CITY OF HOPE MEDICAL CENTER
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DEPARTMENT OF HEMATOLOGY AND HEMATOPOIETIC CELL TRANSPLANTATION

TITLE: PHASE II STUDY OF CLOFARABINE AND HIGH-DOSE MELPHALAN CONDITIONING PRIOR TO ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOR MYELODYSPLASIA OR ACUTE LEUKEMIA IN REMISSION

CITY OF HOPE PROTOCOL NUMBER/VERSION: IRB # 13130 Protocol dated: 06/06/17

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COH Amendment 06	Protocol Dated 10-15-14	Version 06
COH Amendment 07	Title Page Dated 12-22-14	Version 07
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COH Amendment 12	Title Page Dated 11/15/16	Version 12
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COH Amendment 16	Protocol dated 06/06/2017 (tp)	Packet 16
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SITE: Bone Marrow
MODALITY: IV and oral
TYPE: Phase II

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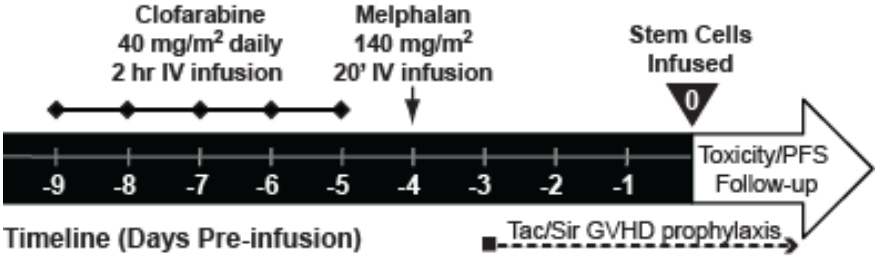
BIOSTATISTICIAN: Joycelynne M. Palmer, Ph.D.

Experimental Design Schema

Schema

This single-institution, single-arm phase II trial is designed to evaluate the anti-leukemic activity of the clofarabine/melphalan alloHCT conditioning regimen, in patients with acute leukemia in remission or high-risk myelodysplasia, as assessed by 2-year progression-free survival (PFS). PFS is defined as time from start of protocol treatment to death, disease relapse/progression, or last contact, whichever occurs first.



***A window of 1-2 days is allowed for stem cell availability**

**** The current dose used on this protocol for Melphalan is 100 mg/m²**

Figure 1. Treatment Schema

Protocol Synopsis

Protocol Title:
Phase II study of clofarabine and high-dose melphalan conditioning prior to allogeneic hematopoietic cell transplantation for myelodysplasia or acute leukemia in remission
Brief Protocol Title for the Lay Public (if applicable):
Phase II study of clofarabine and high-dose melphalan prior to allogeneic hematopoietic cell transplantation for treatment of myelodysplasia or acute leukemia in remission
Study Phase:
Phase II, with patient safety lead-in
Participating Sites:
Single Institution: City of Hope
Rationale for this Study:
While allogeneic hematopoietic cell transplantation (alloHCT) is the only option for possible cure for many patients with hematological malignancies such as acute leukemia and MDS, many patients, especially the older population and younger patients who have been heavily pretreated, cannot tolerate full intensity ablative conditioning regimens. Fludarabine and melphalan has been efficacious as a reduced intensity conditioning regimen for alloHCT for multiple malignancies, but the relapse rate for this regimen remains high. In this study, we are replacing fludarabine with clofarabine, a rationally designed second-generation purine nucleoside analog. Since clofarabine has increased anti-leukemic activity compared to fludarabine *in vitro*, and preliminary City of Hope data are promising (IRB #06114 – Blood 2012, and IRB #12241 – American Society of Hemaology Meeting 2012), we hypothesize that a clofarabine/melphalan conditioning regimen for alloHCT in patients with acute leukemia or MDS will yield improved relapse/progression rates while maintaining a low-toxicity profile.
Objectives:
Primary: Following a patient safety lead-in, evaluate the anti-tumor activity of clofarabine given in combination with high-dose melphalan as assessed by 2-year progression-free survival (PFS). **Secondary:** Estimate overall survival (OS), cumulative incidence (CI) of relapse/progression, and non-relapse mortality (NRM) at 100 days, 1 year and 2 years. Summarize toxicities/complications by organ and severity, including acute and chronic GVHD, and infection.

Study Design:

This is a single-institution, single-arm phase II trial to evaluate the anti-leukemic activity and safety/tolerability of the clofarabine/melphalan alloHCT conditioning regimen in patients with acute leukemia in remission or high-risk myelodysplasia. A *patient safety lead-in* will be conducted to ensure there are no unexpected toxicities. Ultimately a total of 65 patients will be treated and evaluated for progression-free survival at the clofarabine/melphalan dose level considered safe as determined during the *patient safety lead-in* segment of this study.

The first six patients enrolled on this study will be part of a *patient safety lead-in*. Initially, a group of up to 3 patients can be enrolled. Note: no more than 3 patients can be <30 days post stem cell infusion at any time during the patient safety lead-in phase. During the safety lead-in, if >1/6 patients experience unacceptable toxicity within 30 days after stem cell infusion, subsequent patients will receive a de-escalated dose of melphalan at 100 mg/m^2. While not expected, if >1/6 patients experience unacceptable toxicity at the 40mg/m^2 (clofarabine)/100 mg/m^2 (melphalan) dose level, the clofarabine dose will be reduced to 30mg/m^2.

Endpoints:

Primary:

The primary study endpoint is 2-year progression-free survival (PFS). PFS will be estimated from the start of treatment to the date of death, disease relapse/progression, or date of last contact using the Kaplan-Meier method.

The primary endpoint for the patient saftey lead-in segment of the study is toxicity. Toxicity will be scored on both the Bearman Scale and NCI CTCAE v4.0 Scale. Unacceptable toxicity in a given patient is defined as any regimen-related grade III/IV toxicity per Bearman Criteria (Appendix B), or for hematologic toxicities, per NCI CTCAE v4.03 toxicity criteria, any grade 4 neutropenia associated with fever or infection and lasting beyond 3 weeks, or grade 4 neutropenia lasting for more than 28 days, or any other regimen-related cause of death. This unacceptable toxicity definition was established after reviewing two sources of toxicity data (see protocol section 2.1): 1) preliminary data from patients conditioned with clofarabine/melphalan (Forman, 2013 unpublished) and 2) historical data from patients conditioned with fludarabine/melphalan (Forman, 2013 unpublished; Nakamura 2012). Generally, regimen-related grade I/II toxicity per Bearman Criteria occurred in >50% of patients, in at least one toxicity class; this was also true for hematologic toxicity. Note: The NCI CTCAE v4.0 scale will also be used for reporting of adverse events and more detailed reporting.**Secondary:**

Secondary study endpoints include: overall survival (OS), cumulative incidence of relapse/progression and non-relapse mortality, overall toxicity (Bearman Scale and CTCAE v4.0), incidence/severity of acute/chronic GVHD, and infection.

Sample Size:

We expect to treat a total of 65 patients in this phase II study; assuming the 40mg/m^2 (clofarabine)/140 mg/m^2 (melphalan) dose level is well tolerated.

With two dose reductions permissible, the overall maximum study sample size could reach 77.

The first six patients treated will be part of a patient safety lead-in. The safety lead-in will be conducted to ensure there are no unexpected toxicities.

Estimated Duration of the Study

10 years: 5 years accrual (23 patients/year), 5 years follow-up after enrolling last patient.

Summary of Subject Eligibility Criteria:

Inclusion Criteria:

- Ages 18-75 years old
- Karnofsky performance status score ≥70.
- Patients in 1st or 2nd remission with AML or ALL, who are eligible for stem cell transplant
  - remission defined as no circulating blasts, <5% blasts in the bone marrow, normalization of previously detected cytogenetic abnormalities, no extramedullary disease
- High risk MDS
  - Intermediate II and High risk by IPSS
  - Intermediate, High or Very High by WPSS
  - Transfusion dependent
  - Therapy-related MDS or MDS evolved from previous hematological disorder (excepting myelofibrosis)
- Patients with CMML are allowed to be enrolled
- Patients with MDS that has evolved to AML must be in remission
- Patients must not be eligible for fully ablative regimens by the attending physician.
- Patients with AML or MDS arising from myeloproliferative neoplasm can be enrolled after PI approval on case to case basis, depends on the spleen size and degree of bone marrow fibrosis
- Bone Marrow should be done within 28 (+ 4 day window) days prior to registration to confirm remission.
- A pretreatment measured creatinine clearance (absolute value) of ≥ 60 ml/minute.
- Patients must have a serum bilirubin ≤2.0 mg/dl, SGOT and SGPT ≤2.5 times the institutional upper limits of normal.
- Ejection Fraction measured by echocardiogram or MUGA > 50%
- DLCO or FEV1 > 45% predicted.
- Availability of an HLA matched (6/6) sibling donor or 8/8 matched unrelated donor. Donors with mismatch at HLA-A, HLA-B, HLA-C and HLA-DR will be reviewed by MUD committee and allowed if their mismatch with the recipient does not require additional GVHD prophylaxis (other than Tacrolimus and sirolimus), donors with mismatch at HLA-DQ or HLA-DPB are eligible. Donor evaluation according to COH SOP.Donor stem cell source can be either peripheral blood or bone marrow.
- All patients must have a psychosocial evaluation prior to transplant as per COH SOP
- All subjects must have the ability to understand and the willingness to sign a written informed consent.
- ALL or AML patients who received chemotherapy (induction or consolidation) can proceed to transplant once bone marrow cellularity is >10% with no evidence of leukemia.

Exclusion Criteria:

- Patients who have received a prior autologous or allogeneic transplant are excluded
- Patients with suspected or proven CNS leukemia. (Diagnostic lumbar puncture not required

before enrollment)

- Patients with significant hepatic dysfunction (not meeting LFT eligibility criteria)
- Acute promyelocytic leukemia
- Patients with myeloproliferative neoplasms
- Uncontrolled intercurrent illness including, but not limited to ongoing or active or poorly controlled infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, poorly controlled pulmonary disease or psychiatric illness/social situations that would limit compliance with study requirements.
- Pregnant and lactating women are excluded from this study.
- Patients who do not agree to practice effective forms of contraception.
- Patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy. Therefore, HIV-positive patients are excluded from this study.
- Patients are excluded if they are Hepatitis B sAg, Hep B cAb, or Hep C positive. Patients with Hepatitis B cAB positive and Hepatitis B PCR negative are eligible if they started prophylactic treatment prior to registration to trial.
- Patients who received radiation therapy as part of their leukemia treatment may be ineligible and individual cases must be presented to the study PI for eligibility determination.
- Patients with active 2nd malignancies other than AML, ALL, MDS. Excised skin cancer, early stage cervical and prostate cancer allowed.
- Any psychiatric, social or compliance issues that, in the treating physician opinion, will interfere with completion of the transplant treatment and follow up.
- Medical or psychiatric reasons which make the donor unlikely to tolerate or cooperate with G-CSF therapy or leukapheresis or bone marrow harvest
- Known allergies to clofarabine, melphalan sirolimus or tacrolimus
- Cord blood as a donor source is not acceptable.

Investigational Product Dosage and Administration:

Clofarabine will be given as an inpatient at 40 mg/m^2 IV administration over 2 hours on 5 successive days (-9 to-5 pre-transplant). If calculated creatinine clearance falls below 50 ml/min, subsequent doses of clofarabine in the 5 days of clofarabine treatment will be reduced to 30 mg/m^2 in that patient. If calculated creatinine clearance falls below 30 ml/min, subsequent doses of clofarabine will be held and the case will be discussed with the PI.

Melphalan 100 mg/m^2 (current dose) IV will be administered inpatient over 30 minutes on a single day (-4 pre-transplant), allow at least 20 hours post last clofarabine dose. On day 0, patients will have an infusion of allogeneic stem cells (derived from peripheral blood or bone marrow) from HLA-matched related or unrelated donors.

Graft versus host prophylaxis will be tacrolimus 0.02 mg/kg/d IV, beginning day –3, and sirolimus 12 mg oral loading dose on day –3, followed by 4 mg orally as a single morning daily dose (target serum level 3-10 ng/ml by HPLC).

Clinical Observations and Tests to be Performed:

We will perform standard of care post-transplantation observations and tests as per SOP. Follow-up will be twice weekly for the first 100 days post-transplant, twice monthly until 6 months post-transplant, and monthly until the patient is off immunosuppressive therapy without evidence of GVHD. Follow-up will be as clinically indicated through year 5, including bone marrow biopsies

on day 30, 100 (all ± 7 days), and 1 year and 2 years (all ± 14 days) post-transplant, with yearly follow-up through 5 years including data collection on survival and disease status.
Statistical Considerations:
This single-institution, single-arm phase II trial is designed to evaluate the anti-leukemic activity (as assessed by 2-year PFS) and safety/tolerability of the clofarabine/melphalan alloHCT conditioning regimen in patients with acute leukemia in remission or high-risk myelodysplasia. Based on PFS estimates from previously reported studies and COH pilot studies, a total of 65 patients are required. Using the SWOG, single arm non-parametric approach (based on work done by Brookmeyer and Crowley) an increase in two-year PFS from an historical estimate of 62% to 77% would be detectable with approximately 86% power, 1-sided α=0.05. The primary endpoint, PFS, will be calculated using the Kaplan-Meier product-limit method. Secondary endpoints include, toxicity, incidence and severity of acute and chronic GVHD, and infection. Toxicity will be assessed and reported using the Bearman and CTCAE v4.03 scales. The cumulative incidence of acute and chronic GVHD will be estimated after taking into account the competing risks of death, relapse/progression, and graft failure. Additional secondary endpoints will include overall survivival (OS), cumulative incidence of relapse/progression and non-relapse mortaility (NRM). OS will be calculated using the Kaplan-Meier product-limit method. The cumulative incidence of relapse/progression and non-relapse mortality will be calculated as competing risks using the Gray method.
Sponsor/Licensee:
Sanofi-Aventis will provide the clofarabine for the study.
Case Report Forms
Medidata Rave® EDC Application

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Abbreviations

Abbreviation	**Meaning**
AE	Adverse Event

ALL	Acute Lymphoblastic Leukemia
AML	Acute Myelogenous Leukemia
CFR	Code of Federal Regulations
COH	City of Hope
CR	Complete Response
CRA	Clinical Research Associate
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLT	Dose Limiting Toxicity
DNA	Deoxyribonucleic Acid
DSMC	Data Safety Monitoring Committee
EFS	Event-free Survival
FDA	Food and Drug Administration
GCP	Good Clinical Practice
alloHCT	Allogeneic Hematopoietic Cell Transplantation
ICF	Informed Consent Form
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
MDS	Myelodysplastic Syndrome
MRD	Minimal Residual Disease
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NRM	Non-relapse Mortality
OS	Overall Survival
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response

RIC	Reduced Intensity Conditioning
SAE	Serious Adverse Event
SD	Stable Disease

1.0 Goals and Objectives (Scientific Aims)

- Following a patient safety lead-in, determine the anti-tumor activity of clofarabine given in combination with high-dose melphalan as assessed by 2-year progression-free survival (PFS).
- Estimate overall survival (OS), cumulative incidence (CI) of relapse/progression and non-relapse mortality (NRM) at 100 days, 1 year and 2 years.
- Summarize toxicities/complications by organ and severity, including acute and chronic GVHD, and infection.

2.0 Background

2.1 Introduction/Rationale for Development

Allogeneic stem cell transplantation remains the only curative treatment modality for hematologic malignancies such as acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), and myelodysplastic syndrome (MDS) [2-4]. Conditioning regimens for allogeneic transplant serve two purposes: 1) maximal immunosuppression, to prevent transplant rejection, and, 2) killing any remaining tumor cells. There has been an evolution in conditioning regimens over time, with the recognition that immunosuppression may be more important than tumor kill by chemotherapy, given that a critical component of allogeneic transplant is the graft versus tumor effect [5, 6]. Thus, the first reduced intensity regimens were designed, replacing the toxic alkylating agent cyclophosphamide, with the purine nucleoside anti-metabolite, fludarabine, which is a potent immunosuppressant with a substantially milder toxicity profile [7]. The success of these reduced intensity regimens in terms of lower toxicity, with apparently equal efficacy, at least in the case of chronic myelogenous leukemia (CML) and AML in patients over age 50 [8], has allowed the benefits of transplantation to be extended to groups that were previously excluded [9-11]. Some of the most common reduced intensity regimens combine fludarabine with an alkylating agent, either busulfan or melphalan. However, fludarabine is not an active agent against leukemias or MDS; hence it may be beneficial to devise a reduced intensity regimen with greater anti-tumor efficacy.

