Abbreviated Title: Palifermin dose escalation

NIH Protocol #: 15C0067

NCT #: NCT02356159

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Title: A Phase I/II Open Label, Dose Escalation Study of Palifermin (Kepivance) in Persons Undergoing Unrelated Donor Allogeneic Hematopoietic Cell Transplantation

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Investigational/Commercial Agents:

Drug Name:	Palifermin	Cyclophosphamide, Mesna, Filgrastim, Fludarabine, Etoposide, Doxorubicin, Vincristine, Prednisone, Rituximab, Cytarabine, Methotrexate, Sirolimus, Tacrolimus
IND Number:	124506	124506
Sponsor:	Center for Cancer Research	Center for Cancer Research
Manufacturer:	Swedish Orphan Biovitrum (Sobi) AB	Generic
Supplier:	Swedish Orphan Biovitrum (Sobi) AB	CC Pharmacy

PRÉCIS:

Background:

- Graft *versus* host disease (GVHD) and impaired immune reconstitution are major transplant complications and barriers to improving outcomes after allogeneic hematopoietic stem cell transplantation (alloHSCT) for hematologic malignancies. GVHD is initiated when donor T-cells become alloreactive against recipient major or minor histocompatibility antigens. This process may be exacerbated during the transplantation process by exposure of tissue antigens to donor T-lymphocytes after chemotherapy-induced injury.
- Palifermin, a recombinant keratinocyte growth factor-1 (KGF-1), imitates the actions of intrinsic KGF and binds to the FGF receptor 2b, which is expressed in the epidermis, oral mucosa, GI mucosa and urothelium, thereby increasing the regenerative capacity of these tissues. Palifermin has been shown to reduce the duration and severity of oral mucositis after intensive chemo-radiotherapy and autologous HSCT for hematologic cancers and is FDA approved. In pre-clinical studies, palifermin has been shown to have an effect on control of acute or chronic GVHD and immune reconstitution after alloHSCT. However, subsequent clinical studies in alloHSCT indicate that the dose and schedule of palifermin as currently used in humans does not optimize its activity in terms of prevention of GVHD or thymus recovery following alloHSCT.
- We hypothesize that higher doses of palifermin in the immediate pre alloHSCT conditioning setting will lead to enhanced thymopoiesis, decreased chronic GVHD, and improved immune reconstitution. A dose escalation study is necessary to determine safe dosing levels in persons undergoing alloHSCT.

Objectives:

- The primary objective of the phase I portion is to assess the safety and tolerability of the administration of the recombinant keratinocyte growth factor (KGF) palifermin in alloHSCT using unrelated donor peripheral blood stem cells.
- The primary objective of the phase II portion is to determine the incidence of severe chronic GVHD after the addition of palifermin to TMS (tacrolimus, methotrexate and sirolimus) based GVHD prophylaxis delivered in the identical fashion to the NCI 07C0195 study.

Eligibility:

- Adults (≥18 years) with advanced or high risk hematologic malignancies (including AML, ALL, MDS, CLL, NHL, HL, CML, multiple myeloma, and MPN) who lack a suitable HLA-matched sibling donor.
- An unrelated donor matched at a minimum of 8 alleles (HLA-A, -B, -C, and DRB1) by high-resolution typing, identified through the National Marrow Donor Program.
- Karnofsky ≥60 and acceptable organ functions.

Design:

- Patients will receive disease-specific induction chemotherapy (EPOCH-F/R or FLAG) prior to transplant as needed for disease control and immune depletion.
- All patients will receive an identical conditioning regimen consisting of cyclophosphamide 1200 mg/m^2/day IV for 4 days and fludarabine 30 mg/m^2/day for 4 days (transplant days -6 to -3).
- All patients will receive a peripheral blood stem cell product from an unrelated donor matched at HLA-A, -B, -C, -DRB1 (8/8) by high-resolution typing.
- Palifermin will be administered in a phase 1, open label design with the following proposed schedule:
    - Dose level 1: 180 mcg/kg on day -7
    - Dose level 2: 360 mcg/kg on day -7
    - Dose level 3: 540 mcg/kg on day -7
    - Dose level 4: 720 mcg/kg on day -7
- The phase I portion will be conducted in a standard 3+3 design; the maximum possible number of patients accrued to this portion will be 24.
- The maximum tolerated dose (MTD) from the phase I portion of the study will be used to conduct a phase II study. Total accrual on the phase II study will be 27 patients, including 3-6 patients treated at the MTD in the phase I portion of the study.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary objectives

- The primary objective of the phase I portion is to assess the safety and tolerability of administration of the recombinant keratinocyte growth factor (KGF) palifermin in alloHSCT using unrelated donor peripheral blood stem cells.
- The primary objective of the phase II portion is to determine the incidence of severe chronic GVHD after the addition of palifermin to TMS (tacrolimus, methotrexate, sirolimus) based GVHD prophylaxis delivered in the identical fashion of the NCI 07C0195 study.

1.1.2 Exploratory objectives

- Assess the effects of palifermin on immune reconstitution in persons undergoing alloHSCT.
- Monitor the effects of palifermin on thymic function in persons undergoing alloHSCT.
- Assess for post-transplant outcomes including:
  - overall survival
  - failure-free survival
  - non-relapse mortality (death in clinical remission)
  - relapse-related mortality (death without clinical remission)
  - GVHD related mortality
  - rate of achieving remission, malignancy relapse (recurrence after remission)/progression

- incidence of infections
- engraftment of neutrophils and platelets, graft failure
- incidence of starting steroids and steroids administration AUC
- rate of starting secondary therapy for acute and chronic GVHD separately
- time to permanent (>6 months) interruption of immunosuppression
- malignancy remission
- chronic (cGVHD) severity at last yearly follow-up.

- Assess the fraction of patients who experience grade II-IV acute GVHD (aGVHD) and compare it to the fraction from the TMS arm of the NCI 07C0195 study.

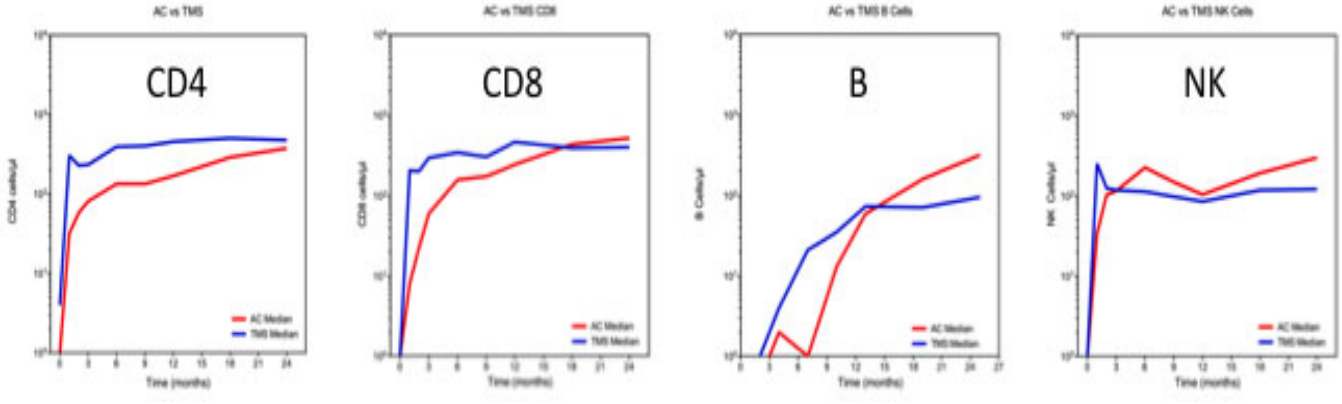
1.2 Background

Allogeneic hematopoietic stem cell transplantation (alloHSCT) is capable of producing sustained remissions in patients with hematologic malignancies. Less than one-third of transplant candidates have an HLA-matched sibling donor (MSD) available. For these persons, HLA-matched adult unrelated donors (URD) have become the preferred stem cell source for alloHSCT and is currently a most prevalent and rapidly growing source of stem cells for alloHSCT [1, 2]. The use of URDs has expanded the number of persons who proceed to alloHSCT but has led to a greater risk of transplant related toxicity, principally GVHD and infections [3]. While advances in HLA typing and supportive care have decreased transplant-related mortality, the problem of higher rates of late complications, especially chronic GVHD after URD alloHSCT, became a major source of morbidity and mortality in hematologic cancer patients who are survivors of this procedure [3, 4].

Multiple methods to reduce the incidence of chronic GVHD in persons undergoing URD alloBMT have been tested in clinical trials. Functionally, these studies have focused on reduction in the T-lymphocyte content of the donor stem cell product or administration of anti-thymocyte globulin in order to reduce alloreactivity.[5-8] These studies remain inconclusive in terms of ultimate benefit for patients in terms of GVHD reduction and survival and major concerns remain relative to malignancy control especially in the reduced intensity conditioning setting[9]. In order to test the hypothesis that T-lymphocyte depletion results in decreased chronic GVHD, we conducted a randomized phase 2 study (NCI 07C0195) whereby persons with hematologic malignancies received an unmodified HLA-matched or single antigen mismatched URD alloHSCT graft. Subjects were randomized to GVHD prophylaxis with alemtuzumab (humanized anti-CD52 antibody) based *in vivo* lymphodepletion followed by cyclosporine monotherapy (AC arm) versus tacrolimus, sirolimus, and methotrexate (TMS arm). Seventy-eight persons have been transplanted at the time of the most recent analysis (January 2014). 69 individuals had survived >100 days post-transplant. The 24-month overall survival (OS) in AC and TMS were similar but favored TMS (69.8% versus 49.6%, p=0.052 by Gray test). The most recent analysis of 78 randomized patients showed no difference in the rate of grade 2-4 acute GVHD (6 month probability: TMS = 50.5%, AC = 44.7%; p=0.74). The cumulative incidence of chronic GVHD at 24 months was 57.5% in TMS arm vs. 21.8% in AC arm (P=0.0004). Persons receiving AC had lower numbers of T- and B-lymphocytes during the first 12 months post alloHSCT

(**Figure 1**, Gress lab), higher rates of total infections and especially CMV. Finally, there was a trend for lower probability of incidence of progressive disease in TMS versus AC at 24 months, 27.6% versus 49.6% (P = 0.052 by Gray test). In summary, these results indicate that *in vivo* T-cell depletion is effective at reducing chronic GVHD but does not improve overall survival likely due to effects related to impaired recipient immune reconstitution.

Figure 1: Peripheral blood lymphocytes (log-10 scale) following URD allogeneic transplantation using AC (grey line) versus TMS (dark line)



One hypothetical approach to reduce acute and chronic GVHD after alloHSCT is cytoprotection of target tissues such as skin, mouth, eye, gut and thymus. Injury to these tissues during conditioning chemotherapy may result in exposure to alloantigens or pathogen antigens that trigger the GVHD cascade or disrupt thymic function in establishing immune tolerance [10-12]. The advantage of such an approach is that it is GVHD specific, hypothetically sparing anti-tumor or anti-pathogen immunity. One pharmacologic mechanism to test this hypothesis is via use of epithelial cell cytoprotectants in the context of URD alloBMT. Of available agents, recombinant human keratinocyte growth factor-1 has accumulated the most preclinical and clinical efficacy.

1.2.1 Recombinant Human Keratinocyte Growth Factor (Palifermin)

Keratinocyte growth factor-1 (KGF) is a 28 kDa endogenous fibroblast growth factor that binds to FGF receptor 2b (FGFR). FGFR is widely expressed on epithelial cell tissues including the oral mucosa, gastrointestinal tract, bladder, skin, salivary gland, and thymus. Binding of KGF to KGFR induces proliferation and anti-apoptosis in target tissues. Hematopoietic cells do not express FGFR and are thus unaffected by KGF. Palifermin (Swedish Orphan Biovitrium AB, Stockholm, Sweden) is a recombinant human KGF-1 that differs from the endogenous human form by a 23 amino acid deletion at the N-terminus to increase stability.

