-establishments

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

**3.2 Exclusion Criteria**

3.2.1 Participants who have had a previous hematopoietic stem cell transplantation

3.2.2 Participants who have had prior treatment with uproleselan

3.2.3 CNS 3 disease at time of admission for HSCT. Patients previously diagnosed CNS 3 disease that has improved (CNS1 or CNS2) will be eligible. (See Section 3.3 for definitions).

3.2.4 Down Syndrome

3.2.5 Fanconi Anemia, Dyskeratosis Congenita and other disorders associated with excess risk for transplant related toxicities

3.2.6 Acute Promyelocytic Leukemia

3.2.7 Multiply relapsed (≥2) disease

3.2.8 Pregnancy (positive serum beta-HCG) or breastfeeding
Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with uproleselan, breastfeeding should be discontinued if the mother is treated with uproleselan. These potential risks also apply to other agents used in this study.

3.2.9 Absolute neutrophil count <300/μL due to treatment (chemotherapy or immunotherapy).

Patients with neutropenia due to disease related marrow dysfunction (refractory disease, underlying myelodysplasia or an underlying marrow failure disorder) will be eligible regardless of the absolute neutrophil count. However, enrolling centers must provide clear evidence that the neutrophil count is not rising, that the patient does not have an inadequately controlled infection (see section 3.2.15), and that the patient is on broad anti-fungal prophylaxis.

Given the serious risk associated with starting conditioning in patients with severe neutropenia, centers are encouraged to delay transplant if they have any reason to believe that the absolute neutrophil count may improve.

3.2.10 Estimated GFR of <60 mL/min/1.73 $m^2$. Estimated GFR may be calculated using the CKD-EPI Creatinine Equation (2009) for patients ≥19 years or creatinine-based Bedside Schwartz equation (2009) for patients <19 years. It is recommended that estimates be determined using the calculators found on the National Kidney Foundation website. the (https://www.kidney.org/professionals/KDOQI/gfr_calculator). Any patient for whom these equations yields a GFR less than 90 mL/min/1.73 $m^2$ should have radionucleotide testing. Measurement of 24-hour urine creatinine clearance is not an acceptable substitute for radionucleotide testing.

3.2.11 Cardiac ejection fraction <50% or shortening fraction <27%

3.2.12 Total bilirubin (with elevated direct bilirubin) or ALT >2 X ULN.

3.2.13 Pulmonary disease with FVC, FEV1 or DLCO (corrected for hemoglobin) <50 % predicted or requiring supplemental oxygen. Children who are developmentally unable to perform pulmonary function testing will be assessed solely on their need for supplemental oxygen

3.2.14 Active hepatitis B or C infection

3.2.15 Active, poorly controlled infections. In patients being treated for infection at the time of enrollment, source documentation of the results of all microbiologic, radiographic and pathology assessments performed for diagnosis and for evaluation of response to treatment will be required.

3.2.16 Patients with a known history of HIV are excluded, unless they meet **all** of the following conditions:

- No history of HIV complications with the exception of CD4 count <200 cells/$mm^3$
- No antiretroviral therapy with overlapping toxicity such as myelosuppression
- CD4 count >500 cells/$mm^3$ prior to the diagnosis of relapsed AML
- HIV viral loads below the limit of detection
- No history of highly active antiretroviral therapy (HAART)-resistant HIV

3.2.17 Patients who have received another investigational drug within 28 days or 5 half-lives (whichever is longer).

## 3.3 Definitions of CNS Disease

**CNS 1:** In cerebral spinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs).

**CNS 2:** CNS2 is defined as any one of the following:
**CNS 2a:** Blasts present in cytospin CSF with CSF WBC <5/μL and CSF RBC < 10/μL (atraumatic tap)
**CNS 2b:** Blasts present in cytospin CSF with CSF WBC <5/μL and CSF RBC ≥ 10/μL (traumatic tap)
**CNS 2c:** Blasts present in cytospin CSF with CSF WBC ≥5/μL and CSF RBC ≥ 10/μL (traumatic tap) in which the WBC/RBC ratio in the CSF is less than twice that in the peripheral blood (see Steinherz/Bleyer calculation method below)

**CNS3:** CNS3 is defined as any one of the following:
**CNS3a:** Blasts present in cytospin CSF with CSF WBC ≥5/uL and CSF RBC <10/uL (atraumatic tap)
**CNS3b:** Blasts present in cytospin CSF with CSF WBC ≥5/uL in a traumatic tap (CSF RBC ≥10/uL) in which the WBC/RBC ratio in the CSF is twice or greater that in the peripheral blood (see Steinherz/Bleyer calculation method below)
**CNS3c:** Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome) OR radiographic evidence of an intracranial or intradural mass consistent with a chloroma. Retinal hemorrhage and extra-ocular orbital masses are not considered CNS leukemia.

STEINHERZ/BLEYER ALGORITHM FOR EVALUATING TRAUMATIC LUMBAR PUNCTURES:
If the patient has leukemic cells in the peripheral blood and the lumbar puncture is

traumatic (CSF RBC ≥10/uL) and the cytospin contains ≥ 5 WBC/μL with blasts, the following equation should be used to distinguish between CNS2 and CNS3 disease:

CNS2c: (CSF WBC ÷ CSF RBC) < 2 x (Blood WBC ÷ Blood RBC)
CNS3b: (CSF WBC ÷ CSF RBC) ≥ 2 x (Blood WBC ÷ Blood RBC)

For example, the following patient would be classified as CNS3:
CSF WBC = 60/μL; CSF RBC = 1500/μL;
blood WBC = 46000/μL;
blood RBC = 3.0 X 106/μL:

$$\frac{60}{1500} = 0.04 > 2X \frac{46000}{3.0 \times 10^6} = 0.015$$

### 3.4 Inclusion of Children and Minorities

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

### 3.5 Co-Enrollment on Other Studies

If seeking to enroll patients on this uproleselan trial who are currently enrolled on another study , centers must indicate any and all studies that the patients are currently enrolled on and the Sponsor-Investigator or their designee will review this information and determine whether the patient can be approved for enrollment. Patients who are not currently enrolled on another study but who seek to enroll in another trial (either while they enroll on the uproleselan trial or after they enroll on the uproleselan trial) must be granted permission by the Sponsor-Investigator or their designee. Patients will be taken off uproleselan if they need to be treated with another investigational product.

## 4. REGISTRATION PROCEDURES

### 4.1 General Guidelines for Dana-Farber/Boston Children's Cancer and Blood Disorders Center

Dana-Farber/Boston Children's study staff will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. The participant will first be registered in OnCore after the screening consent is signed and reviewed by the lead site study staff. The participant will then be fully enrolled on study after the main consent is signed and eligibility criteria is reviewed by the lead site study staff. Registration and enrollment must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria (including confirmed marrow testing eligibility from Hematologics) and will complete and sign the protocol-specific eligibility checklist.

If the subject fails eligibility review, the subject may re-screen at the discretion of the Sponsor-

Investigator.

Following enrollment, participants must begin conditioning within 7 days. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator or designee. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

## 4.2 Registration Process for DF/HCC Institution

Institutions will register eligible participants in the Clinical Trials Management Systems (CTMS) OnCore as required by DF/HCC Policy REGIST-101.

## 4.3 General Guidelines for Other Investigative Sites

The participant will first be registered in OnCore after the screening consent is signed and reviewed by the lead site study staff. The participant will then be fully enrolled on study after the main consent is signed and eligibility criteria is reviewed by the lead site study staff. Registration and enrollment must occur prior to the initiation of protocol therapy.

Eligible participants will be entered on study centrally at the coordinating center by the study coordinator (or designee). All sites should contact the study coordinator to verify dose level availabilities.The coordinating center requires 48 hours to review eligibility and register a participant, and is available business days from 9:00AM- 5:00PM EST.

Following full study enrollment, participants must begin conditioning within 7 days. Issues that would cause treatment delays should be discussed with the Sponsor-Investigator or designee. If a participant does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

## 4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating institution and faxed or e-mailed to the coordinating center: [Email: lead study coordinator or study team at UproTrial@DFCI.Harvard.edu or Fax: 617-632-3977]

- Completed copy of Registration Request and Email/Fax Cover Sheet
- Copy of all screening and eligibility source documents
- Signed informed consent documents (and assents, if applicable) with patient initials and date of birth on every page of the consent
- HIPAA authorization form (if separate from the informed consent document)
- Completed eligibility checklist

The research team at the participating site will then e-mail [UproTrial@DFCI.Harvard.edu] the Coordinating Center Lead Study Coordinator to verify eligibility. To complete the registration process, the coordinator will follow DF/HCC Policy REGIST-101 and register the participant on the protocol. The coordinator will fax or e-mail the participant study number, and if applicable the dose treatment level, to the participating site. The coordinator will also call the research team at the participating site and verbally confirm registration

**Treatment may not begin without confirmation from the coordinating center that the participant has been registered and enrolled on study.**

## 5. TREATMENT PLAN

### 5.1 Treatment Regimen

This is a phase 1/2 trial comprised of a dose-finding phase (phase 1) to determine the RP2D of uproleselan followed by a dose expansion phase (phase 2) to further assess safety and to make a preliminary assessment of efficacy of the RP2D. In both phases, uproleselan will be combined with a high dose busulfan-based myeloablative conditioning regimen (busulfan, fludarabine, and clofarabine) starting with a single dose on day -8, followed by doses q12 hours (H) on conditioning day -7 and ending on day -2 (Table 1).

| **Table 1. Pre-transplant Conditioning** | |
|---|---|
| **Uproleselan with Busulfan, Fludarabine, Clofarabine** | |
| **Days -8 to day -2** | **Uproleselan** will be administered IV over 20 minutes (+/- 2 minutes) per assigned dose level in the phase 1 portion or at the RP2D in the phase 2 portion. The maximum dose of uproleselan is 800mg BID. The 20 minute infusion time should include the line flush to ensure the full dose has been infused.<br>• A single primer dose will be administered on day -8<br>• Beginning on day -7 (with start of fludarabine, clofarabine and busulfan) 12 doses of uproleselan will be administered q 12 H.<br>• The 1st, 3rd, 5th, and 7th of theses 12 doses (not including initial day -8 dose) should be administered sequentially, after clofarabine and just prior to busulfan. Delay of doses should be avoided.<br>• The last dose of uproleselan will be administered 12 hours (+/- 60 minutes) after dose 11. |
| **Days –7 to –4** | **Fludarabine** 10 mg/m$^2$/dose daily IV administered as a 30 minute* infusion immediately prior to clofarabine |
| | **Clofarabine** 30 mg/m$^2$/dose daily IV administered as a 120 minute* infusion immediately after fludarabine and just prior to uproleselan |
| | **Busulfan** IV daily IV over 3 hours administered immediately after uproleselan.<br>• See section 5.2.1 for details |

| | |
|---|---|
| | • Busulfan exposure targeted to 80-100 mg x h/L |
| **Day -1** | **Rest** |
| **Day 0** | **Hematopoietic Stem Cell Infusion** |

*Duration of infusion may be adjusted per institutional standards

**Phase 1 Design**

Patients will be assigned a uproleselan dose level at the time of enrollment (Table 2). The starting dose will be 10 mg/kg/dose (DL1). Dose finding decisions are governed by the standard 3+3 design (see section 13.2.3).

**Table 2. Uproleselan Dosing**

| Dose Level | Uproleselan Dose |
|---|---|
| Level 0 | 5 mg/kg/dose* |
| Level 1 – Starting Dose | 10 mg/kg/dose* |

*The maximum dose of uproleselan is 800mg BID.

**Phase 2 Design**

After completion of the phase 1 portion of the trial, enrollment at the recommended phase 2 dose will be expanded to bring total enrollment at this dose to 20 evaluable patients. Information about patients treated at the RP2D will be used to obtain preliminary estimates of drug exposure and efficacy, and to confirm safety. A one-stage stopping rule will monitor transplant-related mortality (TRM) for the 20 patients treated at the RP2D (see section 13.5).

Once the RP2D has been identified, an additional 14 to 17 evaluable patients will be enrolled at the RP2D. Interim monitoring of treatment related mortality (TRM) of the 20 patients treated at the RP2D (3 to 6 from Phase 1 and 14 to 17 from Phase 2) is described in section 13.5.

Treatment will be administered on an inpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

## 5.2 Agent Administration

Uproleselan will be administered as a 20 minute (+/- 2 minutes) IV infusion (including flush) with dose per assigned level. The maximum dose of uproleselan is 800mg BID. A single priming dose will be administered on Day -8. 12 additional doses will then be administered q 12 H beginning immediately after clofarabine and just before busulfan on Day -7. The dose of uproleselan can be rounded to the nearest whole unit to avoid decimal dosing. Dosing will be based on actual weight. Weight can be collected up to 7 days prior to first dose administration, if weight changes by >10% prior to first dose, dose will be adjusted to current weight. Doses of Uproleselan should not be adjusted once conditioning has started.

Reactions to uproleselan infusions have not been observed to date. Patients should have vital signs just prior to infusion and repeated with the start of the infusion three times at 10-minute (+/- 2 minutes) intervals. Frequent vitals can be discontinued if the patient has not manifested any signs of anaphylaxis or other serious infusion-related reaction. The infusion should be discontinued for any signs of anaphylaxis and the event should be reported immediately to the study team.

### 5.2.1 **Administration of other Conditioning Agents**

Fludarabine Administration

Fludarabine will be administered 10 mg/m$^2$ IV daily as a 30 minute infusion for four days (-7 to -4). Patients with an estimated of GFR < 70 ml/min/1.73 m2 shall receive 8 mg/m2 IV daily. Dosing will be based on AIBW in patients whose actual weight exceeds 125% of their IBW (see formula in section 6.3). Duration of fludarabine infusion may be adjusted per institutional standards.

Clofarabine Administration

Clofarabine will be administered 30 mg/m2 IV daily as a 120 minute infusion for four days (-7 to -4). Dosing will be based on AIBW in patients whose actual weight exceeds 125% of their IBW (see formula in section 6.3). Doses will be administered immediately after completion of fludarabine infusion and just prior to start of uproleselan infusion. Duration of clofarabine infusion may be adjusted per institutional standards

Hydrocortisone 100 mg/m2/dose IV may be administered 30 minutes prior to each clofarabine dose for prevention of capillary leak syndrome.

Busulfan Administration

Busulfan will be administered once daily (q24) IV for four days (-7 to -4) over 3 hours. Pharmacy will provide busulfan at final concentration of 0.5mg/ml. Recommended weight-based initial doses are listed in (Table 3) and based on actual body weight of the patient in kg.

**Table 3-** Estimated initial busulfan dosing in mg/kg by subject weight aimed to target a goal cAUC exposure of 90mg*hr/L (range 80-100mg*hr/L) for patients receiving a regimen containing busulfan/fludarabine/clofarabine.

| Actual Body Weight[1] | Initial Busulfan Dose |
|---|---|
| >10 - 15kg | 4.3 mg/kg/dose |
| >15 - 20kg | 4.2 mg/kg/dose |
| >20 - 25kg | 3.9 mg/kg/dose |
| >25 - 30kg | 3.7 mg/kg/dose |
| >30 - 35kg | 3.5 mg/kg/dose |
| >35 - 40kg | 3.3 mg/kg/dose |
| >40 - 45kg | 3.3 mg/kg/dose |
| >45 - 50kg | 3.2 mg/kg/dose |

| | |
|---|---|
| >50 - 55kg | 3.2 mg/kg/dose |
| >55 - 60kg | 3.1 mg/kg/dose |
| >60 - 65kg | 3.0 mg/kg/dose |
| >65 - 70kg | 2.9 mg/kg/dose |
| >70 - 75kg | 2.8 mg/kg/dose |
| >75 - 80kg | 2.7 mg/kg/dose |
| >80 - 85kg | 2.6 mg/kg/dose |
| >85 - 90kg | 2.5 mg/kg/dose |
| >90 - 95kg | 2.4 mg/kg/dose |
| >95 - 100kg | 2.3 mg/kg/dose |
| >100 - 110kg | 2.2 mg/kg/dose |

These doses are a simplification of the nomogram published by Shukla et al[29].
[1]Actual body weight should be applied in all subjects.

Busulfan Pharmacokinetic (PK) Assessments

Busulfan will be infused at a continuous rate over 3 hours. Blood Sample collections for PK sampling assessment whole blood will be performed with Dose 1 and used to determine dose modifications for subsequent doses, if needed. Recommended times for blood sample collections are based on an individualized optimal validated sampling strategy with for a 3 hour infusion and blood collection times at 15 minutes, 1 hour, 3 hours, and 5 hours post the end of the busulfan infusion. These Dose 1 PK data will be used to calculate cumulative busulfan exposure (cAUC) to guide dose adjustment. The cAUC will be estimated using standard compartmental PK methods in Pumas 2.0 (Pumas-AI, Baltimore) and verified by the study pharmacologist and pharmacometrician.

Irrespective of if a dose modification is needed guided by Dose 1 data, PK sampling will be repeated with a subsequent dose (Doses 2, 3 or 4) for a minimum of two complete PK sets (including Dose 1) per subject. Recommended times for sample collections for a 3 hour infusion are 15 minutes, 1 hour, 3 hours and 5 hours post the *end* of the busulfan infusion.

Estimation of cAUC and dose modifications

After busulfan PK results are provided by the institution's respective lab, individual AUC will be estimated by the standard equation:

$AUC_{(0\text{-infinity})} = Dose_{(mg)} / CL_{(individual)}$ (Equation 1)

Updated individualized doses for Doses 2-4 will be calculated by scaling the previous dose with the ratio of obtained $AUC_{(0\text{-infinity})}$ and predefined $AUC_{target}$ using the equation and solving for new dose as follows:

$Dose_{(mg)} / AUC_{(0\text{-infinity})} = \text{New dose (mg)} / \text{Desired } AUC_{target}$ (Equation 2)

**Busulfan Exposure and Therapeutic Drug Monitoring:** Initial doses for busulfan will be individualized for each subject and aimed to achieve a cumulative area-under-the-curve (cAUC) of 80-100 mg x h/L. Individualized doses will be derived using a validated dose algorithm for busulfan in children.[29] To ensure the cAUC goal is achieved, therapeutic drug monitoring will be performed as standard of care in all patients. Busulfan will be administered once daily infused at a continuous rate over 3 hours. Blood collections for pharmacokinetic (PK) sampling will be performed with dose 1 and used to determine dose modifications for subsequent doses, if needed. Recommended times for blood collections are as followed: 15 minutes, 1 hour, 3 hours, and 5 hours post the *end* of the busulfan infusion. Irrespective of whether modification of the first dose is needed, PK sampling will be repeated following dose 2, 3 or dose 4 in all patients to ensure accurate estimation of cumulative overall exposure. The cAUC will be estimated using standard compartmental PK methods in Pumas 2.0 (Pumas-AI, Baltimore) and verified by the study pharmacologist and pharmacometrician.