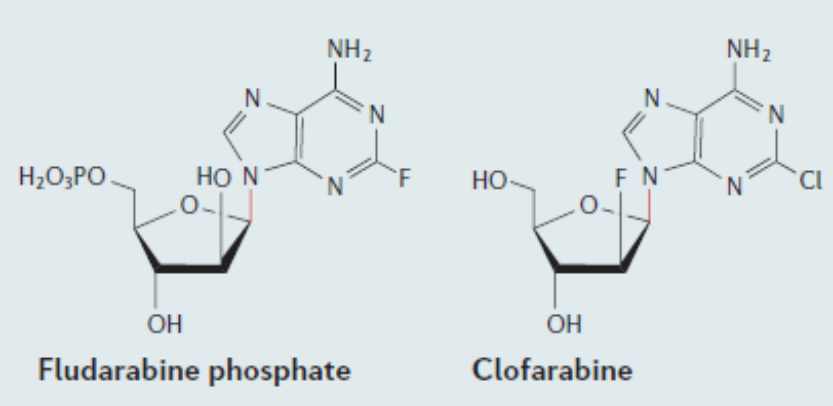


Figure 2. Chemical structures of fludarabine phosphate and clofarabine

Clofarabine is a rationally designed second-generation purine nucleoside analog, and a relative of fludarabine. The double halogen replacement strategy confers resistance to adenosine deaminase, while increasing acid stability and bioavailability (in part through more rapid membrane transport) [12]. Figure 2 diagrams the cellular mechanism of action. Clofarabine enters cells by both facilitated and passive diffusion. Initially, deoxycytidine kinase (dCK) phosphorylates clofarabine, after which additional kinases phosphorylate it to its active form, clofarabine triphosphate. The triphsophate form acts to terminate DNA chain elongation and inhibit repair through incorporation into the DNA chain by competitive inhibition of DNA polymerases [13]. It also inhibits ribonucleotide reductase resulting in reduction of dNTP pools [14], and induces apoptosis both directly and indirectly via mitochondrial damage leading to release of cytochrome c and apoptosis-inducing factor [15]. Clofarabine is more efficient than fludarabine at disrupting mitochondrial function, which explains the increased killing of

non-dividing lymphocytes by clofarabine compared to fludarabine. This advantage would theoretically reduce the likelihood of graft rejection in the allogeneic setting, and perhaps augment the graft-versus-tumor effect of the donor graft.

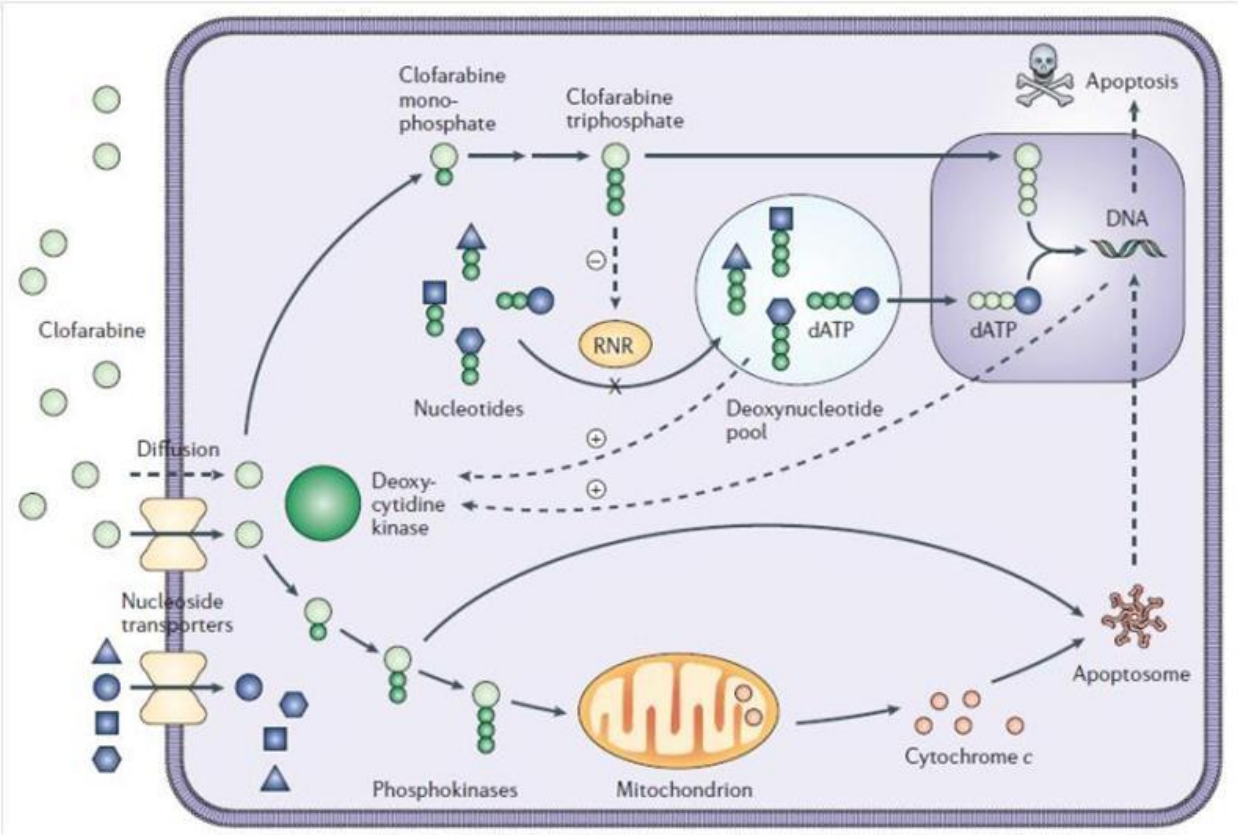


Figure 3. Schematic of clofarabine mechanism of action. Borrowed from Bonate *et al.* [1]

At City of Hope, Kirschbaum *et al.* performed a phase I study of the combination of clofarabine plus melphalan as a reduced intensity conditioning regimen for allogeneic hematopoietic stem cell transplant (alloHCT) in patients with acute myelogenous leukemia [16]. Patients over age 18 in complete remission or with active disease (up to 50% marrow blasts) who had matched-related or -unrelated donors were eligible. The conditioning regimen consisted of escalating doses of clofarabine plus melphalan, followed by allogeneic stem cell transplantation. Sixteen patients, with a median age of 63 years, were treated at three dose levels; 4 were primary induction failures and 3 were in first relapse. One patient at dose level 2 and one at dose level 3 died of multi-organ failure; otherwise no dose limiting toxicities were seen. All other patients at both doses of clofarabine studied showed complete engraftment by day 30, with a median time to absolute neutrophil count recovery of 14 days, and 16 days for platelet recovery. With a median follow-up of 17 months, only two patients relapsed and four patients died. Twelve patients were treated at dose level 2 (40 mg/m^2 clofarabine and 100mg/m^2 melphalan) with a 1-year OS of 73% (95% CI 38%-91%) and EFS of 61% (95% CI 26%-84%). Clofarabine plus melphalan at dose level 2 is a well-tolerated conditioning regimen with activity in advanced AML patients. Dose level 3 in the phase I trial treated only one patient with co-morbid conditions, who died on day 12 post-transplant from multi-organ failure. Since normal engraftment had been achieved at dose level 2, the higher of the tested clofarabine doses, further patients were not accrued at dose level three to avoid possible further toxicity.

Van Besien *et al.* [17] recently published a phase I/II trial of clofarabine, melphalan, and alemtuzumab as conditioning for alloHCT in patients with advanced hematologic malignancies. In the

phase I portion of the trial, clofarabine 40 mg/m^2 with melphalan 140 mg/m^2 and alemtuzumab 20 mg was found to be safe and tolerable with no DLTs in 6 patients, and this dosing was carried forward into the phase II portion. With a median follow-up of 25 months, the 1-yr OS was 59% and 1-yr EFS was45% for phase II patients. During the phase II trial they encountered problems with rapid-onset renal failure, observing grade 3-5 renal toxicity in 16 of 74 patients during the clofarabine treatment, typically starting prior to the melphalan dose. After the first 24 patients, they dose-reduced clofarabine to 30 mg/m^2, and when the problem continued in the next 27 patients, they increased the clofarabine infusion time from 30 minutes to 3 hours. Neither of these adjustments decreased the rate of renal toxicity. Other studies using clofarabine as transplant conditioning with busulfan [18, 19], and our own phase I study have not seen high rates of renal toxicity. Renal complications in the van Besien trial may be particularly related to the co-administration of alemtuzumab and clofarabine, which were given on the same days. Elderly patients and those with impaired baseline renal function were at higher risk for this complication. We do not expect to see an elevated rate of renal toxicity, particularly since the proposed trial will be in less advanced patients with fewer pre-treatments and we are requiring a baseline creatinine clearance of ≥ 60 ml/minute compared to the allowed 50 ml/minute in the van Besien trial.

Since publication of the Van Besien trial, City of Hope has treated 9 additional patients using clofarabine 40 mg/m^2 and melphalan 140 mg/m^2 dosing (dose level 3 in phase I trial) with no grade 3 renal toxicity. We have retrospectively analyzed survival outcomes for all 24 COH patients transplanted using the clofarabine plus melphalan regimen as their first transplant between November 2007 and October 2012. The median follow-up for surviving patients was 23.9 months (range 3.2–52.9). Overall survival at 1 year and 2 years was 83.9% and 76.9% respectively, as depicted in Figure 4. Progression-free survival at 1 and 2 years was the same, at 77.7% (95% CI: 57.7 – 89.1). The cumulative incidence of acute GvHD was 38% (grades I and II only), while 50% of evaluable patients experienced chronic GVHD.

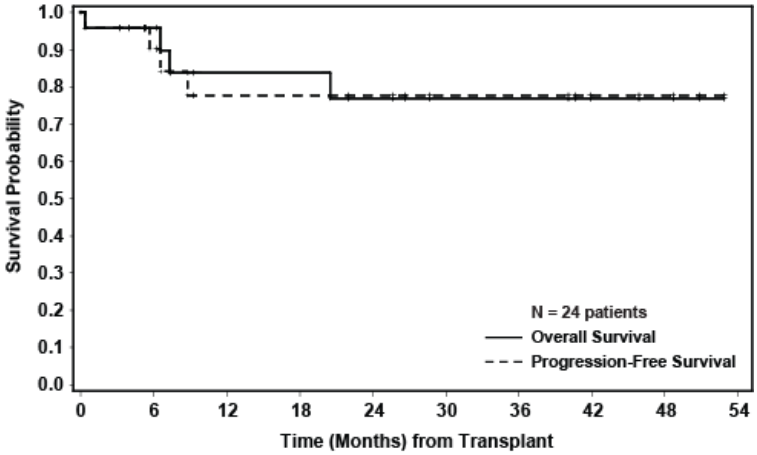


Figure 4. OS and PFS estimates for 24 patients treated with Clo/Mel conditioning with a median follow-up time of 24 months.

Use of clofarabine in place of fludarabine in the reduced intensity conditioning regimen may provide enhanced immunosuppression as well as a probable increased anti-leukemic effect. For this reason, we propose a phase II study of clofarabine combined with high-dose melphalan followed by allogeneic stem cell reinfusion on day 0.

As noted, published historical estimates of 2-year PFS post RIC alloHCT vary from 31% to 65%. For AML patients who underwent RIC alloHCT in complete remission, using various RIC regimens, McClune *et al.* reported 2-year PFS estimates that ranged from 31%-43%, across four age-groups [20]. Marks et al. reported 2-year PFS estimates ranging from 46% (2CR) to 57% (1CR), among ALL patients who were also treated with various RIC alloHCT regimens [21]. For high-risk MDS patients treated with a fludarabine/melphalan RIC regimen, Nakamura *et al.* reported a 2-year PFS estimate of 65.2% [22]. Because the available historical data for AML and ALL are not reflective of our observed RIC fludarabine-melphalan experience (COH PFS estimates are better), 2-year PFS estimates were also generated using data from the COH transplant repository from patients transplanted from 2005 to 2009 using patient selection criteria that match the protocol inclusion criteria. The estimates from these analyses are as follows: AML 2-year PFS 60%, ALL 2-year PFS 51% (Forman, 2013 unpublished). Using these data, the published MDS outcomes (Nakamura et al. [22]) and expected accrual pattern

across disease subgroups, the 2-year PFS estimate for fludarabine/melphalan is 62% (95%CI: 57.7-66.5) (Figure 5).

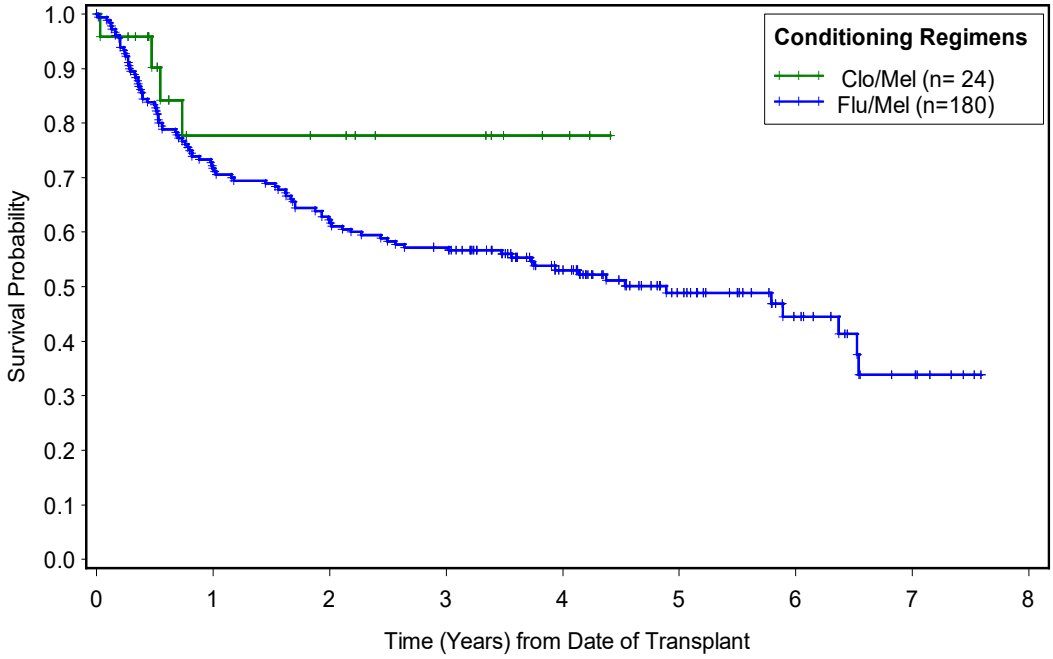


Figure 5. PFS estimates for 24 patients conditioned for RIC alloHCT with Clo/Mel conditioning and 180 patients with Flu/Mel at City of Hope.

2.2 Overview of Proposed Study

We will be examining the safety and clinical activity of a reduced-intensity conditioning regimen of clofarabine and melphalan prior to alloHCT for adult patients with myelodysplastic syndrome, and those with acute myelogenous leukemia or acute lymphoblastic leukemia in remission. The primary endpoint for this phase II trial is 2-year progression-free survival (PFS), estimated from time of start of treatment to death, relapse/progression, or last contact.

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

2.3 Preclinical Studies

2.3.1 Clofarabine in vitro

Clofarabine is a potent inhibitor of DNA polymerase, exhibiting significant growth inhibition and cytotoxic activity in a wide variety of leukemic and solid tumor cell lines, with IC50 values ranging from 0.028-0.29 μM [23]. Of the 60 human tumor cell lines in the National Cancer Institute's Developmental Therapeutics Program panel, clofarabine potently inhibits the growth of 35 of them (GI50 from <0.001-0.45μM [1]. Clofarabine is more efficient than fludarabine at disrupting mitochondrial function, which leads to apoptosis via recruitment of APAF-1 [15]. This mitochondria-triggered apoptosis explains the increased killing of non-dividing lymphocytes by clofarabine compared to fludarabine. Pretreatment of chronic lymphocytic leukemia cells with cyclophosphamide, and alkylating agent that activates DNA excision repair mechanisms, facilitates the incorporation of nucleoside analogues and increases their cytotoxicity [24]; clofarabine produces a cooperative killing effect with cyclophosphamide at a 10-fold lower concentration (5 μM) than does fludarabine (50 μM).

2.3.2 2.3.2 Clofarabine animal studies

Clofarabine demonstrates anti-tumor activity in a wide range of human tumor mouse xenografts [23], and was dose and schedule dependent in human lung and colon cancer mouse models [25]. Oral, intravenous, and intraperitoneal clofarabine administration routes are all effective [1, 26]. With intravenous administration in rats, clofarabine is widely distributed in both normal and tumor tissues and accumulates in the liver and myocardium [27]. The volume of distribution at steady state (Vdss) is approximately 1.4-2.6 L/hg in mice, 3.2-3.6 L/kg in rats, and 0.9-1.2 L/Kg in dogs [1]. IV clofarabine displays non-linear pharmacokinetics, with three exponential phases of elimination, with half-lives of 0.3, 1.3, and 12.8 hours; 80% of the dose is eliminated in urine and 10% in feces [27].