1.2.2 Palifermin Pre-Clinical Studies

1.2.2.1 Effect of Palifermin on Acute GVHD in allogeneic HSCT mouse models

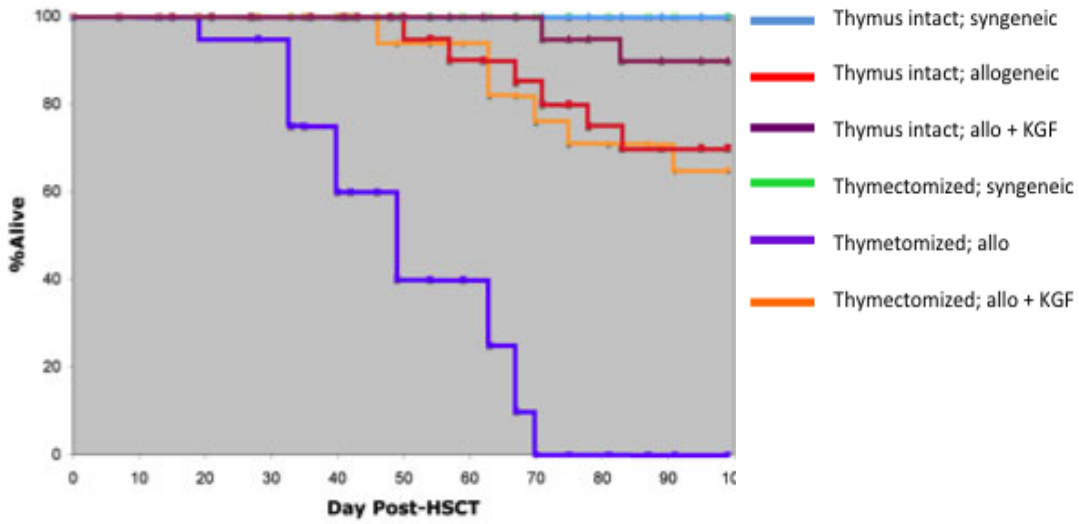
Because palifermin induces cytoprotection of GVHD target tissues, murine studies were performed to determine if peri-transplant palifermin would reduce the incidence of GVHD. Krijanovski and colleagues administered KGF in a murine allo-HSCT transplant model on days -3 to 0 or days -3 to +7. The administration of KGF resulted in decreased mortality ($p<0.01$) in both treatment schedules. There were also decreased levels of inflammatory mediators (lipopolysaccharide [LPS] and TNF-alpha), reduced acute GVHD, and longer leukemia-free survival. The authors found the incidence of recipient alloantigen specific cytotoxic T-lymphocytes was unaffected by KGF, thus suggesting that GVL was not affected by KGF in this model [13]. Panoskaltsis-Mortari and colleagues administered KGF at a dose of 5 mg/kg on Day -6, -5, -4 pre-transplant in a murine allo HSCT model with and without conditioning therapy. The authors noted a beneficial effect on 80 days survival and incidence of acute GVHD independent of the use of conditioning treatment [14].

1.2.2.2 Effect of Palifermin on the Thymus, Chronic GVHD and Immune Reconstitution in allogeneic HSCT mouse models

Min and colleagues tested palifermin at a dose of 5 mg/kg/day for 3 days pre-cytotoxic treatment vs. placebo, and found that KGF-treated mice had improved thymic cellularity, normalized thymic subpopulation and increased peripheral naive T cells [15]. In an allogeneic, non-irradiated allo HSCT mouse model for acute GVHD, Rossi and colleagues administered palifermin 5 mg/kg/day vs. placebo on days -3 to +3. Although the administration of palifermin failed to modulate the incidence of splenic acute GVHD, it was found to be associated with preserved, normal thymic size and cellularity 2 weeks after HSCT [16].

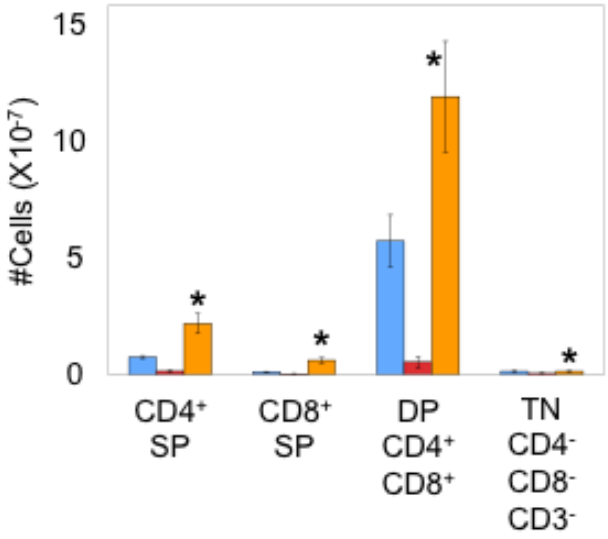
Using a minor antigen mismatch model of chronic graft versus host disease in mice, it was found that KGF protected mice from lethality in a dose dependent pattern (Gress laboratory murine transplant model B10.D2 to BALB/c with or without thymectomy [17]. Mice were lethally irradiated (850 cGy on day 0) using KGF at 5, 10, 50, 100 µg/day on days -4, -3 and -2 prior to total-body irradiation. Cumulative doses were 15, 30, 150 and 300 µg, respectively. There was an effect on immune reconstitution at the latter two doses (150 and 300 µg cumulative dose). At the highest dose (300 µg), 100 day survival in thymus bearing mice receiving KGF was 90% versus 0% survival in carrier treated animals. In thymectomized mice, those receiving KGF had a survival at 100 days of 65% versus 0% in control animals. The difference of enhanced survival between thymus bearing and thymectomized animals (25%) was associated with enhanced thymus activity. While specific dose per kilogram of KGF and attendant toxicities may not translate from mouse to man, the concept that dose may matter in preventing chronic graft versus host disease is potentially of great importance and has not been explored in any rigorous fashion (**Figure 2**).

Figure 2: Survival of mice undergoing transplantation with or without palifermin pretreatment.



Recovery of immune function is an important predictor of survival in persons undergoing alloHSCT [18-21]. Pre-clinical models suggest that palifermin may augment immune reconstitution in this setting. For example, Alpdogan and colleagues found that KGF knockout mice demonstrated defective thymopoiesis and peripheral T-lymphocyte reconstitution after sub-lethal radiation, with increased donor derived CD8 and naïve CD4 T-cells [22]. Volk and colleagues found that KGF enhanced tumor specific cytotoxic T-lymphocytes in a murine post-transplant tumor vaccine model. [23] Finally, Gress and colleagues (unpublished data) demonstrated improved reconstitution of CD4+, CD8+, and double positive T-lymphocyte populations after administration of KGF in a murine transplant model (**Figure 3**).

Figure 3: Improved reconstitution of major T-lymphocyte subpopulations after syngeneic (blue), allogeneic with diluent (red) or allogeneic with KGF (orange) transplantation in mice.



* p< 0.05 for all Syn vs. Allo-Dil and Allo-KGF Groups

In a non-human primate study published by Suggewiss R. et al [24], rhesus macaque monkeys received palifermin 250 µg/kg/day on Day – 5 (n=4) or on days -5, -4, -3, 0, +1, +2 (n=2) of TBI and autologous HSCT rescue. The goal of the study was to determine the effect of palifermin on immune reconstitution, and found that CD4 and CD8 naïve T cell frequencies in lymph nodes were higher in KGF treated animals vs. untreated animals. Animals who had received 6 doses of KGF had higher T-cell receptor excision circle (TREC) levels in CD4 and CD8 T cells at >= 5 month post-transplant. In addition, all 6 KGF treated animals had no or little thymic atrophy, whereas 3 out of the 5 control animals had severe thymic atrophy. This is the only non-human primate study to show the effect of palifermin on thymic function in an autologous transplant setting.

1.2.3 Clinical Experience: Pharmacokinetics, Pharmacodynamics, Safety

The toxicity and pharmacokinetics of palifermin has been extensively studied in humans for prevention of chemo or radiotherapy induced mucositis. Meropol and colleagues tested various doses and administration schedules of palifermin in metastatic colon and rectal cancer patients receiving fluorouracil for 5 days (day +4 to +8) [25]. Palifermin was administered on Day +1 to +3 at escalating doses, starting at 1 to 80 µg/kg/day IV (3 to 240 µg/kg, cumulative dose). Patients receiving palifermin had reduced incidence of grade 2 to 4 mucositis (43% KGF group vs. 67% placebo). The patients who received 20 µg/kg/day or more had the largest decreases in mucositis incidence compared to placebo (25% to 35% decrease in mucositis). Maximum tolerated dose was defined as the highest dose tested for which <=33% of patients received that dose experienced a dose limiting toxicity, defined by grade 3 or greater adverse event or grade 2 adverse event that the subject found intolerable. Twelve subjects were planned per dose level. Three adverse events involving the skin occurred in the 18 patients who received 60 or

80 µg/kg/day. Although the predefined definition of maximum tolerated dose was not met, dose escalation beyond 80 µg/kg/day was halted since there appeared to be evidence of biologic activity based on the reduction in the frequency of grade 2 to 4 mucositis in these two group of patients (60 µg/kg/day: -25% and 80 µg/kg/day: -30%). As for other skin and oral toxicities associated with KGF, they were generally mild to moderate with onset 36 hours after administration and resolution after 7-10 days.

Zia-Amirhosseini and colleagues assessed the pharmacokinetics, pharmacodynamics and safety of palifermin in healthy volunteers after single, escalating doses starting at 60 µg/kg and administered as IV boluses [26]. The pharmacodynamic endpoint selected for this study was a 3-fold or greater increase in oral mucosal epithelial Ki67-stained area per millimeter, as compared to baseline at 48 and 72 hours post-palifermin administration. The $t_{1/2}$ ranged from 4.5 hours at a 60 µg/kg dose to 6.1 hours at 250 µg/kg, and the AUC was approximately linear across this dose range. Results from Ki67 staining showed that 13 out of 16 patients who received doses of 120, 160, 210 or 250 µg/kg achieved the primary endpoint of a 3 fold increase in Ki67-stained area per millimeter, and a greater dose response was observed at 48 hours than at 72 hours. The drug was well tolerated at these dose levels, and all adverse events (AE) recorded were transient and resolved without intervention. No serious AEs were reported and no subject discontinued because of an AE. Erythema, warm sensation, elevated amylase level were more frequently noted at 210 – 250 µg/kg than lower dose levels. Since there was a sufficient pharmacodynamic response at 120 µg/kg and higher doses, as well as in an effort to minimize AEs (erythema, warm sensation), a single dose of 120 to 180 µg/kg was selected for further studies.

A follow-up study by Zia- Amirhosseini and colleagues tested whether a collapsed dose of 180 mcg/kg/day was as effective as 60 µg/kg/day in patients with hematological malignancies [27]. Patients in the 60 µg/kg/day arm received palifermin for 3 consecutive days before and 3 consecutive days after chemoradiotherapy and autologous HSCT. Patients in the 180 µg/kg/day arm received palifermin once before (72 hours before starting conditioning) and once after myeloablative treatment). They found that there was similar overall exposure to palifermin in both groups with approximately dose-linear pharmacokinetics, and both dosing regimens were well tolerated.

1.2.4 Clinical Experience: Autologous HSCT

Palifermin was approved by the Food and Drug Administration for prevention of chemotherapy induced mucositis based on a large, randomized study demonstrating a 35% reduction in persons undergoing a total body irradiation based autologous HSCT. [28]. In this double-blind, randomized study, 212 patients with various hematologic cancers were randomized to palifermin vs. placebo, comparing the development of oral mucositis which was evaluated daily up until day 28 post-autologous HSCT. The dose of palifermin administered was 60 µg/kg/day for three consecutive days pre-conditioning and three days post- autologous HSCT. AEs included rash, pruritus, erythema, mouth and tongue disorders, and taste alterations. All AEs were transient (occurring three days after the third dose of palifermin and lasting approximately three days), and mild to moderate in severity.

1.2.5 Clinical Experience: Allogeneic HSCT

Based on pre-clinical GVHD models Blazar and colleagues conducted a phase I/II randomized, placebo-controlled study examining the effect of palifermin on development of acute GVHD in humans undergoing alloBMT [29]. One-hundred persons were randomized 2:1 to receive palifermin 40-60 µg/kg/day x 3 doses pre-transplant followed by a dose escalation of 60 µg/kg/day up to 9 doses post-transplant. At the highest dose level (60 µg/kg/day x 12 doses total), 65% of persons completed the prescribed treatment regimen. The most common reason for discontinuing palifermin was erythematous skin reaction (3 patients). Other AEs related to palifermin vs. placebo were edema (78% vs. 65%), local pain (88% vs. 77%), and infection (11% vs. 0%). These events did not result in serious injury to any patient and resolved after drug removal. The rate of acute GVHD was not different between the placebo and palifermin arm. Similarly the rate of relapse was not different between the placebo and palifermin arms. A subsequent follow-up study did not show any impact on chronic GVHD either [30]. Thus, the authors concluded that palifermin was tolerated post-transplant but did not result in meaningful benefit at these doses; however, more prolonged palifermin administration was associated with highest incidence of side – effects.

In 2012, Jagasia and colleagues published the results of a phase 3, double-blind, randomized trial in which patient undergoing related donor or unrelated 6/6 matched allo HSCT received either palifermin (n=77) or placebo (n=78) with a primary aim to explore the role of palifermin in reducing the incidence of severe (grade 3-4) acute GVHD [31]. All patients underwent myeloablative conditioning, and the palifermin or placebo dose was 60 µg/kg/day on three days before the start of conditioning and then a single dose of palifermin 180 µg/kg or placebo at least 24 hours after the last dose of conditioning chemotherapy or radiation therapy. No significant difference was noted in the palifermin arm vs. placebo arm in terms of severe acute GVHD incidence (17% vs. 16&), and rates of mucositis were also similar (grade 3-4 mucositis was 73% in palifermin arm vs. 62% in placebo arm). AEs attributed to palifermin were tongue disorders, rash and pruritus, but all AEs were mild to moderate as well as transient.

Unfortunately these results are difficult to interpret in terms of GVHD prevention due to the doses selected in each study. While similar to that approved by the Food and Drug administration for mucositis prevention in the autologous HSCT setting, palifermin dose was comparatively much lower than that used in murine models where a benefit in GVHD, thymic function and immune reconstitution was observed.