Timing of Combination Chemotherapy Administration

The timing of the administration of busulfan, fludarabine and clofarabine may be altered to accommodate busulfan pharmacokinetic testing. The last dose of uproleselan, then, will be administered in the evening of Day -2 or the morning on Day -1, depending on the timing of combination chemotherapy administration.

### 5.2.2 Graft Versus Host Disease Prophylaxis and Treatment

Tacrolimus will be combined with methotrexate for Graft Versus Host Disease Prophylaxis. Mycophenolate will also be substituted for patients not tolerating methotrexate.

**Tacrolimus:** Administration will commence on day –2 (at least 36 hours before the stem cell infusion); tacrolimus route and dosing will be adjusted according to institutional preference to maintain a level of 5-15 ng/ml. The timing and the schedule for tapering will be determined by the treating center.

For dosing modifications, see Section 6.1.

**Methotrexate:** Methotrexate will be given at a dose of 5 mg/m2 IV on Days +1, +3, +6 and +11. Dosing shall be based on actual weight. The Day +1 dose shall not be administered until 24 hours following completion of the stem cell infusion

For dosing modifications, see Section 6.2.

**Mycophenolate mofetil:** For patients where methotrexate needs to be discontinued before the 3rd dose (see section 6.2), mycophenolate mofetil should be started. It will be dosed at 15 mg/kg/dose IV or PO (maximum of 1000 mg/dose) q 8H and administered through at least Day +30. It may be continued longer at the discretion of the treating physician. It should be discontinued without tapering.

**Treatment of acute and chronic GVHD:** Treatment of patients who develop GVHD either acute or chronic, will be prescribed by the treating physician.

### 5.2.3 Hematopoietic stem cell infusion

The donor graft will be infused on Day 0. Institutional guidelines should be followed for preparation and infusion of grafts.

## 5.3 Definition of Dose-Limiting Toxicity (DLT)

As mentioned, uproleselan has been well tolerated when combined with AML chemotherapy in the phase 1/2 trial in adults. No toxicities have been identified to date. In the present trial, DLT will be defined to encompass two forms of transplant related adverse events: Regimen related toxicity (RRT) and failure of engraftment.

RRT is defined using a modification of the Seattle Toxicity Criteria (AKA Bearman Criteria).[30] We have modified the system by adding skin to the list of assessed organs, since both busulfan and clofarabine can cause erythroderma, assessed according to the NCI CTCAE v.5.0 (Table 4). (Appendix C). Thresholds for DLT are included. **Organ toxicity attributable to serious infection, will not be considered RRT.** Patients will be evaluated for RRT from transplant day 0 through post-transplant Day +30.

Failure of engraftment is defined as failure of recovery of an absolute neutrophil count to 500 cells/mm3 by Day +30 or a peripheral blood donor myeloid chimerism (obtained no later than Day +35) of at least 90%.

**Table 4. Regimen-Related Toxicities**

| Organ | Seattle Toxicity Criteria-threshold for DLT | CTCAE Term | CTCAE-threshold for DLT |
|---|---|---|---|
| Renal | ≥3 | | |
| Bladder | ≥3 | | |
| Gastrointestinal | ≥3 | | |
| Hepatic | ≥3 | | |
| Oral | ≥3 | | |

| | | | |
|---|---|---|---|
| Cardiovascular | ≥3 | | |
| Nervous System | ≥2 | | |
| Pulmonary | ≥3 | | |
| Skin | N/A | Erythroderma | ≥4 |

## 5.4 Supportive Care Guidelines

**Growth factor:** The use of filgrastim (G-CSF) or biosimilar may be used at the treating physician's discretion. It is recommended that GCSF be avoided when possible in forms of AML where it may stimulate leukemia (e.g. AML with monosomy 7 or CSF3R mutations).

**Blood Products:** The transfusion of blood products (erythrocytes and platelets) will be administered according to local institutional guidelines. Granulocyte infusions may be used at the discretion of the treating physician for treating serious infection.

**Prophylaxis and Management of Infections:** Strategies to prevent and treat infections (viral, fungal, bacterial, PJP) should be administered according to institution guidelines.

**Busulfan precautions:** Lorazepam (or other suitable benzodiazepine) or levetiracetam should be administered according to institutional guidelines to prevent seizures. If feasible, administration of acetaminophen should be held beginning 72 hours prior to administration of first dose of busulfan through 24 hours after last dose administered.

## 5.5 Criteria for Taking a Participant Off Protocol Therapy

Adherence to protocol therapy is required through Day +30, the time point at which dose limiting toxicity will be assessed, unless one of the following criteria are met.

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant

unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy, the participant's status must be updated in OnCore by the study team at the coordinating center in accordance with REGIST-OP-1. Patients coming off protocol therapy will stay on study.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Sponsor-Investigator or designee.

### 5.6 Duration of Follow Up

Participants will be followed for adverse event reporting from the time of enrollment through when the participant is taken off study (see section 5.7). Participants who experience adverse events associated with study therapy will be followed until resolution or stabilization (new baseline) of the adverse event.

In addition, we are interested in the long-term outcome of patients who participated in the study. Therefore, we plan to follow patients for 2 years (Day +730) from day of transplantation (Day 0) or until death, if the patient dies prior to 2 years post-transplant. Participants will be taken off study at the end of follow-up. We will obtain information regarding participant status via phone call or electronic mailing if no longer followed locally.

### 5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Completion of planned follow-up
- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team at the coordinating center will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1.

## 6. DOSE MODIFICATIONS

Dose modifications will be made as indicated below.

There will be no dosing modifications or delays for uproleselan.

## 6.1 Dosing Modifications for Tacrolimus

Tacrolimus dosing should be modified for for significant renal toxicity, poorly controlled hypertension, neurotoxicity, and other serious toxicities.

## 6.2 Dosing Modifications for Methotrexate

The methotrexate dose should be reduced by 50% in the following situations:

- Serum creatinine 2-3 times baseline.
- Serum ALT 5-10 times the upper limit of normal or direct bilirubin 2.1-4.0 mg/dL.
- Oropharyngeal mucositis that is causing symptomatic, but non-life threatening airway obstruction

The methotrexate dose should be held in the following situations:

- Serum creatinine >3 times baseline.
- Serum ALT >10 times the upper limit of normal or direct bilirubin >4.0 mg/dL.
- Oropharyngeal mucositis that is causing life threatening airway obstruction.
- Significant third-spacing of fluid (i.e. pleural effusion, pericardial effusion, or ascites).

If methotrexate is discontinued before the 3rd dose is administered, mycophenolate should be started in its place.

## 6.3 Dosing Modifications for Obesity

For patients weighing more than 125% of their ideal body weight, dosing for fludarabine, and clofarabine (but not busulfan,uproleselan, or medications used for graft versus host disease prophylaxis) will be based on adjusted ideal body weight as outlined below.

IDEAL AND ADJUSTED IDEAL BODY WEIGHT (IBW AND AIBW) FORMULAS:
Pts ≤60 inches: IBW = [(height) $^2$ x 1.65] / 1000, where ht=cm, IBW=kg

Patients >60 inches:
Males IBW=39.0+[2.27*(ht-60)] where ht=inches, IBW=kg
Females IBW=42.2+[2.27*(ht-60)] where ht=inches, IBW=kg

AIBW = IBW + [(0.25) x (Actual BW - IBW)]

# 7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. This is done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. The following lists of expected toxicities (Section 7.1) and the event characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

## 7.1 Expected Toxicities

### 7.1.1 Adverse Event List for Uproleselan

The adverse events observed with uproleselan in the phase 1/2 clinical trial of uproleselan combined with induction chemotherapy did not differ in type or frequency from those associated with AML induction chemotherapy alone. Likewise, no toxicities have been observed in animal studies. As mentioned, because the drug has been shown to protect normal hematopoietic stem cells from the cytotoxicity of chemotherapy, there is a concern that it could impede engraftment of donor hematopoietic stem cells.

In A Phase I/II, open-label, multicenter trial (GMI-1271-201) as an adjunct to standard chemotherapy in 91 subjects with AML patients were enrolled in both the relapsed/refractory and newly diagnosed populations and added uproleselan to standard induction chemotherapy (MEC or cytarabine + idarubicin '7+3'), with the option in Phase II for consolidation (uproleselan plus chemotherapy) in patients who responded to treatment. In the relapsed/refractory patient population, no additive toxicity was detected and the adverse event (AE) profile was consistent with that normally observed with cytarabine-based chemotherapy. The safety profile observed in the elderly newly diagnosed patient population suggested no additive toxicity in this more vulnerable group (refer to the uproleselan IB). Collectively, the treatment of AML in adult patients up to 75 years of age was not found to raise any concerns for the safety profile of uproleselan when combined with 2 different cytarabine-based induction regimens. Further, while more limited, the patient experience with cytarabine-based consolidation and uproleselan appears to be consistent with this profile.

### 7.1.2 Adverse Event List for Allogeneic HSCT with Busulfan, Fludarabine, Clofarabine Conditioning with Tacrolimus and Methotrexate or Myecophenolate Mofetil GVHD Prophylaxis.

There are many expected AEs of the treatment that uproleselan is being incorporated into. These include, but are not limited to, serious bacterial, viral and fungal infections, veno-occlusive disease of the liver, acute kidney injury, thrombotic microangiopathy, oral and gastrointestinal mucositis, idiopathic pneumonia syndrome, encephalopathy, bleeding, dermatitis, and GVHD. These and other toxicities known to occur in HSCT using these forms of conditioning and GVHD prophylaxis are considered expected.

### 7.1.3 Adverse Event List for Busulfan

| | **Common**<br>Happens to 21-100 children out of every 100 | **Occasional**<br>Happens to 5-20 children out of every 100 | **Rare**<br>Happens to < 5 children out of every 100 |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **Immediate:** Within 1-2 days of receiving drug | Nausea, vomiting, fever, electrolyte changes (hypokalemia, hypomagnesemia, hypocalcemia, hypophosphatemia, and hyponatremia), hyperglycemia, dizziness, rash, pruritus, urticaria, injection site pain and inflammation, back pain, tachycardia, chest pain, edema, insomnia, anxiety, depression, headache, abdominal pain, diarrhea (L) or constipation, anorexia, rectal discomfort, dyspnea, epistaxis | Weight gain, confusion | Seizures (rare with phenytoin prophylaxis), hematemesis, hyperuricemia, arrhythmias other than tachycardia, pleural effusion, alveolar hemorrhage |
| **Prompt:** Within 2-3 weeks | Myelosuppression, asthenia, immunosuppression (L), mucositis, hyperbilirubinemia | Hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD) (L), mild alopecia (L), arthralgia, myalgia, hemorrhagic cystitis, hyperpigmentation (L), elevated creatinine and BUN | Reduced adrenal function (L), esophagitis, radiation recall reactions |
| **Late:** Any time after completion of treatment | Infertility, testicular atrophy and azoospermia, amenorrhea, ovarian failure | | Secondary malignancy, breast enlargement, cataracts, idiopathic pulmonary syndrome (cough, dyspnea, pleural effusion, infiltrates, and hypoxemia), bronchopulmonary dysplasia with interstitial pulmonary fibrosis and pneumonitis, myocardial fibrosis, Osteonecrosis |
| **Unknown Frequency and Timing**: | Fetal toxicities and teratogenic effects of busulfan and its solvent have been noted in animals. Toxicities include: multiple anomalies and low birth weight. It is unknown whether the drug or its solvent is excreted in breast milk. | | |

7.1.4 Adverse Event List for Clofarabine

| Incidence | Toxicities |
|---|---|

| | |
|---|---|
| **Common**<br>(> 20% of patients) | • Tachycardia<br>• Hypotension<br>• Headache<br>• Fever, chills<br>• Fatigue<br>• Anxiety<br>• Skin rash, pruritus<br>• Nausea, vomiting, diarrhea, abdominal pain<br>• Anorexia<br>• Leukopenia<br>• Anemia<br>• Thrombocytopenia<br>• Lymphopenia<br>• Febrile neutropenia<br>• Infection<br>• Epistaxis, petechiae<br>• Aspartate aminotransferase increased<br>• Alanine aminotransferase increased<br>• Bilirubin increased<br>• Serum creatinine increased<br>• Pain |
| **Occasional**<br>(4-20% of patients) | • Capillary leak syndrome<br>• Pericardia effusion<br>• Pleural effusion<br>• Mucositis, gingival or mouth bleeding<br>• Hematuria<br>• Drowsiness, irritability, lethargy, agitation<br>• Tumor lysis syndrome<br>• Respiratory distress, tachypnea, dyspnea<br>• Flushing<br>• Hypertension<br>• Edema<br>• Palmar-plantar erythrodysesthesia, erythema<br>• Sepsis |
| Rare<br>(≤ 3% of patients) | • Sinusoidal obstruction syndrome<br>• Typhlitis<br>• Pancreatitis<br>• Acute renal failure<br>• Hypersensitivity<br>• Gastrointestinal hemorrhage<br>• Hepatitis, hepatic failure<br>• Stevens-Johnson Syndrome (SJS), toxic epidermal necrolysis (TEN) |
| Pregnancy & Lactation | Adverse events were observed in animal reproduction studies. May cause fetal harm if administered to a pregnant woman. Women of childbearing potential should avoid becoming pregnant during therapy. Patients should |

| | |
|---|---|
| | use effective contraception to prevent pregnancy during treatment. It is not known clofarabine is excreted in breast milk. Due to the potential for serious adverse reactions in the nursing infant, breast-feeding is not recommended by the manufacturer. |

7.1.5 Adverse Event List for Fludarabine

| | **Common** Happens to 21-100 subjects out of every 100 | **Occasional** Happens to 5-20 subjects out of every 100 | **Rare** Happens to < 5 subjects out of every 100 |
|---|---|---|---|
| **Immediate:** Within 1-2 days of receiving drug | Fever, fatigue, weakness, pain, nausea, vomiting, anorexia, cough, dyspnea | Edema including peripheral edema, chills, rash, diarrhea, rhinitis, diaphoresis, malaise, abdominal pain, headache, back pain, myalgia, stomatitis, flu-like syndrome | Anaphylaxis, tumor lysis syndrome, dehydration* |
| **Prompt:** Within 2-3 weeks, prior to next course | Myelosuppression (anemia, neutropenia, thrombocytopenia), infection (urinary tract infection, herpes simplex infection, pneumonia, upper respiratory) | Weight loss, gastrointestinal bleeding, hemoptysis, paresthesia, allergic pneumonitis, bronchitis, pharyngitis, visual disturbance, hearing loss, hyperglycemia | Sinusitis, dysuria, opportunistic infections and reactivation of latent viral infections like Epstein-Barr virus (EBV), herpes zoster and John Cunningham (JC) virus (progressive multifocal leukoencephalopathy [PML])[L], EBV associated lymphoproliferative disorder, panycytopenia (can be prolonged), pulmonary hypersensitivity[a] (dyspnea, cough, hypoxia, interstitial pulmonary infiltrate), pulmonary toxicity (acute respiratory distress syndrome [ARDS], pulmonary fibrosis, pulmonary hemorrhage, respiratory distress, respiratory failure), pericardial effusion, skin toxicity (erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigus), liver failure, renal failure, hemorrhage, transfusion-associated graft-versus-host disease has occurred following transfusion of nonirradiated blood products, phlebitis*, sleep disorder*, cerebellar syndrome*, depression*, mentation impaired*, alopecia*, pruritus*, seborrhea*, esophagitis*, constipation*, mucositis*, dysphagia*, hesitancy*, cholelithiasis*, abnormal liver function tests*, osteoporosis*, arthralgia*, abnormal renal function test*, proteinuria*, epistaxis*, hemorrhagic cystitis*, eosinophilia* |

| | | | |
|---|---|---|---|
| **Delayed:** Any time later during therapy, excluding the above conditions | | | Neurotoxicity (increased with high doses): seizures, agitation, confusion, weakness, visual disturbances, optic neuritis, optic neuropathy, photophobia, blindness, paralysis, coma, death, peripheral neuropathy[a]; autoimmune phenomena: thrombocytopenia/thrombocytopenic purpura (ITP), Evans syndrome, hemolytic anemia, acquired hemophilia |
| **Late:** Any time after completion of treatment | | | Myelodysplastic syndrome/acute myeloid leukemia (mainly associated with prior or concomitant or subsequent treatment with other anticancer treatments), skin cancer (new onset or exacerbation) |
| **Unknown Frequency and Timing:** | **Pregnancy Category D** Based on its mechanism of action, fludarabine phosphate can cause fetal harm when administered to a pregnant woman. Fludarabine phosphate was embryolethal and teratogenic in both rats and rabbits. | | |

*(L) Toxicity may also occur later.*
[*] Reported in ≤ 3% of subjects. Since these are not considered life threatening they are not included in the consent.
[a] These effects were not reported in children.