2.4 Human StudiesType equation here.

2.4.1 Clofarabine single agent trials

In the initial phase I study done at MD Anderson, the MTD was established as 40 mg/m^2 daily for 5 days, with the DLT being hepatotoxicity seen at the 55 mg/m^2 dose. This study included all acute leukemias; there was an objective response rate of 16% with 2 complete responses (CR), one in a patient with AML and one with ALL, and an additional 2 complete responses without platelet recovery (CRp), one each in AML and ALL [28]. A phase II study of 62 patients with acute leukemia followed, using the 40 mg/m^2 dose for 5 days [29]. There were 20 CRs, 1 PR, and 9 patients with hematologic improvement. Specifically looking at acute leukemias, CR was seen in 13 of the 31 patients with AML, with another 4 patients showing CRp, for a total overall response rate of 55%. In ALL, one patient out of 12 developed CR. The most frequent toxicities seen in those studies were hyperbilirubinemia and transaminitis, resolving by day 15 of therapy, as well as rashes, hand-foot syndrome, and mucositis [29]. A phase I/II trial of the combination of clofarabine 40 mg/m^2 daily (days 2-6) with ara-C 1 gram/m^2 daily (days 1-5), in 32 patients with relapsed leukemias, reports 7 patients achieving CR, and 5 patients with CRp, all with AML; no patients with ALL in this study attained CR [30]. The lower CR rate seen in the AML patients in the phase II study versus the phase I study was explained as being possibly due to two factors: the phase II study treated a group of patients at lower clofarabine doses during the dose-finding run-in, also, fewer patients in the single agent phase II study had short duration remissions or primary refractory disease.

In the pediatric population, a higher dose was tolerated, with an MTD of 52 mg/m^2 established. Four patients with ALL and one patient with AML achieved CR, with 1 ALL patient and 2 AML patients achieving CR, for a total response rate of 32% [31]. Pediatric phase II studies in ALL using the 52 mg/m^2 dose over 5 days patients are reported [32] with a response rate of 30%: 7 CR, 5 CRs without platelet recovery, and 6 PR. Responses were durable enough for 9 patients to proceed to transplant. Toxicities in these studies were similar to those seen in the adult studies. The agent subsequently received FDA approved for the treatment of relapsed or refractory pediatric ALL.

2.4.2 Trials combining clofarabine with alkylating agents

A phase I trial of clofarabine plus cyclophosphamide (Cy) tested the *in vitro* findings of synergistic activity of these two drugs on leukemia cells [33]. Eighteen patients were treated with Cy (200 mg/m^2) on day 0, escalating doses of clofarabine + cyclophosphamide on day 2. Overall response rates were 50% for dose level 1 (20 mg/m^2); however, DLTs including prolonged marrow aplasia required de-escalation to 10 mg/m^2, where the response rate was 30%. DNA damage was increased after clofarabine + Cy compared to Cy alone as proof of principle. Since the combined regimen has such potent marrow suppression, it is well suited to be an alloHCT conditioning regimen.

Two groups have conducted trials of clofarabine combined with busulfan, another alkylating agent, as part of reduced intensity conditioning for alloHCT. Farag *et al.* [18] treated a total of 15 relapsed and refractory acute leukemia patients in a phase I study using a schedule of 0.8 mg/kg every 6 hours on days -6 to -3 pre-transplant, with escalating clofarabine (30-60 gm/m^2 daily) on days -6 to -2 pre-transplant.

MTD was not reached and grade 3/4 non-hematologic toxicities included vomiting (20%), mucositis (60%), hand-foot syndrome (7%), acute renal failure (7%), and reversible elevation of liver enzymes (67%). One-year EFS was 53% and OS was 60%. Mineishi *et al.* [19] report on alternative dosing of busulfan (once daily at 3.2 mg/kg IV days -5 to -2) combined with escalating clofarabine (20, 30 or 40 mg/m^2 IV on days -6 to -2) in an ongoing phase I/II study in 46 patients with non-remission hematological malignancies [19]. Grade 3-4 non-hematological toxicities include transaminitis (50%), mucositis (26%), hand-foot syndrome (13%), transient hypoxia (13%), nausea/vomiting (11%), diarrhea (11%), hypertension (7%), veno-occlusive disease (4%), hyperbilirubinemia (4%0, hypersensitivity (2%), joint pain (2%) and seizure (2%). There was no renal insufficiency and transaminitis resolved to grade 1 or less within 2 weeks. All patients engrafted. For the 31 AML patients, the median duration of remission was 15.4 months and cumulative incidence of relapse was 38% and overall survival was 50%.

Two groups, including our own have conducted phase I studies of clofarabine combined with the alkylating agent melphalan, as conditioning prior to alloHCT; both of these, Kirschbaum *et al.* [16] and van Besien *et al.* [17] are described in Section 2.1.

3.0 Patient & Donor Eligibility

3.1 Patient Inclusion Criteria

3.1.1 Diagnoses and Disease Status

- Patients in 1st or 2nd remission with AML or ALL, who are eligible for stem cell transplant. Remission defined asno circulating blasts, <5% blasts in the bone marrow, normalization of previously detected cytogenetic abnormalities, no extramedullary disease
- High risk MDS
  - Intermediate II and High risk by IPSS
  - Intermediate, High or very high by WPSS
  - Transfusion dependent
  - Therapy-related MDS or MDS evolved from previous hematological disorder (excepting myelofibrosis)
- Patients with CMML are allowed to be enrolled
- Patients with MDS that has evolved to AML must be in remission
- Patients must not be eligible for full ablative regimens by the attending physician.
- Patients with AML or MDS arising from myeloproliferative neoplasm can be enrolled after PI approval on case to case basis, depends on the spleen size and degree of bone marrow fibrosis

3.1.2 Age Criteria, Performance Status and Life Expectancy

Patients must be ages 18-75 years of age with a performance status of ≥70% on the Karnofsky scale (Appendix A). Children will not be treated with this regimen until safety and dose have been established in adults.

3.1.3 Child Bearing Potential

The effects of allogeneic transplantation on the developing fetus are highly toxic. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence] prior to study entry and for six months following duration of study participation. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.1.4 Protocol-Specific Criteria

- Bone marrow and peripheral blood studies must be available for confirmation of diagnosis. Cytogenetics, flow cytometry, and molecular studies (such as Flt-3 status) will be obtained as per standard practice.
- Bone marrow aspirates/biopsies should be performed within 28 (+ 4 day window) days from registration to confirm disease remission status.
- A pretreatment measured creatinine clearance (absolute value) of ≥ 60 ml/minute.
- Patients must have a serum bilirubin ≤2.0 mg/dl, SGOT and SGPT ≤2.5 times the institutional upper limits of normal.
- Ejection fraction measured by echocardiogram or MUGA > 50%
- DLCO or FEV1 > 45% predicted.
- Availability of an HLA matched (6/6) sibling donor or 8/8 matched unrelated donor. Donors with mismatch at HLA-A, HLA-B, HLA-C and HLA-DR will be reviewed by MUD committee and allowed if their mismatch with the recipient does not require additional GVHD prophylaxis (other than Tacrolimus and sirolimus), donors with mismatch at HLA-DQ or HLA-DPB are eligible. Donor evaluation according to COH SOP.
- Donor stem cell source can be either peripheral blood or bone marrow.
- All patients must have a psychosocial evaluation prior to transplant as per COH SOP

3.1.5 Informed Consent/Assent

All subjects must have the ability to understand and the willingness to sign a written informed consent.

3.1.6 Prior Therapy

- ALL or AML patients who received chemotherapy (induction or consolidation) can proceed to transplant once bone marrow cellularity is >10% with no evidence of leukemia.

3.2 Patient Exclusion Criteria

- Patients who have received a prior autologous or allogeneic transplant are excluded
- Patients with significant hepatic dysfunction (not meeting LFT eligibility criteria in Section 3.1.4)
- Patients with MDS evolved into AML that is not in remission
- Patients with acute promyeloctyic leukemia
- Patients with myeloproliferative neoplasms
- Patients with suspected or proven CNS leukemia. (Diagnostic lumbar puncture not required before enrollment)
- Uncontrolled intercurrent illness including, but not limited to ongoing or active or poorly controlled infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, poorly controlled pulmonary disease or psychiatric illness/social situations that would limit compliance with study requirements.
- Pregnant and lactating women are excluded from this study.
- Patients who do not agree to practice effective forms ofcontraception as described in section 3.1.3.

- Patients with immune deficiency are at increased risk of lethal infections when treated with marrow-suppressive therapy. Therefore, HIV-positive patients are excluded from this study.
- Patients are excluded if they are Hepatitis B sAg, Hep B cAb, or Hep C positive. Patients with Hepatitis B cAB positive and Hepatitis B PCR negative are eligible if they started prophylactic treatment prior registration to trial.
- Patients who have received radiation therapy as part of their leukemia treatment may be ineligible and individual cases must be presented to the study PI for determination of eligibility.
- Any psychiatric, social or compliance issues that, in the treating physician opinion, will interfere with completion of the transplant treatment and follow up.
- Medical or psychiatric reasons which make the donor unlikely to tolerate or cooperate with G-CSF therapy or leukapheresis or bone marrow harvest
- Known allergies to clofarabine, melphalan sirolimus or tacrolimus
- Patients with other active malignancies (besides AML, ALL, MDS) requiring treatment or where there is concern of progression are ineligible for this study. However, patients with previously treated skin cancer, early stage cervical or prostate cancer may be eligible if there is no evidence of residual disease.
- Cord blood as a donor source is not acceptable.

3.2.1 Non-Compliance

Subjects, who in the opinion of the investigator, may not be able to comply with the safety monitoring requirements of the study.

3.3 Donor Eligibility Criteria

Donor evaluation and eligibility will be assessed as per current City of Hope SOP.

3.4 Inclusion of Women and Minorities

The study is open to anyone regardless of gender or ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which plans to accrue approximately 65 subjects (maximum 77), a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand, and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

4.0 Screening and Registration Procedures

4.1 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were performed for clinical indications within 30 days prior to registration (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Patients who received chemotherapy, immunotherapy or radiation need at least a 2 week' washout period prior to registration from the last dose of therapy. See Section 10.0 – Study Calendar for a complete list of screening procedures. Lab values required for eligibility are listed in the Patient Eligibility section 3.1. Patient screening tests for eligibility will be based on City of Hope Standard of Care transplant work up.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record.

4.3 Registration Requirements/Process

See Section 10.0 – Study Calendar for a complete list of pre-study tests.

Registration is to occur within 4 days of the start of conditioning.

Registration

Patients who received chemotherapy , immunotherapy or radiation need at least a 2 week washout period prior to registration from the last dose of therapy. To register a patient, the treating physician should contact the protocol nurse or the responsible Clinical Research Coordinator (CRC) in Clinical Trial Office (CTO) to complete the eligibility/registration form. The protocol nurse or CRC will contact the Data Coordinating Center at the City of Hope (626-256-4673, ext. 64267 or e-mail dcc@coh.org), EMAIL a copy of the completed eligibility checklist, required pre-study tests (per protocol – and may include laboratory, CT and pathology reports), signed Informed Consent, signed Patients' Bill of Rights and HIPAA authorization form to dcc@coh.org.

Registration Process

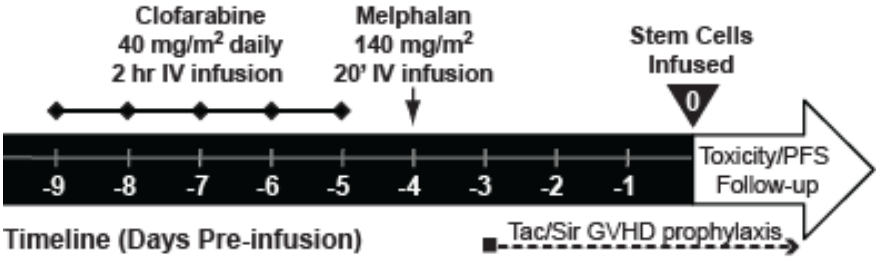
The patient registration process will be handled by the Department of Clinical Research Information Support (CRIS) Data Coordinating Center (DCC) at City of Hope. The steps below are to be taken when registering a patient:

- The research staff must assure they have the most current and updated version of the protocol and informed consent prior to enrolling a patient. If a question arises, please contact the Data Coordinating Center at 626-256-4673 extension 64267 or via email at dcc@coh.org.
- The study staff must assure that all pre-study laboratory tests, scans and x-rays have been completed prior to registration according to the study calendar
- The study staff must assure that the patient has signed an approved informed consent prior to registration/randomization, including the Experimental Subject Bill of Rights and appropriate HIPAA authorization.
- The study staff must confirm that the patient meets all inclusion and exclusion eligibility criteria for the protocol. The eligibility checklist (provided by the COH DCC) must be completed in its entirety.
- Registration is to occur within 4 days of the start of conditioning. A patient failing to meet all protocol requirements may not be registered.
- Once a patient is eligible, all the pre-study requirements have been fulfilled, and the informed consent obtained, the research nurse or the study coordinator will inform the COH Data Coordinating Center at (626) 256-4673, extension 64267; email dcc@coh.org a copy of the patient's signed informed consent, , completed eligibility checklist and corresponding source documentation confirming eligibility (including pathology reports, lab reports, x-ray reports, etc.).

The City of Hope Data Coordinating Center will:

- Review all materials/source documentation to ensure the patient is eligible.
- Ensure the consent form is valid and is signed correctly by all parties. If additional information is needed or should there be any questions, the Data Coordinating Center will immediately contact the participating institution and registration will not occur until all issues are resolved.
- If there are questions regarding exceptions to the eligibility criteria, please contact he study Principal Investigator, as well as the COH DCC. Documentation of IRB approval of exception will need to be submitted as well as the COH DCC.
- Confirmation of Registration will be emailed to the study staff noting the patient's study number within 24 hours post receipt of a complete eligibility packet.
- The COH DCC will call the research nurse or study coordinator and verbally confirm the registration (if needed).
- If the patient does not receive protocol therapy following registration, the patient's registration on the study may be cancelled. The COH DCC should be notified of cancellations as soon as possible.

5.0 Treatment Program



5.1 Treatment Overview

***A window of 1-2 days is allowed for stem cell availability**

****The current dose used on this protocol for Melphalan is 100 mg/m²**

Figure 3. Treatment Schema

5.1.1 Donor Mobilization and Leukapheresis

Donor stem cells will be mobilized and leukapheresed as per current COH SOP.

5.1.2 Patient Treatment Schedule

Clofarabine will be administered in the inpatient setting at 40 mg/m^2 IV over 2 hours on 5 successive days (-9 to-5 pre-transplant). Prior to each dose of clofarabine, patients will be pre-medicated with 100 mg hydrocortisone as an antiemetic and for prevention of capillary leak syndrome. If one or more signs of capillary leak syndrome occurs (unexplained hypotension, tachycardia, and/or tachypnea) during infusion of clofarabine, administration of clofarabine will be interrupted and the patient will be administered 12 mg dexamethasone (8 mg/m^2 if weight is less than 50 kg). After the patient is free of symptoms, clofarabine administration may resume with caution and dexamethasone will be administered at 20 mg IV

daily prior to all subsequent doses of clofarabine. If during the course of the 5 days of clofarabine, the calculated creatinine clearance falls below 50 ml/min, subsequent doses of clofarabine will be reduced to 30 mg/m^2.

Melphalan will be administered inpatient at 100 mg/m^2 IV administration over 30 minutes on a single day (-4 pre-transplant), allow at least 20 hours post last clofarabine dose. Prior to administration of melphalan, patients will be pre-medicated with 10 mg IV dexamethasone as part of anti-emetic prophylaxis.

On day 0, patients will receive an infusion of allogeneic stem cells from an HLA-matched related or unrelated donor as per current COH SOP.

Graft versus host prophylaxis will be with tacrolimus 0.02 mg/kg/d continuous IV, beginning on day –3 and converting to oral dosing when the patient is able to tolerate and absorb oral medications. Sirolimus will be administered at a 12 mg oral loading dose on day –3, followed by 4 mg orally as a single morning daily dose. Target serum levels for both tacrolimus and sirolimus are 5-10 ng/ml for each by HPLC. In the absence of GVHD, the immunosuppressive taper will be as per COH SOP.

For a tabular view of the treatment, monitoring, and follow-up schedule, see study calendar in Section 10.

5.2 Planned Duration of Therapy

Initial therapy will require ~4 weeks of inpatient treatment during transplantation. A standard schedule of follow-up visits for alloHCT will be used with some additional visits for MRD assessment (see Study Calendar Section 10.0) with study follow-up extending 5 years beyond the date of stem cell infusion. Patients will be co-enrolled in the COH long-term follow-up protocol for allogeneic transplantation (protocol #00029).

5.3 Criteria for Removal from Treatment

Disease relapse or progression, unacceptable toxicity, non-compliance with protocol

5.4 Subject Follow-Up

Follow-up will be at least once weekly for the first 60 days post-transplant, day 100 (+/- 14 days), day 180 (+/- 28 days), one year (+/- 28 days) and yearly follow-up afterward up to year 5 post-transplant. Follow-up will be as clinically indicated through year 5, including bone marrow biopsies on day 30, 100, and 1 year and 2 years post-transplant, with yearly follow-up through 5 years including data collection on survival and disease status.