1.2.6 Palifermin dose effects in patients undergoing chemotherapy +/- radiation for solid tumors

Palifermin has been tested in cancer patients using larger doses. Two studies tested palifermin at a dose of 180 µg/kg/week x 8 weeks in persons undergoing chemoradiotherapy for head/neck squamous cell carcinoma. The first, conducted by Le and colleagues, was a randomized, placebo controlled study in 188 persons receiving 70 Gy of radiotherapy in combination with cisplatin 100 mg/m2 on days 1, 22, and 43. [32]. Palifermin was administered weekly at 180 mcg/kg/week for 7 weeks, for a cumulative dose of 1260 µg/kg. The drug was tolerated well, with the most common grade 3-4 adverse events (AE) reported in the palifermin group being skin rash (9%),

flushing (5%) and dysguesia (5%). One episode of severe pancreatitis was observed. A reduction in grade 3-4 mucositis was observed in the palifermin group compared to placebo (54% vs. 69%, p=0.041).

The second study, conducted by Henke and colleagues, randomized 186 persons undergoing 60-66 Gy of radiotherapy plus a similar dose of cisplatin to placebo versus palifermin 180 μg/kg/week for up to 8 weeks, for a total cumulative dose of 840 – 1440 μg/kg [33]. Total adverse events in the palifermin versus control arms included dysphagia (35% versus 21%), diarrhea (12% versus 5%), and leukopenia (5% versus 13%). Elevations in amylase and/or lipase were noted in about 50% of persons receiving palifermin but these abnormalities resolved spontaneously in the first three weeks of treatment. In both trials greater than 75% of persons completed the study drug. A reduction was seen in the incidence of grade 3-4 mucositis in the palifermin vs. placebo group (51% vs. 67%, p = 0.027). The authors of these studies conclude that this dose of palifermin is tolerable in persons undergoing chemoradiotherapy.

A summary of the clinical experience with palifermin described in this section, in addition to several other prospective and retrospective studies involving the use of palifermin in the allogeneic setting are detailed in **Table 1** (please see below).

Table 1: Summary of the clinical studies carried out in humans

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
Phase 1									
Meropol, N. 2003 [25]	Metastatic colon or rectal cancer N = 54 KGF N=27 placebo	FU for 5 days (day +4 to +8)	Palifermin IV mcg/kg/ day on Day +1 to +3. Dosing: 1, 10, 20, 40, 60, 80 mcg/kg/ day	3, 30, 60, 120, 180, 240 mcg/kg (over 3 days)	18 rash, 6 flushing, 13 pruritus, 2 edema, 43 oral symptoms, 19 nausea, 48 pts Amylase and lipase elevated 18 DLT skin			Grade 2-4: 43% KGF vs 67% placebo Decrease in OM incidence in KGF vs. placebo: 10mcg:12% 20mcg:35% 40mcg:35% 60mcg:25% 80mcg:30%	
Zia-Amirhosseini, P et al 2006 [26]	Healthy volunteers Initially age 18 to 55	none	Single dose IV palifermin, randomized, double blind Starting at 60 mcg N= 63 palifermin N=16 placebo	Max tolerated dose 250 mcg/kg. Majority of palifermin exposure in first 24 hours, >95% AUC $_{0\text{-inf}}$ i AUC 3x from 60 to 250 (4x)	1 midcycle abd pain, Asymptomatic elevated lipase levels (pts > 30 yo, non-doses related), 16erythema, 10warm sensation, 21 access pain, 17 amylase, 8 lipase				

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
Zia-Amirhosseini, P et al. 2007 [27]	Auto HSCT N = 13 standard dose group N=12 collapsed dose group	TBI 12 Gy Day -8 to -5, Etoposide Day -4 Cyclophosphamide Day -2	Standard dose group: palifermin 60 mcg/kg/d IV on Day -11, -10, -9, 0, +1, +2). Collapsed dose group: 180 mcg/kg IV on day -11 and 0	Standard dose: 360 mcg/kg Collapsed dose: 360 mcg/kg Mean AUC on D-11 and D0 were 4 fold higher for collapsed-dose	Standard vs. collapsed: Rash 9vs.8, Diarrhea 13 vs.12, nausea 13 vs.12, pruritus 9 vs.5, abd pain 9 vs.2, vomiting 8 vs.6, anxiety 7 vs.2, increase amylase/ lipase.				
Srinivasan A, et al. 2012 [34]	N = 12, pediatric undergoing allogeneic HSCT	Cy/TBI Or TBI/Cy/ATG GVHD prophylaxis MMF or TMS or calcineurin/pentostatin	IV bolus: Day -9 to -7 or Day -11 to -9 pre-conditioning and Day +1, +2, +3 3 dose levels: 40, 60, 90mcg/kg/day	dose levels: 240 mcg/kg 360 mcg/kg 540 mcg/kg	8 Rash, 1 facial edema, 2 abd pain, 1 vomiting, 1 diarrhea, 1 headache, 4 elevated AST, 2 elevated ALT. No lipase /amylase changes.		83% survival at median follow-up 27%, 75% without progression of disease	(25%) mucositis.	
Phase 2									

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
Blazar et al. 2006 [29] Follow-up report: Levine/Blazar, BBMT 2008 UMinn, UMich	Palifermin: N=69 Control: N=31 Allogeneic 6/6 HLA matched sibling donor	Cy/TBI or Bu/Cy GVHD prophylaxis: MTX (D+1, 3, 6, 11)+ calcineurin	Pre-conditioning (D-11,-10 ,-9) Cohort 1: 40-60 mcg/kg/d) (D 0, +1, + 2) Cohort 2: 60 mcg/kg /day)(D0, +1, +2 and D+7 to 9) Cohort 3: 60 mcg/kg /day)(D0, +1, +2 and D+7 to 9, D+14to 16	Cohort 1: 240 – 360 mcg/kg Cohort 2: 540 mcg/kg Cohort 3: 720 mcg/kg	patients with DLT: 2 resp event, 3 skin reaction, 1 cardiac event, 1 hepatic enzyme elevation AEs palifermin vs. placebo: edema 78%vs65%, infection 11%vs.0%, local pain 88%vs77%, rash 94%vs68%	No difference in incidence or severity aGVHD Follow-up: cGVHD at 2 years (55 vs. 53%)	No difference in survival (84 vs. 82% at day 100) or Day 100 relapse Follow-up: long term survival at 2 yrs. (palifermin 46% vs. placebo 58%, p = 0.42).	Overall severity 2.8 vs. 2.3, p=0.01 Cy/TBI had grade 3-4 mucositis (100% placebo to 81% palifermin, p=0.05) No difference in Bu/Cy group (50% vs 44%mean severity 2.4 vs. 2.0)	Follow-up: no difference in CMV or invasive fungal infections at 1 year Median ALC at day 30, 60, 100, after transplant did not significantly differ
Rosen, LS et al. 2006 [35]	Colo-rectal, metastatic N = 28 palifermin N= 36 placebo	FU/ leucovorin	40 mcg/kg IV for 3 consecutive days	120 mcg/kg	Palifermin vs. placebo: skin rash 67 vs 54%, tongue issues 64% vs. 44%, Amylase 39% vs. 6% lipase 64% vs. 17%			Grade 2-4: 29% palifermin vs. 61% placebo in cycle 1, 11 vs. 47% cycle 2	

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
Nasilowska-Adamska, B et al 2007 [36] Follow-up: Nasilowska-Adamska, B et al 2011 [37]	N = 53 palifermin (n=29 auto, n = 24 allo) N= 53 control, (retrospective, n=29 auto, n=24 allo)	Myeloablative regimens No T depleted grafts	60 mcg/kg/d IV for 3 days pre-conditioning and 3 days post conditioning (total 6 doses)	360 mcg/kg	15 rash, 15 taste change, 14pruritus, 13 erythema, 10 general edema, 13 white film on tongue	Palifermin vs. control group: 25 % vs. 50% control group (p=0.14) Liver aGVHD 8.3% vs. 37.5%, p=0.036 cGvHD: 41% vs. 53.8% (p=0.70)	OS at 36 months f/u: 62.3% palifermin vs. 72.8% control, p=0.13	Auto: Grade 1-4 45% palifermin vs. 96% control (p<0.001), Grade 3-4 (7% palifermin vs. 28% control, p=0.037) Allo: Grade 1-4: 75% palifermin vs. 92% control (p=0.04), Grade 3-4 21% palifermin vs. 63% control (p=0.003)	
Retrospective									
Langner S,et al 2008 [38]	N = 30 palifermin alloHSCT	Myeloablative transplant Cy/TBI or Bu/Cy GVHD prophylaxis CYA and	60 mcg/kg/d for 3 consecutive days before initiation of conditioning therapy, and three days after		10 Rash, 10 erythema, 5 white coating on tongue, 4 taste alteration, 1 painful swelling gum,	Grade 2-4 aGVHD 31% vs. 30%, grade 3-4 aGVHD: 17 vs. 20%	OS day 100 similar: 90 vs. 86%	Grade 2-4: 60% palifermin group, 86% control group (p=0.04) Grade 3-4: 37%	

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
		MTX, +/- ATG	graft infusion		tongue, lips, mammary			palifermin vs. 53% control (p=0.19)	
Goldberg JD, et al 2013 [39]	N = 251, allo HSCT (n=154 receiving peri-transplant palifermin) N=121 TBI based, 64% received palifermin N=130 chemo based, 59% received palifermin	60 mcg/kg/d in three daily doses pre-transplant, and then 3 additional daily doses 60 mcg/kg/d starting 6 hours after PSCT		64 rash, 22 oral hyperplasia/ discoloration, 11 edema of hands or feet	No effect on aGVHD or cGVHD	No effect on relapse. No effect on OS or EFS	If TBI, PCA (palifermin 7 vs. control 12 days, p=0.023), TPN (7 palifermin vs. control 13 , p=0.0002). For non-TBI: no benefit observed		
Phase 3									
Spielberger R. et al 2004 [28]	Auto HSCT N = 106 palifermin N= 106 placebo	TBI 1200 cGy, Etoposide 60mg/kg on Day-4, and cyclophosphamide 100 mg/kg on Day -2	60 mcg/kg/day IV for 3 days pre-conditioning (i.e. starting 3 days before initiation of TBI)	180 mcg/kg (over 3 days)	Palifermin vs placebo: Rash 55vs 46%, pruritus 50vs32%, erythema 33vs.30%, edema 27vs.17%, taste change 22vs9%			Grade 3-4, 63% vs. 98% (p<0.001) Grade 4 (20% vs. 62%, P<0.001)	Incidence febrile neutropenia (72 vs. 95%, p<0.001)

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
Vadhan-Raj S, et al 2010 [40]	Sarcoma patients, multi-cycle chemo N= 32 palifermin N=16 placebo	Doxorubicin-based chemotherapy (90mg/m2 over 3 days) Max 6 cycles	180 mcg/kg as single dose IV 3 days before each chemo cycle	180 mcg/kg	Palifermin vs. placebo: thickness oral mucosa 72vs.31%, altered taste 44 vs. 19%, flushing 38 vs.13%, film in mouth 34 vs. 13%, gum sensitivity 28 vs. 6%			Grade 2-4: 44% palifermin vs. 88% placebo (p<0.001). Grade 3-4: 13% palifermin vs. 51% placebo (p=0.002)	
Henke M, et al 2011 [33]	Stage II to VIB ENT cancer with post-op CRT N=92 palifermin N=94 placebo	60 or 66 cGY, with cisplatin 100 mg/m2 Day 1, 22, 43	120 mcg/kg or placebo once weekly from - 3 days and throughout radio/chemo therapy	840 mcg/kg – 960 mcg/kg	Palifermin vs. placebo: dysphagia 35 vs. 21%, diarrhea 12 vs. 5%, asthenia 14 vs. 8%, headache 10 vs. 4%, Grade 3 or 4 amylase levels 50% vs. 42%		25% deaths overall, disease relapse 27% palifermin vs. 24% placebo	Grade 3-4: 51% palifermin vs. 67% placebo (p=0.027) Decreased duration (median 4.5 vs. 22 days)	
Le QT, et al 2011 [32]	Stage III to IVb ENT cancer, definitive CRT N= 94 palifermin,	70 Gy + Cisplatin (mean duration of radiation 50 days)	180 mcg/kg IV or placebo before radio/chemo and weekly for 7 weeks	1440 mcg/kg	Palifermin vs placebo: rash 9 vs. 2%, flushing 5 vs. 0%, dysgeusia 5vs1%,			Grade 3-4: 54% palifermin vs. 69% placebo arm (p=0.041)	

Ref.	No. of Persons	Conditioning /chemotherapy	Dosing	Cumulative dose	Toxicities	aGVHD	Survival	Mucositis	Immune reconstit.
	N= 94 placebo	100 mg/m2 on Day 1, 22, 43			nausea 4vs1%. 1 necrotic pancreatitis				
Jagasia MH. Et al 2012 [31]	Related donor or unrelated 6/6 matched allo HSCT N=77 palifermin N = 78 placebo	Myeloablative: D-11 to -2. Cy + TBI +/- etoposide, TBI + etoposide Melphalan + TBI Bu/Cy Busufan/ melphalan or fludarabine melphalan GVHD: MTX day 1, 3, 6, 11 + calcineurin	Placebo or Palifermin 60 mcg/kg/d IV on 3 days pre-condition + palifermin 180 mcg/kg IV or placebo 24h post-myeloablative Tx	360 mcg/kg	Palifermin vs. placebo AEs: tongue disorders, 6 vs. 0 Rash, 11 vs. 6 Pruritus in 10 vs. 4 Erythema 4 vs. 3	Grade 3-4 aGvHD similar, 17% vs. 16%		Grade 3-4 mucositis no difference (73% vs. 62%) Grade 2-4 mucositis 88 vs.90% TPN 49 vs 69%	