### 7.1.6 Adverse Event List for Tacrolimus

| | **Common** Happens to 21-100 subjects out of every 100 | **Occasional** Happens to 5-20 subjects out of every 100 | **Rare** Happens to < 5 subjects out of every 100 |
|---|---|---|---|
| **Immediate:** Within 1-2 days of receiving drug | | Nausea, vomiting, diarrhea, abdominal pain, rash, pruritus. | Anaphylaxis, allergic reaction, dyspnea, bloating, dyspepsia, taste perversion, QT prolongation, torsade de pointes, headache, dizziness, seizure. |
| **Prompt:** Within 2-3 weeks, prior to next course | | Elevated ALT/AST | Stevens-Johnson syndrome and toxic epidermal necrolysis, hypokalemia, hypercholesterolemia, hypertriglyceridemia, leucopenia including neutropenia and agranulocytosis, eosinophilia, thrombocytopenia[L], hepatitis, cholestasis, fulminant liver failure |
| **Delayed:** Any time later during therapy, excluding the above conditions | | | Alopecia with prolonged used |
| **Unknown Frequency and Timing:** | Pregnancy Category C. There are no adequate and well controlled studies in pregnant women. There have been reports of multiple congenital abnormalities in infants whose mothers were being treated for 3 or more months with high dose (400-800 mg/day) of fluconazole. The relationship between fluconazole use and these events is unclear. Fluconazole should be used in pregnancy only if the potential benefit justifies the possible risk to the | | |

| | |
|---|---|
| | fetus. |

*(L) Toxicity may also occur later.*

### 7.1.7 Adverse Event List for Methotrexate (IV Only)

| | **Common** Happens to 21-100 children out of every 100 | **Occasional** Happens to 5-20 children out of every 100 | **Rare** Happens to <5 children out of every 100 |
|---|---|---|---|
| **Immediate:** Within 1-2 days of receiving drug | Transaminase elevations | Nausea, vomiting, anorexia | Anaphylaxis, chills, fever, dizziness, malaise, drowsiness, blurred vision, acral erythema, urticaria, pruritus, toxic epidermal necrolysis, Stevens-Johnson Syndrome, tumor lysis syndrome, seizures[1], photosensitivity |
| **Prompt:** Within 2-3 weeks, prior to the next course | | Myelosuppression, stomatitis, gingivitis, photosensitivity, fatigue | Alopecia, folliculitis, acne, renal toxicity (ATN, increased creatinine/BUN, hematuria), enteritis, GI ulceration and bleeding, acute neurotoxicity[1] (headache, drowsiness, aphasia, paresis, blurred vision, transient blindness, dysarthria, hemiparesis, decreased reflexes) diarrhea, conjunctivitis |
| **Delayed:** Any time later during therapy, excluding the above conditions | | Learning disability[1] (L) | Pneumonitis, pulmonary fibrosis (L), hepatic fibrosis (L), osteonecrosis (L), leukoencephalopathy[1] (L), pericarditis, pericardial effusions, hyperpigmentation of the nails |
| **Late:** Any time after the completion of therapy | | | Progressive CNS deterioration[1] |
| **Unknown Frequency and Timing**: | Methotrexate crosses the placenta. Fetal toxicities and teratogenic effects of methotrexate have been noted in humans. The toxicities include: congenital defects, chromosomal abnormalities, severe newborn myelosuppression, low birth weight, abortion, and fetal death. Methotrexate is excreted into breast milk in low concentrations. | | |

*[1] May be enhanced by HDMTX and/or cranial irradiation.*
*(L) Toxicity may also occur later.*

### 7.1.8 Adverse Event List for Myecophenolate Mofetil

| Incidence | Toxicities |
|---|---|
| **Common** (>20% of patients) | Hypertension, edema (face, limbs, trunk), rash maculo-papular, cholesterol high, hyperglycemia, hyperkalemia, hypocalcemia, hypokalemia, hypomagnesemia, abdominal pain, constipation, diarrhea, nausea, vomiting, anorexia, dyspepsia, anemia, white blood cell decreased, platelet count decreased, back pain, anxiety, generalized muscle weakness, dizziness, headache, insomnia, tremor, creatinine increased, dyspnea, cough, fever, pleural effusion, alanine aminotransferase increased, alkaline phosphatase increased, aspartate aminotransferase increased, blood bilirubin increased, GGT increased, pain, paresthesia, infection[1] |
| **Occasional** (4-20% of patients) | Sepsis, urinary tract pain, urinary frequency, phlebitis (IV only), thrombosis (IV only) |
| **Rare** (≤3% of patients) | Neoplasms benign, malignant and unspecified (including cysts and polyps) - Other, [Malignant epithelial neoplasm of skin, non-melanoma; lymphoproliferative disease or lymphoma], gastric ulcer, gastrointestinal hemorrhage, gastric perforation, mucositis oral, thromboembolic event, infective endocarditis, renal calculi, pulmonary fibrosis, pneumonitis, neutrophil count decreased, leukoencephalopathy, colitis, pancreatitis, pure red cell aplasia |
| **Pregnancy & Lactation** | **Pregnancy Category D**<br>**Mycophenolate is associated with an increased risk of congenital malformations and spontaneous abortions when used during pregnancy.** Adverse events have been reported in animal studies at doses less than the equivalent recommended human dose. Data from the National Transplantation Pregnancy Registry (NTPR) have observed an increase in structural malformations (including ear malformations) in infants born to mothers taking mycophenolate during pregnancy. Spontaneous abortions have also been noted. Females of childbearing potential should have a negative pregnancy test within 1 week prior to beginning therapy. Two reliable forms of contraception should be used beginning 4 weeks prior to, during, and for 6 weeks after therapy. The effectiveness of hormonal contraceptive agents may be affected by mycophenolate.<br><br>It is unknown if mycophenolate is excreted in human milk. Due to potentially serious adverse reactions, the decision to discontinue the drug or discontinue breast-feeding should be considered. Breast-feeding is not recommended during therapy or for 6 weeks after treatment is complete. |

*[1]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.*

## 7.2 Adverse Event Characteristics

Investigators are to provide the following adverse event characteristics for all adverse events experienced from the time of enrollment through when the participant is taken off study (see section 5.7).

In this trial, in which uproleselan, an investigational agent is being administered in combination with commercial agents (fludarabine, clofarabine, and busulfan), the combination is considered investigational and expedited reporting of adverse events will follow the guidelines for investigational agents. Attribution and expectedness for adverse events will not be evaluated with respect to uproleselan alone; these evaluations will be done based on the combination of uproleselan and the above commercial agents as a single study treatment.

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

**Expectedness** of the AE: In addition to term, grade, attribution, and seriousness, are to be designated as "expected" or "unexpected." For the purposes of determining expectedness, all the adverse events listed in Sections 7.1.1-7.1.8 or in the consent documents are to be considered expected. Any adverse events not listed in sections Sections 7.1.1-7.1.8 or in the consent documents are to be considered unexpected.

**Attribution** of the AE:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

As stated above, attribution and expectedness for adverse events will not be evaluated with respect to uproleselan alone; these evaluations will be done based on the combination of uproleselan and the above commercial agents as a single study treatment.

## 7.3 Adverse Event Collection

All Grade 3 and higher non-hematologic adverse events occurring through transplant day +30 (regardless of attribution or expectedness) will be routinely reported. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. Adverse events that require collection are defined in Tables 5 and Table 6 and must be reported in routine study data submissions on the toxicity case report forms. This includes adverse events previously reported in an expedited manner (see section 7.5).

| Table 5. Hematologic Adverse Event Routine Collection Requirements | |
|---|---|
| **Grade 1-4** | Short-term, reversible grades 1-4 hematologic laboratory abnormalities are expected during transplant and do not require collection or reporting. |
| **Grade 5** | All grade 5 AEs should be collected and reported. |

| Table 6. Non-Hematologic Adverse Event Routine Collection Requirements | |
|---|---|
| **Grade 1** | Grade 1 AEs do not need to be collected unless they otherwise meet criteria for a serious adverse events (SAE) as described in Section 7.4. |
| **Grade 2** | Unexpected grade 2 AEs will be collected with the following exceptions:<br>• *Abnormal laboratory values or test results that are not associated with clinical signs or symptoms and are not considered clinically significant (i.e., require no treatment or further diagnostic testing)* |
| **Grade 3** | All grade 3 non-hematologic AEs should be collected and reported. |
| **Grade 4** | All grade 4 non-hematologic AEs should be collected and reported. |
| **Grade 5** | All grade 5 non-hematologic AEs should be collected and reported. |

Between Day +30 – Day +100 the following adverse events should be collected and reported to the Sponsor:

- Events that are deemed to be Serious and Unexpected
- The following adverse events of special interest would be deemed SAEs: VOD/SOS; graft failure; graft rejection; and > or = grade 3 pulmonary toxicity per CTCAE criteria.

These events should be reported to the Sponsor Investigator within 5 business days of discovery.

### 7.4 Serious Adverse Events

A serious adverse event (SAE) is any adverse event, regardless of causality that:

- Results in death (Grade 5).
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires inpatient hospitalization or prolongation of existing hospitalization ≥ 24 hours. Hospitalizations for routine treatment or monitoring not associated with deterioration in condition, elective or pre-planned treatment for a pre-existing condition unrelated to indication under study, and social reasons and respite care in absence of patient's general condition should not be reported as serious adverse events.
- Requires intensive inpatient medical interventions, including mechanical ventilation, pressor support, and/or fluid resuscitation, or emergent surgical operations.
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect.

- Is an important medical event (IME) when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. An example of such a medical event is allergic bronchospasm requiring intensive treatment in an emergency room or in the hospital.
- Any event that is unexpected and related to study drug.

## 7.5 Expedited Reporting

All SAEs must be reported to the Sponsor Investigator immediately, no later than 24 hours of first becoming aware of the event. All SAEs must be reported despite grade or whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

The Sponsor Investigator (Dr. John Horan) and DFCI study team (UproTrial@DFCI.Harvard.edu) should be informed by email within 24 hours of all SAEs that occur after the patient has provided informed consent, during treatment, or until the participant is taken off study. In the event that the participating investigator does not become aware of the SAE immediately, the participating investigator is to report the event within 24 hours of learning of it and document the time of his or her first awareness of the event.

The study site will have 5 business days from the discovery of the SAE to send a complete expedited report to the Sponsor Investigator.

In addition to SAEs, per DFCI's Expedited Reporting requirements, the following events also require expedited reporting unless they are specifically excluded in Section 7.3:

- Unexpected grade 2 or 3 with a possible, probable, or definite attribution
- Unexpected grade 4 toxicities regardless of attribution
- Dose limiting toxicities that are described in section 5.3

*Pregnancies*: To ensure patient safety, each pregnancy in a patient on study treatment must be reported to the Sponsor Investigator. Pregnancy follow-up should include an assessment of thepossible relationship to the study treatment any pregnancy outcome. Any SAE experienced during pregnancy must be reported. Additionally, pregnancy outcomes should be collected for the female partners of any male study participants. Consent to report information
regarding these pregnancy outcomes should be obtained from the pregnant partner. Sites should follow their institutional policies regarding consenting pregnant partners.

*Unanticipated problems or life-threatening complications:* In the event of an unanticipated problem or life-threatening complications that places subjects or other persons at greater risk of harm than previously recognized occurs, treating investigators must immediately notify the Sponsor-Investigator.

Sponsor-Investigator contact information:

- Dr. John Horan
- Email: john_horan@dfci.harvard.edu
- Telephone: (617) 582-7742
- DFCI lead study coordinator or study team: UproTrial@DFCI.Harvard.edu

Outside institutions will report expedited AEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. These sites will also complete a DF/HCC AE Reporting form and submit to the Sponsor-Investigator and DFCI study team. The Sponsor-Investigator and DFCI study team will then submit the DF/HCC AE Reporting form from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

### 7.6 Reporting to the Food and Drug Administration (FDA)

The Sponsor-Investigator will be responsible for all communications with the FDA. The Sponsor-Investigator will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA. All adverse events reported to the FDA will simultaneously be forwarded to GlycoMimetics, Inc.

Unexpected fatal or life-threatening events associated with the use of the investigational treatment will be reported to the FDA as soon as possible but no later than 7 calendar days after initial receipt of the information.

All other serious unexpected events associated with the use of the investigational treatment will be reported to the FDA as soon as possible but no later than 15 calendar days after initial receipt of the information.

Findings from other clinical, animal, or in-vitro studies that suggest significant human risk will be reported to the FDA as soon as possible but no later than 15 calendar days after initial receipt of the information.

### 7.7 Reporting to GlycoMimetics, Inc.

As the holder of the IND for the Study, DF/HCC Sponsor, John Horan, MD, MPH, will be responsible for all required regulatory reporting obligations and will submit Adverse Event reports to the FDA in accordance with applicable laws and regulations.

Reports will be submitted to GlycoMimetics, Inc. by the following guidelines:

- Sponsor will send a final copy of all Suspected Unexpected Serious Adverse Reaction (SUSAR) reports to GlycoMimetics, Inc. in parallel to submission to FDA
- Sponsor will send a final copy of all Serious Adverse Event reports to GlycoMimetics, Inc. within 15 days of Sponsor awareness of the event
- All SAE and SUSAR reports should be e-mailed to IQVIA Biotech: Safety-inbox.biotech@iqvia.com.

Any additional information pertaining to the SAE and SUSAR, including complications, progression of the initial event, and recurrent episodes, will be reported as follow up to the original episode.

### 7.8 Reporting to Hospital Risk Management

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

## 8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

### 8.1 Uproleselan Injection
(50 mg/mL)

#### 8.1.1 Description

Uproleselan is a synthetic, glycomimetic molecule and E-selectin (CD62E) antagonist, with potential anti-thrombotic, antineoplastic and chemo-potentiating activities. Upon administration, uproleselan binds to E-selectin expressed on endothelial cells and prevents their interaction with selectin-E ligand-expressing cancer cells. It has a terminal half-life of approximately 3 hours, and is excreted unchanged in the urine.

Its chemical name is sodium (1R, 3R, 4R, 5S)-3-({2-N-acetylamino-2-deoxy-3-O-[(1S)-1-carboxylato-2-cyclohexylethyl]-β-D-galactopyranosyl} oxy)-4-({6-deoxy-α-L-galactopyranosyl}oxy)-5-ethyl-cyclohexan-1-yl-(38-oxo-2,5,8,11,14,17,20,23,26,29, 32,35-dodecaoxa-39-azahentetracontan-41-yl) carboxamide.

Uproleselan is also known as GMI-1271.The molecular formula is C60H108N3NaO27 and the molecular weight is 1326.5.

#### 8.1.2 Form

Uproleselan will be supplied by GlycoMimetics, Inc. as a ready to use vial (800 mg Uproleselan in 16 ml).

#### 8.1.3 Storage and Stability

Uproleselan injection 50 mg/mL is stored per the specified conditions on the label, refrigerated (2°C to 8 °C), prior to administration. Vials of uproleselan will be refrigerated and should be brought to room temperature and gently inverted 4 to 5 times before dose preparation.

Uproleselan is to be kept in a locked and secured storage facility, accessible only to those individuals authorized by the principal investigator (PI).

### 8.1.4 **Compatibility**

Uproleselan has not been studied for compatibility with other medications, and should be administered in a separate line.

### 8.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of uproleselan in a self-contained and protective environment.

### 8.1.6 **Availability**

Uproleselan will be provided directly to participating centers by GlycoMimetics, Inc.

### 8.1.7 **Preparation**

Uproleselan must be prepared in one of two ways to maintain stability requirements. Sites should use the practice that best fits within their specific institutional preparation protocol.

1. Uproleselan may be prepared at the time of administration in syringes or IV bags. Only one dose of uproleselan can be prepared when using this method. Allow prepared dose to reach room temperature, and then administer to subject. Storage expiration at controlled room temperature (CRT) is a maximum of 24 hours.

**OR**

2. Uproleselan may be prepared up to 72 hours before administration in syringes or IV bags. Up to two doses of uproleselan may be prepared in advance for each subject (e.g. morning and evening doses). Store prepared doses in refrigerated conditions (+2°C to +8°C). Allow prepared doses to reach room temperature, and then administer to subject.

Dilutions are acceptable for ease of administration, but are not required. Uproleselan may be administered undiluted at a concentration of 50mg/mL or may be further diluted with Sodium Chloride, 0.9% to a final concentration in the range of 5 to 10mg/mL.

Example: If diluting with a 100 mL normal saline (NS) IV bag, remove 36 mL NS from the IV bag. Add the entire contents of uproleselan vial (16 mL) to the remaining 64 ml to reach the final volume of 80 mL/10 mg/mL (concentration). If dilution is performed, the

amount of dilution should be recorded.

Intravenous lines consisting of PVC with DEHP should be avoided when possible. If PVC with DEHP administration sets must be used they should be primed with uproleselan solution no more than 2 hours before dosing. It is highly recommended that uproleselan prepared prior to administration be refrigerated until 1 hour prior to dosing.

### 8.1.8 **Administration**

Uproleselan injection should be administered IV into a peripheral line, a central catheter, or a peripherally inserted central line catheter (PICC).

Infusion should take place at a steady rate over a period of 20 minutes (including flush if used) using a syringe pump or IV pump. Microbore tubing is preferred. In-line filtration (0.2 micron) is highly recommended.

Compatibility with other therapeutic agents has not been determined; therefore, uproleselan injection should be administered via a separate IV line and should not be administered concurrently with anything other than saline. If a flush is used, saline flush is preferred. The flush should be included in the 20 minute infusion.

### 8.1.9 **Ordering**

Individual sites will order uproleselan directly from GlycoMimetics, Inc.

### 8.1.10 **Accountability**

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

### 8.1.11 **Destruction and Return**

All participating centers will return unused uproleselan to GlycoMimetics, Inc. after the last participant enrolled has completed uproleselan.

## **8.2 Busulfan**

Injection (Busulfex®) NSC #750

### 8.2.1 **Description**

Busulfan is a non-cell course specific bifunctional alkylating agent. In aqueous media, busulfan hydrolyzes to release methanesulfonate groups. This produces reactive carbonium ions that interact with cellular thiol groups and nucleic acids to form DNA

cross-links. Busulfan injection is 100% bioavailable by definition of intravenous administration. The elimination of busulfan appears to be independent of renal function, presumably reflecting the extensive metabolism of the drug in the liver, since less than 2% of the administered dose is excreted in the urine unchanged within 24 hours. The drug is metabolized by enzymatic activity to at least 12 metabolites, among which tetrahydrothiophene, tetrahydrothiophene 12 oxide, sulfolane, and 3-hydroxysulfolane were identified. These metabolites do not have cytotoxic activity. Irreversible binding to plasma proteins (primarily albumin) is approximately 32.4%. Busulfan has a plasma terminal elimination half-life (t1/2) of about 2.6 hours and demonstrates linear kinetics. It is rapidly distributed into tissue and crosses the blood-brain and the placental barriers. CSF concentrations are approximately equal to those in plasma. Itraconazole reduced busulfan clearance by up to 25% in patients receiving itraconazole compared to patients who did not receive itraconazole. Higher busulfan exposure due to concomitant itraconazole could lead to toxic plasma levels in some patients. Fluconazole had no effect on the clearance of busulfan.