5.5 Supportive Care

The following supportive care measures will be administered as per City of Hope Standard Operating Procedures:

- Dietary requirements
- Anti-infective prophylaxis for bacterial, viral, fungal and pneumocystis infections as per COH transplant SOP. *Exception:* to avoid renal toxicity, Bactrim should **not** be given during the pre-transplant period but should be administered only during the post transplant period as indicated by COH transplant SOP.
- Blood product support (including immunoglobulins)

5.6 Additional Studies

NA

5.6.1 Laboratory Studies

See Section 10.0 – Study Calendar for a complete list of required laboratory procedures. Studies or procedures that were performed for clinical indications within 30 days prior to registration (not

exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Lab values required for eligibility are listed in the Patient Eligibility section 3.1.

6.0 Dose Delays/Modifications

Patients, with a measured creatinine clearance <100 ml/min at screening, will receive a 25% dose reduction of their assigned clofarabine dose. For patients receiving the full planned assigned dose, if calculated creatinine clearance falls below 50 ml/min, subsequent doses of clofarabine in the 5 days of clofarabine treatment will be reduced by 25%. In either case if the calculated creatinine clearance falls below 30 ml/min, subsequent doses of clofarabine will be held and the case will be discussed with the PI.

7.0 Data and Safety Monitoring

7.1 Definition of Risk Level

This is a Risk Level 3 study, as defined in the "City of Hope Data and Safety Monitoring Plan", http://www.coh.org/dsmc/Pages/forms-and-procedures.aspx because it is a phase II clinical trial where the risks are at least balanced by the potential benefit to subjects and the importance of the knowledge that may result.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the PI, Collaborating Investigator, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of any stopping rules for safety and efficacy.

This study will utilize the Phase I tracking log to monitor data and safety for dose escalation, recording doses administered, and resultant adverse events *in the safety lead-in segment*. The tracking log will contain dose levels administered, DLT-defining adverse events, and documentation that the data from a dose level is complete before dose escalation. Those data and safety elements will be reported to the COH DSMC as applicable within the PMT report, which will be submitted quarterly or semi-annually from the anniversary date of activation, as noted in Table 1 below. Protocol specific data collection will include the following items: unacceptable toxicity (as defined in Section 12.1.1), progression-free survival, overall survival (OS), cumulative incidence of relapse/progression and non-relapse mortality, overall toxicity (Bearman Scale and CTCAE v4.0), incidence/severity of acute/chronic GVHD, and infection.

Table 1: City of Hope PMT Reporting Timelines for the DSMC

Risk Level	Phase	Standard Reporting Requirement
RL 1, RL2, and Expanded Access Studies	No reports required	
3	I	Every 3 months from activation date, as indicated in MIDAS

3	Pilot, Feasibility, II-IV	Every 6 months from activation date, as indicated in MIDAS
4	Pilot, Feasibility, I-IV	Every 3 months from activation date, as indicated in MIDAS

For the Phase II segment of the study, data and safety will be reported to the COH DSMC using the PMT report and submitted according to the timelines in Table 1 above. Protocol specific data collection will include the following items: progression-free survival, overall survival (OS), cumulative incidence of relapse/progression and non-relapse mortality, overall toxicity (Bearman Scale and CTCAE v4.0), incidence/severity of acute/chronic GVHD, and infection.

7.3 Definitions

Adverse event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Unexpected Adverse Event [21 CFR 312.32 (a)] - An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Serious Adverse Event (SAE) [21 CFR 312.32] - defined as *any expected or unexpected adverse event* that results in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in term nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

4.

7.4 Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems - Most unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf. Any unanticipated problem that occurs during the study conduct will be reported to the DSMC and IRB by submitting electronically in iRIS (http://iris.coh.org).

Serious Adverse Events - All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to definitions and guidelines at http://www.coh.org/policy/Policies%20and%20Procedures/REVIEWING_AND_REPORTING_UNANTICIPATED_PROBLEMS.pdf and Table 2 below. Those SAEs that require expedited reporting will be submitted electronically in iRIS (http://iris.coh.org).

Adverse Events - Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of *serious* OR are not unanticipated problems will be reported only in the protocol continuation reports and PMT report (see Table 2 below).

Table 2: City of Hope Adverse Event and Unanticipated Problem Reporting Timelines for the DSMC and IRB

DSMC Risk Level 3 and Risk Level 4 Protocol Reporting Timelines

Required Reporting Timeframe to the DSMC		
Attribution	**Unexpected**	**Expected**
Death while on active treatment or within 30 days of last day of treatment		
Possibly, Probably, Definitely	5 calendar days	
Unlikely, Unrelated		
Death after 30 days of last active treatment/therapy		
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
Within 30 days of last active treatment/therapy		

Required Reporting Timeframe to the DSMC		
Attribution	**Unexpected**	**Expected**
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	5 calendar days	10 calendar days
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	10 calendar days	10 calendar days
After 30 days of last active treatment/therapy		
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	10 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required

DSMC Risk Level 1 and Risk Level 2 Protocol Reporting Timelines

Required Reporting Timeframe to DSMC		
Attribution	**Unexpected**	**Expected**
	Death while on active treatment or within 30 days of last day of treatment	
Possibly, Probably, Definitely	5 calendar days	5 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Death after 30 days of last active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days	No reporting required
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required
	Grades 1 and 2 AND resulting in "hospitalization"	
Possibly, Probably, Definitely	5 calendar days	10 calendar days
Unlikely, Unrelated	No reporting required	No reporting required

COH IRB Adverse Event Reporting Timelines

Required Reporting Timeframe to COH IRB		
Attribution	**Unexpected**	**Expected**
	Death while on active treatment/therapy or within 30 days of the last day of active treatment/therapy	
Possibly, Probably, Definitely	5 calendar days[1]	Annual
Unlikely, Unrelated	Annual	Annual
	Grades 3 and 4	
Possibly, Probably, Definitely	5 calendar days[1]	Annual
Unlikely, Unrelated	Annual	Annual
	Grade 1 and 2	
Possibly, Probably, Definitely	5 calendar days[1]	Annual[2]
Unlikely, Unrelated	Annual[2]	Annual[2]

[1] These events must be reported in the time frame if they meet the definition of an unanticipated problem.
[2] For studies that are not first in human, Phase I and first in pediatric trials, only grades 3-5 must be reported at annual review.

7.5 Additional Pharmacovigilance requirements by Sanofi (clofarabine supplier)

7.5.1 **Definitions**

Serious adverse event (SAE): any untoward medical occurrence that at any dose:

- Results in death,
- Is life threatening, (Note: the term "life-threatening" refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/ reaction which hypothetically might have caused death if it were more severe),
- Requires inpatient hospitalization or results in prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect, or
- Is a medically important event or reaction. Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above.

Related Adverse Event, i.e. Adverse Drug Reaction (ADR): There is a reasonable possibility according to the IST/ISS sponsor that the product may have caused the event.

Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product or package insert/summary of product characteristics for an approved product). An expected ADR with a fatal outcome should be considered unexpected unless the local/regional product labeling specifically states that the ADR might be associated with a fatal outcome.

AESI: An adverse event of special interest (AESI) is an adverse event (serious or non-serious) of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor may be appropriate. Such events may require further investigation in order to characterize and understand them. AESIs may be added or removed during a study by protocol amendment.

7.5.2 **Obligations and responsibilities of the IST/ISS sponsor**

- The IST/ISS sponsor warrants that the study will be performed in compliance with all applicable local and international laws and regulations, including without limitation ICH E6 guidelines for Good Clinical Practices.
- The IST/ISS sponsor shall be responsible for the respect of all obligations required by applicable local and international laws and regulations.
- The sponsor shall be responsible for ensuring submission of required expedited and periodic reports to the appropriate Health Authority (HA), the Ethics Committee and investigators of each country participating in the IST/ISS (based on applicable regulations).
- The IST/ISS sponsor is responsible for providing any "Dear Investigator Letter" (DIL) for new safety finding received from Sanofi group entity to the investigators and Ethics Committee in each country participating in the study.
- The sponsor must report the following information in English to the Sanofi group entity Pharmacovigilance contact:
    1. Routine transmission of: Only related SAEs must be transmitted within 1 working day of the Investigator's awareness or identification of the event.
    2. Other events or periodic reports (e.g. Development Safety Update Report (DSUR)), submitted to Regulatory Authority must be transmitted at the time of submission.
    3. Other significant safety issues or findings in a study pertaining to safety of product must be transmitted within 1 working day. (e.g., Data Safety Monitoring Board recommendations)
    4. The study report of any IST/ISS must contain a section describing safety review and conclusion.

1. 5. The reference safety information to be used by the IST/ISS sponsor for evaluation of expectedness of adverse events shall be the current approved product label available in the country.

7.5.3 **Sanofi Group Entity Pharmacovigilance Contact**

IST/ISS Investigators will notify Sanofi via fax or email, attention Sanofi Pharmacovigilance (PV):

Fax: 908-203-7783

E-mail: USPVmailbox@sanofi-aventis.com.

8.0 Agent Information and Risks

8.1 Concomitant / Prohibited Drugs

Management of interacting medications, monitoring of whole blood concentrations and appropriate dosage adjustments of immunosuppressant drugs (tacrolimus and sirolimus) will be based on COH Hematology SOPs for GVHD prophylaxis.

8.1.1 Tacrolimus/Sirolimus-interacting: P450 inducers causing *decreased* blood levels

Rifampin, rifabutin, magnesium-aluminum-hydroxide, caspofungin, carbamazepine, phenobarbital, phenytoin, rifapentine, St. John's Wort (Hypericum perforatum)

8.1.2 Tacrolimus/Sirolimus-interacting: P450 inhibitors causing *increased* blood levels

Grapefruit juice, ketoconazole, itraconazole, posaconazole, erythromycin, telithromycin, clarithromycin, voriconazole, telaprevir, nelfinavir, boceprevir, bromocriptione, cimetidine, cisapride, clotrimazole, danazol, diltiazem, fluconazole, HIVprotease inhibitors (e.g., ritonavir, indinavir), metoclopramide, nicardipine, troleandomycin, verapamil

Due to extreme interactions with sirolimus, voriconazole is not recommended during sirolimus therapy. However, in the event of suspected or documented fungal infection, voriconazole may be used if deemed clinically necessary. In such cases, sirolimus dose should be reduced to 10% of the current dose, and tacrolimus dose should be reduced to 30- 50% of the current dose. Consulting with the pharmacist is highly recommended for immunosuppressant dose adjustment for drug-drug interactions.

8.1.3 Vaccination

Immunosuppressants may affect response to vaccination. Therefore, during treatment with tacrolimus and sirolimus vaccination may be less effective. The use of live vaccines should be avoided; live vaccines may include, but are not limited to, the following: measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid.

8.2 **Clofarabine** (from Clolar® full prescribing information)

8.2.1 Clofarabine Drug Description

Clofarabine (Clolar®) Injection is a purine nucleoside metabolic inhibitor indicated for the treatment of pediatric patients 1 to 21 years old with relapsed or refractory acute lympho blas tic leukemia after at least two prior regimens, which received initial US FDA approval in 2004. Drug structure and mechanism of action are detailed in the background section (2.1) of this protocol.

8.2.2 Toxicology

Clofarabine has not been tested for carcinogenic potential.

Clofarabine showed clastogenic activity in the *in vitro* mammalian cell chromosome aberration assay (CHO cells) and in the *in vivo* rat micronucleus assay. It did not show evidence of mutagenic activity in the bacterial mutation assay (Ames test).

Studies in mice, rats, and dogs have demonstrated dose-related adverse effects on male reproductive organs. Seminiferous tubule and testicular degeneration and atrophy were reported in male mice receiving

intraperitoneal (IP) doses of 3 mg/kg/day (9 mg/m$_2$/day, approximately 17% of clinical recommended dose on a mg/m$_2$ basis). The testes of rats receiving 25 mg/kg/day (150 mg/m$_2$/day, approximately 3 times the recommended clinical dose on a mg/m$_2$ basis) in a 6-month IV study had bilateral degeneration of the seminiferous epithelium with retained spermatids and atrophy of interstitial cells. In a 6-month IV dog study, cell degeneration of the epididymis and degeneration of the seminiferous epithelium in the testes were observed in dogs receiving 0.375 mg/kg/day (7.5 mg/m$_2$/day, approximately 14% of the clinical recommended dose on a mg/m$_2$ basis). Ovarian atrophy or degeneration and uterine mucosal apoptosis were observed in female mice at 75 mg/kg/day (225 mg/m$_2$/day, approximately 4-fold of recommended human dose on a mg/m$_2$ basis), the only dose administered to female mice. The effect on human fertility is unknown.

8.2.3 Adverse Reactions

The most common adverse reactions with clofarabine are (≥ 10%): nausea, vomiting, diarrhea, febrile neutropenia, headache, rash, pruritus, pyrexia, fatigue, palmar-plantar erythrodysesthesia syndrome, anxiety, flushing, and mucosal inflammation, gastrointestinal disorders (such as enterocolitis, neutropenic colitis, cecitis and C. difficile colitis).

The least common adverse reactions with clofarabine are (_<10%): Hemorrhage (may be fatal), metabolism and nutrition disorders (such as hyponatremia). Also, skin and subcutaneous tissue disorders (such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN)) may be fatal.

8.2.4 Warnings

Hematologic Toxicity

- Monitor complete blood counts and platelet counts during clofarabine therapy. (5.1)

Infections

- Clofarabine use is likely to increase the risk of infection, including severe sepsis, as a result of bone marrow suppression. Monitor patients for signs and symptoms of infection and treat promptly. (5.2)

Hyperuricemia (Tumor Lysis)

- Take precautions to prevent and monitor patients for signs and symptoms of tumor lysis syndrome, as well as signs and symptoms of cytokine release. (5.3)

Systemic Inflammatory Response Syndrome (SIRS) or Capillary Leak Syndrome

- Discontinue clofarabine immediately in the event of signs or symptoms of SIRS or Capillary Leak Syndrome
- SIRS and Capillary Leak Syndrome may occur. Evaluate and monitor patients undergoing treatment for signs and symptoms of cytokine release. Consider use of steroids. (5.4)

Hepatic Enzymes

- Monitor and discontinue treatment if necessary. (5.5)

Hepatic/Renal Impairment

- Use with caution in patients with hepatic or renal impairment. Monitor hepatic and renal function.

Use in Pregnancy

- Fetal harm can occur when administered to a pregnant woman. Women should be advised to avoid becoming pregnant when receiving clofarabine

8.2.5 Pharmacology – Handling, Storage, Dispensing and Disposal

The population pharmacokinetics of clofarabine were studied in 40 pediatric patients aged 2 to 19 years (21males/19 females) with relapsed or refractory acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML). At the given 52 mg/m^2 dose, similar concentrations were obtained over a wide range of body surface areas (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 28.8 L/h/m^2 and 172 L/m^2, respectively. The terminal half-life was 5.2 hours. No apparent difference in pharmacokinetics was observed between patients with ALL and AML or between males and females. No relationship between clofarabine or clofarabine triphosphate exposure and toxicity or response was found in this population.

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%). The pathways of non-hepatic elimination remain unknown. The pharmacokinetics of clofarabine have not been evaluated in patients with renal or hepatic dys function.

Clofarabine (Clolar®) Injection is supplied in single-use flint vials containing 20 mg of clofarabine in 20 mL of solution. Each box contains one Clolar® vial (NDC 58468-0100-1) or four Clolar® vials (NDC 58468-0100-2). The 20 mL flint vials contain 20 mL (20 mg) of solution. The pH range of the solution is 4.5 to 7.5.

Vials containing undiluted clofarabine should be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

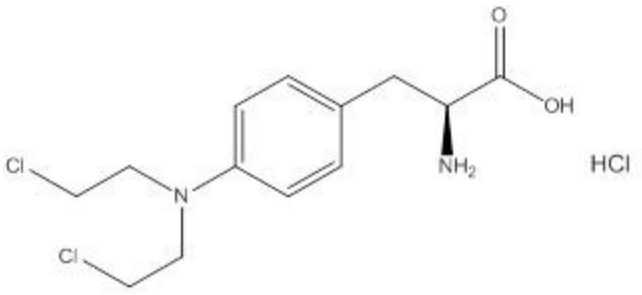
Diluted admixtures may be stored at room temperature, but must be used within 24 hours of preparation. Procedures for proper handling and disposal should be utilized. Handling and disposal of clofarabine should conform to guidelines issued for cytotoxic drugs.

8.3 Melphalan (from Evomela® full prescribing information)

This formulation is currently being used as of this amendment.

8.3.1 Melphalan Description

Melphalan (Evomela®) contains melphalan hydrochloride, an alkylating drug, as the active ingredient. The chemical name of melphalan hydrochloride is 4-[bis(2-chloroethyl)amino]-Lphenylalanine hydrochloride. Its molecular formula is $C_{13}H_{18}Cl_2N_2O_2$ • HCL and the molecular weight is 341.67. The structural formula is:



Melphalan hydrochloride is a white to off-white powder, with a melting range of 199°C - 201°C. It is practically insoluble in water, but freely soluble in 1 N HCL and methanol.