1.3 SUMMARY AND RATIONALE FOR THE STUDY

In numerous pre-clinical studies, palifermin has been shown to have an effect on control of GVHD and immune reconstitution after alloHSCT. Palifermin has been FDA approved in the autologous HSCT setting after being found to decrease the incidence of mucositis, but in the allogeneic HSCT setting the two prospective studies completed thus far have not shown any improvement in the incidence of acute or chronic GVHD or clinical immune recovery although thorough clinical immunology studies were lacking. One possibility is that these studies with palifermin in the allogeneic HSCT setting yielded no clinical effect on GVHD or immune reconstitution due to the fact that the doses of palifermin were much lower than those used in animal models. The doses of palifermin in these human studies were selected based on different pharmacodynamic endpoints, namely the effect on mucosal protection through the epithelial protection mechanism, and not based on thymic or immunologic protection. In human studies, the AUC of palifermin linearly increases with collapsed dosing, increasing exposure to the drug in target tissues such as the thymus. In non-human primate autologous HSCT setting, doses of 1500 µg/kg given over the course of a week in 250 µg/kg single doses peri-transplant were well tolerated and there was a cumulative dose-effect on immunological reconstitution and thymic protection. In addition, the animal chronic GVHD mouse model by Chu et al [17] showed better survival and better lymphocyte recovery which was dose dependent. Single doses of 180 µg/kg in the allogeneic HSCT setting and cumulative doses of 720 µg/kg over 1-2 weeks or 1440 µg/kg over 8 weeks have been given in the chemoradiotherapy solid tumor setting with good tolerance.

We hypothesize that there is a dose effect of palifermin on control of GVHD and immune reconstitution after alloHSCT and low dose administration of palifermin is not effective at reaching these endpoints. Therefore we propose to administer higher dose palifermin in the pre alloHSCT setting. A single dose of 180 µg/kg is well tolerated in humans, therefore we propose to start at this dose level and increase palifermin in a stepwise fashion in the pre-conditioning chemotherapy administration period. The primary endpoint of the phase I study is to establish a safe and well-tolerated dose of palifermin. We will then expand the study in a phase II portion to test the hypothesis that the higher dosing schema will result in an improved rate of chronic GVHD in this study population. Results of this study will be compared to those of our previous study (NCI-07C0195), where an identical conditioning (Flu/Cy) and GVHD prophylaxis (TMS) regimen was used without palifermin, to instruct the rationale for a larger, multicenter study outside of the NIH. Exploratory endpoints of this study include the examination of the effects of palifermin on acute GVHD and donor allograft immune reconstitution.

2 ELIGIBILITY ASSESSMENT & ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Induction Phase Inclusion Criteria

Patients meeting below eligibility criteria are eligible to receive suitable disease specific therapy for the purposes of disease control while the donor search takes place

2.1.1.1 The patient is ≥18 years of age.

2.1.1.2 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.1.3 Karnofsky performance score ≥ 60.

2.1.1.4 No suitable HLA matched sibling donor is available and the patient has one or more potentially suitable HLA matched unrelated donor(s) in the National Marrow Donor Registry or other available registry.

- The evaluation of donors shall be in accordance with existing NMDP Standard Policies and Procedures (See **Appendix E**)
- HLA-matched donors are defined by allele matching at HLA-A, -B, -C, and DRB1 (8/8).

2.1.1.5 There is a high likelihood that the patient, in the opinion of the PI or LAI, will meet the research phase eligibility criteria and proceed to transplant after induction phase therapy is completed.

2.1.1.6 Diagnosis of hematologic malignancy meeting **at least one** of the disease status criteria outlined in the table below. Diagnosis must be confirmed by the NCI laboratory of pathology.

Table 2: Disease Status Criteria in HSCT Recipients

Disease	Disease Status
B-Chronic Lymphocytic Leukemia (CLL)	○ Relapse/progression after two previous regimens. ○ No response or progression ≤6 months after fludarabine or bendamustine based regimen. ○ Progression within 24 months of a fludarabine and alkylator combination regimen. ○ Progression after any regimen in the presence of del17p or mutated TP53. ○ Persons with high grade lymphoma transformation.

Disease	Disease Status
Prolymphocytic Leukemia (PLL) T-CLL	○ T-PLL: Treatment failure after Campath-1H and at least one other regimen ○ B-PLL: Treatment failure after fludarabine and at least one other salvage regimen
Hodgkin Lymphoma	○ Primary treatment failure ineligible for autologous HSCT. ○ Relapse/progression after autologous HSCT.
Follicular Lymphoma, Marginal Zone Lymphomas (splenic, nodal, or extranodal/MALT type)	○ Primary refractory disease. ○ Relapse after ≥ 2 prior regimens. ○ Relapse/progression after autologous HSCT.
Burkitt or Lymphoblastic Lymphomas	○ High-risk disease in remission. ○ Progression after ≥1 previous regimen. ○ Non-CR after salvage regimen.
Diffuse Large B-cell Lymphoma, Follicular Large cell Lymphoma, Mantle cell Lymphoma, Anaplastic Large Cell Lymphoma	○ Primary refractory disease. ○ Relapse/progression after autologous HSCT. ○ Non-CR after salvage regimen. ○
Cutaneous T-cell Lymphomas (Mycosis Fungoides, Sézary Syndrome)	○ ≥Stage III ○ Disease progression ≥ 2 prior regimens, including at least one systemic therapy.
Multiple Myeloma*	○ Relapse/progression after autologous HSCT ○ Plasma cell leukemia ○ Adverse cytogenetics: e.g. del(13q) or 11q translocation. ○ *at the time of enrollment, MM must be in complete remission, see section **2.1.1.11** for details
Acute Myelogenous Leukemia	○ In first complete remission (CR1) in addition to one of the criteria outlined in section **2.1.1.7**. ○ Second or greater complete remission. ○ Secondary AML from antecedent myeloid neoplasm, myelodysplasia, or previous chemo or radiotherapy.
Acute Lymphocytic Leukemia	○ In complete first remission (CR1) in addition to one of the criteria outlined in section **2.1.1.8**. ○ Second or greater complete remission.

Disease	Disease Status
Myelodysplastic Syndrome	○ RAEB I or II ○ Intermediate, high or very-high risk per RIPSS ○ Secondary MDS
Myeloproliferative neoplasms (MPN) and myelodysplastic/myeloproliferative overlap neoplasms	○ Myelofibrosis with adverse-risk features as outlined in section **2.1.1.9** ○ Polycythemia vera ○ Essential thrombocythemia ○ Chronic myelomonocytic leukemia
Chronic Myelogenous Leukemia	○ Chronic phase CML having progressed after treatment with BCR-ABL kinase inhibitor or with evidence of T315I BCR-ABL mutation. ○ Accelerated or blast phase CML. ○ Not eligible for myeloablative allogeneic HSCT.
NK Cell Neoplasms	○ First CR for patients with high risk natural killer cell neoplasms including myeloid/NK cell precursor acute leukemia, blastic NK-cell lymphoma, aggressive NK-cell leukemia and nasal-type extranodal NK-cell lymphoma in first complete remission. ○ Second or greater CR.
Mature T-cell Non-Hodgkin Lymphoma (see WHO for specific malignancies)	○ First CR ○ Relapse after greater than or equal to 1 prior regimen

2.1.1.7 Recipients with AML in CR1 must have one of the following:

2.1.1.7.1 Adverse cytogenetics (as evaluated by history) as defined as complex karyotype (> 3 abnormalities); inv(3) or t(3;3); t(6;9); t(6;11); monosomy 7; trisomy 8, alone or with an abnormality other than t(8;21), t(9;11), inv(16) or t(16;16); or t(11;19)(q23;p13.1) or adverse-risk per European LeukemiaNet (ELN) 2017 criteria. [41, 42]

2.1.1.7.2 Intermediate-risk disease, such as cytogenetically normal AML (CN-AML) with mutations in FMS-like tyrosine kinase 3 (FLT3), DNA methyl transferase 3A (DMNT3A), or additional sex coombs like 1 (ASXL1) or per ELN 2017 criteria. [42-45]

2.1.1.7.3 Primary induction failure, defined as failure to achieve CR with primary induction chemotherapy. [46]

2.1.1.7.4 Secondary AML, defined as AML related to antecedent myeloid neoplasm or cytotoxic chemotherapy.

2.1.1.7.5 Hyperleukocytosis, WBC > 100,000, at diagnosis.

2.1.1.8 Recipients with ALL in CR1 must have one of the following:

2.1.1.8.1 Adverse cytogenetics defined as translocations involving t(4;11), t(1;19), t(8;14), 11q23, t(9;22) or bcr-abl rearrangement, Philadelphia chromosome-like (Ph-like ALL), or complex cytogenetic abnormalities.

2.1.1.8.2 Presence of minimal residual disease using multicolor flow cytometry or other analytic technique after primary induction chemotherapy.

2.1.1.8.3 Primary induction failure, defined as failure to achieve CR with primary induction chemotherapy [47].

2.1.1.9 Recipients with myelofibrosis must have at least 2 of the following features, or be DIPSS intermediate-2 or high risk [48-50]:

2.1.1.9.1 Hemoglobin < 10 g/dl, or > 10 g/dl with transfusion dependence.

2.1.1.9.2 WBC < 4,000 or > 30,000/mm3 or requires cytoreductive therapy to maintain WBC < 30,000/mm3.

2.1.1.9.3 Abnormal cytogenetics.

2.1.1.10 Patients with lymphoma must ideally have at least stable disease from last therapy, however if the PI or LAI believes there is a high likelihood of response to induction chemotherapy (EPOCH-F+/-R), then the patient may be enrolled on the induction phase arm. For enrollment on the research phase arm, the patient must have at least stable disease which is defined as:

2.1.1.10.1 absence of disease progression for at least 8 weeks after previous therapy or 12 weeks after autologous transplantation.

2.1.1.10.2 Patients who are less than 8 weeks from previous therapy or 12 weeks from autologous transplantation may participate in the study at the discretion of the PI or LAI as long as they do not have progressive disease.

2.1.1.11 Multiple myeloma in complete remission is defined as per Durie BG et al. [51]:

2.1.1.11.1 Negative immunofixation on the serum and urine and disappearance of any soft tissue plasmacytomas and <=5% plasma cells in the bone marrow. CR requires two consecutive assessments by serum and urine immunofixation made at any time prior to enrollment. CR also requires no known evidence of progressive or new bone lesions if radiographic studies are performed. Confirmation with repeat bone marrow is not needed.

2.1.1.12 The recipient has acceptable end organ function as defined by:

2.1.1.12.1 DLCO > 50% of the expected value (using USA-ITS-NIH equation) when corrected for Hgb (DLCO Adj.)

2.1.1.12.2 24 hour creatinine clearance or calculated (using the Cockcroft-Gault formula) creatinine clearance > 60 mL/min/1.73 m^2 (induction phase only)

2.1.1.12.3 Left ventricular ejection fraction > 45%

2.1.1.12.4 Serum total bilirubin less than 2.5 mg/dl, and serum ALT and AST values less than or equal to 2.5 times the upper limit of normal. Patients with elevations of serum total bilirubin up to 10 mg/dl and/or ALT or AST up to 10

times the upper limit of normal may be considered for participation if such elevations are thought to be due to liver involvement by malignancy. However, in these latter patients, if the BR level does not decrease to less than or equal to 2.5 mg/dl, or AST/ALT do not decrease to less than or equal to 2.5 times the upper limit of normal after induction chemotherapy, eligibility for the transplant (research) phase will be at the discretion of the PI.

2.1.1.13 Patients who are hepatitis B core antibody positive and or have positive hepatitis B surface antigen will require hepatology consultation. The risk/benefit profile of transplant and hepatitis B will be discussed with the patient and eligibility determined by the PI or the LAI.

2.1.1.14 Patient may have a hepatitis C infection. However, each patient will require a hepatology consultation. The risk/benefit profile of transplant and hepatitis C will be discussed with the patient and eligibility determined by the PI or the LAI.

2.1.1.15 Palifermin has had embryotoxic and fetotoxic effects in animal studies. For this reason and because the other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of active study therapy. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.2 Research Phase Inclusion Criteria

2.1.2.1 Verification of donor eligibility (clearance must be received from the NMDP)

- Donors are evaluated by NMDP affiliated donor centers per NMDP Standards (see **Appendix E**).
    - Donors who are medically suitable, but ineligible by FDA guidelines may still donate PBSC with documentation of urgent medical need by the PI.
    - Patients who receive stem cell products from ineligible donors will be informed of any increase in risk of transfusion-related diseases prior to initiation of conditioning chemotherapy.
- Donors who are ineligible or unwilling to donate bone marrow will not be eligible to donate to study recipients. However, in the event that the patient has already begun conditioning chemotherapy and a donor PBSC collection is terminated early for donor-related medical concerns, a bone marrow graft may be infused. Should this occur, the recipient will continue to be managed on this protocol for all transplant-related care and complications. Patient will stay on study but clinical outcomes will not be eligible for the statistics and end point calculations.