8.2.2 **Form**

Each ampoule or vial of busulfan injection contains 60 mg (6 mg/mL) of busulfan, N,N dimethylacetamide (DMA) 33% vol/vol and polyethylene glycol 400, 67% vol/vol.

8.2.3 **Storage and Stability**

Store refrigerated at 2°- 8°C, (36°- 46°F).

8.2.4 **Compatibility**

Busulfan injection contains N,N-dimethylacetamide, which is incompatible with many closed-system transfer devices (CSTDs); the plastic components of CSTDs may dissolve and result in subsequent leakage and potential infusion of dissolved plastic into the patient.

8.2.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of busulfan in a self-contained and protective environment.

8.2.6 **Availability**

Commercially available from various manufacturers. See package insert for further information.

8.2.7 **Preparation**

Dilute busulfan injection to a final concentration of approximately 0.5 mg/mL with NS or

D5W (the diluent quantity should be 10 times the volume of busulfan). The drug should not be infused with any other drug or IV solution other than NS or D5W. Always add the busulfan to the diluent, not the diluent to the busulfan injection. Mix thoroughly by inverting several times. Do not use polycarbonate syringes or filter needles with busulfan injection. Busulfan injection diluted in NS or D5W is stable at room temperature (25°C) for up to 8 hours but the infusion must be completed within that time. Busulfan injection diluted in NS is stable at refrigerated conditions 2°- 8°C (36°-46°F) for up to 12 hours but the infusion must be completed within that time. Busulfan 0.54 mg/mL solution in 0.9% sodium chloride injection was physically and chemically stable for 30 hours when stored in 50 mL polypropylene syringes at 2–8°C (36°-46°F) and protected from light. (Guichard N, et al. Am J Health-Syst Pharm. 2017; 74:1887-94)

#### 8.2.8 **Administration**

Busulfan injection should be administered by IV infusion through a central venous catheter. Patients receiving busulfan in a conditioning regimen for bone marrow transplant must receive seizure prophylaxis with anticonvulsants (e.g., benzodiazepines, phenytoin, valproic acid or levetiracetam) to prevent seizures reported with the use of high dose busulfan. Administer anticonvulsants 12 hours prior to busulfan to 24 hours after the last dose of busulfan.

In dose-finding studies of busulfan where patients received concomitant busulfan and phenytoin, phenytoin reduced busulfan plasma AUC by approximately 15%. After an initial dose of busulfan injection, blood levels are monitored with bone marrow transplant patients in order to achieve a target area-under-the-curve (AUC) plasma concentration.

### 8.3 Clofarabine

(Cl-F-Ara-A, CAFdA, Clolar™, Evoltra®) NSC# 606869

#### 8.3.1 **Description**

Clofarabine (2-chloro-9-[2′-deoxy-2′-fluoro-β-D-arabinofuranosyl]-9H-purine-6-amine) is a second-generation purine nucleoside analog designed as a hybrid molecule to overcome the limitations and incorporate the best qualities of fludarabine and cladribine. Clofarabine has several advantages over other nucleoside analogues: (1) increased resistance to deamination and phosphorolysis, hence better stability; (2) higher affinity to deoxycytidine kinase; (3) prolonged retention of the triphosphate compound in leukemic blasts; and (4) potent inhibition of DNA synthesis and of ribonucleotide reductase (RNR). Clofarabine is S-phase specific and cell cycle phase non-specific. Like other nucleoside analogs, clofarabine must be serially phosphorylated, first by deoxycytidine kinase and then by other kinases, to be active within cells. The primary cytotoxic effect of clofarabine is likely due to its inhibition of DNA synthesis. The triphosphate form of clofarabine is an inhibitor of both DNA polymerase and ribonucleotide reductase. These effects lead to depletion of intracellular deoxynucleotide triphosphate pools, and inhibition of elongation of DNA strands during synthesis. Clofarabine has been shown to disrupt the integrity of the mitochondrial membrane leading to the release of pro-

apoptotic mitochondrial factors and to programmed cell death. In addition to its anti-leukemic activity as a single agent, in vitro studies supported a role for clofarabine in biochemical modulation strategies to enhance the efficacy of other nucleoside analogs such as cytarabine. Clofarabine has been approved by the FDA for the treatment of pediatric patients with relapsed or refractory acute lymphoblastic leukemia (ALL) after at least two prior regimens.

The population pharmacokinetics of clofarabine were studied in 40 pediatric patients aged 2 to 19 years (21 males/19 females) with relapsed or refractory ALL or acute myelogenous leukemia (AML). At the given 52 mg/m$^2$ dose, similar concentrations were obtained over a wide range of BSAs. Clofarabine was 47% bound to plasma proteins, predominantly to albumin. Based on non-compartmental analysis, systemic clearance and volume of distribution at steady-state were estimated to be 28.8 L/h/m$^2$ and 172 L/m$^2$, respectively. The terminal half-life was estimated to be 5.2 hours. No apparent difference in pharmacokinetics was observed between patients with ALL and AML or between males and females. Based on 24-hour urine collections in the pediatric studies, 49-60% of the dose is excreted in the urine unchanged.

In vitro studies using isolated human hepatocytes indicate very limited metabolism (0.2%), therefore the pathways of non-renal elimination remain unknown. The pharmacokinetics of clofarabine have not been evaluated in patients with hepatic dysfunction. Reduce clofarabine starting dose by 50% in patients with CrCl of 30 to 60 mL/min. There is insufficient information to make a dosage recommendation in patients with CrCl less than 30 mL/min or in patients on dialysis.

### 8.3.2 **Form**

Clofarabine (1 mg/mL) is supplied in a 20 mL, single-use flint vial. Each vial contains 20 mg clofarabine in 20 mL of solution.

### 8.3.3 **Storage and Stability**

The pH range of the solution is 4.5 to 7.5. Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

### 8.3.4 **Compatibility**

In vitro studies suggested that clofarabine undergoes limited metabolism and does not inhibit or induce major CYP enzymes. CYP inhibitors and inducers are unlikely to affect the metabolism of clofarabine. Clofarabine is unlikely to affect the metabolism of CYP substrates. However, no in vivo drug interaction studies have been conducted.

An in vitro transporter study suggested that clofarabine is a substrate of human transporters OAT1, OAT3, and OCT1. A preclinical study using perfused rat kidney demonstrated that the renal excretion of clofarabine was decreased by cimetidine, an inhibitor of the hOCT2. Although the clinical implications of this finding have not been

determined, signs of clofarabine toxicity should be monitored when administered with other hOAT1, hOAT3, hOCT1 and hOCT2 substrates or inhibitors.

### 8.3.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of clofarabine in a self-contained and protective environment.

### 8.3.6 Availability

Commercially available. See package insert for further information.

### 8.3.7 Preparation

Filter clofarabine through 0.2 micron syringe filter prior to dilution. Dilute clofarabine with 5% dextrose injection or 0.9% sodium chloride injection to a final concentration of between 0.15 mg/mL and 0.4 mg/mL. The resulting admixture may be stored at room temperature but must be used within 24 hours of preparation.

### 8.3.8 Administration

Infuse over 2 hours. May be infused over 1 hour or per institutional guidelines in some protocols, especially when lower doses are used. Continuous IV fluids are encouraged to decrease adverse events and tumor lysis effects. Consider prophylactic corticosteroids (see Treatment section) to prevent signs/symptoms of capillary leak syndrome or systemic inflammatory response syndrome (SIRS). Hypotension may be a sign of capillary leak syndrome or systemic inflammatory response syndrome (SIRS). Discontinue if the patient becomes hypotensive during administration; may consider therapy re-initiation with 25% dose reduction after return to baseline. An inline filter is not needed for administration. Do not administer any other medications through the same IV line.

Since clofarabine is primarily excreted through the kidneys, drugs with known renal toxicity should be avoided during clofarabine administration. In addition, since the liver is a known target organ for clofarabine toxicity, concomitant use of medications known to induce hepatic toxicity should be avoided.

## 8.4 Fludarabine

(Fludara®, fludarabine phosphate, 2-fluoro-ara-AMP) NSC# 312887

### 8.4.1 Description

Fludarabine phosphate is a synthetic purine nucleoside. It differs from the physiologic nucleosides, adenosine, in that the sugar moiety is arabinose instead of ribose, and by the addition of a fluorine atom to the purine base adenine. Fludarabine is also a fluorinated

nucleotide analog the antiviral agent vidarabine, (ara-A). The addition of fluorine results in increased aqueous solubility and resistance to enzymatic degradation by adenosine deaminase. Fludarabine (2-fluoro-ara-A) is commercially available as the monophosphate salt (2-fluoro-ara-AMP). The monophosphorylation increases the drug's aqueous solubility while maintaining pharmacologic activity. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono β-D-arabino-furanosyl) (2-fluoro-ara-AMP) and the molecular weight is 365.2.

Fludarabine is a purine antagonist antimetabolite. In vivo, fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then it is phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Phase 1 studies in humans have demonstrated that within several minutes after intravenous infusion, fludarabine phosphate is converted to the active metabolite, 2-fluoro-ara-A and becomes undetectable. Therefore, pharmacokinetics studies have focused on 2-fluoro-ara-A. Fludarabine phosphate 25 mg/$m^2$ infused intravenously over 30 minutes to adult cancer patients, showed a moderate accumulation of 2-fluoro-ara-A. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2.

Fludarabine is widely distributed. The volume of distribution at steady state ($V_{ss}$) reported after daily administration of 25 mg/$m^2$ for 5 days to adults averaged at 96-98 L/$m^2$. Tissue distribution studies in animals indicate that the highest concentrations of the drug are in liver, kidney, and spleen. Although the extent to which fludarabine and/or its metabolites distribute into the CNS in humans has not been determined to date, severe neurologic toxicity (e.g., blindness, coma) has been reported in patients receiving the drug, particularly in high dosages. There is evidence from animal studies that fludarabine distributes into the CNS and that a toxic metabolite (2-fluoroadenine, possibly formed by bacteria in the GI tract), can be absorbed systematically via enterohepatic circulation and distributed into CSF. According to in vitro data, about 19-29% of fludarabine is bound to plasma proteins.

Following IV administration, fludarabine phosphate is dephosphorylated rapidly to fludarabine. Plasma concentrations of fludarabine decline in a linear, dose-independent manner. The elimination profile of fludarabine also has been reported to be either biphasic or triphasic; however, reported terminal elimination half-lives have been similar. In adult cancer patients receiving fludarabine 25 mg/$m^2$ as a 30-minute IV infusion daily for 5 days, a terminal half-life of about 20 hours was reported. In a limited number of pediatric patients, the plasma concentration profile of fludarabine exhibited both monoexponential and biexponential decay, with a mean $t_{1/2}$ of 10.5 hours in patients with monoexponential elimination and a $t_{1/2}$ of 1.2-1.4 and 12.4-19 hours, respectively, in patients with biexponential elimination.

Renal clearance accounts for about 40% of the total body clearance of fludarabine. Renal elimination appears to become more important at high dosages of the drug. The dose of fludarabine needs to be adjusted in patients with moderate renal impairment.

### 8.4.2 Form

Fludarabine phosphate injection is available as sterile lyophilized powder and in solution. Each single dose vial of powder contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. After reconstitution, the pH range for the final product is 7.2-8.2. The single-dose solution vial contains 25 mg/mL, 2 mL of fludarabine phosphate. It may contain mannitol and is preservative-free.

### 8.4.3 Storage and Stability

Fludarabine phosphate vials should be stored refrigerated at 2-8ºC (36-46ºF).

### 8.4.4 Compatibility

The use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.

### 8.4.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of fludarabine in a self-contained and protective environment.

### 8.4.6 Availability

Commercially available from various manufacturers. See package insert for further information.

### 8.4.7 Preparation

Fludarabine phosphate powder should be reconstituted with 2 mL of Sterile Water for Injection. The solid cake should fully dissolve in 15 seconds or less. The resulting concentration is 25 mg/mL. When reconstituted to a final concentration of 25 mg/mL, the drug is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the solution be used within 8 hours after reconstitution.

### 8.4.8 Administration

Prior to administration, fludarabine 25 mg/mL solution or the reconstituted 25 mg/mL solution should be further diluted in 100 mL or 125 mL of D5W or NS. Concentrations of 0.25 to 1 mg/mL have been used in clinical trials. When diluted to a final concentration

of 1 mg/mL, fludarabine is stable for at least 16 days at room temperature and normal light conditions. The manufacturer recommends that the diluted solution be used within 8 hours after preparation. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

## 8.5 Tacrolimus

(FK-506, Prograf®) NSC #717865

### 8.5.1 Description

Tacrolimus is a macrolide immunosuppressant produced by Streptomyces tsukubaensis. Tacrolimus is a potent immunosuppressive agent which prolongs the survival of the host and transplanted grafts in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (immunosuppression).

Additionally, tacrolimus may inhibit cellular activities such as nitric oxide synthetase activation and apoptosis, and may potentiate the action of corticosteroids in these processes. Tacrolimus activity is primarily due to the parent drug. The plasma protein binding of tacrolimus is approximately 99% and is independent of concentration over a range of 5-50 ng/mL. Tacrolimus is bound mainly to albumin and alpha-1-acid glycoprotein, and has a high level of association with erythrocytes. The t½ in adult patients ranges from 11-19 hours. The pharmacokinetics of tacrolimus have been studied in pediatric liver transplant patients (0.7 to 13.2 years of age). Following the IV administration of a 0.037 mg/kg/day dose to 12 pediatric patients, mean terminal half-life, volume of distribution and clearance were 11.5 ± 3.8 hours, 2.6 ± 2.1 L/kg and 0.138 ± 0.071 L/hr/kg, respectively. Following oral administration to 9 pediatric patients, the absolute bioavailability was 31 ± 21%. Whole blood trough concentrations from 31 patients less than 12 years old showed that pediatric patients needed higher doses than adults to achieve similar tacrolimus trough concentrations. Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A) in the liver and to a lesser extent in the intestinal mucosa. The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. The main route of elimination is via the biliary tract and excretion in feces. The mean clearance in renal dysfunction and mild hepatic dysfunction is the same as normal volunteers. Severe hepatic dysfunction (Pugh score > 10) led to a substantially decreased clearance. A retrospective comparison of Black and Caucasian kidney transplant patients indicated that Black patients required higher tacrolimus doses to attain similar trough concentrations; there were no gender-based differences. The absorption of tacrolimus from the gastrointestinal tract is incomplete and variable exhibiting large intra- and inter-patient variability. Administration with food significantly decreases the rate and extent of absorption Drugs that stimulate or inhibit hepatic p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.

### 8.5.2 **Form**

*Injection*: Tacrolimus is available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus per 1 mL. Each mL also contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80% v/v.

*Oral:* Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg, or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide.

### 8.5.3 **Storage and Stability**

*Injection*: Store between 5°C and 25°C (41°F and 77°F).

*Oral:* Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).

### 8.5.4 **Compatibility**

Drugs that stimulate or inhibit hepatic p-450 enzymes will alter clearance of tacrolimus and close attention to potential drug interactions is crucial.

### 8.5.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of tacrolimus in a self-contained and protective environment.

### 8.5.6 **Availability**

Commercially available from various manufacturers. See package insert for further information.

### 8.5.7 **Preparation**

See Treatment (section 5) and Dose Modifications (section 6.1) sections of the protocol.

*Injection:*

Tacrolimus injection must be diluted with NS or D5W before use to a concentration between 0.004 mg/mL and 0.02 mg/mL. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The polyoxyethylated castor oil contained in the concentrate for intravenous infusion can cause phthalate stripping from PVC. It is strongly recommended that non-PVC tubing be used to minimize patient exposure to DEHP. Due to the chemical instability of tacrolimus in alkaline media, tacrolimus injection should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir).

Tacrolimus 0.001 mg/mL solution stored in polyolefin bags at room temperature (20–25°C) was stable for 24 hours when prepared in NS and for at least 48 hours when prepared in D5W. Solutions of 0.01 and 0.1 mg/mL prepared in either NS or D5W were stable for at least 48 hours at room temperature in polyolefin bags (Lee JH, et al. Am J Health-Syst Pharm. 2016;73:137-42).

### 8.5.8 **Administration**

*Injection:* Monitor closely for an acute allergic reaction for the first 30 minutes and at frequent intervals thereafter.

*Oral:* Administer at a consistent time of day and at consistent intervals with regard to meals. Tacrolimus may be given with food as long as it is given the same way each time, however, administration with food significantly decreases the rate and extent of

absorption. Grapefruit or grapefruit juice should be avoided during the entire course of tacrolimus administration.

## 8.6 Methotrexate

IV Only (MTX, amethopterin) NSC #000740

### 8.6.1 Description

A folate analogue which reversibly inhibits dihydrofolate reductase, the enzyme that reduces folic acid to tetrahydrofolic acid. Inhibition of tetrahydrofolate formation limits the availability of one carbon fragments necessary for the synthesis of purines and the conversion of deoxyuridylate to thymidylate in the synthesis of DNA and cell reproduction. The polyglutamated metabolites of MTX also contribute to the cytotoxic effect of MTX on DNA repair and/or strand breaks. MTX cytotoxicity is highly dependent on the absolute drug concentration and the duration of drug exposure. MTX is actively transported across cell membranes. At serum methotrexate concentrations exceeding 0.1 μmol/mL, passive diffusion becomes a major means of intracellular transport of MTX. The drug is widely distributed throughout the body with the highest concentration in the kidney, liver, spleen, gallbladder, and skin. Plasma concentrations following high dose IV MTX decline in a biphasic manner with an initial half-life of 1.5-3.5 hours, and a terminal half-life of 8-15 hours. About 50% is bound to protein. MTX is excreted primarily by the kidneys via glomerular filtration and active secretion into the proximal tubules. Renal clearance usually equals or exceeds creatinine clearance. Small amounts are excreted in the feces. There is significant entero-hepatic circulation of MTX. The distribution of MTX into third-space fluid collections, such as pleural effusions and ascitic fluid, can substantially alter MTX pharmacokinetics. The slow release of accumulated MTX from these third spaces over time prolongs the terminal half-life of the drug, leading to potentially increased clinical toxicity.