Evomela (melphalan) for injection is supplied as a sterile white to off-white lyophilized powder in a single dose vial for intravenous use. Each vial contains 50 mg melphalan free base equivalent to 56 mg melphalan hydrochloride and 2700 mg Betadex Sulfobutyl Ether Sodium, NF..

8.3.2 Toxicology

Carcinogenesis, Mutagenesis, Impairment of Fertility: Adequate and well-controlled carcinogenicity studies have not been conducted in animals. However, intraperitoneal (IP) administration of melphalan in rats (5.4 to 10.8 mg/m2) and in mice (2.25 to 4.5 mg/m2) 3 times per week for 6 months followed by 12 months post-dose observation produced peritoneal sarcoma and lung tumors, respectively.

Intramuscular administration of melphalan at 6 and 60 mg/m² produced structural aberrations of the chromatid and chromosomes in bone marrow cells of Wistar rats.

8.3.3 Adverse Reactions

Hematologic: The most common side effect is bone marrow suppression. For patients receiving Evomela as part of a conditioning regimen, myeloablation occurs in all patients. Do not begin the conditioning regimen if a stem cell product is not available for rescue. Monitor complete blood counts, provide supportive care for infections, anemia and thrombocytopenia until there is adequate hematopoietic recovery.

For patients receiving Evomela as palliative treatment, if the bone marrow has been compromised by prior irradiation, prior chemotherapy or is recovering from chemotherapy, the risk of severe myelosuppression with Evomela is increased. Perform periodic complete blood counts during the course of treatment with Evomela. Provide supportive care for infections, bleeding, and symptomatic anemia.

Gastrointestinal: For patients receiving Evomela as part of a conditioning regimen, nausea, vomiting, mucositis, and diarrhea may occur in over 50% of patients. Use prophylactic antiemetic medication. Provide supportive care for nausea, vomiting, diarrhea, and mucositis. The frequency grade 3 / 4 mucositis in clinical studies was 13%. Provide nutritional support and analgesics for patients with severe mucositis.

For patients receiving Evomela as palliative treatment, nausea and vomiting, diarrhea, and oral ulceration may occur. Use prophylactic antiemetics. Provide supportive care for nausea, vomiting, diarrhea and mucositis.

Hepatotoxicity: Hepatic disorders ranging from abnormal liver function tests to clinical manifestations such as hepatitis and jaundice have been reported after treatment with melphalan. Hepatic veno-occlusive disease has also been reported. Monitor liver chemistries.

Hypersensitivity: Acute hypersensitivity reactions including anaphylaxis have occurred in approximately 2% of patients who received an intravenous formulation of melphalan. Symptoms may include urticarial, pruritis, edema, and skin rashes and, in some patients, tachycardia, bronchospasm, dyspnea, and hypotension. Discontinue treatment with Evomela for serious hypersensitivity reactions.

Secondary Malignancies: Melphalan has been shown to cause chromatid or chromosome damage in humans. Secondary malignancies such as myeloproliferative syndrome or acute leukemia have been reported in multiple myeloma patients treated with melphalan-containing chemotherapy regimens. The potential benefit of Evomela therapy must be considered against the possible risk of the induction of a secondary malignancy.

Embryo-Fetal Toxicity: Based on its mechanism of action, Evomela can cause fetal harm when administered to a pregnant woman. Melphalan is genotoxic, targets actively dividing cells, and was embryolethal and teratogenic in rats. Advise females of reproductive potential to avoid pregnancy during and after treatment with Evomela. If this drug is used during pregnancy or if the patient becomes pregnant while taking this drug, advise the patient of potential risk to the fetus.

Infertility: Melphalan-based chemotherapy regimens have been reported to cause suppression of ovarian function in premenopausal women, resulting in persistent amenorrhea in approximately 9% of patients. Reversible or irreversible testicular suppression has also been reported.

Miscellaneous: Other reported adverse reactions include diarrhea, nausea, fatigue, hypokalemia, vomiting, hypophosphatemia, decreased appetite, pyrexia, constipation, febrile neutropenia, mucosal inflammation, dizziness, edema peripheral, stomatitis, abdominal pain, dysgeusia, The most common serious adverse reactions were pyrexia, hematochezia, febrile neutropenia, and renal failure.

8.3.4 Pharmacology – Handling, Storage, Dispensing and Disposal

Evomela is supplied in a single carton containing one (1) vial. Each 50 mg vial contains a white to off-white lyophilized powder in single-dose vial for reconstitution (after reconstitution the solution is clear and colorless to light yellow). Each vial contains 50 mg melphalan free base equivalent to 56 mg melphalan hydrochloride.

NDC 68152-109-00: Individual carton of Evomela 20 mL single-dose vial containing 50 mg melphalan free base.

Store at controlled room temperature 15° to 30°C (59° to 86°F) and protect from light.

Parenteral drug products should be visually inspected for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs, do not use this product.

Retain in original carton until use.

Melphalan is a cytotoxic drug. Follow special handling and disposal procedures.

Preparation and Administration

Evomela is a cytotoxic drug. Follow applicable special handling and disposal procedures.

Evomela is light sensitive. Retain in original carton until use.

Do not mix Evomela with other melphalan hydrochloride for injection drug products.

Reconstitution and Infusion Instructions:

1. Use normal saline (0.9% Sodium Chloride Injection, USP) (8.6 mL as directed) to reconstitute Evomela and make a 50 mg/10 mL (5 mg/ mL) nominal concentration of melphalan. The normal saline used to reconstitute each vial should appears to be assisted or pulled into the vial by the negative pressure (partial vacuum) present in the vial. Discard any vial (and replace with another vial) if there is no vacuum present when reconstituting the vial with normal saline.

The reconstituted Evomela drug product is stable for 24 hours at refrigerated temperature (5°C) without any precipitation due to the high solubility.

The reconstituted Evomela drug product is stable for 1 hour at room temperature.

2. Calculate the required volume of Evomela needed for a patient's dose and withdraw that volume from the vial(s).

3. Add the required volume of Evomela to the appropriate volume of 0.9% Sodium Chloride Injection, USP to a final concentration of 0.45 mg/mL. The Evomela admixture solution is stable for 4 hours at room temperature in addition to the 1 hour following reconstitution.

4. Infuse over 30 minutes via an injection port or central venous catheter.

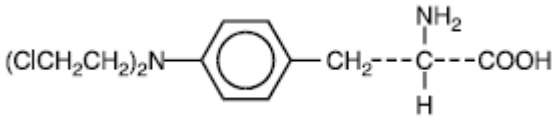
Evomela may cause local tissue damage should extravasation occur. Do not administer by direct injection into a peripheral vein. Administer Evomela by injecting slowly into a fast-running IV infusion via a central venous access line.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

8.4 Melphalan (from Alkeran® full prescribing information) This formulation was used with previous patients enrolled prior to this amendment.

8.4.1 Melphalan Description

Melphalan (Alkeran®), also known as L-phenylalanine mustard, phenylalanine mustard, L-PAM, or L-sarcolysin, is a phenylalanine derivative of nitrogen mustard. Melphalan is a bifunctional alkylating agent that is active against selected human neoplastic diseases. It is known chemically as 4-[bis(2-chloroethyl)amino]-*L*-phenylalanine. The molecular formula is $C_{13}H_{18}Cl_2N_2O_2$ and the molecular weight is 305.20. The structural formula is:



Melphalan is an alkylating agent of the bischloroethylamine type. As a result, its cytotoxicity appears to be related to the extent of its interstrand cross-linking with DNA, probably by binding at the N_7 position of guanine. Like other bifunctional alkylating agents, it is active against both resting and rapidly dividing tumor cells.

8.4.2 Toxicology

Carcinogenesis: Secondary malignancies, including acute nonlymphocytic leukemia, myeloproliferative syndrome, and carcinoma, have been reported in patients with cancer treated with alkylating agents (including melphalan). Some patients also received other chemotherapeutic agents or radiation therapy. Precise quantitation of the risk of acute leukemia, myeloproliferative syndrome, or carcinoma is not possible. Published reports of leukemia in patients who have received melphalan (and other alkylating agents) suggest that the risk of leukemogenesis increases with chronicity of treatment and with cumulative dose. In one study, the 10-year cumulative risk of developing acute leukemia or myeloproliferative syndrome after oral melphalan therapy was 19.5% for cumulative doses ranging from 730 to 9,652 mg. In this same study, as well as in an additional study, the 10-year cumulative risk of developing acute leukemia or myeloproliferative syndrome after oral melphalan therapy was less than 2% for cumulative doses under 600 mg. This does not mean that there is a cumulative dose below which there is no risk of the induction of secondary malignancy. The potential benefits from melphalan therapy must be weighed on an individual basis against the possible risk of the induction of a second malignancy.

Adequate and well-controlled carcinogenicity studies have not been conducted in animals. However, intraperitoneal (IP) administration of melphalan in rats (5.4 to 10.8 mg/m2) and in mice (2.25 to 4.5 mg/m2) 3 times per week for 6 months followed by 12 months post-dose observation produced peritoneal sarcoma and lung tumors, respectively.

Mutagenesis: Melphalan has been shown to cause chromatid or chromosome damage in humans. Intramuscular administration of melphalan at 6 and 60 mg/m^2 produced structural aberrations of the chromatid and chromosomes in bone marrow cells of Wistar rats.

Impairment of Fertility: Melphalan causes suppression of ovarian function in premenopausal women, resulting in amenorrhea in a significant number of patients. Reversible and irreversible testicular suppression have also been reported.

Pregnancy: Pregnancy Category D. Melphalan may cause fetal harm when administered to a pregnant woman. While adequate animal studies have not been conducted with IV melphalan, oral (6 to 18 mg/m^2/day for 10 days) and IP (18 mg/m^2) administration in rats was embryolethal and teratogenic. Malformations resulting from melphalan included alterations of the brain (underdevelopment, deformation, meningocele, and encephalocele) and eye (anophthalmia and microphthalmos), reduction of the mandible and tail, as well as hepatocele (exomphaly). There are no adequate and well-controlled studies in pregnant women. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

8.4.3 Adverse Reactions

Hematologic: The most common side effect is bone marrow suppression. White blood cell count and platelet count nadirs usually occur 2 to 3 weeks after treatment, with recovery in 4 to 5 weeks after treatment. Irreversible bone marrow failure has been reported.

Gastrointestinal: Gastrointestinal disturbances such as nausea and vomiting, diarrhea, and oral ulceration occur infrequently. Hepatic disorders ranging from abnormal liver function tests to clinical manifestations such as hepatitis and jaundice have been reported. Hepatic veno-occlusive disease has been reported.

Hypersensitivity: Acute hypersensitivity reactions including anaphylaxis were reported in 2.4% of 425 patients receiving ALKERAN for Injection for myeloma (see WARNINGS). These reactions were characterized by urticaria, pruritus, edema, and in some patients, tachycardia, bronchospasm, dyspnea, and hypotension. These patients appeared to respond to antihistamine and corticosteroid therapy. If a

hypersensitivity reaction occurs, IV or oral melphalan should not be readministered since hypersensitivity reactions have also been reported with oral melphalan.

Miscellaneous: Other reported adverse reactions include skin hypersensitivity, skin ulceration at injection site, skin necrosis rarely requiring skin grafting, vasculitis, alopecia, hemolytic anemia, allergic reaction, pulmonary fibrosis, and interstitial pneumonitis.

8.4.4 Warnings

As with other nitrogen mustard drugs, excessive dosage will produce marked bone marrow suppression. Bone marrow suppression is the most significant toxicity associated with melphalan in most patients. Therefore, the following tests should be performed at the start of therapy and prior to each subsequent dose of melphalan: platelet count, hemoglobin, white blood cell count, and differential. Thrombocytopenia and/or leukopenia are indications to withhold further therapy until the blood counts have sufficiently recovered. Frequent blood counts are essential to determine optimal dosage and to avoid toxicity. Dose adjustment on the basis of blood counts at the nadir and day of treatment should be considered.

Hypersensitivity reactions including anaphylaxis have occurred in approximately 2% of patients who received the IV formulation. These reactions usually occur after multiple courses of treatment. Treatment is symptomatic. The infusion should be terminated immediately, followed by the administration of volume expanders, pressor agents, corticosteroids, or antihistamines at the discretion of the physician. If a hypersensitivity reaction occurs, IV or oral melphalan should not be readministered since hypersensitivity reactions have also been reported with oral melphalan.

Carcinogenesis: Secondary malignancies, including acute nonlymphocytic leukemia, myeloproliferative syndrome, and carcinoma, have been reported in patients with cancer treated with alkylating agents (including melphalan). See Toxicology Section 8.2.2 for more details.

Mutagenesis: Melphalan has been shown to cause chromatid or chromosome damage in humans. Intramuscular administration of melphalan at 6 and 60 mg/m^2 produced structural aberrations of the chromatid and chromosomes in bone marrow cells of Wistar rats.

Impairment of Fertility: Melphalan causes suppression of ovarian function in premenopausal women, resulting in amenorrhea in a significant number of patients. Reversible and irreversible testicular suppression have also been reported.

Pregnancy: Pregnancy Category D. Melphalan may cause fetal harm when administered to a pregnant woman. See Toxicology Section 8.2.2 for more details.

8.4.5 Pharmacology – Handling, Storage, Dispensing and Disposal

The pharmacokinetics of melphalan after IV administration has been extensively studied in adult patients. Following injection, drug plasma concentrations declined rapidly in a biexponential manner with distribution phase and terminal elimination phase half-lives of approximately 10 and 75 minutes, respectively. Estimates of average total body clearance varied among studies, but typical values of approximately 7 to 9 mL/min/kg (250 to 325 mL/min/m^2) were observed. One study has reported that on repeat dosing of 0.5 mg/kg every 6 weeks, the clearance of melphalan decreased from 8.1 mL/min/kg after the first course, to 5.5 mL/min/kg after the third course, but did not decrease appreciably after the third course. Mean (±SD) peak melphalan plasma concentrations in myeloma patients given IV melphalan at doses of 10 or 20 mg/m^2 were 1.2 ± 0.4 and 2.8 ± 1.9 mcg/mL, respectively.

The steady-state volume of distribution of melphalan is 0.5 L/kg. Penetration into cerebrospinal fluid (CSF) is low. The extent of melphalan binding to plasma proteins ranges from 60% to 90%. Serum albumin is the major binding protein, while ⟨$_1$-acid glycoprotein appears to account for about 20% of the

plasma protein binding. Approximately 30% of the drug is (covalently) irreversibly bound to plasma proteins. Interactions with immunoglobulins have been found to be negligible.

Melphalan is eliminated from plasma primarily by chemical hydrolysis to monohydroxymelphalan and dihydroxymelphalan. Aside from these hydrolysis products, no other melphalan metabolites have been observed in humans. Although the contribution of renal elimination to melphalan clearance appears to be low, one study noted an increase in the occurrence of severe leukopenia in patients with elevated BUN after 10 weeks of therapy.

ALKERAN for Injection is supplied in a carton containing one single-use clear glass vial of freeze-dried melphalan hydrochloride equivalent to 50 mg melphalan and one 10-mL clear glass vial of sterile diluent (NDC 0173-0130-93).

Store at controlled room temperature 15° to 30°C (59° to 86°F) and protect from light.

Parenteral drug products should be visually inspected for particulate matter and discoloration prior to administration whenever solution and container permit. If either occurs, do not use this product.

Preparation for Administration/Stability

1. ALKERAN for Injection must be reconstituted by rapidly injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder using a sterile needle (20-gauge or larger needle diameter) and syringe. Immediately shake vial vigorously until a clear solution is obtained. This provides a 5-mg/mL solution of melphalan. Rapid addition of the diluents followed by immediate vigorous shaking is important for proper dissolution.

2. Infuse undiluted over 20 minutes.

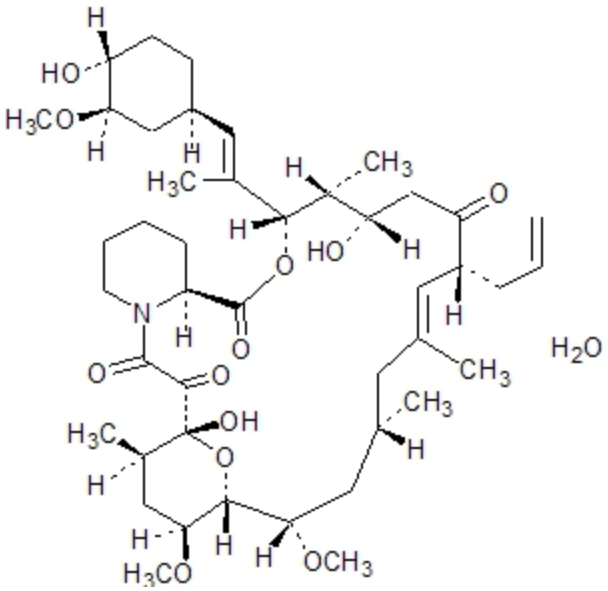
The time between reconstitution/dilution and administration of ALKERAN should be kept to a minimum because reconstituted and diluted solutions of ALKERAN are unstable. Over as short a time as 30 minutes, a citrate derivative of melphalan has been detected in reconstituted material from the reaction of ALKERAN with Sterile Diluent for ALKERAN. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolyzes every 10 minutes.