- Inadequate stem cell collection from the selected donor is defined as less than or equal to 2 x 10^6 CD34+ cells/kg. In most cases, donor cell collections are infused fresh. If a fresh collection is found to have an inadequate cell count, the cells will still be infused, but the recipient will be removed from the study, and managed clinically for all transplant-related care and complications on this protocol. If the patient fails to engraft, the donor may be requested for a second collection or an emergency bone marrow harvest at the discretion of the PI and NMDP Medical Director. In the event of an inadequate collection obtained prior to patient conditioning, the donor may be asked to donate a second time, or another eligible donor may be requested.

2.1.2.2 Renal and hepatic function continues to meet eligibility criteria, reassessed as follows:

- 24 hour creatinine clearance or calculated (using the Cockcroft-Gault formula) creatinine clearance > 60 mL/min/1.73 m2 (induction phase only)
  - $\frac{(140-\text{age})*\text{mass(kg)}}{72*\text{serum creatinine}(\frac{\text{mg}}{\text{dL}})}$ x 1.73 m2/ patients BSA
- If the patient is female, multiply the above by 0.85
- In patients with suspected liver disease, bilirubin must be ≤ 2.5 mg/dL, AST and ALT must be ≤ 2.5 times institutional ULN

2.1.2.3 The malignancy must be restaged prior to research phase and must not have progressed during induction chemotherapy (stable disease or better). Persons with acute leukemia, MDS/RAEB-I or –II or CML with previous accelerated or blast phase must have <5% blasts in the bone marrow. Persons with chronic phase CML may have up to 10% blasts in the bone marrow.

2.1.3 Exclusion Criteria

(applies to all phases of this protocol)

2.1.3.1 Active infection that is not responding to antimicrobial therapy.

2.1.3.2 Active CNS involvement by malignancy (patients with known positive CSF cytology or parenchymal lesions visible by CT or MRI).

2.1.3.3 Previous other malignancies unless they have undergone curative intent therapy for that malignancy and (1) have had no evidence of that disease for 5 years, and/or (2) be deemed at low risk for recurrence (less than or equal to 20% at 5 years).

2.1.3.4 HIV positive patients are ineligible as allogeneic stem cell transplant is not yet a proven approach in this patient population, and patients are at increased risk of lethal infections when treated with marrow suppressive therapy. [52]

2.1.3.5 Pregnant women are excluded from this study because palifermin has been shown to be embryotoxic and fetotoxic in animal studies. Because there is an

unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with palifermin, breastfeeding should be discontinued for the duration of active study therapy. These potential risks may also apply to other agents used in this study.

2.1.3.6 History of psychiatric disorder or any other condition which may compromise compliance with transplant protocol or expose patient to unnecessary risk as determined by principal investigator or lead associate investigator.

2.1.4 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites (i.e., clinicaltrials.gov) and on NIH social media platforms.

2.2 SCREENING EVALUATION

The following clinical, laboratory and radiologic assessments will be performed within 30 days of enrollment unless otherwise specified. Patients will be screened on protocol 01C0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols):

- Complete medical history (including Karnofsky performance status assessment) and physical examination.
- Antibody screen for hepatitis A, B, and C; HIV.
- CBC with differential, acute care panel, hepatic panel, mineral panel, PT, PTT, and ABO typing
- Urine or serum HCG in women of childbearing potential
- Gonadal Assessment: Female: FSH/LH, and Estrogen. Male: Testosterone (free and total)
- Urinalysis and urine protein creatinine (UPC)
- Creatinine clearance by 24 hour urine collection or calculated creatinine clearance by Cockcroft Gault formula.
- Risk of relapse documentation (Kahl scale) [53]
- Comorbidity index HCT-CI documentation [54]
- Unilateral bone marrow aspirate and biopsy as clinically indicated based on underlying disease type and patient history. Flow cytometry, cytogenetics, and molecular testing via polymerase chain reaction (PCR) should be performed on marrow aspirates as deemed clinically appropriate for specific diseases. Each patient will have a MRD (minimal residual disease) marker documented at study entry designated as: “flow cytometry”, “cytogenetics”, “molecular” or “other (specify)”. If bone marrow biopsies are done within the last 4 months outside of NIH (by the referring physician), then these slides will be requested and the bone marrow biopsy does not need to be repeated based on clinical judgment. Disease status assessments, which must be done at least 18 days beyond the start of cycle of therapy prior to enrollment:

  - CT scans of the chest, abdomen, pelvis +/- neck in order to evaluate disease status (with or without IV contrast depending on disease type and patient's renal function). Brain imaging (CT or MRI) may also be performed if clinically indicated.
  - FDG-PET scan for recipients with lymphomas or if clinically indicated to evaluate disease status.
  - Skeletal survey (for multiple myeloma patients only).
  - Recipients with multiple myeloma will also be tested for Blood: Total serum protein, serum protein electrophoresis (with immunofixation), CRP, immunoglobulin free light chains, and beta-2 microglobulin; Urine: 24 hour urine protein electrophoresis (with immunofixation).
- Electrocardiogram and 2D ECHO or MUGA scan to evaluate cardiac function
- Pulmonary function tests including DLCO adjusted for Hemoglobin (USA-ITS-NIH score)
- Hepatology consult in patients with chronic active Hepatitis B and/or Hepatitis C infection
- Optional lumbar puncture and CSF analysis (patient and disease specific) may be performed at the discretion of the principal investigator.
- Tests of DNA mini-satellite regions for STR chimerism profile.

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

2.3.1 Protocol Entry

The patient's entry date on protocol is considered to be the day that consent form has been signed by the recipient. The treatment start date is considered to be the day the recipient begins his/her initial induction cycle. Donors are not enrolled on the CCR protocol.

2.3.2 Registration

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

2.3.3 Treatment Assignment and Randomization/Stratification Procedures for Registration Purposes:

Cohorts

Number	Name	Description
1	Phase I: Dose escalation cohort	Up to 24 evaluable patients requiring an allogeneic stem cell transplant for hematologic malignancy enrolled to determine the MTD of palifermin

2	Phase II cohort	Patients requiring an allogeneic stem cell transplant for hematologic malignancy enrolled after the MTD of palifermin has been determined

Arms

Number	Name	Description
1	Phase I: Dose escalation arm	Induction chemotherapy, then palifermin at escalating doses, then conditioning chemotherapy, then allogeneic stem cell transplant, then immunosuppression
2	Phase II arm	Induction chemotherapy, then palifermin at the MTD determined in Phase 1, then conditioning chemotherapy, then allogeneic stem cell transplant, then immunosuppression

Stratifications

Not applicable to this study.

Randomization and Arm Assignment

This protocol does not utilize randomization. Patients in cohort 1 will be directly assigned to arm 1, and patients in cohort 2 will be directly assigned to arm 2.

2.4 BASELINE EVALUATION

The following clinical, laboratory and radiologic assessments will be performed on the patient (recipient) within 30 days before the initiation of Induction chemotherapy and the research phase (Day-7) unless otherwise specified. These tests can also be performed during screening. It will be at the PI's discretion not to repeat the test if he feels there has been no change in the patient's condition. In addition, all screening tests should be repeated if done > 30 days from Day-7. See Section **2.2**

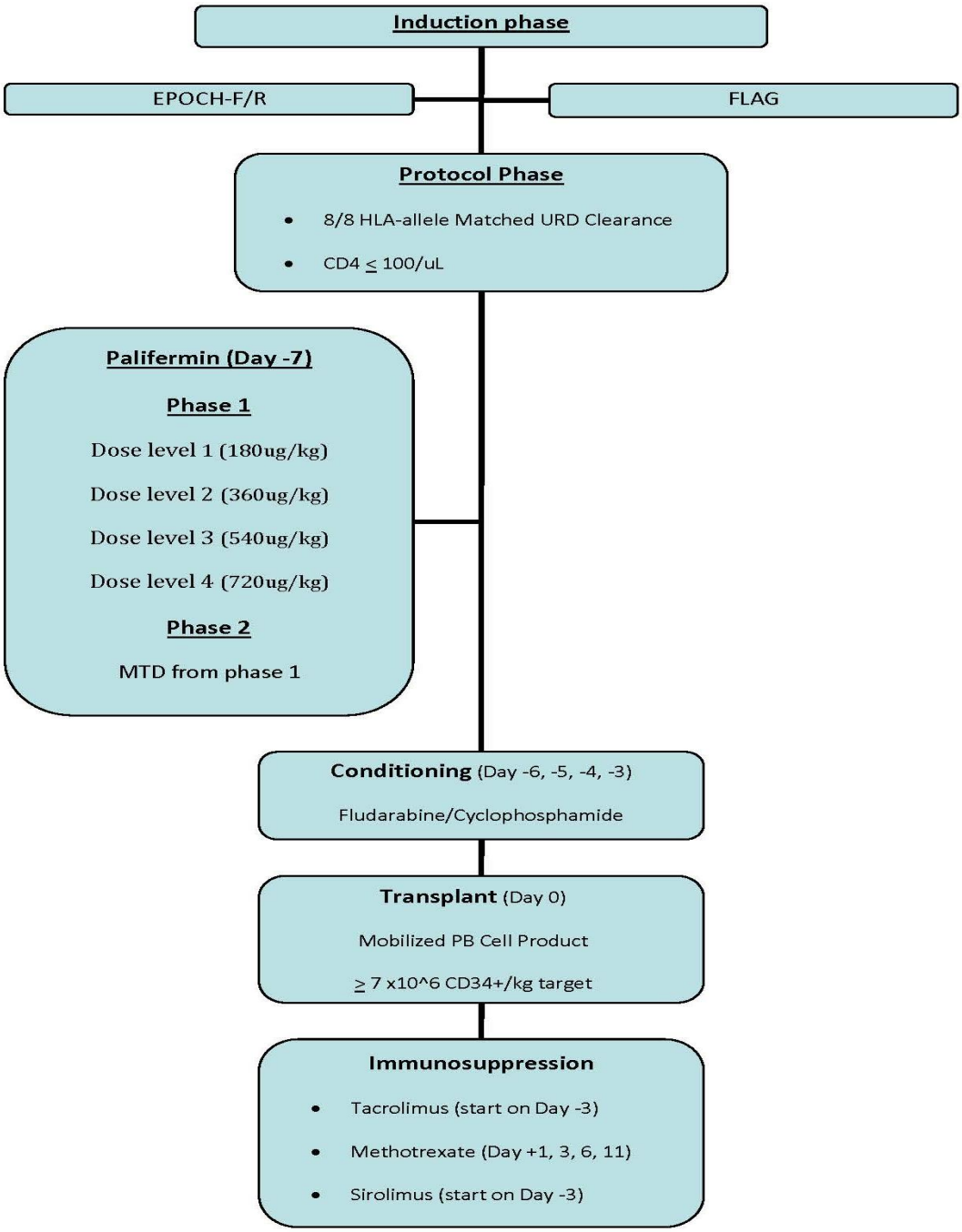
Antibody screen for T. Cruzi, HTLV-I/II, HSV, toxoplasma, varicella (VZV), West Nile Virus, Syphilis screen (RPR) and

- CMV and EBV PCR testing
- QuantiFERON-TB Gold or PPD test
- Amylase, lipase
- Serum ferritin, TSH, T3 (Triiodothyronine) and T4 (Thyroxine, Free), Lipid profile
- Flow cytometric analysis of peripheral blood CD3, CD4, CD8, and CD56 positive lymphocyte populations (TBNK).
- Serum immunoglobulins, quantitative (IgG, IgM, IgA)
- Nutritional assessment
- Dental consultation to assess need for teeth cleaning or removal (valid for 90 days)
- Social work consultation

- Ophthalmology consultation (valid for 90 days)
- Gynecology consultation (for pre-and peri-menopausal women) (valid for 90 days)
- Chemosensitivity status as previously defined by Salit R. et al [55]: chemotherapy-sensitive disease, defined by as CR or PR to their most recent prior regimen, chemo-refractory disease: SD or PD to their most recent prior regimen.

3 STUDY IMPLEMENTATION

3.1 STUDY SCHEMA



3.2 INDUCTION PHASE

- The following items should be obtained within 48 hours before starting induction chemotherapy. The tests need not be repeated if obtained in the appropriate timeframe at screening or baseline:

  - Adequate central venous access is required for all patients undergoing transplantation on this protocol.
  - CBC with differential
  - Acute care, hepatic, and mineral panel.
  - Urine or serum beta-HCG in women of childbearing potential.
- The purpose of induction chemotherapy prior to transplant conditioning is to establish host immune depletion. Individuals who meet the following criteria secondary to previous treatment will not require further immune depletion and may proceed directly to the research phase.
  - ≤100 CD4 cells/μl of blood assessed at least 18 days beyond the start of last cycle of prior therapy. The investigator may allow proceeding without induction if the CD4 count is within the statistical margin of error (≤115 cells/μL) or if dictated by the patient best interest (such excessive risk of infection, toxicity or malignancy relapse), at the discretion of the PI.
  - Demonstration of disease response/control that is at least 28 days beyond the start of cycle of therapy prior to enrollment.
- Induction Chemotherapy - After study enrollment, recipients will typically receive either EPOCH-F/R (every 21 days, maximum 3 cycles) or FLAG (every 28 days, maximum 2 cycles) according to the underlying diagnosis, as indicated in the following table. If other therapy is found more appropriate for disease, this will be allowed per the discretion of PI or LAI.
- Number of cycles of induction chemotherapy will be determined by the quantity of host immune CD4 cells/μL remaining after each cycle or if maximum cycles have been completed or if dictated by the patient best interest (such excessive risk of infection, toxicity or malignancy relapse).
- The CD4 count must be measured at least 48 hours after the cycle's last dose of filgrastim and within three days before the next scheduled cycle.
- Recipients will undergo a CBC two times per week (typically at least 3 days apart) to determine the absolute neutrophil count (ANC) during the neutrophil nadir post EPOCH-F/R or FLAG.
- Recipients with progressive malignancy between the final cycle treatment and transplantation (Research phase) may be removed from study per PI discretion.