### 8.6.2 Form

Methotrexate for injection is available as a lyophilized powder for injection in 1000 mg vials. The powder for injection contains approximately 7 mEq sodium in the 1000 mg vial. Methotrexate for Injection is also available as a 25 mg/mL solution in 2, 4, 8, 10, and 40 mL preservative free vials and 2 and 10 mL vials with preservative. The 2, 4, 8, 10, and 40 mL solutions contain approximately 0.43 0.86, 1.72, 2.15, and 8.6 mEq sodium per vial, respectively. The preserved vials contain 0.9% benzyl alcohol as a preservative.

### 8.6.3 Storage and Stability

Sterile methotrexate powder or solution is stable at 20º-25ºC (68º-77ºF); excursions permitted to 15º-30ºC (59º- 86 Fº). Protect from light.

### 8.6.4 Compatibility

Avoid sulfamethoxazole/trimethoprim, probenecid, penicillins, cephalosporins, aspirin, proton pump inhibitors, and NSAIDS as renal excretion of MTX is inhibited by these agents.

#### 8.6.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of methotrexate in a self-contained and protective environment.

#### 8.6.6 **Availability**

Commercially available from various manufacturers. See package insert for further information.

#### 8.6.7 **Preparation**

*For IV use:* Powder for injection: Dilute 1000 mg vial with 19.4 mL of preservative free SWFI, D5W or NS to a 50 mg/mL concentration. The powder for injection may be further diluted in NS or dextrose containing solutions to a concentration of ≤ 25 mg/mL for IV use.

Do not use the preserved solution for high dose methotrexate administration due to risk of benzyl alcohol toxicity. Methotrexate dilutions are chemically stable for at least 7 days at room temperature but contain no preservative and should be used within 24 hours. Diluted solutions especially those containing bicarbonate exposed to direct sunlight for periods exceeding 4 hours should be protected from light.

#### 8.6.8 **Administration**

See Treatment (section 5) and Dose Modifications (section 6.2) sections of protocol.

### **8.7 Mycophenolate Mofetil**
(Cellcept®, MMF, RS-61443) NSC# 724229

#### 8.7.1 **Description**

Mycophenolate mofetil is the 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent. Side effects include diarrhea, leukopenia, sepsis, and vomiting, as well as a higher risk of certain infections.

MMF has been used in a variety of solid organ and hematopoietic stem cell transplant settings for the prevention of acute rejection. MMF is a prodrug which, after oral administration, is rapidly and primarily hydrolyzed by the liver to the biologically active metabolite mycophenolic acid. MPA is metabolized principally by glucuronyl

transferase to form the pharmacologically inactive phenolic glucuronide of MPA (MPAG). In vivo, MPAG is converted to MPA via enterohepatic recirculation. Mycophenolic acid inhibits nucleic acid synthesis and produces a potent, noncompetitive, and reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), blocking the de novo synthesis of guanosine nucleotides without being incorporated into DNA. Both T and B lymphocytes rely on this de novo pathway for purine synthesis. As a result, the proliferative responses of T and B lymphocytes to both mitogenic and allospecific stimulation are inhibited. Other rapidly dividing cell lines are capable of recycling purine nucleotides via the "salvage" pathway, which is not blocked by mycophenolic acid.

In vitro and in vivo studies have demonstrated the ability of mycophenolic acid to block proliferative responses of T and B lymphocytes, inhibit antibody formation and the generation of cytotoxic T-cells, and suppress antibody formation by B lymphocytes. Mycophenolic acid prevents the glycosylation of lymphocyte and monocyte glycoproteins that are involved in intercellular adhesion of these cells to endothelial cells, and may inhibit recruitment of leukocytes into sites of inflammation and graft rejection. Antirejection effects have been attributed to decreased recruitment of activated lymphocytes to the graft site.

### 8.7.2 **Form**

Commercially available for oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/l. It is also available in an intravenous formulation as a hydrochloride salt in vials containing 500 mg of mycophenolate mofetil.

### 8.7.3 **Storage and Stability**

Oral formulations should be stored at 25°C (77°F); excursions are permitted to 15°C to 30°C (59°F to 86°F). Protect from moisture and light. Once reconstituted, the oral solution may be stored at room temperature or under refrigeration. Do not freeze. The mixed suspension is stable for 60 days.

Store intact vials and diluted solutions at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F). Do not freeze. Begin infusion within 4 hours of reconstitution.

### 8.7.4 **Compatibility**

The mean absolute bioavailability of oral mycophenolate mofetil relative to intravenous mycophenolate mofetil (based on MPA AUC) was 94% in a small sample of healthy, adult volunteers. In this group the mean (±SD) apparent volume of distribution of MPA was approximately 3.6 (±1.5) and 4.0 (±1.2) L/kg following intravenous and oral administration, respectively. At clinically relevant concentrations, MPA is 97% bound to

plasma albumin. MPAG is 82% bound to plasma albumin at MPAG concentration ranges that are normally seen in stable renal transplant patients; however, at higher MPAG concentrations (eg, patients with renal impairment), the binding of MPA may be reduced as a result of competition between MPAG and MPA for protein binding. A negligible amount of the agent (< 1% of dose) is excreted as MPA in the urine. Most of the administered dose (~87%) is excreted in the urine as MPAG. Bile acid sequestrants (e.g., cholestyramine) reduce the AUC of MPA by interfering with the enterohepatic circulation of the drug.

### 8.7.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of mycophenolate mofetil in a self-contained and protective environment.

### 8.7.6 **Availability**

Commercially available from various manufacturers. See package insert for further information.

### 8.7.7 **Preparation**

To prepare the oral suspension, add 47 mL of water to the bottle and shake well for approximately 1 minute. Add another 47 mL of water to the bottle and shake well for an additional minute. The final concentration is 200 mg/mL of mycophenolate mofetil. Avoid inhalation or direct contact with skin or mucous membranes of the dry powder or the constituted suspension. If such contact occurs, wash thoroughly with soap and water; rinse eyes with water.

To prepare the intravenous injection, reconstitute the contents of each vial with 14 mL of D5W. Dilute the contents of a vial with D5W to a final concentration of 6 mg/mL. Each vial is vacuum-sealed; if a lack of vacuum is noted during preparation, the vial should not be used.

### 8.7.8 **Administration**

Oral formulations of MMF should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter). Do not mix the oral suspension with other medications. Some products may contain phenylalanine; refer to the package labeling for additional details.

## 9. PHARMACOKINETIC AND BIOMARKER STUDIES (REQUIRED)

The pharmacokinetic (PK) and Pre-transplant bone marrow AML blast E-selectin ligand expression will be performed on all patients across the study. Patient samples will be collected as

indicated below (Table 7).

For all samples discussed below, all aliquots will be disposed of either at the conclusion of testing or the end of the study, whichever is later. Samples from this study will not be banked for future use.

Specimen collection and shipment will be supported by GlycoMimetics, Inc.

**Table 7. Research Sample Collection Plan**

| | | **PK Subjects ≥6 years** | **PK Subjects <6 years** | **E-selectin Ligand** |
|---|---|---|---|---|
| | | 2 mL/sample | 1 mL/sample | 2-3 mL/sample |
| | | Na Citrate | Na Citrate | Na Heparin |
| | | PB | PB | Bone Marrow Aspirate |
| **Day** | **Time point** | | | |
| Pre-Conditioning | At time of clinical bone marrow, after screening consent | | | X |
| Day -8<br><br>(1st dose of uproleselan) | Immediately Predose | X | X | |
| | 30 minutes (±5 minutes)[a] | X | X | |
| | 1 hours (±20 minutes)[a] | X | X | |
| | 2 hours (±20 minutes)[a] | X | | |
| | 4 hours (±60 minutes)[a] | X | X | |
| Day -4<br><br>(1st dose of day) | Immediately Pre-dose | X | X | |
| | 30 minutes (±5 minutes)[a] | X | X | |
| | 1 hours (±20 minutes)[a] | X | X | |
| | 2 hours (±20 minutes)[a] | X | | |
| | 4 hours (±20 minutes)[a] | X | X | |

PK = pharmacokinetic
[a] After start of the infusion

## 9.1 Pharmacokinetic Assessment

Pharmacokinetic testing for uproleselan will be done on plasma samples collected on a sparse-sampling basis across all subjects in the trial. **Both the start and end times** of investigational drug administration and sample collection times must be accurately recorded in the subject's records. Sample times for PK collection are in relation to the start of investigational drug infusion. Actual date and time of collection is to be recorded for every sample for an accurate PK assessment. See Table 7 for sparse-sampling times and volumes.

**On days of PK testing, a dedicated CVL lumen should be used for uproleselan infusion and a separate dedicated CVL lumen should be used PK sampling. Uproleselan will not stick to the inside of the catheter lumens so the lumens dedicated for infusion and PK testing on day -8 and day -4 may differ.**

### 9.1.1 PK Sampling and Processing

Blood will be drawn into sodium citrate tubes, inverted 3-4 times and stored in an ice bath until centrifugation. Samples will be centrifuged at 2000-2500 rpm at 4°C for 10 minutes. Ideally, samples should be immediately centrifuged following collection. If institutional staffing and/or logistics do not allow for this, samples may be stored (at approximately 4°C) for 48-72 hours before centrifugation.

After centrifugation, pipette half of plasma into an appropriately labelled (participant ID, day/time of collection and primary vs back-up) cryovial (primary aliquot). Pipette the remaining plasma into an additional appropriately labelled cryovials (back-up aliquot).

The aliquots will be stored in a freezer set at -70 to -80°C until shipped for analysis.

### 9.1.2 PK Sample Shipments

Samples will be shipped after each subject collection is complete. The primary and back-up aliquots will be shipped on separate days to mitigate loss (Monday - Wednesday only), via overnight carrier with the appropriate amount of dry ice.

Pyxant Labs
4720 Forge Road
Colorado Springs, Colorado 80907
Contact: Emily Munk
E-mail: emunk@pyxant.com and SampleReceipt@pyxant.com
Tel.: +1 (719) 593-1165 X50

Upon shipment of the samples, an e-mail will be sent containing a sample manifest, the name of the courier, the airway bill number, and a confirmation of the number of samples in the shipment.

### 9.1.3 PK Interim Analysis

An interim analysis will be performed at the completion of phase 1, as phase 2 enrollment commences. Data to be included in the interim analysis include, but is not limited to, subject ID, demogrpahics, vital signs, baseline laboratory values (hematology, full and basic chemistry, coagulation, urinalysis), uproleselan administration (dose level, volume administered, actual dates/times of collection and dilution of amount if used), PK collection dates/times.

Depending on the results of the analysis, the phase 2 dosing may be adjusted and the protocol amended.

## 9.2 Biomarker Assessments

Pre-transplant bone marrow AML blast E-selectin ligand expression will be assessed by multidimensional flow cytometry and by gene expression profiling via RNA analysis.

Residual disease will also be measured as part of the flow cytometry assessment.

### 9.2.1 Multidimensional flow cytometry (eligibility), E-selectin ligand expression, and gene expression profiling sampling and processing

1. Perform pre-transplant bone marrow procedure, as per institutional procedure
2. Collect 2-3 ml bone marrow aspirate in a 5 ml Sodium Heparin tube
   a) If aspirate is unable to be obtained (i.e., dry tap), a fresh bone marrow biopsy can be provided. Biopsy samples can be collected in either: 15mL conical tube, adding enough RPMI to ensure the sample is submerged at all times during transit or NaHep 4.5mL hemoguard tube, adding enough RPMI to ensure the sample is submerged at all times during transit, and adding parafilm on the top of the tube for safety. Biopsy samples will be shipped at ambient temperature.
3. Immediately after sample is drawn, gently invert the tube 180° and back, 8-10 times.
   a) Keep sample at AMBIENT TEMPERATURE and ship immediately.

*No additional specimen will be required for RNA testing. The sample collected for multidimensional flow cytometry will provide sufficient material for RNA aliquoting.*

**Note: If the subject's pre-transplant disease evaluation is coordinated at another center, the study site should attempt to conduct a screening consent prior to the bone marrow. If the subject consents to the bone marrow samples, the study site should coordinate collection and shipment with procedure center for sample receipt at Hematologics, Inc.*

### 9.2.2 Multidimensional flow cytometry (eligibility), E-selectin ligand expression, and gene expression profiling shipments

Samples should be shipped ambient on the day of collection. Samples can be accepted Monday through Saturday.

Hematologics, Inc.
3161 Elliott Ave., Suite 200
Seattle, Washington 98121
Contact: Wayne Fritschle
E-mail: wayne@hematologics.com
Tel.: +1 8008600934 or +1 206 223 2700

Prior to shipping samples an email notification should be sent to laboratory@hematologics.com, including the protocol number, subject ID number and carrier tracking number.
Each sample should be labeled with collection date and time obtained (for fresh samples), protocol number, subject ID number, and site code. Corresponding information must be placed on the Hematologics Laboratory Test Requistion Form. This form must be scanned and emailed to laboratory@hematologics.com and study coordinator, the original retained at the site, and a copy included in the packaging.

## 10. STUDY CALENDAR AND REQUIRED ROUTINE TESTING/CLINICAL EVALUATIONS

| **Table 8.** Study Calendar | **Day Relative to Transplant** | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | **+30** | **+50** | **+100** | **+180** | **+365** | **+730** | |
| **Routine Testing and Clinical Evaluations** | **Baseline[a]** | **-8** | **-4** | **0** | **+14** | +/- 5 days | +/-7 days | +/- 7 days | +/- 14 days | +/- 30 days | +/- 90 days | **InForm Timepoints** |
| Informed consent/assent | X | | | | | | | | | | | Baseline |
| Demographics | X | | | | | | | | | | | Baseline |
| Medical history | X | X | | | | | | | | | | Baseline, Day -8 |
| Physical exam | X[b] | X | | X | | X | | X | | X | X | Baseline, Days -8, 0, +30, +100, +365, and +730 |
| Performance status | X | X | | X | | X | | X | | X | X | Baseline, Days -8, 0, +30, +100, +365, and +730 |
| Serum Pregnancy Test | X | | | | | | | | | | | Baseline |
| Estimated GFR | X | | | | | | | | | | | Baseline |
| Echocardiography | X | | | | | | | | | | | Baseline |
| Pulmonary Function[c] | X | | | | | | | | | | | Baseline |
| Testing Hep B and Hep C infection | X | | | | | | | | | | | Baseline |
| PK collection[d] | | X | X | | | | | | | | | Day +30 |
| | | | | | | | | | | | | |
| E-selectin ligand BM collection[d] | X | | | | | | | | | | | Baseline |
| Disease re-evaluation[e] | X | | | | | X | | | | | | Baseline, Day +30 |
| Blood chemistries[f] | X | Twice weekly through Day +50 | | | | | | Per institutional guidelines | | | | Baseline, Day +30, Day +100 |
| CBC | X | Per institutional guidelines | | | | | | | | | | Baseline, Day +30, Day +100 |
| Coagulation studies | X | | | | | | | | | | | Baseline |
| Urinalysis | X | | | | | | | | | | | Baseline |
| TNC dose, CD3+ and CD34+ counts on infused product | | | | X | | | | | | | | Day 0 |
| Peripheral blood chimerism[g] | | | | | | X | | | | | | Day +30 |
| RRT evaluation | | | | D0 - D+30 Daily while inpatient, or 3x weekly if outpatient | | | | | | | | Day +30 |
| MBI-LCBI | | | | D0 - D+30 Daily while inpatient, or 3x weekly if outpatient | | | | | | | | Day +30 |
| VOD/SOS evaluation | | | | D0 - D+30 Daily while inpatient, or 3x weekly if outpatient | | | | | | | | Day +30 |
| Oral and Intestinal Mucositis Evaluation | | | | Daily D0 - D+14 | | | | | | | | Day +30 |
| Acute and Chronic GVHD Evaluation | | | | Per institutional guidelines through Day +365 | | | | | | | | All |
| Relapse and Survival | Per institutional guidelines through Day +730 | | | | | | | | | | | All |

a. All clinical and laboratory studies to determine eligibility must be performed within 28 days prior to enrollment unless otherwise indicated in Section 3
b. Baseline includes height and weight
c. For patients developmentally unable to perform pulmonary function tests, documentation of oxygen saturation on room air will suffice
d. Refer to Table 7 for timing and other instructions for collection
e. Includes marrow testing and re-assessment of any extramedullary disease, including imaging and/or CSF, as indicated, +/- 5 days from Day +30 assessment

f. Creatinine, bilirubin, alkaline phosphatase, ALT, AST, and other blood chemistries, after Day +50 obtained per institutional practice
g. Sorted for T cells (CD3+) and myeloid cells (CD33+ or CD15+), +/- 5 days

## 10.1 Pre-Transplant

10.1.1 As per eligibility criteria. See section 3.

10.1.2 Medical history, physical exam, performance status on Day -8.

10.1.3 PK collection and E-selectin ligand BM collection as per Table 7.

## 10.2 Post-Transplant Laboratory Testing

10.2.1 Total nucleated cell count, $CD3^+$, and $CD34^+$ counts on the infused product on Day 0.

10.2.2 Physical exam and performance status on Day 0.

10.2.3 Peripheral blood chimerism analysis to assess donor engraftment at Day +30 +/- 5 days Samples shall be sorted for T cells ($CD3^+$) and myeloid cells ($CD33^+$ or $CD15^+$).

10.2.4 Disease re-evaluation will be performed at Day +30 +/- 5 days. It should include marrow testing and re-assessment of any extramedullary disease, including imaging and/or CSF, as indicated.

10.2.5 Complete blood counts with a differential shall be obtained according to institutional practice to monitor myeloid recovery and to determine transfusion needs.

10.2.6 Creatinine, bilirubin, alkaline phosphatase, ALT, AST, and other blood chemistries shall be performed at least twice weekly through Day +50 and otherwise obtained according to institutional practice.