A precipitate forms if the reconstituted solution is stored at 5°C. DO NOT REFRIGERATE THE RECONSTITUTED PRODUCT.

8.5 Tacrolimus (from Prograf® full prescribing information)

8.5.1 Tacrolimus Description

Tacrolimus, a calcineurin inhibitor previously known as FK506, is a macrolide immunosuppressant produced by *Streptomyces tsukubaensis.* Tacrolimus has an empirical formula of $C_{44}H_{69}NO_{12}•H_2O$ and a formula weight of 822.03. The chemical structure of tacrolimus is:



Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental vidence 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 (i.e., immunosuppression).

Tacrolimus prolongs the survival of the host and transplanted graft in animal transplant models of liver, kidney, heart, bone marrow, small bowel and pancreas, lung and trachea, skin, cornea, and limb.

In animals, tacrolimus has been demonstrated to suppress some humoral immunity and, to a greater extent, cell-mediated reactions such as allograft rejection, delayed type hypersensitivity, collagen-induced arthritis, experimental allergic encephalomyelitis, and graft versus host disease.

8.5.2 Toxicology

Carcinogenicity studies were conducted in male and female rats and mice. In the 80-week mouse study and in the 104-week rat study, no relationship of tumor incidence to tacrolimus dosage was found. The highest doses used in the mouse and rat studies were 1.2 to 3.3 times (mice) and 4.0 to 10.8 times (rats) the clinical dose range of 0.075 to 0.2 mg/kg/day when corrected for body surface area.

No evidence of genotoxicity was seen in bacterial (*Salmonella* and *E. coli*) or mammalian (Chinese hamster lung-derived cells) in vitro assays of mutagenicity, the in vitro CHO/HGPRT assay of mutagenicity, or in vivo clastogenicity assays performed in mice; tacrolimus did not cause unscheduled DNA synthesis in rodent hepatocytes.

Tacrolimus given orally at 1.0 mg/kg (0.8 to 2.2 times the clinical dose range of 0.075 to 0.2 mg/kg/day based on body surface area) to male and female rats, prior to and during mating, as well as to dams during gestation and lactation, was associated with embryolethality and adverse effects on female reproduction. Effects on female reproductive function (parturition) and embryolethal effects were indicated by a higher rate of pre-implantation loss and increased numbers of undelivered and nonviable pups. When given at 3.2 mg/kg (2.6 to 6.9 times the clinical dose range based on body surface area), tacrolimus was associated with maternal and paternal toxicity as well as reproductive toxicity including marked adverse effects on estrus cycles, parturition, pup viability, and pup malformations.

8.5.3 Adverse Reactions

- Kidney Transplant: The most common adverse reactions (≥ 30%) were infection, tremor, hypertension, abnormal renal function, constipation, diarrhea, headache, abdominal pain, insomnia, nausea, hypomagnesemia, urinary tract infection, hypophosphatemia, peripheral edema, asthenia, pain, hyperlipidemia, hyperkalemia, anemia
- Liver Transplant: The most common adverse reactions (≥ 40%) were tremor, headache, diarrhea, hypertension, nausea, abnormal renal function, abdominal pain, insomnia, paresthesia, anemia, pain, fever, asthenia, hyperkalemia, hypomagnesemia, and hyperglycemia
- Heart Transplant: The most common adverse reactions (≥ 15%) were abnormal renal function, hypertension, diabetes mellitus, CMV infection, tremor, hyperglycemia, leukopenia, infection, anemia, bronchitis, pericardial effusion, urinary tract infection and hyperlipemia

8.5.4 Warnings

- Lymphoma and Other Malignancies: Risk of lymphomas, including post transplant lymphoproliferative disorder (PLTD); appears related to intensity and duration of use. Avoid prolonged exposure to UV light and sunlight.
- Serious infections: Increased risk of bacterial, viral, fungal and protozoal infections, including opportunistic infections: combination immunosuppression should be used with caution
- Polyoma Virus Infections: Serious, sometimes fatal outcomes, including polyoma virus-associated nephropathy (PVAN), mostly due to BK virus, and JC virus-associated progressive multifocal leukoencephalopathy (PML); consider reducing immunosuppression
- Cytomegalovirus (CMV) Infections: Increased risk of CMV viremia and disease; consider reducing immunosuppression
- New Onset Diabetes After Transplant: Monitor blood glucose
- Nephrotoxicity: Acute and/or chronic; reduce the dose; use caution with other nephrotoxic drugs
- Neurotoxicity: Risk of Posterior Reversible Encephalopathy Syndrome, monitor for neurologic abnormalities; reduce or discontinue tacrolimus and other immunosuppressants
- Hyperkalemia: Monitor serum potassium levels. Careful consideration should be given prior to use of other agents also associated with hyperkalemia
- Hypertension: May require antihypertensive therapy. Monitor relevant drug-drug interactions
- Anaphylactic Reactions with IV formulation: Observe patients receiving tacrolimus for signs and symptoms of anaphylaxis
- Use with Sirolimus:. For GVHD prophylaxix in hematopoietic cell transplant, use with sirolimus may increase the risk of thrombotic microangiopathy (TMA) and levels should be monitored carefully
- Myocardial Hypertrophy: Consider dosage reduction or discontinuation
- Immunizations: Use of live vaccines should be avoided
- Pure Red Cell Aplasia: Discontinuation should be considered

8.5.5 Pharmacology – Handling, Storage, Dispensing and Disposal

Pharmacokinetic parameters (mean ± SD) of tacrolimus in healthy human volunteers

Population	N	Route (Dose)	Parameters					
			C_{max} (ng/mL)	T_{max} (hr)	AUC (ng•hr/mL)	$t_{1/2}$ (hr)	CI (L/hr/kg)	V (L/kg)
Healthy Volunteers	8	IV (0.025 mg/kg/4hr)	[a]	[a]	598[b] ± 125	34.2 ± 7.7	0.040 ± 0.009	1.91 ± 0.31
	16	PO (5 mg)	29.7 ± 7.2	1.6 ± 0.7	243[c] ± 73	34.8 ± 11.4	0.041[d] ± 0.008	1.94[d] ± 0.53

Absorption of tacrolimus from the gastrointestinal tract after oral administration is incomplete and variable. The absolute bioavailability of tacrolimus was 17±10% in adult kidney transplant patients (N=26), 22±6% in adult liver transplant patients (N=17), 23±9% in adult heart transplant patients (N=11) and 18±5% in healthy volunteers (N=16).

The rate and extent of tacrolimus absorption were greatest under fasted conditions. The presence and composition of food decreased both the rate and extent of tacrolimus absorption when administered to 15 healthy volunteers. The effect was most pronounced with a high-fat meal (848 kcal, 46% fat): mean AUC and C_{max} were decreased 37% and 77%, respectively; T_{max} was lengthened 5-fold. A high-carbohydrate meal (668 kcal, 85% carbohydrate) decreased mean AUC and mean C_{max} by 28% and 65%, respectively. Tacrolimus capsules should be taken consistently every day either with or without food because the presence and composition of food decreases the bioavailability of tacrolimus.

Tacrolimus is extensively metabolized by the mixed-function oxidase system, primarily the cytochrome P-450 system (CYP3A). The major metabolite identified in incubations with human liver microsomes is 13-demethyl tacrolimus. In in vitro studies, a 31-demethyl metabolite has been reported to have the same activity as tacrolimus.

The mean clearance following IV administration of tacrolimus is 0.040, 0.083, and 0.053, and 0.051 L/hr/kg in healthy volunteers, adult kidney transplant patients, adult liver transplant patients, and adult heart transplant patients, respectively. In man, less than 1% of the dose administered is excreted unchanged in urine.

Tacrolimus for IV must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a concentration between 0.004 mg/mL and 0.02 mg/mL prior to use. Diluted infusion solution should be stored in glass or polyethylene containers and should be discarded after 24 hours. The diluted infusion solution should not be stored in a PVC container due to decreased stability and the potential for extraction of phthalates. In situations where more dilute solutions are utilized (e.g., pediatric dosing, etc.), PVC-free tubing should likewise be used to minimize the potential for significant drug adsorption onto the tubing.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Due to the chemical instability of tacrolimus in alkaline media, tacorlimus should not be mixed or co-infused with solutions of pH 9 or greater (e.g., ganciclovir or acyclovir).

The initial dose of tacrolimus is 0.02 mg/kg/day by continuous infusion to start on day minus three. The orders should stipulate that the tacrolimus infusion should not be interrupted for more than one hour per day. When the patient has recovered from the gastrointestinal toxicity of the conditioning regimen and can tolerate and absorb oral medications, tacrolimus should be converted to oral formulation. When converting patients from IV to oral, the dose conversion ratio is approximately 1:3 (IV:oral) divided into 2 doses. Oral tacrolimus is supplied in 0.5 and 1 mg capsules and the dose must be rounded to the nearest 0.5 mg.

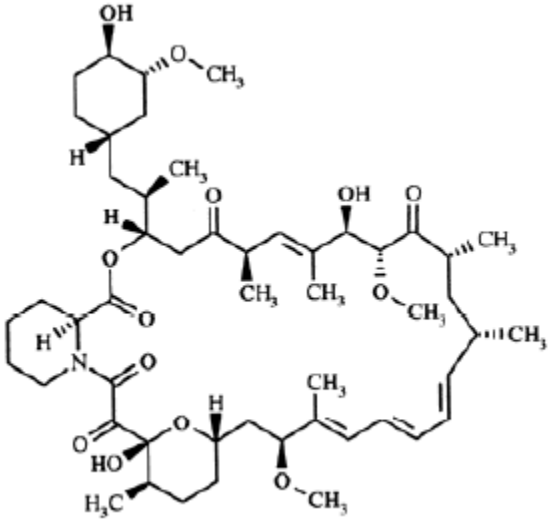
Tacrolimus dose is adjusted to maintain a whole blood trough plasma concentration of 5 - 10 ng/ml when used in combination with sirolimus. Sequentially rising levels (especially if levels are approaching 10 ng/ml) and the presence of symptoms of toxicity may be indications for dose reduction. Management of interacting medications, monitoring of whole blood concentrations and appropriate dosage adjustments of immunosuppressant drugs (tacrolimus and sirolimus) will be based on Hematology SOPs for GVHD prophylaxis.

8.6 **Sirolimus** (from Rapamune® full prescribing information)

8.6.1 Sirolimus Description

Rapamune (sirolimus) is an immunosuppressive agent. Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus*. Its molecular formula is C51H79NO13 and its molecular weight is 914.2.

The structural formula of sirolimus is illustrated as follows.



Sirolimus inhibits T-lymphocyte activation and proliferation that occurs in response to antigenic and cytokine (Interleukin [IL]-2, IL-4, and IL-15) stimulation by a mechanism that is distinct from that of other immunosuppressants. Sirolimus also inhibits antibody production. In cells, sirolimus binds to the immunophilin, FK Binding Protein-12 (FKBP-12), to generate an immunosuppressive complex. The sirolimus:FKBP-12 complex has no effect on calcineurin activity. This complex binds to and inhibits the activation of the mammalian Target Of Rapamycin (mTOR), a key regulatory kinase. This inhibition suppresses cytokine-driven T-cell proliferation, inhibiting the progression from the G1 to the S phase of the cell cycle.

8.6.2 Toxicology

Carcinogenicity studies were conducted in mice and rats. In an 86-week female mouse study at sirolimus doses 30 to 120 times higher than the 2 mg daily clinical (adjusted for body surface area), there was a statistically significant increase in malignant lymphoma at all dose levels compared with controls. In a second mouse study at dosages that were approximately 3 to 16 times the clinical dose (adjusted for body surface area), hepatocellular adenoma and carcinoma in males were considered sirolimus-related. In the 104-week rat study at dosages equal to or lower than the clinical dose of 2 mg daily (adjusted for body surface area), there were no significant findings.

Sirolimus was not genotoxic in the *in vitro* bacterial reverse mutation assay, the Chinese hamster ovary cell chromosomal aberration assay, the mouse lymphoma cell forward mutation assay, or the *in vivo* mouse micronucleus assay.

Fertility was diminished slightly in both male and female rats following oral administration of sirolimus at doses approximately 10 times or 2 times, respectively, the clinical dose of 2 mg daily (adjusted for body surface area). In male rats, atrophy of testes, epididymides, prostate, seminiferous tubules and/or reduction in sperm counts were observed. In female rats, reduced size of ovaries and uteri was observed. Reduction of sperm count in male rats was reversible upon cessation of dosing in one study. Testicular tubular degeneration was also seen in a 4-week intravenous study of sirolimus in monkeys at doses that were approximately equal to the clinical dose (adjusted for body surface area).

8.6.3 Adverse Reactions

The most common (> 30%) adverse reactions are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, creatinine increased, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, arthralgia, pain, and thrombocytopenia

8.6.4 Warnings

Increased Susceptibility to Infection and the Possible Development of Lymphoma. Increased susceptibility to infection and the possible development of lymphoma and other malignancies, particularly of the skin, may result from immunosuppression. The rates of lymphoma/lymphoproliferative disease observed in Studies 1 and 2 were 0.7-3.2% (for sirolimus-treated patients) versus 0.6-0.8% (azathioprine and placebo control). Oversuppression of the immune system can also increase susceptibility to infection, including opportunistic infections such as tuberculosis, fatal infections, and sepsis. Only physicians experienced in immunosuppressive therapy and management of organ transplant patients should use sirolimus. Patients receiving the drug should be managed in facilities equipped and staffed with adequate laboratory and supportive medical resources. The physician responsible for maintenance therapy should have complete information requisite for the follow-up of the patient.

Hypersensitivity Reactions. Hypersensitivity reactions, including anaphylactic/anaphylactoid reactions, angioedema, exfoliative dermatitis and hypersensitivity vasculitis, have been associated with the administration of sirolimus.

Angioedema. Sirolimus has been associated with the development of angioedema. The concomitant use of sirolimus with other drugs known to cause angioedema, such as ACE-inhibitors, may increase the risk of developing angioedema.

Fluid Accumulation and Wound Healing. There have been reports of impaired or delayed wound healing in patients receiving sirolimus, including lymphocele and wound dehiscence. mTor inhibitors such as sirolimus have been shown *in vitro* to inhibit production of certain growth factors that may affect angiogenesis, fibroblast proliferation, and vascular permeability. Lymphocele, a known surgical complication of renal transplantation, occurred significantly more often in a dose-related fashion in patients treated with Rapamune. Appropriate measures should be considered to minimize such complications. Patients with a body mass index (BMI) greater than 30 kg/m^2 may be at increased risk of abnormal wound healing based on data from the medical literature. There have also been reports of fluid accumulation, including peripheral edema, lymphedema, pleural effusion and pericardial effusions (including hemodynamically significant effusions and tamponade requiring intervention in children and adults), in patients receiving sirolimus.

Hyperlipidemia. Increased serum cholesterol and triglycerides requiring treatment occurred more frequently in patients treated with sirolimus compared with azathioprine or placebo controls. There were increased incidences of hypercholesterolemia (43-46%) and/or hypertriglyceridemia (45-57%) in patients receiving sirolimus compared with placebo controls (each 23%). The risk/benefit should be carefully

considered in patients with established hyperlipidemia before initiating an immunosuppressive regimen including sirolimus. Any patient who is administered sirolimus should be monitored for hyperlipidemia. If detected, interventions such as diet, exercise, and lipid-lowering agents should be initiated as outlined by the National Cholesterol Education Program guidelines. In clinical trials, the concomitant administration of sirolimus and HMG-CoA reductase inhibitors and/or fibrates appeared to be well-tolerated. During Rapamune therapy with cyclosporine, patients administered an HMG-CoA reductase inhibitor and/or fibrate should be monitored for the possible development of rhabdomyolysis and other adverse effects, as described in the respective labeling for these agents.

Renal Function. Renal function should be closely monitored during the co-administration of sirolimus with cyclosporine, because long-term administration of the combination has been associated with

deterioration of renal function. Patients treated with cyclosporine and sirolimus were noted to have higher serum creatinine levels and lower glomerular filtration rates compared with patients treated with cyclosporine and placebo or azathioprine controls. The rate of decline in renal function in these studies was greater in patients receiving sirolimus and cyclosporine compared with control therapies. Appropriate adjustment of the immunosuppressive regimen, including discontinuation of sirolimus and/or cyclosporine, should be considered in patients with elevated or increasing serum creatinine levels. In patients at low-to-moderate immunologic risk, continuation of combination therapy with cyclosporine beyond 4 months following transplantation should only be considered when the benefits outweigh the risks of this combination for the individual patients. Caution should be exercised when using agents (e.g., aminoglycosides and amphotericin B) that are known to have a deleterious effect on renal function. In patients with delayed graft function, sirolimus may delay recovery of renal function.