FLAG	EPOCH-F/R
Acute myelogenous leukemia (AML) Secondary AML Acute lymphocytic leukemia (ALL) Lymphoblastic lymphoma Myelodysplastic syndrome Myeloproliferative disorder (MPD) Chronic myelogenous leukemia (CML)	Hodgkin lymphoma Non-Hodgkin lymphoma (NHL) (except lymphoblastic lymphoma) Chronic lymphocytic leukemia (CLL) Prolymphocytic leukemia (PLL) Multiple myeloma

3.2.1 EPOCH-F/R

3.2.1.1 Drug Administration

Drug	Dose	Days
Rituximab+*	375 mg/m^2 IV infusion (Patients with CD20+ malignancies only)	Day 1 only
Fludarabine	25 mg/m^2 per day IV infusion over 30 minutes, daily for 4 days	Days 1, 2, 3, 4
Etoposide	50 mg/m^2 per day continuous IV infusion over 24 hours daily for 4 days	Days 1, 2, 3, 4
Doxorubicin	10 mg/m^2 per day continuous IV infusion over 24 hours daily for 4 days	Days 1, 2, 3, 4
Vincristine	0.4 mg/m^2 per day continuous IV infusion over 24 hours daily for 4 days	Days 1, 2, 3, 4
Cyclophosphamide	750 mg/m^2 IV infusion over 30 minutes	Day 5 only
Prednisone	60 mg/m^2 per day PO daily for 5 days	Days 1, 2, 3, 4, 5
Filgrastim	5 μg/kg per day SC or IV (see section **13.3.4**)	Daily from day 6 until ANC > 1000/μl x 2 or > 5000/μL x 1 following nadir.
*Recipients with a malignancy that does not express CD20 will not receive Rituximab. +Subjects will be premedicated 30 -60 minutes prior to rituximab infusion. Please see section **13.7.4**		

3.2.1.2 Dose modifications to EPOCH-F/R

The above table describes dose level 1. For cycles 2 and 3 patients may receive dose adjustment according to the following guidelines:

- Cyclophosphamide, doxorubicin, etoposide dose adjustments

Dose Level	-2	-1	1	2	3
Doxorubicin (mg/m^2/day)	10	10	10	12	14.4
Etoposide (mg/m^2/day)	50	50	50	60	72
Cyclophosphamide (mg/m^2/day)	480	600	750	900	1080

  - Drug doses are based on the duration of ANC nadir during the previous cycles (as determined by consecutive twice weekly CBC/differential tests).

If ANC < 500/μl on 1 or fewer measurements AND there is no other grade 3+ non-hematologic toxicity	→	Increase cyclophosphamide, doxorubicin, and etoposide 1 dose level above previous cycle.
If ANC < 500/μl on 2 measurements	→	Same dose level as previous cycle.
If ANC < 500/μl on 3 measurements or if there is a grade 4 non-hematologic toxicity	→	Decrease cyclophosphamide, doxorubicin, and etoposide 1 dose level below previous cycle.

- Vincristine Dose Adjustment (for EPOCH-F/R):
  a) For grade 2 sensory or motor neuropathy reduce vincristine to 50%
  b) For grade 3 sensory or motor neuropathy omit vincristine

- Fludarabine will be reduced by 20% in recipients with a 24 hour or calculated creatinine clearance of < 70 mL/min/1.73 m^2 (Cockcroft-Gault formula).
- There are no dose modifications for rituximab; however adverse events may be managed by changes in the infusion rate as described in section **13.7.4**.

All other patients will continue at the same dose of EPOCH-F/R for the subsequent cycle.

3.2.2 FLAG

3.2.2.1 Drug Administration

Drug	Dose	Days
Fludarabine	25 mg/m^2 per day IV infusion over 30 minutes, Daily for 5 days	Days 1, 2, 3, 4, 5
Cytarabine	2,000 mg/m^2 IV infusion over 4 hours, Daily for 5 days	Days 1, 2, 3, 4, 5
Filgrastim	5 μg/kg per day SC beginning 24 hours PRIOR to initiation of chemotherapy	Daily from day 0 until ANC >1000/μl x 2 following nadir

- Corticosteroid ophthalmic drops will be administered every 6 hours starting the day before and continuing until the day after cytarabine therapy.
- In the event of signs of CNS toxicity the cytarabine infusion will be discontinued. No cytarabine will be administered in recipients with history of cytarabine neurotoxicity.

3.2.2.2 FLAG dose modification for cycle 2:

- If the first cycle of FLAG is associated with a grade 4 non-hematologic toxicity the second cycle of FLAG will be dose reduced by eliminating the fifth day of drug infusion to the above regimen.
- Fludarabine will be reduced by 20% for recipients with 24 hour or calculated creatinine clearance < 70 mL/min/1.73 m2 (Cockcroft-Gault formula – section **2.1.2.2**).
- Patients with malignancies positive for BCR-ABL mutation may continue use of BCR-ABL tyrosine kinase inhibitors during therapy as deemed appropriate by the investigator.

3.3 RESEARCH PHASE

3.3.1 Palifermin / Conditioning chemotherapy / HSCT

- Recipients may begin the research phase (administration of palifermin followed by conditioning and peripheral blood hematopoietic stem cell transplant) 22 or 28 days after the most recent cycle of EPOCH-F/R or FLAG induction therapy, respectively.
- Disease restaging must be done no more than 28 days before initiation of research phase, with testing done per patient's primary disease and investigator discretion:

  - Unilateral bone marrow aspirate and biopsy. Flow cytometry, cytogenetics, and molecular testing via polymerase chain reaction (PCR) should be performed on marrow aspirates as deemed clinically appropriate for specific diseases. Each patient will have a MRD (minimal residual disease) marker documented at study entry designated as: “flow cytometry”, “cytogenetics”, “molecular” or “other (specify)”
  - CT scans of the chest, abdomen, pelvis +/- neck in order to evaluate disease status in Lymphoma patients (with or without IV contrast depending on disease type and patient’s renal function)
  - Brain imaging (CT or MRI) may also be performed if clinically indicated.
  - FDG-PET scan if clinically indicated to evaluate disease status (e.g., some lymphoma patients).
  - Recipients with multiple myeloma will also have the following tests: Total serum protein, serum protein electrophoresis (with immunofixation), immunoglobulin free light chains; 24 hour urine protein electrophoresis (with immunofixation).
  - Persons with acute leukemia, MDS/RAEB-I or –II or CML with previous accelerated or blast phase must have peripheral blood flow cytometry for determination of blast count performed.
- CT scan of the chest without IV contrast to determine baseline thymus measurements. (If CT chest with IV contrast is being done to determine disease status, this may be used instead for thymus measurement.)
- GI consultation prior to administration of palifermin (to document any pre-existing conditions) within 30 days
- Collection of Dental Research Samples (See Section **5.1.3.1**)
- The following studies will be obtained from the recipient within 7 days prior to starting palifermin:
  - CBC with differential, acute care panel, mineral panel, and hepatic panel, serum amylase and lipase, urine or serum beta-HCG in women of childbearing potential.
  - Recording of baseline symptoms prior to administration of palifermin.

3.3.1.1 Palifermin Administration

- Palifermin will be administered as a single intravenous bolus injection at the appropriate dose levels on day -7 relative to HSCT. Co-administration with heparin should be avoided.

3.3.1.2 Phase 1

3.3.1.2.1 Dose Escalation

- The initial phase I study will incorporate a 3+3 type design using the following dose levels:

Dose Level	Dose palifermin (mcg/kg) IV on day -7
1	180
2	360
3	540
4	720

- Three patients will be enrolled on each dose level. If no dose limiting toxicities occur in the first cohort after three patients (and they have reached the 30 day post-transplant time point), subsequent patients will be enrolled in the next dose level.
- If one patient experiences a dose limiting toxicity, an additional three patients will be enrolled on this dose level. If no further dose limiting toxicities occur, subsequent patients will be enrolled in the next dose level.
- If a second dose limiting toxicity (DLT) occurs, this dose level will be closed and the previous dose level dosing will be selected as the maximum tolerated dose (MTD).
- In summary: The MTD is the dose level at which no more than 1 (of ≤ 6) patients who experience DLT, and the dose below that at which at least 2 (of ≤6) patients have DLT as a result of the drug.

3.3.1.2.2 Dose Limiting Toxicity

The following events are considered dose limiting toxicities secondary to palifermin administration:

- Non-relapse mortality (see Section **8** for definition) before day 30 post transplantation regardless of attribution to palifermin. Persons who expire from malignancy related causes are not considered DLTs.
- Non-hematologic CTCAE ≥ grade 4 adverse events (AEs), occurring within 14 days after administration of palifermin that are determined by the investigator to be at least possibly related to the study drug. An isolated laboratory value is not considered an AE unless it meets the guidelines described in section **8**.

Note: Patients will not be removed from study therapy due to palifermin toxicity as only 1 dose is administered. DLT criteria are established only to determine dose levels for subsequent patients.

3.3.1.3 Phase 2 Guidelines

- The maximum tolerated dose (MTD) from the phase I portion of the study will be used to conduct a phase II study. If no MTD is reached, the highest dose level will be used for the phase II portion of the study
- The maximum number of individuals enrolled on the phase II study portion is 27 (see section **8**). Individuals treated at the MTD in the phase I portion are included in the phase II portion.

- The phase II portion of the study will be conducted with a stopping rule based on treatment related mortality as described in section **8**.

3.3.2 Conditioning Chemotherapy

The conditioning chemotherapy will consist of:

Drug	Dose	Days
Fludarabine	30 mg/m^2 per day IV infusion over 30 minutes, daily for 4 days	Transplant Days –6, -5, -4, -3
Cyclophosphamide	1200 mg/m^2 per day IV infusion over 2 hours, daily for 4 days	Transplant Days –6, -5, -4, -3
Mesna	1200 mg/m^2 per day Continuous IV infusion, Daily for 4 days Concurrent with cyclophosphamide	Transplant Days –6, -5, -4, -3
Furosemide	20 mg IV flat dose	Transplant days -6, -5, -4, -3

- Hydration and dose modifications associated with conditioning chemotherapy include:
    - Hydration will consist of 0.9% Sodium Chloride at a rate of 100 mL/hour starting 12 hours prior to initiation of cyclophosphamide and continuing until 24 hours post the final dose of cyclophosphamide.
    - Furosemide may be increased at the investigators' discretion.
    - Fludarabine will be reduced by 20% in recipients with 24 hour or calculated creatinine clearance < 70 mL/min/1.73 m^2 (Cockcroft-Gault formula – section **2.1.2.2**).
- Filgrastim will be administered at a dose of 10 mcg/kg recipient body weight beginning one day after donor cell infusion and continuing until the recipient has an ANC of >1000/mcL for at least two days or > 5000/mcL once.

3.3.3 Graft versus Host Disease (GVHD) prophylaxis

3.3.3.1 Tacrolimus

- Tacrolimus will be initiated on day -3 before the transplant. Tacrolimus will be administered continuous intravenous infusion at 0.02 mg/kg/day. Tacrolimus dose will be adjusted to achieve a therapeutic goal of 5 to 10 ng/ml according to trough levels monitored at least two times per week and/or upon symptoms or alterations in renal function. Trough levels may be monitored more frequently if clinically indicated.

- When the recipient is able to take oral medications (typically 10 to 21 days after transplantation), tacrolimus will be converted to an equivalent oral dose. The total daily dose will be divided into two equal doses, one dose given approximately every 12 hours.
- This dose of tacrolimus will continue until day +60 (+/- 7 days), and then reduced by one-third as long as the severity of GVHD is at minimum less than grade 2 and patient is not requiring systemic steroids. Tacrolimus will be subsequently reduced by one third on day +100 (+/- 7 days). Tacrolimus will then be completely discontinued by day +180 (i.e. 6 months) if there are no signs of GVHD or as clinically indicated.