10.2.7 Routine clinical monitoring for CMV and other infections shall be performed according to institutional practice.

10.2.8 Immune reconstitution testing shall be performed according to institutional practice.

## 10.3 Other Post-Transplant Evaluations

10.3.1 Mucosal barrier injury-associated laboratory-confirmed bloodstream infection (MBI-LCBI)

MBI-LCBI will be assessed from day 0 through Day +30 and defined according to CDC criteria: https://www.cdc.gov/nhsn/pdfs/pscmanual/4psc_clabscurrent.pdf*-

Oral mucositis severity will be assessed daily from Day 0 through Day +14, using CTCAE v5 criteria.

Intestinal mucositis severity will be assessed daily from Day 0 through Day +14, using

CTCAE v5 criteria for enterocolitis.

10.3.2 Hepatic veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS) will be will be diagnosed and graded according to the pediatric (<18 years) and adult (≥18 years) European Society for Blood and Marrow Transplantation (EBMT) criteria (Appendix C)[31,32].

All reported cases of sinusoidal obstruction syndrome will be adjudicated (diagnosis and grading) by a physician with expertise in this area.

10.3.3 Acute GVHD will be assessed through 1 year according to the Blood and Marrow Transplant Clinical Trials Network Manual of Procedures. https://web.emmes.com/study/bmt2/public/MOP/BMTCTNTechnicalMOPv3.pdf

10.3.4 Physical exam and performance status collected at least on Day 0, +30, +100, +365, and +730. May be collected more often as per institutional guidelines.

10.3.5 Chronic GVHD will be assessed through 1 year using the NIH consensus criteria.[33]

10.3.6 Relapse will be assessed per institutional guidelines through 2 years post transplant.

## 11. MEASUREMENT OF EFFECT

### 11.1 Refractory Disease

Applies to patients who were not in remission at the time of transplant.

At Day +30 (+/-5 days) post-transplant disease evaluation, there is persistence of >1% leukemic blasts by multidimensional flow cytometry (MDF) or there is persistence of EMD.

### 11.2 Leukemic Relapse

Applies to patients who were in remission at the time of transplant and pateints not in remission at the time of transplant who achieve remission post-transplant (based on day +30 assessment)

11.2.1 Bone Marrow Relapse

Defined in accordance with the Children's Oncology Group's AAML1831 trial protocol criteria:

A single bone marrow sample (aspirate or biopsy) showing ≥5% leukemic blasts confirmed by flow cytometry, FISH testing, ICH, or other molecular method.

OR

A single bone marrow sample with at least two tests showing ≥1% leukemic blasts.

Examples of tests include:

- Flow cytometry showing leukemia ≥1% by multidimensional flow cytometry (MDF) in a CLIA-approved laboratory.

- Karyotypic abnormality (must display at least 1 metaphase similar/identical to diagnosis; central cytogenetic review by COG required).

- FISH abnormality identical to one present at diagnosis (must be above level of sensitivity of specific FISH probe; central cytogenetic review by COG required).

- PCR or NGS-based demonstration of validated pathogenic leukemogenic lesion (e.g., fusion, mutation) in a CLIA-approved laboratory that matches initial diagnosis and is quantifiable as ≥1%.

OR

Two serial marrows with at least two tests showing a one log increase of leukemic involvement with the second tests quantified as ≥0.1%. Examples of tests include:

- MDF in CLIA-approved laboratory

- PCR or NGS-based demonstration of validated leukemogenic lesion (e.g., fusion, mutation) that matches diagnosis and is quantifiable where the positive result is at or above the assay sensitivity and meets the criteria above (one log increase and second test quantified as ≥0.1%)

## 11.3 Extramedullary Relapse

Defined as radiographic or biopsy proven extramedullary disease after documented CR or evidence of CSF recurrence.

### 11.3.1 CNS Relapse

A single CSF sample with CNS3 status (Rel-CNS3a/b).

OR

Clinical signs of CNS leukemia such as facial nerve palsy, brain/eye involvement, or hypothalamic syndrome (Rel-CNS3c). Competing explanations for clinical symptoms should be ruled out.

OR

Radiographic evidence of an intracranial, intradural mass consistent with a chloroma (Rel-CNS3c).

Equivocal CNS Relapse (Eq-CNS)
A single CSF sample with CNS2 status

In the case of equivocal relapse, CSF evaluation should be repeated at least 1 week and at most 4 weeks later. For repeat CSF assessment, flow cytometric testing is strongly recommended and FISH (if a diagnostic FISH marker is available) should be sent

To convert to definitive relapse, the repeat CSF or clinical status must:

- Meet criteria for definitive relapse (Rel-CNS3a/b/c)
- Re-demonstrate CNS2 status, but with myeloblasts confirmed by flow cytometry and/or FISH (Rel-CNS2)
- For Rel-CNS2, the two CSF samples MUST BE CONSECUTIVE

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7 (Adverse Events: List and Reporting Requirements).

### 12.1 Data Reporting

12.1.1 Method

The DF/HCC Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ). All data will be entered within two weeks of the indicated time point.

### 12.2 Data Safety Monitoring

This study will be centrally reviewed by the Data Safety and Monitoring Committee (DSMC) of the Pediatric Transplant and Cellular Therapy Consortium (PTCTC). This DSMC is chartered to monitor trials enrolling both children and adult patients. The DSMC is a standing committee, composed of a chair, patient advocate, biostatistician, nurse representative and 2 bone marrow transplant physicians with procedures and processes as defined in the PTCTC DSMC Charter. The DSMC will review the study protocol prior to study activation and IRB review, and will continue to review the study on a regular basis according to the committee rules. The DSMC will meet at regular intervals to review all adverse events and deaths and determine whether any patient safety problems necessitate protocol modifications or discontinuation of the trial. The DSMC will also meet on an ad hoc basis to review the results of interim analyses or if unexpected safety events occur that may necessitate study suspension or closure. The DSMC will discontinue the review of outcomes when administration of the study product has ended for all

subjects. The DSMC has the authority to unblind study personnel prior to study closure if, in their opinion, doing so will be critical to patient safety.

Before each regularly scheduled DSMC meeting, the study coordinator will submit a report including tabular summaries of all reported SAEs, AEs and deaths on study to date. The report will also include a brief summary of each previously unreported SAE and death, including an assessment of whether the event was unexpected or related to the study. If the DSMC recommends protocol or informed consent changes during the study, the recommendations will be reviewed by the Protocol Chair and incorporated into the protocol as deemed appropriate. The protocol with incorporated changes will be distributed to the participating PIs after approval by the Dana Farber Cancer Institute IRB. It is the responsibility of each Transplant Center PI to forward the distributed communications from the DSMC to their local IRB.

### 12.3 Multicenter Guidelines

This protocol will adhere to DF/HCC Policy MULTI-100 and the requirements of the DF/HCC Multi-Center Data and Safety Monitoring Plan. The specific responsibilities of the Sponsor-Investigator, Coordinating Center, and External Sites and the procedures for auditing are presented in Appendix B.

## 13. STATISTICAL CONSIDERATIONS

This is a prospective, multicenter, single treatment arm phase 1/2 clinical trial. Phase 1 will proceed according to a standard 3+3 design. Phase 2 will expand enrollment to N=20 patients at the RP2D identified in Phase 1 to confirm safety and estimate preliminary efficacy. In phase 2, interim monitoring will be performed for transplant-related mortality within 100 days of transplant (see section 13.5).

### 13.1 Definition of Evaluability

13.1.1 Definition of Evaluability for Dose Escalation Evaluation (Phase 1)

Eligible patients who are evaluable for the dose-determining analysis are those who either:
A) meet the following minimum exposure criterion and complete the DLT observation period without experiencing a DLT;
*or*
B) receive at least 1 dose of uproleselan and experience a DLT during the DLT observation period.

A patient is considered to have met the minimum exposure criterion if he/she received at least 10 of the 12 of planned doses of uproleselan in the first cycle of dosing. Patients who do not meet criteria for inclusion in the dose-determining analysis population will be replaced in the study accrual.

13.1.2 Definition of Evaluability for estimation of efficacy (Phase 2)

Eligible patients will be evaluable for estimation of efficacy if they have received at least one dose of uproleselan at the RP2D. Patients who do not receive any uproleselan will be replaced in the trial accrual.

13.1.3 Patients to be included in safety analysis (Phase 1 and 2)

Eligible patients will be included in reporting toxicities if they have received at least one dose of uproleselan.

## 13.2 Study Design/Endpoints

13.2.1 Primary Endpoints

Phase 1. The occurrence of a DLT as defined in section 5.3.
Phase 2. The occurrence of a DLT as defined in section 5.3.

13.2.2 Secondary Endpoints

Leukemia-free survival: The time from transplant to the first occurrence of an event (leukemic relapse [section 11.2] or death from any cause), or censored on date of last contact, if no event occurs.

Overall survival: The time from transplant to death from any cause, or censored on date of last contact, if no death occurs. **Cause of death will be classified according to CIBMTR consensus criteria**.[34]

Non-relapse mortality: The time from transplant to death while in continuous complete remission, or censored on date of last contact, if no death occurs.

Relapse: The time to leukemic relapse.

Oral and gastrointestinal mucositis endpoint. The occurrence and severity of oral or gastrointestinal mucositis (between day 0 and post-transplant day 14) as graded by CTCAEv5.0.

Neutrophil engraftment: time to the first of 3 consecutive days following the nadir that the absolute neutrophil count is at least 500/µl.

Platelet engraftment: time to the first day following the nadir that the platelet count is at least 20,000/µl without a transfusion in the preceding 7 days.

Mucosal barrier injury-associated laboratory-confirmed bloodstream infection (MBI-LCBI) : cumulative incidence of MBI-LCBI by Day +30.

13.2.3 Phase 1 Design

Dose escalation will proceed according to the standard 3+3 design (Table 9). Dose limiting toxicities (DLT) are defined in section 5.3. The DLT observation window is from transplant Day 0 through post-transplant Day +30. Cohorts of three patients will be enrolled on the current dose level. If 0 out of 3 patients experience a DLT, the RP2D has been identified. If ≥2 out of 3 patients experience a DLT, assessment of this dose levelwill stop. If this occurs at dose level 1, the dose will be de-escalated to level 0. If this occurs at dose level 0, the trial will be terminated. If 1 out of 3 patients experience a DLT, 3 additional patients will be enrolled at the current dose level. If 0 out of these 3 additional patients experience a DLT, the RP2D has been identified. If ≥ 1 out of these 3 additional patients experience a DLT, assessment of this dose level will stop. If this occurs at dose level 1, the dose will be de-escalated to level 0.If this occurs at dose level 0, the trial will be terminated.

The maximum tolerated dose (MTD) is declared as the highest dose level at or below the maximally administered dose where ≤1 out of 6 patients experienced a DLT.

The RP2D is the dose recommended by the study team after the declaration of the MTD and review of the study data.

**Table 9. Dose Escalation Schema**

| **Number of Participants with DLT at a Given Dose Level** | **Escalation Decision Rule** |
|---|---|
| 0 out of 3 | This is the RP2D |
| ≥2 out of 3 | If occurs at dose level 1, then de-escalate to dose level 0. If occurs at dose level 0, trial will be terminated. |
| 1 out of 3 | Enter 3 more participants at this dose level.<br>• If 0 of these 3 participants experience DLT, this is the RP2D.<br>• If 1 or more of this group suffer DLT, then assessment at this level is stoppedIf this occurs at dose level 1, then de-escalate to dose level 0. If this occurs at dose level 0, the trial will be terminated. |
| ≤1 out of 6 at highest dose level at or below the maximally administered dose | This will be the RP2D. |

13.2.4 Phase 2 Design

Once the RP2D has been identified, an additional 14 to 17 evaluable patients will be enrolled at the RP2D. Interim monitoring of treatment related mortality (TRM) of the 20 patients treated at the RP2D is described in section 13.5 below.

## 13.3 Sample Size, Accrual Rate and Study Duration

During Phase 1, a minimum of 3 evaluable patients will be required (if 0 out of 3 patients at dose level 1 experience a DLT, the RP2D has been identified). A maximum of 12 evaluable patients will be required (6 patients at dose level 1 followed by 6 patients at dose level 0).

During Phase 2, an additional 14 to 17 evaluable patients will be required to reach a total of 20 evaluable patients treated at the RP2D. A sample size of 20 evaluable patients achieves an exact 95% confidence interval width equal to 0.379 assuming that the observed proportion of patients experiencing a DLT is 0.20.

Total Sample Size. A minimum of 3 and a maximum of 26 evaluable patients will be required. To account for up to 2 potentially unevaluable patients, a maximum sample size of up to 28 patients is anticipated.

Study Duration. Assuming an accrual rate of 1 patient per month, accrual of the up to 28 patients will take about 2 years and 4 months, plus 2 years of follow-up on the last patient, for a total study duration of about 4 years 4 months.

## 13.4 Stratification Factors

There are no stratification factors in this trial.

## 13.5 Interim Monitoring Plan

### 13.51 Monitoring rule for TRM

A one-stage stopping rule will monitor transplant-related mortality (TRM) for the N=20 patients treated at the RP2D. TRM is defined as the occurrence of death from any cause from Day -7 pre-transplant to post-transplant Day +100.

The one-stage interim monitoring rule for TRM is presented in Table 10. If there are 3 or more transplant-related deaths in the 20 patients treated at the RP2D, enrollment to the trial will be suspended so that DSMC can consider terminating the trial. If there are 2 or fewer transplant-related deaths in the 20 patients treated at the RP2D, uproleselan will be declared as safe for further investigation in pediatric patients.

**Table 10. One-stage stopping rule for treatment-related mortality for patients treated at the RP2D**

| Decision Rule | Action |
|---|---|
| ≥3 transplant-related deaths out of 20 patients | Trial termination recommended. |
| ≤2 transplant-related deaths out of 20 patients | Uproleselan is safe for further investigation in pediatric patients |

This one-stage rule has 92.5% power with an 9.1% type 1 error rate to test the null hypothesis that $P>0.25$ versus the alternative that $P<0.05$, where P is the probability of a transplant-related

death. The choice of the null and alternative hypothesis is based on an observed non-relapse mortality of 10.3% at 3 years for the BFC trial of pediatric patients in the Netherlands[23].

### 13.52 Monitoring rule for RRT

A one-stage stopping rule will monitor regimen-related toxicity (RRT) for the N=20 patients treated at the RP2D. RRT is defined as the occurrence of dose limiting toxicities as outlined in Table 4, from Day -7 pre-transplant to post-transplant Day +30.

The one-stage interim monitoring rule RRT is presented in Table 11. If there are 3 or more patients with dose limiting RRT in the 20 patients treated at the RP2D, enrollment to the trial will be suspended so that DSMC can consider terminating the trial. If there are 2 or fewer patients with RRT in the 20 patients treated at the RP2D, this is evidence in support of safe use of uproleselan for further investigation in pediatric patients.

The choice of the null and alternative hypothesis is derived from two early phase clinical trials of conditioning with busulfan and a purine analogue. In a trial of fludarabine and busulfan in adolescents and adults transplanted for hematologic malignancies, where busulfan exposure was escalated, 5 of 40 patients (12.5%) receiving busulfan targeted to an average AUC 6, 000 μMol-min developed grade 4 regimen related toxicity (based on CTCAE criteria, mucositis and/or transaminitis){Perkins, 2012 #76}. In a phase 1/2 trial in children and adults transplanted for hematologic malignancies, 1 of 22 patients (4.5%) receiving fludarabine 10 mg/m2, clofarabine 30 mg/m2 and busulfan targeted to an average AUC 6, 000 μMol-min developed grade 4 regimen related toxicity (based on CTCAE criteria){Andersson, 2011 #59}.

**Table 11. One-stage stopping rule for RRT for patients treated at the RP2D**

| Decision Rule | Action |
|---|---|
| ≥3 RRTs out of 20 patients | Trial termination recommended. |
| ≤2 RRT out of 20 patients | Evidence supports safety of uproleselan for further investigation in pediatric patients |

This one-stage rule has 94% power with an 9.3% type 1 error rate to test the null hypothesis that $P>0.185$ versus the alternative that $P<0.020$, where P is the probability of a transplant-related death. The choice of the null and alternative hypothesis is based on sinusoidal obstruction syndrome accounting for the majority of severe RRT in HSCT and an expected incidence of severe VOD of approximately 10%[35]

## 13.6 Analysis of Primary Endpoints

To address primary objective in section 1.3 Phase 1, the RP2D will be identified through the application of the 3+3 design. Accrual will be temporarily suspended after the enrollment of each cohort of 3 patients on a dose level, until all 3 patients have either completed the DLT observation period (30 days) or experienced a DLT.

To address primary objective in section 1.3 Phase 2, all observed toxicities will be summarized

using frequencies, proportions, and 95% confidence intervals by type (organ affected or laboratory determination), severity (by CTCAEv5.0), attribution, and expectedness. Only the maximum grade for each type of toxicity will be tabulated for each patient.

## 13.7 Analysis of Secondary Endpoints

To address secondary objective 1.3.2.1, additional pharmacokinetic parameters, including clearance, Cmax, and terminal half-life, for uproleselan will be calculated using standard methods and reported descriptively, in aggregate and by assigned dose level.

To address secondary objective 1.3.2.2, Kaplan-Meier curves of leukemia-free survival (LFS), overall survival (OS), and non-relapse mortality (NRM) will be generated for the cohort treated at the RP2D, and the point estimates of 1-year LFS, OS, and NRM will be calculated, with standard error according to Greenwood.

To address secondary objective 1.3.2.3, the proportion (and corresponding 95% exact confidence interval [CI]) of patients who experience oral and/or gastrointestinal mucositis will be summarized by severity.

## 13.8 Analysis of Exploratory Endpoints

To address exploratory objective 1.3.3.1, the median proportion (and corresponding 95% exact confidence interval [CI]) of pre-transplant marrow blasts with detectable E-selectin ligand (EsL) for the cohort treated at the RP2D will be summarized by leukemia-free survival (LFS) status (yes/no) 1-year post-transplant.

To address exploratory objective 1.3.3.2, LFS status at 1 year (yes/no) for the cohort treated at the RP2D, will be described by level of expression (low, high, intermediate) of the EsL related glycan synthesis genes ST3GAL4 and FUT7 (as defined in section 2.8).