Proteinuria. Periodic quantitative monitoring of urinary protein excretion is recommended. In a study evaluating conversion from calcineurin inhibitors (CNI) to sirolimus in maintenance renal transplant patients 6-120 months post-transplant, increased urinary protein excretion was commonly observed from 6 through 24 months after conversion to sirolimus compared with CNI continuation. Patients with the greatest amount of urinary protein excretion prior to sirolimus conversion were those whose protein excretion increased the most after conversion. New onset nephrosis (nephrotic syndrome) was also reported as a treatment emergent adverse event in 2.2% of the sirolimus conversion group patients in comparison to 0.4% in the CNI continuation group of patients. Nephrotic range proteinuria (defined as urinary protein to creatinine ratio > 3.5) was also reported in 9.2% in the sirolimus conversion group of patients in comparison to 3.7% in the CNI continuation group of patients. In some patients, reduction in the degree of urinary protein excretion was observed for individual patients following discontinuation of sirolimus. The safety and efficacy of conversion from calcineurin inhibitors to sirolimus in maintenance renal transplant patients have not been established.

Interstitial Lung Disease. Cases of interstitial lung disease (including pneumonitis, bronchiolitis obliterans organizing pneumonia [BOOP], and pulmonary fibrosis), some fatal, with no identified infectious etiology have occurred in patients receiving immunosuppressive regimens including sirolimus. In some cases, the interstitial lung disease has resolved upon discontinuation or dose reduction of sirolimus. The risk may be increased as the trough sirolimus concentration increases.

Increased Risk of Calcineurin Inhibitor-Induced Hemolytic Uremic Syndrome/Thrombotic Thrombocytopenic Purpura/Thrombotic Microangiopathy (HUS/TTP/TMA). The concomitant use of sirolimus with a calcineurin inhibitor may increase the risk of calcineurin inhibitor-induced hemolytic uremic syndrome/thrombotic thrombocytopenic purpura/thrombotic microangiopathy (HUS/TTP/TMA).

Antimicrobial Prophylaxis. Cases of *Pneumocystis carinii* pneumonia have been reported in patients not receiving antimicrobial prophylaxis. Therefore, antimicrobial prophylaxis for *Pneumocystis carinii* pneumonia should be administered for 1 year following transplantation. Cytomegalovirus (CMV) prophylaxis is recommended for 3 months after transplantation, particularly for patients at increased risk for CMV disease.

Skin Cancer Events. Patients on immunosuppressive therapy are at increased risk for skin cancer. Exposure to sunlight and ultraviolet (UV) light should be limited by wearing protective clothing and using a sunscreen with a high protection factor.

8.6.5 Pharmacology – Handling, Storage, Dispensing and Disposal

Mean ± SD Steady State Sirolimus Pharmakokinetic parameters in low- to moderate-immunologic risk adult renal transplant patients following 2 mg daily dosing

C_{max} (ng/mL)	14.4 ± 5.3	15.0 ± 4.9
t_{max} (hr)	2.1 ± 0.8	3.5 ± 2.4
AUC (ng•h/mL)	194± 78	230 ± 67
C_{min} (ng/mL)[c]	7.1 ± 3.5	7.6± 3.1
CL/F (mL/h/kg)	173 ± 50	139 ± 63

a. In presence of cyclosporine administered 4 hours before Rapamune dosing
b. Based on data collected at months 1 and 3 post-transplantation.
c. Average C_{min} over 6 months.

Whole blood trough sirolimus concentrations, as measured by LC/MS/MS in renal transplant patients, were significantly correlated with AUC τ ,ss. Upon repeated, twice-daily administration without an initial loading dose in a multiple-dose study, the average trough concentration of sirolimus increases approximately 2- to 3-fold over the initial 6 days of therapy, at which time steady-state is reached. A loading dose of 3 times the maintenance dose will provide near steadystate concentrations within 1 day in most patients.

To minimize variability in sirolimus concentrations, sirolimus should be taken consistently with or without food. In healthy subjects, a high-fat meal (861.8 kcal, 54.9% kcal from fat) increased the mean total exposure (AUC) of sirolimus by 23 to 35%, compared with fasting. The effect of food on the mean sirolimus C_{max} was inconsistent depending on the sirolimus dosage form evaluated.

Management of interacting medications, monitoring of whole blood concentrations and appropriate dosage adjustments of immunosuppressant drugs (tacrolimus and sirolimus) will be based on Hematology SOPs for GVHD prophylaxis.

Distribution. The mean (± SD) blood-to-plasma ratio of sirolimus was 36 ± 18 in stable renal allograft patients, indicating that sirolimus is extensively partitioned into formed blood elements. The mean volume of distribution (Vss/F) of sirolimus is 12 ± 8 L/kg. Sirolimus is extensively bound (approximately 92%) to human plasma proteins, mainly serum albumin (97%), α1-acid glycoprotein, and lipoproteins.

Metabolism. Sirolimus is a substrate for both CYP3A4 and P-gp. Sirolimus is extensively metabolized in the intestinal wall and liver and undergoes counter-transport from enterocytes of the small intestine into the gut lumen. Inhibitors of CYP3A4 and P-gp increase sirolimus concentrations. Inducers of CYP3A4 and P-gp decrease sirolimus concentrations . Sirolimus is extensively metabolized by O-demethylation and/or hydroxylation. Seven (7) major metabolites, including hydroxy, demethyl, and hydroxydemethyl, are identifiable in whole blood. Some of these metabolites are also detectable in plasma, fecal, and urine samples. Sirolimus is the major component in human whole blood and contributes to more than 90% of the immunosuppressive activity.

Excretion. After a single dose of [14C] sirolimus oral solution in healthy volunteers, the majority (91%) of radioactivity was recovered from the feces, and only a minor amount (2.2%) was excreted in urine. The mean ± SD terminal eliminatioCorrelative/Special Studies

9.0 Correlative/Special Studies

9.1 Pharmacokinetics

9.1.1 Clinical Pharmacology Objectives

Optional Pharmacokinetics samples are being collected to compare the plasma pharmacokinetics of clofarabine in patients with varying degrees of renal impairment. Optional research samples will be collected from those patients that provide consent to optional research samples. PK samples will be collected for a total of 12 patients with varying degrees of renal impairment, 6 patients from each arm: Arm 3- Clofarabine 40mg/m^2 and Melphalan 100mg/m^2 and Arm 4- Clofarabine 30mg/m^2 and Melphalan 100mg/m^2. If a participant's sample is not considered "evaluable" another participant may be consented until 12 evaluable patients have been completed.

9.1.2 Blood Sample Collection

Blood samples will be collected from an indwelling venous catheter or by venipuncture. The PK collection should be done at a separate anatomic site as drug infusion e.g., in the opposite arm of the drug infusion or from catheter when drug is infused peripherally. PK blood draws are allowed to be drawn from the same central line if the patient has a central line where there is more than one lumen port. The PK draws should not be drawn from the same lumen port to where the study agent is being administered.

At each time point indicated in the table below, peripheral blood will be collected into a single 7 ml green-top tube (sodium or lithium heparin) containing deoxycoformycin to prevent blood clotting and ex vivo deamination of clofarabine. Tubes will be inverted several times and then immediately placed on ice for transportation to the CICSL, the processing laboratory.

Blood samples will be collected at the following times during the first cycle:

Table 9.2.1 Time Points of Blood Sample Collection for PK/PD Studies

Time Point	Sample Type	Day	Hour:minute (h:m) of collection (24-hour clock)
Pretreatment(immediately prior to Clofarabine infusion) *Document start time of infusion	Peripheral blood	-8	
End of clofarabine infusion (within 15 min) *Document end time of infusion	Peripheral blood	-8	
2 ± 0.5 hr (after completion of the infusion)	Peripheral blood	-8	
4 ± 0.5 hr (after completion of the infusion)	Peripheral blood	-8	
Pretreatment: (immediately prior to Clofarabine infusion)	Peripheral blood	-7	

*Document start time of infusion			
Pretreatment: (immediately prior to Clofarabine infusion) *Document start time of infusion	Peripheral blood	-5	
End of clofarabine infusion (within 15 min) *Document end time of infusion	Peripheral blood	-5	
2 ± 0.5 hr (after completion of the infusion)	Peripheral blood	-5	
4 ± 0.5 hr (after completion of the infusion)	Peripheral blood	-5	
24 hrs from Start Time of Day -5 Clofarabine Dose	Peripheral blood	-4	

9.1.3 Sample Processing

Anti-coagulated whole blood samples (one 10 ml green-top tube that contains deoxycoformycin specific for correlative research sample testing) will be processed by centrifugation for 10 minutes at 1500 x g at 4^0 C. The resulting plasma will be transferred to appropriately labeled polypropylene tubes and frozen at < -70^0 C until analysis.

9.1.5 Pharmacokinetic Analytic Method

For analysis of clofarabine in human plasma, a sensitive and selective LC/MS/MS method will be developed and validated in the Analytical Pharmacology Core Facility at the City of Hope.

9.1.6 Pharmacokinetic Data Analysis Methods

Plasma clofarabine PK data will be analyzed using non-compartmental methods to derive the following secondary parameters; C_{max}, C_{trough}, $AUC_{0\text{-}24hr}$, and CL_{sys}). The individual non-compartmental PK parameters will be summarized for each renal function cohort and compared using standard statistical methods.

10.0 Study Calendar

10.1 Pre-transplant study calendar (Conditioning Phase)

Procedure	Pre-Study screening	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1
Informed Consent	x									
Demographic information	x									
History and Physical	x	x								
Vital signs	x	x	x	x	x	x	x	x	x	x
Height/Weight****	x	x								
KPS	x	x								
CBC with differential AND ANC	x	x		x		x	x	x	x	x
PT/PTT	x									
CMP and Mg	x	x	x	x	x	x	x	x	x	x
LDH	x	x		x		x	x	x	x	x
Ferritin	x									
Phos, Uric Acid	x	x		x		x	x	x	x	x
24 hour urine creatinine clearance	x									
Urine analysis	x									
Pregnancy test for female patients*	x	x								
Pulmonary function tests	x									
Echocardiogram/MUGA scan	x									
Stress test (if applicable)	x									
EKG	x									
CT chest	x									
Chest X-ray**										
HSV 1 Antibody	x									
Hepatitis B Panel (Hepatitis B surface antigen, core antibody and surface antibody)	x									
Hepatitis C total antibody; if positive, Hepatitis C RNA quantitation	x									
Syphilis testing by RPR	x									
HIV-1 antigen or HIV NAT testing, HIV 1 and 2 antibody	x									
CMV igG antibody	x									
ABO/Rh type and antibody screen	x									

Immunoglobulin G levels * **	x									
Lumbar puncture WITH MTX if indicated	x									
Bone Marrow Biopsy and Aspiration	x									
Cytogenetics	x									
TREATMENT										
Hydrocortisone (pre-clofarabine)		x	x	x	x	x				
Clofarabine		x	x	x	x	x				
Dexamethasone (pre-melphalan)							x			
Melphalan							x			
Tacrolimus								x	x	x
Sirolimus								x	x	x
Toxicity Monitoring		x	x	x	x	x	x	x	x	x

*with childbearing potential; **CXR done weekly during transplant hospitalization; ***Immunoglobulin level checked as per SOP; ****Height is not required after the screening visit

10.2 Post-transplant Study Calendar (Stem Cell Infusion and Follow-up)

Procedure	Day 0	Day 30 ± 7 days	Day 100 ± 14 days	Day 180 ± 28 days	***1 year ± 28 days	2 years ± 45 days	3 years ± 45 days	4 years ± 45 days	5 years ± 45 days
History and Physical		x	x	x	x	x	x	x	x
Vital signs	x	x	x	x	x	x	x	x	x
Height/Weight**		x	x	x	x	x	x	x	x
KPS		x	x	x	x	x	x	x	x
CBC with differential AND ANC	x	x	x	x	x	x	x	x	x
CMP and Mg	x	x	x	x	x	x			
LDH	x	x	x	x	x	x			
Pulmonary function tests					x	x			
Echocardiogram/MUGA scan					x	x			
EKG					x	x			
Immunoglobulin levels*		x	x		x	x			
Bone Marrow Biopsy and Aspiration		x	x	x	x	x			
Cytogenetics		x	x	x	x	x			
Chimerism study		x	x	x	x	x			
TREATMENT									
Stem Cell Infusion	x								
Tacrolimus	X ----------†								

Sirolimus	**X ----------†**								
Acute/Chronic GVHD Assessment ‡		**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**
CMV qPCR		**x**	**x**	**x**					
Toxicity Monitoring	**x**	**x**	**x**						

*Immunoglobulin level will be checked as per SOP; †Tacrolimus and sirolimus will be tapered as per COH SOP based on donor match and presence of GVHD; ‡GVHD assessment will be done weekly during admission and at indicated clinic visits post-transplant; **Height is not required after the screening visit; ***May be done as per treating physician discretion.

Procedure	Day 0	Day 30 ± 7 days	Day 100 ± 14 days	Day 180 ± 28 days	***1 year ± 28 days	2 years ± 45 days	3 years ± 45 days	4 years ± 45 days	5 years ± 45 days
History and Physical		x	x	x	x	x	x	x	x
Vital signs	x	x	x	x	x	x	x	x	x
Height/Weight***		x	x	x	x	x	x	x	x
KPS		x	x	x	x	x	x	x	x
CBC with differential AND ANC	x	x	x	x	x	x	x	x	x
CMP and Mg	x	x	x	x	x	x			
LDH	x	x	x	x	x	x			
Pulmonary function tests					x	x			
Echocardiogram/MUGA scan					x	x			
EKG					x	x			
Immunoglobulin levels**		x	x		x	x			
Bone Marrow Biopsy and Aspiration		x	x	x	x	x			
Cytogenetics		x	x	x	x	x			
Chimerism study		x	x	x	x	x			
TREATMENT									
Stem Cell Infusion	x								
Tacrolimus	X ----------†								
Sirolimus	X ----------†								
Acute/Chronic GVHD Assessment ‡		x	x	x	x	x	x	x	x
CMV qPCR		x	x	x					
Toxicity Monitoring	x	x	x						

*CXR done weekly during transplant hospitalization; **Immunoglobulin level will be checked as per SOP; †Tacrolimus and sirolimus will be tapered as per COH SOP based on donor match and presence of GVHD; ‡GVHD assessment will be done weekly during admission and at indicated clinic visits post-transplant; ***Height is not required after screening; **** May be done as per treating physician discretion.

11.0 Data Reporting/Protocol Deviations

11.1 Data Reporting

11.1.1 Confidentiality and Storage of Records

Electronic data capture forms will be stored in encrypted, password protected, secure computers/servers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

11.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

11.1.3 Data Collection Forms and Submission Schedule

All data will be collected within 1-2 weeks using standard Medidata Electronic Data Capture (EDC) case report forms. Data will be collected and stored on secured computers as indicated in Section 11.1.1.

11.1.3.1 Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and electronically signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

11.1.3.2 Prior Therapy Forms and On-Study Forms

Within 1-2 weeks of registration, the clinical research associate will submit case report forms.

11.2 Protocol Deviations

11.2.1 Deviation Policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy" located at http://www.coh.org/dsmc/Documents/Institutional%20Deviation%20Policy.pdf.

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. In addition, if contractually obligated, the sponsor must also approve the deviation. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered to be deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

11.2.2 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

11.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

12.0 Endpoint Evaluation Criteria/Measurement of Effect

12.1 Primary Endpoints

12.1.1 Safety Lead-in Segment

Toxicity is the primary endpoint for the patient safety lead-in segment of this study. Toxicity will be scored on both the Bearman Scale and NCI CTCAE v4.03 Scale. We expect Bearman Gr I/II tox in all categories and grade 3, 4 CTCAE hematological tox since it is myeloablative conditioning. All grades of Bearman toxicities are described in the Appendix B, and the CTCAE v4.3 is available on the internet at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.
Unacceptable toxicity in a given patient is defined as any regimen-related grade III/IV toxicity per Bearman Criteria, or for hematologic toxicities, per NCI CTCAE v4.03 toxicity criteria, any grade 4 neutropenia associated with fever or infection and lasting beyond 3 weeks, or grade 4 neutropenia lasting for more than 28 days, or any other regimen-related cause of death.

This unacceptable toxicity definition was established after reviewing two sources of toxicity data (see protocol section 2.1): 1) preliminary data from patients conditioned with clofarabine/melphalan (Forman, 2013 unpublished) and 2) historical data from patients conditioned with fludarabine/melphalan (Forman, 2013 unpublished; Nakamura 2012). Generally, regimen-related grade I/II toxicity per Bearman Criteria occurred in >50% of patients, in at least one toxicity class; this was also true for hematologic toxicity.
Note: The NCI CTCAE v4.0 scale will also be used for reporting of adverse events and more detailed reporting.

12.1.2 Overall Trial

The primary endpoint for the trial is 2-year progression-free survival (PFS). Patients are considered a failure for this endpoint if they die (regardless of cause) or experience disease progression/relapse. Progression-free survival is estimated from the start of treatment to the date of death, disease relapse/progression, or last follow-up whichever occurs first.