3.3.3.2 Methotrexate

- Methotrexate will be given at 5 mg/m2 IV on days +1, +3, +6, and +11. Each day's dose of methotrexate will not be administered until approved that day by the transplant attending after the transplant team has evaluated the patient.
- Doses will be withheld for the development of grade III or IV mucositis or clinical evidence of veno-occlusive disease. Administration of the combination of trimethoprim and sulfamethoxazole (i.e., Bactrim®) or non-steroidal anti-inflammatory drugs is contraindicated during methotrexate administration.
- Methotrexate administration will be assessed on each planned day of administration, and dose adjustments will be guided by the following criteria, however can be adjusted based on the discretion of the attending physician, P.I., and/or LAI:

Creatinine	Total Bilirubin	Methotrexate Dose
< 2.0 mg/dL	<5.0 mg/dL	100%
2.0 – 3.0 mg/dL	----	50%
> 3.0 mg/dL	> 5.0 mg/dL	Hold Dose

- Methotrexate may also be held or dose adjusted at the discretion of attending physician, P.I., and/or LAI for severe mucositis, third space fluid collection (e.g. pleural effusion, ascites, or edema).
- Leucovorin (folinic acid) rescue may be considered under the direction of the attending physician or PI. The recommended dose regimen of leucovorin is 10mg IV or PO every 6 hours starting 24 hours after methotrexate administration and continued until undetectable methotrexate levels or upon the discretion of the attending physician, P.I., and/or LAI.
- Leucovorin should be held 12 hours prior to the any subsequent methotrexate dose and not resumed (if indicated) for 24 hours after a methotrexate dose.

3.3.3.3 Sirolimus

- Sirolimus will be initiated on day –3 before the transplant procedure. Sirolimus will be administered by mouth as sirolimus tablets at an initial "loading dose" of 6 mg, p.o., on day –3 of transplantation.
- Subsequently, sirolimus dosing will be 2 mg, p.o., each day.
- Sirolimus dose will be adjusted to achieve a therapeutic goal of 3 to 12 ng/ml according to trough levels monitored at least two times a week and/or upon symptoms or alterations in renal function. Trough levels may be monitored more frequently if clinically indicated.
- This dose of sirolimus will continue until day +60 (+/- 7 days), and then reduced by one-third as long as the severity of GVHD is at minimum less than grade 2 and patient is not requiring systemic steroids. Sirolimus will be subsequently reduced by one third on day +100 (+/- 14 days). Sirolimus will then be completely discontinued by day +180 (+/-14 days) if there are no signs of GVHD or as clinically indicated.

3.3.4 Hematopoietic Stem Cell Transplant

Hematopoietic stem cell transplant will be performed per the standards of the NIH CC Department of Transfusion Medicine. Please see section **13.13** for protocol specific donor cell processing procedures.

3.3.5 Evaluation Post Transplant

3.3.5.1 Clinical Response

- The following studies and clinical response evaluations will be obtained in the recipient during post transplantation:
  - CBC and differential count daily during transplant hospitalization, then as clinically indicated.
    - Persons with minor ABO mismatch will have their CBC checked twice daily from Day +4 to Day +14 post-transplant and transfused for a goal hemoglobin level of ≥ 9.5 g/dL.
  - Acute care, mineral panel and hepatic panel, daily during transplant hospitalization, then as clinically indicated.
  - GGT twice a week through day 100 and then as clinically indicated.
  - Amylase, lipase daily during transplant hospitalization until Day +6, then twice weekly until day +28. After Day +28, amylase and lipase will be drawn weekly until Day +100, then at each timepoint.
  - CMV weekly through day +180 post-transplant, then as clinically indicated (monitoring will continue longer if patients are continued on immunosuppression).
  - EBV weekly until day +180 post-transplant, then as clinically indicated (longer if patient deemed high risk).

- Type and screen every 4 days during transplant hospitalization, then as clinically indicated.
- Toxoplasmosis weekly on positive patients during inpatient hospitalization, then as clinically needed.
- Tacrolimus and sirolimus trough level at least twice weekly, for first 100 days, or as clinically indicated. Sirolimus trough level will need to be collected on day -2 (one day after loading dose).
- Of note, all lab tests done between day +100 and Day +180 (namely CBC, acute care panel, mineral panel, hepatic panel, CMV, EBV, tacrolimus and sirolimus levels) can be obtained at patient's home institution since beyond Day +100 as patients are usually stable enough to go home and no longer require all lab testing to be obtained at NIH. The frequency of the labs will be at the discretion of the patient's home physician.
- TBNK at day +14 +/-7 days, +28(+/- 7 days), +60 (+/- 7 days), +100(+/- 14 days), +180(+/- 14 days), +270(+/-14 days), +365(+/- 30 days), 18 months (+/- 30 days) and 24 months (+/- 30 days) then yearly until year 5 in follow-up.
- Recipient peripheral blood lymphoid, myeloid, and total chimerism on day +14 (+/- 7 days), +28(+/- 7 days), +60 (+/- 7 days), +100 (+/- 14 days), +180(+/- 14 days). Chimerism may be measured at other time-points if clinically indicated (e.g., to determine effect of manipulating immune suppression in order to increase donor chimerism).
- Peripheral blood or bone marrow flow cytometry, cytogenetics, and/or molecular studies to determine minimal residual disease as is clinically indicated day +28 (+/- 7 days), +100(+/- 14 days), +180(+/- 14 days), +365(+/- 30 days), 24 months (+/- 30 days) and yearly thereafter as clinically indicated.
- Bone marrow biopsy and aspiration (including cytogenetics and molecular studies) on day +28 (+/- 7 days), +100(+/- 14 days), +180(+/- 14 days), +365(+/- 30 days) and yearly thereafter in patients with prior bone marrow disease up until year three post-transplant. Confirm with the PI if cytogenetics and/or molecular studies are needed. Bone marrow biopsy and aspirates can also be done at any time if clinically indicated.
- Recipient bone marrow chimerism on days +28 (+/-7 days), +100(+/- 14 days), and +180 (+/- 14 days). Lymphoid & myeloid subset chimerism will also be measured at these time-points if donor chimerism was < 95% on the previous study. Chimerism may be measured at other time-points if clinically indicated.
- Optional lumbar puncture and CSF analysis (patient and disease specific) if the principal investigator determines it is needed on day +28 (+/- 7 days), +100(+/- 14 days), +180(+/- 14 days), +365(+/- 30 days) and yearly thereafter in patients with prior CNS disease up until year three post-

transplant. Confirm with the PI for the appropriateness and need for concurrent intrathecal chemotherapy administration (see section **4.7**).

- CT scan of the chest (without IV contrast) at day +28(+/- 7 days), +180 days (+/- 14 days), +365 days (+/- 30 days), 24 months (+/- 30 days) and 36 months (+/- 60 days) post -transplant for determination of palifermin effect on thymus measurements [15, 24, 56-59] . At each timepoint the thymus volume (cm^3), thymus size grading score (Score 0-5, ref: McCune et al [56]) and thymic density grading score (Score 1 to 5, ref: Simanovsky [59]). If a CT chest is being done with IV contrast for other reasons, then this scan can be used to evaluate thymus measurements as well.
- For assessment of disease, CT scans (with contrast if medically appropriate) of chest, abdomen, and pelvis (neck included if measurable disease is present), and/or PET and/or cranial imaging will be performed at the discretion of the PI or LAI at day 28(+/-7 days), day 100 (+/- 14 days), day 180(+/- 14 Days) , and yearly (+/- 30 Days) thereafter as clinically indicated.
- For multiple myeloma patients, repeat MM workup at 28 days (+/- 7 days), +100(+/- 14 days), +180(+/- 14 days), +365(+/- 30 days), 24 months (+/- 30 days) and yearly thereafter as clinically indicated.

- Evaluation and documentation for acute GVHD at time point clinic visits at days +28, +100, and +180. The date of acute GVHD diagnosis and stage/grade will be recorded at diagnosis (+/-30 days from time point, due to clinic schedule).
- Evaluation and documentation for chronic GVHD at time-point clinic visits at days +28, +100, +180, +270, +365, +18 months, 24 months and every year thereafter up until 5 years (+/-30 days from time point, due to clinic schedule).
- The recipient will be seen in follow-up at the Clinical Center at Day+28 (+/- 7 days, if Outpatient) this visit will count as a 30 day post palifermin safety visit , Day +60 (+/- 7 days), Day +100 (+/- 14 days), Day +180 (+/- 14 days), Day +270 (+/- 14 days), 12 months (+/- 30 days), 18 months (+/- 30 days), 24 months (+/- 30 days) and then annually post-transplant until 5 years, unless an earlier evaluation is clinically indicated. Evaluations beyond 5 years will be done only if clinically indicated.

3.3.5.2 Other Studies

- Other studies: Patients may obtain these studies within 2 months of desired date unless otherwise specified and these may be done in other institutions than NIH with documentation. Please note that the following can be performed either as part of this protocol or at an outside clinic.
  - PFTs on day +100 (+/- 14 days), +180 (+/- 14 days), +270 (+/- 14 days), +365 (+/- 30 days), 18 months (+/- 30 days), and 24 months (+/- 30 days) then yearly until 5 years.

- Serum immunoglobulins (IgG, IgM, IgA) at day +28 (+/- 7 days), +60 (+/- 7 days), +100 (+/- 14 days), +180 (+/- 14 days), +270 (+/- 14 days), +365 (+/- 30 days), 18 (+/- 30 days) and 24 months (+/- 30 days) then yearly until 5 year follow-up
- Eye clinical exam at 1 year and later if clinically indicated
- Dental evaluation at day 60 (+/- 7 days), +180 (+/- 14 days), +365 (+/- 30 days), and later if clinically indicated. Buccal biopsy, saliva collection and clinical oral photo series at day 60 (+/- 7 days) and +180 (+/- 14 days). Note: Dental consult and biopsies/saliva collection/photo series will be obtained only if the patient's medical status allows for these outpatient evaluations to take place. The buccal biopsy is an elective procedure and is not required for this protocol.
- Dermatology evaluation and skin biopsy at day +60 (+/- 7 days) and +180 (+/- 14 days). Additional dermatology evaluations if clinically indicated. Dermatology consult will be obtained if the patient's medical status allows for these outpatient evaluations to take place. Additional skin biopsies will be taken at the time of suspected GVHD for diagnosis and/or research. Skin biopsy is an elective procedure and is not required for this protocol.
- Serum ferritin measured in patient's day +365 (+/- 30 days) then as clinically indicated
- DEXA scan at day +365 (+/- 30 days) post-transplant then as clinically indicated
- Thyroid function test (TSH, T3 (Triiodothyronine), T4 (Thyroxine, Free)) at 1 year (+/- 30 days) and then yearly thereafter until 5 years or as clinically indicated.
- Echocardiogram at day +100 (+/- 14 days), then as clinically indicated.
- Clinical and gonadal assessment of women 1 year after transplant then yearly until 5-year follow-up if clinically indicated (GYN consult, FSH, LH, estrogen levels)
- Clinical and gonadal assessment of men at 1 year after transplant then yearly until 5- year follow-up if clinically indicated (testosterone)
- Urinalysis day +180 (+/- 14 days), +365 (+/- 30 days), then yearly until 5 years.

3.3.5.3 Relapse or malignancy progression:

If a patient relapses they will be followed clinically for survival and occurrence of Graft versus Host Disease; no research blood or protocol-directed tests or procedures are planned. Patients will continue to be evaluated as close as possible to protocol driven timepoints, these evaluations can occur at NIH or at home (via medical records review and/or phone call). Further clinical evaluation will be at the discretion of the PI.

3.4 COST AND COMPENSATION

3.4.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.4.2 Compensation

Participants will not be compensated on this study.

3.4.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.5 TELEHEALTH

Telemedicine is the use of interactive audio, video, audio-visual, or other telecommunications or electronic technology by a licensed health care practitioner to deliver clinical services. This protocol will allow the team to practice telemedicine to communicate with patients in real time, to be able to monitor and collect data, as well as the ability to share the patients' health information with other health professionals. Providers may include primary providers, specialists/consultants and nurses. Other members of the healthcare team may also be present to aid with the communication devices, scheduling or records management. These visits may include the following: patient history, verbal exam, symptom reporting, and education.

The patient or patient's legal representative will be informed prior to the use of a telemedicine encounter and consent will be obtained as outlined in the Consent Process and Documentation (section **11.3**).

Telemedicine visits will be arranged through our NIH Clinical Center Health Information Management Department and will be scheduled using NIH-approved remote platforms. Telemedicine visits may be used for follow-up visits if deemed appropriate by the PI. All telemedicine visits must be documented in CRIS like a normal onsite visit and the note should indicate that this visit was performed virtually.

Remote visits will be conducted in compliance with NIH guidelines and FDA regulations.

Local Evaluations

A patient may be asked to come to the NIH CC for an in-person assessment or be referred to their local provider or outside lab, at the discretion of the investigator. All physical exams, assessments, labs, and imaging used for follow-up or restaging visits may also be performed with the patient's local physician or completed at outside labs. For laboratory evaluations conducted with local providers, interlaboratory variability is not a concern. In the case of any visits with participants' local providers or outside labs, records will be obtained for the research records.

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of Palifermin .

3.6.1 Criteria for Removal from Protocol Therapy

- Failure to meet pre-transplant eligibility criteria after completion of induction therapy
- Patient requests to be withdrawn from protocol therapy
- PI Discretion.
- Relapse Disease
- Once the post-Palifermin safety follow-up visit has been conducted

3.6.2 Off Study Criteria

- Failure to meet pre-transplant eligibility criteria (See section **2.1.2**).
- The recipient requests to be removed from the study.
- Patient lost to follow up
- Investigator discretion.
- After completing 5 years of follow-up per protocol per discretion of the Principal Investigator
- Death

3.6.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit 3 times and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 INFECTION PROPHYLAXIS

All Concomitant medications will be listed in the study database with just the start and stop dates. See **Appendix G** for further details.