## 14. REFERENCES

1. Barbier V, Erbani J, Fiveash C, et al. Endothelial E-selectin inhibition improves acute myeloid leukaemia therapy by disrupting vascular niche-mediated chemoresistance. *Nat Commun*. Apr 27 2020;11(1):2042. doi:10.1038/s41467-020-15817-5
2. DeAngelo DJ, Jonas BA, Liesveld JL, et al. GMI-1271 improves efficacy and safety of chemotherapy in R/R and newly diagnosed older patients with AML: results of a phase 1/2 study. *Blood*. 2017;130(suppl 1):894-894.
3. Sonis ST, Elting LS, Keefe D, et al. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer*. May 1 2004;100(9 Suppl):1995-2025. doi:10.1002/cncr.20162
4. Winkler IG, Barbier V, Nutt HL, et al. Administration of E-selectin antagonist GMI-1271 improves survival after high-dose chemotherapy by alleviating mucositis and accelerating neutrophil recovery. American Society of Hematology Washington, DC; 2013.
5. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant*. Oct 2009;15(10):1143-238. doi:10.1016/j.bbmt.2009.06.019
6. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. May 2 2009;373(9674):1550-61. doi:10.1016/S0140-6736(09)60237-3
7. Fogler WE, Daniel D, Barnes S, Wong A, Draper D, Magnani JL. Enhanced survival of lethally-irradiated mice with HSC reconstitution in combination with the E-selectin antagonist, GMI-1271 (uproleselan). AACR; 2020.
8. Beier R, Albert MH, Bader P, et al. Allo-SCT using BU, CY and melphalan for children with AML in second CR. *Bone Marrow Transplant*. May 2013;48(5):651-6. doi:10.1038/bmt.2012.204
9. Bunin NJ, Davies SM, Aplenc R, et al. Unrelated donor bone marrow transplantation for children with acute myeloid leukemia beyond first remission or refractory to chemotherapy. *J Clin Oncol*. Sep 10 2008;26(26):4326-32. doi:10.1200/JCO.2008.16.4442
10. Horan JT, Alonzo TA, Lyman GH, et al. Impact of disease risk on efficacy of matched related bone marrow transplantation for pediatric acute myeloid leukemia: the Children's Oncology Group. *J Clin Oncol*. Dec 10 2008;26(35):5797-801. doi:10.1200/JCO.2007.13.5244
11. Maher OM, Silva JG, Wu J, et al. Outcomes of children, adolescents, and young adults following allogeneic stem cell transplantation for secondary acute myeloid leukemia and myelodysplastic syndromes-The MD Anderson Cancer Center experience. *Pediatr Transplant*. May 2017;21(3)doi:10.1111/petr.12890
12. Quarello P, Fagioli F, Basso G, et al. Outcome of children with acute myeloid leukaemia (AML) experiencing primary induction failure in the AIEOP AML 2002/01 clinical trial. *Br J Haematol*. Nov 2015;171(4):566-73. doi:10.1111/bjh.13611
13. Roux C, Tifratene K, Socie G, et al. Outcome after failure of allogeneic hematopoietic stem cell transplantation in children with acute leukemia: a study by the societe Francophone de greffe de moelle et de therapie cellulaire (SFGM-TC). *Bone Marrow Transplant*. May 2017;52(5):678-682. doi:10.1038/bmt.2016.360
14. de Lima M, Porter DL, Battiwalla M, et al. Proceedings from the National Cancer Institute's Second International Workshop on the Biology, Prevention, and Treatment of Relapse After Hematopoietic Stem Cell Transplantation: part III. Prevention and treatment of relapse

after allogeneic transplantation. *Biol Blood Marrow Transplant*. Jan 2014;20(1):4-13. doi:10.1016/j.bbmt.2013.08.012
15. Bredeson C, LeRademacher J, Kato K, et al. Prospective cohort study comparing intravenous busulfan to total body irradiation in hematopoietic cell transplantation. *Blood*. Dec 5 2013;122(24):3871-8. doi:10.1182/blood-2013-08-519009
16. de Lima M, Couriel D, Thall PF, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood*. Aug 1 2004;104(3):857-64. doi:10.1182/blood-2004-02-0414
17. Harris AC, Boelens JJ, Ahn KW, et al. Comparison of pediatric allogeneic transplant outcomes using myeloablative busulfan with cyclophosphamide or fludarabine. *Blood Adv*. Jun 12 2018;2(11):1198-1206. doi:10.1182/bloodadvances.2018016956
18. Russell JA, Tran HT, Quinlan D, et al. Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. *Biol Blood Marrow Transplant*. 2002;8(9):468-76. doi:10.1053/bbmt.2002.v8.pm12374451
19. Scott BL, Pasquini MC, Logan BR, et al. Myeloablative Versus Reduced-Intensity Hematopoietic Cell Transplantation for Acute Myeloid Leukemia and Myelodysplastic Syndromes. *J Clin Oncol*. Apr 10 2017;35(11):1154-1161. doi:10.1200/JCO.2016.70.7091
20. Alatrash G, Thall PF, Valdez BC, et al. Long-Term Outcomes after Treatment with Clofarabine ± Fludarabine with Once-Daily Intravenous Busulfan as Pretransplant Conditioning Therapy for Advanced Myeloid Leukemia and Myelodysplastic Syndrome. *Biol Blood Marrow Transplant*. Oct 2016;22(10):1792-1800. doi:10.1016/j.bbmt.2016.06.023
21. Andersson BS, Valdez BC, de Lima M, et al. Clofarabine ± fludarabine with once daily i.v. busulfan as pretransplant conditioning therapy for advanced myeloid leukemia and MDS. *Biol Blood Marrow Transplant*. Jun 2011;17(6):893-900. doi:10.1016/j.bbmt.2010.09.022
22. Andersson BS, Thall P, Valdez BC, et al. A Randomized Study of Pretransplant Conditioning Therapy for AML/MDS with Fludarabine ± Clofarabine and Once Daily IV Busulfan with Allogeneic Hematopoietic Transplantation for AML and MDS. *Blood* 2020;136(Supplement 1):37-38.
23. Verslujis AB, Koning C, Lankester AC, et al. Combining Clofarabine/Fludarabine with Exposure Targeted Busulfan for Pediatric Leukemia Is an Effective, Low Toxic TBI-Free Conditioning Regimen. *Biol Blood Marrow Transplant*. 2020;26(3, Supplement):S34.
24. Konopleva M, Benton CB, Thall PF, et al. Leukemia cell mobilization with G-CSF plus plerixafor during busulfan-fludarabine conditioning for allogeneic stem cell transplantation. *Bone Marrow Transplant*. Jul 2015;50(7):939-946. doi:10.1038/bmt.2015.58
25. Gergis U, Roboz G, Shore T, et al. A phase I study of CPX-351 in combination with busulfan and fludarabine conditioning and allogeneic stem cell transplantation in adult patients with refractory acute leukemia. *Biol Blood Marrow Transplant*. Jul 2013;19(7):1040-5. doi:10.1016/j.bbmt.2013.04.013
26. Walter RB, Buckley SA, Pagel JM, et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation for AML in first and second complete remission. *Blood*. Sep 5 2013;122(10):1813-21. doi:10.1182/blood-2013-06-506725
27. Jacobsohn DA, Loken MR, Fei M, et al. Outcomes of Measurable Residual Disease in Pediatric Acute Myeloid Leukemia before and after Hematopoietic Stem Cell Transplant: Validation of Difference from Normal Flow Cytometry with Chimerism Studies and Wilms

Tumor 1 Gene Expression. *Biol Blood Marrow Transplant*. Oct 2018;24(10):2040-2046. doi:10.1016/j.bbmt.2018.06.010
28. Leonti AR, Pardo L, Alonzo TA, et al. Transcriptome profiling of glycosylation genes defines correlation with e-selectin ligand expression and clinical outcome in AML. American Society of Hematology Washington, DC; 2019.
29. Shukla P, Goswami S, Keizer RJ, et al. Assessment of a Model-Informed Precision Dosing Platform Use in Routine Clinical Care for Personalized Busulfan Therapy in the Pediatric Hematopoietic Cell Transplantation (HCT) Population. *Front Pharmacol*. 2020;11:888. doi:10.3389/fphar.2020.00888
30. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. *J Clin Oncol*. 1988;6(10):1562-8.
31. Corbacioglu S, Carreras E, Ansari M, et al. Diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in pediatric patients: a new classification from the European society for blood and marrow transplantation. *Bone Marrow Transplant*. Feb 2018;53(2):138-145. doi:10.1038/bmt.2017.161
32. Mohty M, Malard F, Abecassis M, et al. Revised diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in adult patients: a new classification from the European Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. Jul 2016;51(7):906-12. doi:10.1038/bmt.2016.130
33. Chien SS, Dai J, Magnani JL, et al. E-Selectin Ligand Expression By Leukemic Blasts Is Associated with Prognosis in Patients with AML. *Blood*. 2018;132(Supplement 1):1513-1513.
34. Hahn T, Sucheston-Campbell LE, Preus L, et al. Establishment of Definitions and Review Process for Consistent Adjudication of Cause-specific Mortality after Allogeneic Unrelated-donor Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant*. Sep 2015;21(9):1679-1686. doi:10.1016/j.bbmt.2015.05.019
35. Corbacioglu S, Cesaro S, Faraci M, et al. Defibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial. *Lancet*. Apr 7 2012;379(9823):1301-9. doi:10.1016/S0140-6736(11)61938-7

**APPENDIX A PERFORMANCE STATUS SCALES/SCORES**

**Performance Status Criteria**

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

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

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

**APPENDIX B DANA-FARBER/HARVARD CANCER CENTER MULTI-CENTER DATA AND SAFETY MONITORING PLAN**

## INTRODUCTION

The Dana-Farber/Harvard Cancer Center Multi-Center Data and Safety Monitoring Plan (DF/HCC DSMP) outlines the procedures for conducting a DF/HCC Multi-Center research protocol. The DF/HCC DSMP serves as a reference for any sites external to DF/HCC that are participating in the research protocol.

### Purpose

To establish standards that will ensure that a Dana-Farber/Harvard Cancer Center Multi-Center protocol will comply with Federal Regulations, Health Insurance Portability and Accountability Act (HIPAA) requirements and applicable DF/HCC Policies and Operations.

## GENERAL ROLES AND RESPONSIBILITIES

For DF/HCC Multi-Center Protocols, the following general responsibilities apply, in addition to those outlined in DF/HCC Policies for Sponsor-Investigators:

### DF/HCC Sponsor

The DF/HCC Sponsor, John Horan, MD, MPH, will accept responsibility for all aspects of conducting a DF/HCC Multi-Center protocol which includes but is not limited to:

- Oversee the coordination, development, submission, and approval of the protocol as well as subsequent amendments.
- Ensure that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol.
- Include the Multi-Center Data and Safety Monitoring Plan as an appendix to the protocol.
- Ensure all Participating Institutions are using the correct version of the protocol.
- Ensure that each participating investigator and study team member receives adequate protocol training (and/or a Site Initiation Visit prior to enrolling participants) and throughout trial's conduct as needed.
- Ensure the protocol will be provided to each participating site in a language understandable to all applicable site personnel when English is not the primary language.
- Monitor progress and overall conduct of the study at all Participating Institutions.
- Ensure all DFCI Institutional Review Board (IRB), DF/HCC and other applicable (FDA) reporting requirements are met.
- Review data and maintain timely submission of data for study analysis.
- Act as the single liaison with the FDA (investigator-held IND trials) as applicable.
- Ensure compliance with all requirements as set forth in the Code of Federal Regulations, applicable DF/HCC requirements, HIPAA requirements, and the approved protocol.
- Commit to the provision that the protocol will not be rewritten or modified by anyone

other than the DF/HCC Sponsor.
- Identify and qualify Participating Institutions and obtain accrual commitments prior to extending the protocol to that site.
- Monitor accrual and address Participating Institutions that are not meeting their accrual requirements.

**Coordinating Center (Dana-Farber Cancer Institute)**

The general responsibilities of the Coordinating Center may include but are not limited to:

- Assist in protocol development.
- Maintain CTEP, FDA or OBA correspondence, as applicable.
- Review registration materials for eligibility and register participants from Participating Institutions in the DF/HCC clinical trial management system (CTMS).
- Distribute protocol and informed consent document updates to External Sites as needed.
- Oversee the data collection process from External Sites.
- Maintain documentation of Serious Adverse Event (SAE) reports and deviations/violation submitted by External Sites and provide to the DF/HCC Sponsor for timely review and submission to the IRB of record, as necessary.
- Distribute serious adverse events reported to the DF/HCC Sponsor that fall under the reporting requirements for the IRB of record to all External Sites.
- Provide External Sites with information regarding DF/HCC requirements that they will be expected to comply with.
- Carry out plan to monitor External Sites either by on-site or remote monitoring.
- Maintain Regulatory documents of all External Sites which includes but is not limited to the following: local IRB approvals/notifications from all External Sites, confirmation of Federalwide Assurances (FWAs) for all sites, all SAE submissions, Screening Logs for all sites, IRB approved consents for all sites
- Conduct regular communications with all External Sites (conference calls, emails, etc) and maintain documentation all relevant communications.

**External Site**

An External Site is an institution that is outside the DF/HCC and DF/PCC consortium that is collaborating with DF/HCC on a protocol where the sponsor is a DF/HCC investigator. The External Site acknowledges the DF/HCC Sponsor as having the ultimate authority and responsibility for the overall conduct of the study.

Each External Site is expected to comply with all applicable DF/HCC requirements stated within this Data and Safety Monitoring Plan and/or the protocol document.

The general responsibilities for each External Site may include but are not limited to:
- Document the delegation of research specific activities to study personnel.
- Commit to the accrual of participants to the protocol.

- Submit protocol and/or amendments to their IRB of record. For studies under a single IRB, the Coordinating Center will facilitate any study-wide submissions.
- Maintain regulatory files as per ICH GCP and federal requirements.
- Provide the Coordinating Center with regulatory documents or source documents as requested.
- Participate in protocol training prior to enrolling participants and throughout the trial as required.
- Update Coordinating Center with research staff changes on a timely basis.
- Register participants through the Coordinating Center prior to beginning research related activities when required by the sponsor.
- Submit Serious Adverse Event (SAE) reports to sponsor, Coordinating Center, and IRB of record as applicable, in accordance with DF/HCC requirements.
- Submit protocol deviations and violations to the Sponsor, Coordinating Center, and IRB of record as applicable.
- Order, store and dispense investigational agents and/or other protocol mandated drugs per federal guidelines and protocol requirements.
- Participate in any quality assurance activities and meet with monitors or auditors at the conclusion of a visit to review findings.
- Promptly provide follow-up and/or corrective action plans for any monitoring queries or audit findings.
- Notify the sponsor immediately of any regulatory authority inspection of this protocol at the External Site.

## DF/HCC REQUIREMENTS FOR MULTI-CENTER PROTOCOLS

Certain DF/HCC Policy requirements apply to External Sites participating in DF/HCC research. The following section will clarify DF/HCC requirements and further detail the expectations for participating in a DF/HCC Multi-Center protocol.

### Protocol Revisions and Closures

The External Sites will receive notification of protocol revisions and closures from the Coordinating Center. When under a separate IRB, it is the individual External Site's responsibility to notify its IRB of these revisions.

**Protocol revisions:** External Sites will receive written notification of protocol revisions from the Coordinating Center. All protocol revisions should be IRB approved and implemented within a timely manner from receipt of the notification.

**Non-life-threatening revisions:** Participating Institutions will receive written notification of protocol revisions regarding non-life-threatening events from the Coordinating Center. Non-life-threatening protocol revisions must be IRB approved and implemented within 90 days from receipt of the notification.

**Revisions for life-threatening causes:** Participating Institutions will receive immediate notification from the Coordinating Center concerning protocol revisions required to protect lives

with follow-up by fax, mail, e-mail, etc. Life-threatening protocol revisions will be implemented immediately followed by IRB request for approval.

**Protocol closures and temporary holds:** External Sites will receive notification of protocol closures and temporary holds from the Coordinating Center. Closures and holds will be effective immediately. In addition, the Coordinating Center, will update the External Sites on an ongoing basis about protocol accrual data so that they will be aware of imminent protocol closures.

The following (but not limited to) must be on file with the Coordinating Center:

- Initial approval letter of the Participating Institution's IRB.
- Copy of the Informed Consent Form(s) approved by the Participating Institution's IRB.
- Participating Institution's IRB approval for all amendments.
- Annual approval letters by the Participating Institution's IRB.

**Informed Consent Requirements**

The DF/HCC approved informed consent document will serve as a template for the informed consent for External Sites. The External Site consent form must follow the consent template as closely as possible and should adhere to specifications outlined in the DF/HCC Guidance Document on Model Consent Language for Investigator-Sponsored Multi-Center Trials. This document will be provided separately to each External Site upon request.

External Sites must send their version of the informed consent document to the Coordinating Center for sponsor review and approval. If the HIPAA authorization is a separate document, please submit to the sponsor for the study record. Once sponsor approval is obtained, the External site may submit to their IRB of record, as applicable. In these cases, the approved consent form must also be submitted to the Coordinating Center after approval by the local IRB for all consent versions.

The Principal Investigator (PI) at each External Site will identify the appropriate members of the study team who will be obtaining consent and signing the consent form for protocols. External Sites must follow the DF/HCC requirement that for all interventional drug, biologic, or device research, only attending physicians may obtain initial informed consent and any re-consent that requires a full revised consent form.

**IRB Re-Approval**

Verification of IRB re-approval for the External Sites is required in order to continue research activities. There is no grace period for continuing approvals.

The Coordinating Center will not register participants if a re-approval letter is not received for the External Site on or before the anniversary of the previous approval date.

**DF/HCC Multi-Center Protocol Confidentiality**

All documents, investigative reports, or information relating to the participant are strictly confidential. Whenever reasonably feasible, any participant specific reports (i.e. Pathology Reports, MRI Reports, Operative Reports, etc.) submitted to the Coordinating Center should be de-identified. It is recommended that the assigned protocol case number be used for all participant specific documents. Participant initials may be included or retained for cross verification of identification.

**Participant Registration and Randomization**

See detailed registration instructions in protocol Section 4.4.