12.2 Secondary Endpoints

12.2.1 Overall Survival (OS)

Patients are considered a failure for this endpoint if they die, regardless of cause. The time to this event is the time from start of treatment until death, or last follow-up, whichever comes first.

12.2.2 Relapse/Progression (CIR)

The event is relapse/progression. The time to this event is measured from start of treatment. Deaths without relapse/progression are considered a competing risk. Surviving patients with no history of relapse/progression are censored at time of last follow-up.

12.2.3 Non-Relapse Mortality (NRM)

Non-relapse mortality (NRM) is defined as death occurring in a patient from causes other than relapse or progression. NRM is measured from start of treatment until non-disease related death, or last follow-up, whichever comes first. Deaths from relapse/progression are considered a competing risk.

12.2.4 Toxicities and Adverse Events

Safety Lead-In Segment and Phase II Study: The worst grade of all toxicities will be collected from day -9 to day -1, and again from day 0 to 30 post-transplant. Toxicity will be assessed and reported using the Bearman [34] and CTCAE v4.03 scales [35]. Safety Lead-In Segment: From day 31 to 100, all post-transplant toxicities that are considered serious adverse events will be collected. Phase II Study: From day 31 to day 100 post-transplant, only toxicities that are considered serious adverse events that are at least possibly related to the study drug (clofarabine/melphalan) will be collected.

12.2.5 Acute Graft versus Host Disease (aGVHD) of grades 2-4 and 3-4

Acute graft versus host disease is graded according to the Consensus Grading [36] and grading and staging tables are included in Appendix C. The first day of acute GVHD onset at a certain grade will be used to calculate cumulative incidence curves for that GVHD grade. The endpoint will be evaluated from day 0 through 100 days post transplant.

12.2.6 Chronic Graft versus Host Disease (cGVHD)

Chronic graft versus host disease is scored according to NIH Consensus Staging [37] as shown in Appendix D. The first day of chronic GVHD onset will be used to calculate cumulative incidence curves.

12.2.7 Infection

Microbiologically documented infections will be reported by site of disease, date of onset, severity and resolution, if any. This data will be captured via case report form and will be collected from day -9 to day 100 post-transplant and will follow the same data collection intervals as the toxicity and adverse event data.

12.3 Disease Status Assessment

12.3.1 Complete Remission

Remission is defined as <5% blasts with no morphological characteristics of acute leukemia (e.g. Auer rods) or myelodysplasia in a bone marrow with >20% cellularity, peripheral blood counts showing ANC >1000/μL (including patients in CR with incomplete platelet recovery). In addition, normalization of cytogenetics must be demonstrated by the absence of previous cytogenetic abnormality identified prior to transplantation in the bone marrow aspirate without appearance of new abnormalities.

12.3.2 Relapse

Any of the following indicates relapse:

- The reappearance of blast cells in the peripheral blood
- >5% blasts in the marrow, not attributable to another cause (e.g., bone marrow regeneration)
- The appearance of new dysplastic changes within the bone marrow
- Development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid
- Reappearance of cytogenetic abnormalities present prior to transplantation

12.3.3 Disease Persistence /Progression:

Patient does not achieve CR upon repeat bone marrow examination after therapy and displays persistent evidence of disease by cytogenetics in blood or bone marrow consistent with pre-transplant features, or consistent with progression of MDS to AML.

12.4 Study Termination

All patients will be followed for survival until death or 5 years post transplant, whichever occurs first. For patients who relapse/progress, the date of relapse/progression will be recorded, but these patients will no longer follow the study calendar for required disease monitoring, however patients will continue to be followed for survival.

All reasons for discontinuation of treatment must be documented.

Patients may withdraw from the study at any time for any reason.

13.0 Statistical Considerations

13.1 Study Design

This study is a single-center, single-arm phase II trial to evaluate the anti-leukemic activity and safety of clofarabine (40 mg/m^2), given in combination with high-dose melphalan (140 mg/m^2), in patients with acute myeloid leukemia/acute lymphoid leukemia in remission or myelodysplastic syndrome. The first six patients treated on this study will be part of a patient safety lead-in.

Given that the primary endpoint for this trial is 2-year progression-free survival, we recognize that a randomized design would be the design of choice where patients would be randomized to receive either fludarabine/melphalan or clofarabine/melphalan or one of two doses of clofarabine. We believe that the results achieved at COH with the clofarabine/melphalan regimen combination (Figure 4) suggest that the results from a single arm trial will be scientifically compelling. This regimen would fit in the category of agents that "provide evidence of extraordinary and unanticipated activity compared to prior agents in the same class" a category of agents which Cannistra indicates is publishable as a single arm trial with a stable historical control [38]).

13.2 Patient Safety Lead-in Segment

The patient safety lead-in segment of this study will be conducted to ensure there are no unexpected toxicities; ultimately we plan to treat 6 patients at what is deemed to be a safe dose level. At the initial dose 'level' up to 3 patients will be treated with 40mg/m^2 of clofarabine and 140 mg/m^2 of melphalan. Note: no more than 3 patients can be <30 days post stem cell infusion at any time during the patient safety lead-in phase. If ≤ 1/6 patients experience an unacceptable toxicity event, the trial will enroll up to an additional 59 patients. If, however, > 1/6 patients experience unacceptable toxicity, the dose of melphalan will be reduced to 100 mg/m^2, with subsequent patients be treated at 40mg/m^2 (clofarabine)/100 mg/m^2 (melphalan) dose 'level'.

While not expected, if >1/6 patients at the 40mg/m^2 (clofarabine)/100 mg/m^2 (melphalan) dose level experience unacceptable toxicity, the clofarabine dose will be reduced to 30mg/m^2 with subsequent patients treated at 30mg/m^2 (clofarabine)/100 mg/m^2 (melphalan) dose 'level'. Additional dose levels below the 30/100 dose level will not be considered. Ultimately a total of 65 patients will be treated at the clofarabine/melphalan dose level considered safe as determined during the *patient safety lead-in* segment of this study. With two dose reductions permissible, the overall maximum study sample size could reach 77.

Toxicities will be scored using both the Bearman Scale and the NCI CTCAE v4.0 Scale. To be evaluable for toxicity, a patient must start treatment and be observed for 30 days from stem cell infusion or have experienced an unacceptable toxicity. All patients who are not evaluable for toxicity will be replaced. Unacceptable toxicity in a given patient is defined as any regimen-related grade III/IV toxicity per Bearman Criteria, or for hematologic toxicities, per NCI CTCAE v4.0 toxicity criteria, any grade 4 neutropenia associated with fever or infection and lasting beyond 3 weeks, or grade 4 neutropenia lasting for more than 28 days, or any other regimen-related cause of death. Note: The NCI CTCAE v4.0 scale will also be used for reporting of adverse events and more detailed reporting.

13.3 Stopping Rules for Excessive Toxicity

The following table will be consulted as relevant toxicities are encountered following the patient safety lead-in. The early stopping rule for safety/toxicity will be assessed for each patient at day +30 post transplant/stem cell infusion. The expected rate of unacceptable toxicity should not be ≥33%. See the table below for detailed early stopping rules. Patients with ongoing toxicity (beyond day +30) will be followed until resolution or stability. If more than the specified number of patients has significant treatment related toxicities, patient accrual will be halted and a full review of the data by the Data Safety Monitoring Committee will be mandated. Patient accrual will not resume until approved by the Data Safety Monitoring Committee to do so.

# of patients treated at phase II dose	# of patients with unacceptable toxicity to halt enrollment[1]	Cumulative probability of early stopping given a toxicity rate of:		
		15%	33%	45%
6	2	0.22	0.64	0.84
12	4	0.24	0.73	0.92
18	6	0.25	0.78	0.95
24	8	0.25	0.81	0.97
30	10	0.25	0.83	0.98
36	12	0.25	0.84	0.99
42	14	0.25	0.85	0.99
48	16	0.25	0.86	0.99
54	18	0.25	0.87	0.99
60	20	0.25	0.88	0.99
[1]: For each unacceptable toxicity, halt enrollment and evaluate if the cumulative # of patients reaches or exceeds the specified limits.				

13.4 Stopping Rules for Activity

After 20 patients have the opportunity to achieve one year of follow-up, the observed PFS rate will be compared to our hypothesized alternative rate of 77% using a single sample Log-Rank test. If the alternative is rejected at the 0.02 level, patient accrual will be halted and a full review of the data by the Data Safety Monitoring Committee will be mandated. Patient accrual will not resume until approved by the Data Safety Monitoring Committee to do so. This test is similar is spirit to that of test of futility in the SWOG two stage design [39]. Note: The actual discouraging rate will be determined by the proportion of AML, ALL and MDS patients accrued and the encouraging rate will be taken as 15% higher.

13.5 Sample Size

Historical Estimates, Fludarabine/Melphalan RIC alloHCT:

As noted in the Background section, because the available historical data are not reflective of our observed RIC fludarabine-melphalan experience (COH PFS estimates are better), 2-year PFS estimates were also generated using data from the COH transplant repository from patients transplanted from 2005 to 2009 using patient selection criteria that match the protocol inclusion criteria. The estimates from these analyses are as follows: AML 2-year PFS 60%, ALL 2-year PFS 51% (Forman, 2013 unpublished). Using these data, the published MDS outcomes (Nakamura *et al.* [22]) and expected accrual pattern across disease subgroups, the 2-year PFS estimate for fludarabine/melphalan is 62% (95%CI: 57.7-66.5).

Preliminary Estimate, Clofarabine/Melphalan RIC alloHCT:

From a similar patient population treated at City of Hope (Khaled 2013, pending publication of ASBMT Oral Abstract), the estimated two-year PFS probability for patients conditioned with clofarabine (30/40 mg/m^2) and high dose melphalan (100/140 mg/m^2) as part of alloHCT, was reported to be 77.7% (95% CI: 57.7 – 89.1). Based on these preliminary estimates, a total of 65 patients are required to detect a 15% increase in two-year PFS from the historical COH fludarabine/melphalan estimate of 62% to 77%, with approximately 86% power and a 1-sided α=0.05, using the SWOG, single arm non-parametric approach (based on work done by Brookmeyer and Crowley [40]).

Smaller trials (patient subgroups) like the one proposed here are more sensitive to patient variation. The sample size calculation used to estimate number of patients and expected difference between historical fludarabine-based/RIC patients and clofarabine/melphalan may be impacted if the patients (AML, ALL and MDS) are not sufficiently homogeneous. The patient population eligible for this trial will be standard alloHCT-eligible patients (except that patients up to age 75 will be allowed) in CR1 or CR2 only. This is a healthier population than those treated so far in the phase I study. For this reason, we expect outcomes to be comparable to, or somewhat better than those seen in the patients treated with this regimen thus far at City of Hope.

Accrual Estimates:

Annually City of Hope treats 13 MDS, 26 AML (1CR/2CR), and 5 ALL (1CR/2CR) patients with RIC alloHCT using a fludarabine/melphalan preparative regimen. Based on these numbers, if half of these patients enrolled, this study expects to accrue roughly 23 patients per year, just under 2 per month. The study is expected to complete the enrollment phase of the study in 5 years, with an additional 5 years of follow-up required from the time the last patient is enrolled to complete the analysis.

Statistical Analysis Plan:

Survival estimates will be calculated using the Kaplan-Meier method. The cumulative incidence of relapse/progression and non-relapse mortality will be calculated as competing risks using the Gray method. Toxicity information recorded will include the type, severity, and the probable association with the study regimen. Tables will be constructed to summarize the observed incidence by severity and type of toxicity. Baseline information (e.g. the extent of prior therapy) and demographic information will be presented, to describe the patients treated in this study.

14.0 Human Subject Issues

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects

Patients will be recruited from among candidates for reduced intensity HCT at City of Hope Cancer Center for acute leukemia or MDS. Individuals will be recruited from the COH campus and from the San Gabriel Valley and environs (~25 mile radius) using the City of Hope Website and a quarterly e-newsletter sent to local hematologists/oncologists listing our ongoing clinical trials.

14.3 Advertisements

No advertisements will be used to recruit potential study subjects.

14.4 Study location and Performance Sites

This study will be performed at COH.

14.5 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record toxicity, disease response, survival and toxicity data, and this will be linked to the subject's identity using a coded study number. In addition, results of screening tests to rule out HIV, HBC, and HCV performed in the Department of Transfusion Medicine will be used with the participant's permission if performed in the past 4 months. The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

14.6 Financial Obligations and Compensation

The investigational drug clofarabine, will be provided free of charge by Sanofi-Aventis. Should this drug become commercially available during the course of treatment, the research participant and/or the insurance carrier may be asked to pay for the costs of the clofarabine.

The standard of care drug(s) and procedures provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.7 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Before signing the study consent form, HIPAA authorization form and the Experimental Subject's Bill of Rights, research subjects will undergo an assessment of their comprehension of the study by the Research Subject Advocate. Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the questionnaire repeated until a satisfactory result is obtained.

Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

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Appendix A: Karnofsky Performance Status Scale

Karnofsky Scale %	Karnofsky Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity, minor symptoms or signs of disease.
80	Normal activity with effort, some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of own needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization is indicated although death is not imminent.
20	Hospitalization necessary, very sick, active supportive treatment necessary.
10	Moribund, fatal processes
Dead	

Appendix B: Modified Bearman Scale [34]

	Grade I	Grade II	Grade III
Cardiac toxicity	Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on chest x-ray with no clinical symptoms	Moderate EKG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestiveheart 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 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days 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 chest x-ray changes not caused by infection or congestive heart failure; or chest x-ray showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	Chest x-ray with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO2 (> 10% from baseline) but not requiring mechanical ventilation or > 50% O2 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 bilirubin ≥ 2.0 mg/dL and ≤ 6.0 mg/dL or weight gain > 2.5% and < 5% from baseline, of non-cardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning	Moderate hepatic dysfunction with bilirubin > 6.0 mg/dL and < 20 mg/dL; or SGOT increase > 5-fold from preconditioning; or clinical ascitis or image documented ascitis > 100 mL; or weight gain > 5% from baseline of non-cardiac origin	Severe hepatic dysfunction with bilirubin > 20 mg/dL; or hepatic encephalopathy; or ascitis 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 day not related to infection	Watery stools > 2,000 mL every day 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

Appendix C: Acute GVHD Grading and Staging [36]

Extent of Organ Involvement			
Stage	**Skin**	**Liver**	**Gut**
1	Rash on <25% of skin[a]	Bilirubin 2-3 mg/dl[b]	Diarrhea > 500 ml/day[c] or persistent nausea[d]
2	Rash on 25-50% of skin	Bilirubin 3-6 mg/dl	Diarrhea >1000 ml/day
3	Rash on >50% of skin	Bilirubin 6-15 mg/dl	Diarrhea >1500 ml/day
4	Generalized erythroderma with bullous formation	Bilirubin >15 mg/dl	Severe abdominal pain with or without ileus

Grade[e]			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	—	Stage 2-3 or	Stage 2-4
IVf	Stage 4	Stage 4	—

a. Use “Rule of Nines” (Table 4) or burn chart to determine extent of rash.
b. Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
c. Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if an additional cause of diarrhea has been documented.
d. Persistent nausea with histological evidence of GVHD in the stomach or duodenum.
e. Criteria for grading given as minimum degree of organ involvement required to confer that grade.
f. Grade IV may also include lesser organ involvement with an extreme decrease in performance status.

Appendix D: Chronic GVHD Scoring [37]

	Score 0	Score 1	Score 2	Score 3
Performance score: ☐ KPS ECOG LPS	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
Skin Clinical features: ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritis ☐ Hair involvement ☐ Nail involvement ☐ **% BSA involved**	☐ No symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA OR deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
Mouth	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
Eyes **Mean tear test(mm):** ☐ >10 ☐ 6-10 ☐ ≤10 ☐ Not done	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops >3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI tract	☐ No symptoms	☐ Symptoms such as dysphagia, nausea, vomiting, abdominal pain or diarrhoea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** oesophageal dilation
Liver	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dL or Bilirubin enzymes 2-5 x ULN	☐ Bilirubin or enzymes >5 x ULN
Lungs ☐ FEV1 ☐ DLCO	☐ No symptoms ☐ FEV1 >80% **OR** LFS = 2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring O2) ☐ FEV1 ≤39% **OR** LFS 10-12
Joints and fascia	☐ No symptoms	☐ Mild tightness of arms and legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms and legs OR joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
Genital tract	☐ No symptoms	☐ Symptomatic with mild signs of examination **AND** no effect on coitus and minimal discomfort with gynaecological examination	☐ Symptomatic with moderate signs on examination **AND** with mild dyspareunia or discomfort with gynaecological examination	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Organ scoring of chronic GVHD. *AP may be elevated in growing children, and not reflective of liver dysfunction. Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchioloitis obliteransas already been established. The percent predicted FEV1 and DLCO (adjusted for haematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12. GVHD indicates graft versus host disease; ECOG, Eastern Cooperative Oncology Group; KPS, Karnofsky Performance Status; LPS, Lansky Performance Status; BSA, body surface area; ADL, activities of daily living; LFTs, liver function tests; AP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotrasferase; ULN, upper limit of normal.