**Initiation of Therapy**

Participants must be registered with the DF/HCC CTMS before the initiation of treatment or other protocol-specific interventions. Treatment and other protocol-specific interventions may not be initiated until the External Site receives confirmation of the participant's registration from the Coordinating Center. The DF/HCC Sponsor and IRB of record must be notified of any violations to this policy.

**Eligibility Exceptions**

No exceptions to the eligibility requirements for a protocol without IRB approval will be permitted. All External Sites are required to fully comply with this requirement. The process for requesting an eligibility exception is defined below.

**Data Management**

DF/HCC Inform develops case report forms (CRF/eCRFs), for use with the protocol. These forms are designed to collect data for each study. DF/HCC provides a web-based training for all eCRF users.

**Data Forms Review**

Data submissions are monitored for timeliness and completeness of submission. If study forms are received with missing or questionable data, the submitting institution will receive a written or electronic query from the DF/HCC Office of Data Quality, Coordinating Center, or designee.

Responses to all queries should be completed and submitted in a timely manner..

If study forms are not submitted on schedule, the External Sites will periodically receive a Missing Form Report from the Coordinating Center noting the missing forms.

**Protocol Reporting Requirements**

**Protocol Deviations, Exceptions and Violations**

Federal Regulations require an IRB to review proposed changes in a research activity to ensure that researchers do not initiate changes in approved research without IRB review and approval, except when necessary to eliminate apparent immediate hazards to the participant. DF/HCC requires all departures from the defined procedures set forth in the IRB approved protocol to be reported to the DF/HCC Sponsor and to the IRB of record.

For reporting purposes, DF/HCC uses the terms "violation", "deviation" and "exception" to describe departures from a protocol. All Participating Institutions must adhere to requirements for reporting to the DF/HCC Sponsor and will follow their institutional policy for reporting to their local IRB.

**Definitions**

Protocol Deviation: Any departure from the defined procedures set forth in the IRB-approved protocol which is *prospectively approved* prior to its implementation.
Protocol Exception: Any protocol deviation that relates to the eligibility criteria, e.g. enrollment of a participant who does not meet all inclusion/exclusion criteria.
Protocol Violation: Any protocol departure that was not *prospectively approved* by the IRB prior to its initiation or implementation.

**Reporting Procedures**

DF/HCC Sponsor: is responsible for ensuring that clear documentation is available in the medical record and/or regulatory documents to describe all protocol exceptions, deviations and violations. The DF/HCC Sponsor will also be responsible for ensuring that all protocol violations/deviations are promptly reported per DFCI IRB guidelines.

Participating Institutions: Protocol deviations require prospective approval from the DFCI IRB. The Participating Institution must submit the deviation request to the Coordinating Center who will then submit the deviation request to the DFCI IRB. Upon DFCI IRB approval the deviation is submitted to the Participating Institution IRB, per institutional policy. A copy of the Participating Institution's IRB report and determination will be forwarded to the Coordinating Center within 10 business days after the original submission. The deviation may not be implemented without all required approvals.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

Coordinating Center: Upon receipt of the violation/deviation report from the Participating Institution, the Coordinating Center will submit the report to the DF/HCC Sponsor for review. Subsequently, the Participating Institution's IRB violation/deviation report will be submitted to the DFCI IRB for review per DFCI IRB reporting guidelines.

**Reporting Procedures**

Requests to deviate from the protocol require approval from the IRB of record and the sponsor.

All protocol violations must be sent to the Coordinating Center in a timely manner. The Coordinating Center will provide training for the requirements for the reporting of violations.

**Guidelines for Processing IND Safety Reports**

The DF/HCC Sponsor will review all IND Safety Reports per DF/HCC requirements, and ensure that all IND Safety Reports are distributed to the External Sites as required by DF/HCC Policy. External Sites will review/submit to the IRB according to their institutional policies and procedures.

## MONITORING: QUALITY CONTROL

The Coordinating Center, with the aid of the DF/HCC Office of Data Quality, provides quality control oversight for the protocol.

**Ongoing Monitoring of Protocol Compliance**

The Participating Institutions may be required to submit participant source documents to the Coordinating Center for monitoring. Participating Institution may also be subject to on-site monitoring conducted by the Coordinating Center.
The Coordinating Center will implement ongoing monitoring activities to ensure that Participating Institutions are complying with regulatory and protocol requirements, data quality, and participant safety. Monitoring practices may include but are not limited to source data verification, and review and analysis of eligibility requirements, informed consent procedures, adverse events and all associated documentation, review of study drug administration/treatment, regulatory files, protocol departures reporting, pharmacy records, response assessments, and data management.

**Site Qualification**

Sites are invited to participate in this protocol if they are familiar with and have participated in prior Phase I studies, have an appropriate volume of patients to be able to contribute to study enrollment goals, and have appropriate resources, including IRB support, investigational pharmacists, clinical research coordinators, research nurses, and experienced physician investigators to conduct Phase I research. DF/HCC Site/Protocol Feasibility Questionnaires must be completed by each site. The Sponsor will assess that the investigators, study team members, and Participating Institutions are qualified and appropriately resourced to conduct the protocol. The DF/HCC Sponsor will have final approval of any participating sites on this trial.

**Regulatory Documents:**

DFCI will collect all required regulatory documentation prior to study activation. The study team will work with all participating sites to ensure all updated / revised regulatory documentation is maintained at each site and forwarded to DFCI and maintained in the Trials Master File.

Once a proposed Participating Institution receives institutional IRB approval, all approval documentation and IRB-approved related documents will be emailed to the Coordinating Center study staff and an amendment to add a site will be submitted to the DF/HCC IRB. Once approval is received to add the Participating Institution study materials will be sent to the Participating Institution. Study materials will include, but are not limited to, regulatory documents and participant tracking forms.

All Participating Institutions must maintain and update all essential regulatory documents in a regulatory binder. A designated member of the DF/HCC Lead Institution will be responsible for maintaining the Trial Master File which will include copies of all regulatory documentation for the Lead Site and all Participating Institutions.

All Participating Institutions are required to submit all institutional IRB correspondences and approvals to be retained in the Trial Master File at the Lead Institution. The Lead Institution will review all working study documents and ensure that the most current IRB-approved protocol and study documents are being used by the Participating Institutions.

**Delegation of Authority/Responsibility:**

Participating Institutions will be instructed to complete a Delegation of Responsibility/ Authority Log which will be reviewed/approved by the DF/HCC Sponsor. A copy of this document will be retained in the Trial Master File. Participating Institutions will be instructed to inform the Coordinating Center immediately should there be a change in personnel. An updated training and Delegation of Authority/Responsibility log will need to be completed as soon as possible.

**Site Initiation Visit:**

A Site Initiation Visit (SIV) will be conducted with all participating sites prior to enrolling participants. The SIV will cover study objectives and rationale, study design, eligibility, registration, required data, treatment schedule, side effects/AEs, SAE reporting, and dose modification. It will be led by the DFCI Overall Principal Investigator, John Horan, MD, MPH. It is expected that the Participating Institution Site PI and all study staff will attend.

**Ongoing Communication and Training:**

Regularly scheduled teleconferences will occur between the Coordinating Center and Participating Institutions. This will be a forum to discuss and study related issues not limited to the following: protocol amendments, study accrual, SAE's/AE's experienced, clinical response and deviations/violations. An agenda will be sent to all sites at the time the teleconference is scheduled. Minutes will be taken and distributed to the participating institutions after each teleconference.

**Virtual Monitoring**

The Coordinating Center will request source documentation from Participating Institutions as needed to complete virtual monitoring activities. Participating Institutions will be asked to

forward de-identified copies of participants' medical record and source documents to the Coordinating Center to aid in source data verification.

**On-Site Monitoring**

On-Site monitoring may occur on an as needed basis. On-site source documentation verification (SDV) will be conducted by having access to participants' complete medical record and source documents. In addition, upon request from a monitor or auditor, Participating Institutions should provide access to regulatory documents, pharmacy records, local policies related to the conduct of research, and any other trial-related documentation maintained by the participating site. If there are concerns for protocol compliance, issues that impact subject safety or the integrity of the study are found, or trends identified based on areas of need, additional monitoring visits may be scheduled.

**Adverse Event Reporting:**

Documentation of all adverse events must be included as part of source documentation. Documentation that a physician determined attribution of each event must also be included. AEs and SAEs must be reported as instructed in the protocol with the log serving as record for all events at each Participating Institution. The DF/HCC Sponsor, John Horan, MD, MPH, will be notified/report all AEs SAEs as per protocol.

**Drug Accountability:**

Participating pharmacies will be required to submit Drug Accountability Logs at the time of monitoring documenting receipt and shipment of drug supply, dispensing/ordering of supply, and destruction of unused study medication and/or damaged or expired drug.

**Monitoring Reports**

The DF/HCC Sponsor will review all monitoring reports to ensure protocol compliance. The DF/HCC Sponsor may increase the monitoring activities at External Sites that are unable to comply with the protocol, DF/HCC Sponsor requirements or federal and local regulations.

**Accrual Monitoring**

Prior to extending a protocol to an external site, the DF/HCC Sponsor will establish accrual requirements for each External Site. Accrual will be monitored for each External Site by the DF/HCC Sponsor or designee. Sites that are not meeting their accrual expectations may be subject to termination.

It is expected that each External Site will accrue 2 - 5 patients over the duration of the trial.

**AUDITING: QUALITY ASSURANCE**

**DF/HCC Internal Audits**

All External Sites are subject to audit by the DF/HCC Office of Data Quality (ODQ). Typically, approximately 3-4 participants would be audited at the site over a 2-day period. If violations which impact participant safety or the integrity of the study are found, more participant records may be audited.

**Audit Notifications**

It is the External Site's responsibility to notify the Coordinating Center of all external audits or inspections (e.g., FDA, EMA, NCI) that involve this protocol. All institutions will forward a copy of final audit and/or re-audit reports and corrective action plans (if applicable) to the Coordinating Center, within 12 weeks after the audit date.

**Audit Reports**

The DF/HCC Sponsor will review all final audit reports and corrective action plans, if applicable. The Coordinating Center must forward any reports to the DF/HCC ODQ per DF/HCC policy for review by the DF/HCC Audit Committee. For unacceptable audits, the DF/HCC Audit Committee would forward the final audit report and corrective action plan to the IRB as applicable.

**External Site Performance**

The DF/HCC Sponsor and the IRB of record are charged with considering the totality of an institution's performance in considering institutional participation in the protocol.

External Sites that fail to meet the performance goals of accrual, submission of timely and accurate data, adherence to protocol requirements, and compliance with state and federal regulations, may be put on hold or closed.

**APPENDIX C HEPATIC SOS/VOD DIAGNOSTIC AND GRADING CRITERIA**

# Pediatric EBMT diagnostic criteria for hepatic SOS/VOD

**The presence of two or more of the following:**

| Criterion | Comments |
|---|---|
| **Unexplained consumptive and transfusion-refractory thrombocytopenia** | ⩾one platelet transfusion/day. |
| **Otherwise unexplained weight gain on three consecutive days despite the use of diuretics or a weight gain >5% above baseline value** | |
| **Hepatomegaly** | Best if confirmed by imaging above baseline value* |
| **Ascites** | Best if confirmed by imaging above baseline value* |
| **Rising bilirubin from a baseline value on 3 consecutive days or bilirubin ⩾2 mg/dL within 72 h** | |

*Suggested: imaging (US, CT or MRI) immediately before HCT to determine baseline value for both hepatomegaly and ascites.

EBMT criteria for SOS/VOD diagnosis in adults

| *Classical SOSNOD*<br>In the first 21 days after HSCT | *Late onset SOSNOD*<br>> 21 Days after HSCT |
|---|---|
| Bilirubin ≥2 mg/dl and two the following criteria must be present:<br>Painful hepatomegaly<br>Weight gain > 5%<br>Ascites | Classical VOD/SOS beyond day 21 of<br>OR<br>Histologically proven SOS/VOD OR<br>Two or more of the following criteria must be present:<br>Bilirubin ≥ 2 mg/dl (or 34 µmol/L)<br>Painful hepatomegaly<br>Weight gain> 5% Ascites<br>AND Hemodynamical or/and ultrasound evidence of SOS/VOD |

Abbreviations: EBMT = European Society for Blood and Marrow Transplantation;

SOS= sinusoidal obstruction syndrome; VOD = veno-occlusive disease. These symptoms/signs should not be attributable to other causes.

**EBMT criteria for grading the severity of suspected hepatic SOS/VOD in children**[a]

| *CTCAE* | *Mild* | *Moderate* | *Severe* | *Very severe MOD/MOF* |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| **LFT**[b] **(ALT, AST, GLDH)** | ⩽2 × normal | >2 and ⩽5 × normal | >5 | |
| **Persistent RT**[b] | <3 days | 3–7 days | >7 days | |
| **Bilirubin (mg/dL)**[b, c] | <2 | ⩾2 | | |
| **Bilirubin (µmol/L)** | <34 | ⩾34 | | |
| **Ascites**[b] | Minimal | Moderate | Necessity for paracentesis (external drainage) | |
| **Bilirubin kinetics** | | | Doubling within 48 h | |
| **Coagulation** | Normal | Normal | Impaired coagulation | Impaired coagulation with need for replacement of coagulation factors |
| **Renal function GFR (mL/min)** | 89–60 | 59–30 | 29–15 | <15 (renal failure) |
| **Pulmonary function (oxygen requirement)** | <2 L/min | >2 L/min | Invasive pulmonary ventilation (including CPAP) | |
| **CNS** | Normal | Normal | Normal | New onset cognitive impairment |

Abbreviations: ALT=alanine transaminase; AST=aspartate transaminase; CNS=central nervous system; CPAP=continuous positive airway pressure; CTCAE=Common Terminology Criteria for Adverse Events; GFR=glomerular filtration rate; GLDH=glutamate dehydrogenase; LFT=liver function test; MOD/MOF=multi-organ dysfunction/multi-organ failure; RT=refractory thrombocytopenia; SOS/VOD, sinusoidal obstruction syndrome/veno-occlusive disease.

[a]If patient fulfills criteria in different categories they must be classified in the most severe category. In addition, the kinetics of the evolution of cumulative symptoms within 48 h predicts severe disease.

[b]Presence of ⩾2 of these criteria qualifies for an upgrade to CTCAE level 4 (very severe SOS/VOD).

[c]Excluding pre-existent hyperbilirubinemia due to primary disease.

EBMT criteria for severity grading of a suspected SOS/VOD in adults

| | *Mild*[a] | *Moderate*[a] | *Severe* | *Very severe - MOD/MOF*[b] |
|---|---|---|---|---|
| Time since first clinical symptoms of SOS/VOD[c] | > 7 Days | 5-7 Days | ≤4 Days | Any time |
| Bilirubin (mg/dl)<br>Bilirubin (µmol/L) | ≥2 and <3<br>≥34 and <51 | ≥3 and< 5<br>≥51 and< 85 | ≥5 and<8<br>≥85and <136 | ≥8<br>≥136 |
| Bilirubin kinetics | | | Doubling within 48 h | |
| Transaminases | ≤2x normal | > 2 and ≤ 5 x normal | > 5 and ≤8 x normal | >8x Normal |
| Weight increase | < 5% | ≥5% and < 10% | ≥5% and < 10% | ≥10% |
| Renal function | < 1.2 X baseline at transplant | ≥1.2 and < 1.5 x baseline at transplant | ≥1.5 and < 2 x baseline at transplant | ≥2 X baseline at transplant or others signs of MOD/MOF |

Abbreviations: EBMT = European society for Blood and Marrow Transplantation; MOD= multi-organ dysfunction; MOF = multiorgan failure; SOS= sinusoidal obstruction syndrome; VOD = veno-occlusive disease. Patients belong to the category that fulfills two or more criteria. If patients fulfill two or more criteria in two different categories, they must be classified in the most severe category. Patients weight increase ≥5% and < 10% is considered by default as a criterion for severe SOS/VOD; however. if patients do not fulfill other criteria for severe SOS/VOD, weight increase ≥5% and < 10% is therefore considered as a criterion for moderate SOS/VOD. [a]In the case of presence of two or more risk factors for SOS/VOD, patients should be in the upper grade. [b]Patients with multi-organ dysfunction must be classified as very severe. [c]Time from the date when the first signs/symptoms of SOSNOD began to appear (retrospectively determined) and the date when the symptoms fulfilled SOS/VOD diagnostic criteria.

## APPENDIX D BEARMAN SCALE

| | Grade I | Grade II | Grade III |
|---|---|---|---|
| Cardiac toxicity | Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms | Moderate EKG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics | Severe EKG abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention; or decrease in voltage by more than 50% |
| Bladder toxicity | Macroscopic hematuria after 2 d from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection | Macroscopic hematuria after 7 d from last chemotherapy dose not caused by infection; or hematuria after 2 d with subjective symptoms of cystitis not caused by infection | Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure |
| Renal toxicity | Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning) | Increase in creatinine above twice baseline but not requiring dialysis | Requirement of dialysis |
| Pulmonary toxicity | Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure | CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of $PO_2$ (>10% from baseline) but not requiring mechanical ventilation or >50% $O_2$ on mask and not caused by infection or CHF | Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF |
| Hepatic toxicity | Mild hepatic dysfunction with 2.0 mg% ≤ bilirubin ≤ 6.0 mg%; or weight gain >2.5% and <5% from baseline, of noncardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning | Moderate hepatic dysfunction with bilirubin >6 mg% <20 mg%; or SGOT increase >5-fold from preconditioning; or clinical ascites or image documented ascites >100 mL; or weight gain >5% from baseline of noncardiac origin | Severe hepatic dysfunction with bilirubin >20 mg%; or hepatic encephalopathy; or ascites compromising respiratory function |
| CNS toxicity | Somnolence but the patient is easily arousable and oriented after arousal | Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection | Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding |
| Stomatitis | Pain and/or ulceration not requiring a continuous IV narcotic drug | Pain and/or ulceration requiring a continuous IV narcotic drug (morphine drip) | Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation |
| GI toxicity | Watery stools >500 mL but <2,000 mL every d not related to infection | Watery stools >2,000 mL every d not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection | Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion |

NOTE. Grade IV regimen-related toxicity is defined as fatal toxicity.
Abbreviations: CXR, chest x-ray; IV, intravenous.