- All infection prophylaxis medications will be administered as per NIH Bone Marrow Transplant Consortium guidelines viewed at: (http://intranet.cc.nih.gov/bmt/education/supportive-care.shtml) Pneumocystis jiroveci pneumonia prophylaxis will be instituted at the time of induction chemotherapy (EPOCH-F/R or FLAG) and continue for at minimum six months after transplant immunosuppression is discontinued. If trimethoprim/sulfamethoxazole is used, it should be held during the engraftment period immediately after donor cell infusion.
- Trimethoprim/Sulfamethoxazole double strength will be administered twice a day starting on the evening of day -7 for 5.5 days for a total of 11 doses (stopping with the evening dose on day-2) for gut decontamination pre-transplant. This was a strategy used in the 07C0195 patients; thus we will continue this for the current protocol for consistency.
- Yeast prophylaxis will begin during induction chemotherapy and continue until at least 100 days post transplantation. Typically, during induction chemotherapy, fluconazole will be used, except it will be held during EPOCH-F/R infusion due to interaction with vincristine. During conditioning chemotherapy, micafungin will be used.
- Persons on extended schedules (>1 week) of high-dose corticosteroids (>0.5 prednisone/kg) may be changed to an alternate anti-fungal regimen at the investigator's discretion.
- Persons with an absolute neutrophil count of <500/μL post conditioning chemotherapy shall receive ceftazidime or appropriate alternative regimen (as recommended by infectious disease consultant) until neutrophils have recovered.
- All recipients will receive herpes virus prophylaxis starting with induction chemotherapy and continuing for at minimum six months until immunosuppression is discontinued after transplantation.
- All recipients will undergo weekly peripheral blood mononuclear cell DNA analysis for CMV and EBV at least until day 100 post transplantation and then as clinically indicated thereafter.
- Management of CMV viremia is according to the NIH Bone Marrow Transplant Consortium guidelines. (http://intranet.cc.nih.gov/bmt/education/supportive-care.shtml)
- Vaccines will be administered on the schedule provided in the appendices, as clinically appropriate.

- Patient on immunosuppression for chronic graft versus host disease should take penicillin VK 500 mg twice daily or appropriate alternative regimen (as recommended by infectious disease consultant) for prevention of bacterial infections.

4.2 Blood Product Support

- Packed red blood cells will be infused in recipients at any time the serum hemoglobin concentration falls below 8.0 g/dL or if symptoms of anemia are present.
- Platelets may be infused at the investigator's discretion to prevent bleeding or if the platelet count falls below 10,000/μL.
- Leukocyte filters and irradiation will be used for all blood and platelet products according to the NIH Department of Transfusion Medicine standard policies.

4.3 Menses Suppression

- All pre- or peri-menopausal women who have not undergone hysterectomy will be referred at study entry for gynecology consult to receive counseling about menses suppression, contraception and fertility preservation per NIH Blood and Marrow Consortium supportive care guidelines. (http://intranet.cc.nih.gov/bmt/education/supportive-care.shtml)
- Male patients will be advised to use abstinence or barrier methods (condoms) for pregnancy prevention for at least one year post transplantation.

4.4 Engraftment Syndrome

- Engraftment syndrome is an inflammatory disorder that may occur within 96 hours of neutrophil recovery after transplantation. [60]
- Recipients may receive corticosteroids for engraftment syndrome at a starting dose of at least 1 mg/kg/d of prednisone or equivalent. The steroid taper may be performed at the investigator's discretion.

4.5 Graft Versus Host Disease (GVHD)

- Grading and diagnosis:
    - Acute GVHD will be graded according to the 1994 Consensus Conference Working Criteria. [61]
        - Diagnosis of acute GVHD is a clinical diagnosis and is therefore at the discretion of the investigator. The diagnosis will include review of tissue biopsy whenever clinically feasible.
    - Chronic GVHD will be graded according to the 2014 NIH Consensus Criteria. [62]
- Therapy:

- Grade 2 or greater acute GVHD typically requires systemic therapy. Corticosteroids will be initiated in these persons at a dose and schedule to be determined by the investigator.
- Persons with more than mild chronic GVHD may be treated with systemic therapy at the investigator's discretion.
- If the patient is eligible for a therapeutic study that is available at the NIH for either acute or chronic GVHD this is the preferred treatment option.

4.6 DONOR LYMPHOCYTE INFUSIONS (DLI)

- Persons with relapsed primary malignancy, development of EBV related lymphoproliferative disorder (EBV-PTLD), or those who fail to achieve full donor derived hematopoietic cell engraftment (mixed chimerism) are eligible for a DLI.
- Mobilized, cryopreserved donor lymphocytes may be used if there are remaining samples from the original donor collection; otherwise, a request will be made to NMDP for subsequent lymphocyte collection from the original donor.
- Donor and recipient hematopoiesis (chimerism) will be assessed after DLI infusion if deemed necessary.
- Withdraw of immune suppression should be attempted before DLI if the donor lymphoid chimerism is >50%. However, DLI may be performed before withdrawal of immune suppression at the investigator's discretion
- Recipients with declining donor chimerism despite withdrawal of immune suppression or with donor lymphoid chimerism <50% are eligible for a DLI.
- The initial dose of the DLI is at the discretion of the investigator.
- Persons must have < grade 2 acute GVHD or < moderate chronic GVHD in order to receive a DLI.
- DLIs will not be administered more frequently than every 28 days
- Subsequent DLIs will be at the discretion of the PI

4.7 OTHER THERAPIES

- Chemotherapy, cytokines, radiotherapy, monoclonal antibodies, or any other available therapy may be administered to persons with progressive primary malignancy, or EBV-PTLD post-allogeneic transplant in order to establish a remission. These therapies are administered at the discretion of the investigator. If the patient is eligible for another study ongoing at the NIH, this approach is preferred.
- Intrathecal chemotherapy may be administered as deemed clinically appropriate per PI.

5 CORRELATIVE STUDIES FOR RESEARCH

Note: Once a patient is taken off "protocol therapy" and the safety follow-up visit has been conducted, then no further research samples will be collected.

Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1 RESEARCH STUDIES OF TRANSPLANT BIOLOGY

5.1.1 Immune Reconstitution of Blood

5.1.1.1 Recovery of T cell receptor repertoire diversity by CDR3 spectratyping and assessment of recent thymic emigrants by TREC PCR.

5.1.1.1.1 50 mL of blood will be drawn from the recipient at Baseline (pre induction) and, following transplant, at 6 months, 12 months and 24 months and placed into yellow top ACD tubes. 50 million lymphapheresis cells from the donor PBSC product will be sent to the Blood Processing Core (Dr. Figg's Lab).

This sample permits calculation of spectratype complexity index. (Blood to be sent to the Blood Processing Core (Dr. Figg's Lab).

5.1.1.1.2 Studies performed: Donor and recipient samples will be sorted to separate CD4+ and CD8+ T cells and monocytes. T cell subsets will be divided for storage by snap freezing (DNA) or in Trizol (RNA). After completion of patient accrual, RNA will be isolated and RT-PCR followed by Vβ spectratype analysis will be performed to assess T cell receptor repertoire diversity. DNA will be used in PCR assays to assess the frequency of T cell receptor rearrangement circles (TREC) relative to total genomic DNA (compared to a single gene standard, RNAseP).

5.1.1.2 Phenotypic and functional assessment of lymphocyte reconstitution. (Blood to be sent to the Blood Processing Core (Dr. Figg's Lab).

5.1.1.2.1 Collection

- 16ml of whole blood, collected in the form of 1 green top (heparin) tube and 1 SST (serum) tube will be sent at Day-7 and the day of PBSC infusion (Day 0 +/- 1 Day) to the Blood Processing Core (Dr. Figg's Lab). These will be used to track depletion of lymphocyte populations during conditioning and as a source of plasma and serum for assessing cytokine changes during conditioning.
- 50 ml of whole blood, collected in the form of 1 SST and 5 red & green CPT tubes will be sent at serial time points to the Blood Processing Core (Dr. Figg's Lab). These time points include: baseline (before start of respective (EPOCH-F/R or FLAG) induction chemotherapy); days +7(+/- 3 Days), +14 (+/- 7 Days), +28 (+/- 7 Days), +60 (+/- 7 Days), +100 (+/- 14 Days); 6 months (+/- 14 Days), 9 months (+/- 14 Days), 12 months (+/- 30 days), then 18 months (+/- 30 Days), 24 months (+/- 30 Days), annually thereafter (+/- 30 days). These will be used to track reconstitution of lymphocyte populations following transplant and as a source of plasma and serum for assessing cytokine changes after transplant.

- An additional 50 ml (6 R&G CPT) will be sent at the onset of acute and/or chronic GVHD, prior to start of systemic GVHD treatment, whenever clinically possible.

5.1.1.2.2 Studies performed

- For all recipients, peripheral blood subsets will be serially monitored by multiparameter FACS analysis on fresh whole blood, to assess T, B and NK subsets. Peripheral blood mononuclear cells (PBMC) will be cryopreserved in liquid nitrogen (vapor phase) for later assays by flow cytometry of T lineage commitment (based on cytokine production and transcription factors) and expression of markers indicative of hematopoietic lineage, state of activation and anergy and capacity for migration (adhesion and chemokine receptors). Heparinized plasma will be separately stored at -80°C as the main source of plasma for assays of chemokines, cytokines and other factors relevant to studies of immune dysfunction after transplantation.
- Cryopreserved PBMC cells may be activated in vitro with a number of different stimuli including specific antigens and mitogens which are known to activate distinct pathways of T lymphocyte function. Assays may include T cell proliferation, cytokine production and gene expression, cytotoxic T lymphocyte generation, and antigen presenting cell function.
- The specific assays to be used in the on-going data analyses are subject to be modified, deleted or replaced as technology and knowledge in the field evolve during the course of the study without constituting a change in research aims. These assays apply also to those samples received from outside institutions. If a significant departure from this “Immune Characterization” is contemplated based on accumulated data, then the protocol and consent will be amended accordingly to cover the new line of investigation and its potential risks to subjects.

5.1.2 Immune Reconstitution of Bone Marrow

The bone marrow is a critical site in allogeneic transplantation because it is (1) the site of hematopoietic progenitors and early differentiation of lymphocyte and myeloid lineages, as well as (2) a potential site of persistent or relapsed malignancy. For all patients, an additional bone marrow aspirate (up to 5ml in a heparinized syringe) will be obtained at the times scheduled for bone marrow examination as per protocol. The aspirate will be collected following marrow aspirates required for clinical assessments.

5.1.3 GVHD Pathogenesis

The pathogenesis of chronic GVHD is poorly understood and no studies have rigorously evaluated cellular and molecular changes that occur in the target tissues of chronic GVHD. Heterogeneity in patient and treatment related factors complicate group comparisons. Prospectively designed study with planned sequential sample collection is the ideal format that would give the best chance of success in study of this complex problem.

5.1.3.1 Collection

- We will focus on the skin and oral cavity as the two most common target organs of chronic GVHD. Standard 4-6 mm punch biopsies will be performed in the skin and buccal mucosa in all patients at the beginning of immunosuppressive taper at day 60 (+/- 7 days) and at 6 months (+/- 14 days). A research biopsy may also be performed at the onset of clinical chronic GVHD (skin or oral mucosa) at additional time points. Research skin and oral biopsies are optional and will not be collected if the patient declines this procedure. If a patient's medical status is unstable, then these research biopsies will not be collected.
    - Preferably, skin biopsies should be taken from upper back, biceps area or mid-abdomen. However, consideration should be that day 60 and day 180 biopsy will be done of the same anatomical area.
- Whole saliva will be collected at pre- Palifermin (Post induction and before Palifermin administration on Day – 7) and at day 60 (+/- 7 days) and at day 180 (+/- 14 days) to evaluate the changes in the salivary proteome, microbiome and cytokine content at the onset of chronic GVHD.
- Swabs of the buccal mucosa, tongue and gums will be performed at Pre-Palifermin (Post induction and before KGF administration on Day-7) and at Day 60 (+/- 7 days) and at day 180 (+/- 14 days) to evaluate changes in oral microbiome.

5.1.3.2 Studies Performed

Analysis of changes in immune cell populations and molecular events in the target tissues of chronic GVHD (Biospecimen Processing Core).

The tissue biopsy samples may be analyzed using a variety of methods including immunofluorescence and confocal microscopy, gene expression profiles, and protein based assays in order to better understand the reconstitution of resident immune cell populations following HSCT and how they change with the onset of chronic GVHD.

5.1.4 Exploring status of Endocannabinoids/CB1R system and iNOS

5.1.4.1 Background

Endocannabinoids are lipid-signaling molecules that act on the same cannabinoid receptors - CB_1 and CB_2 - that recognize and mediate the effects of marijuana. Arachidonoylethanolamide (anandamide) and 2-arachidonlyglycerol (2AG) are the major endocannabinoids. Endocannabinoids acting via CB_1R promote inflammation and fibrosis progression in multiple organs including liver, kidney, heart, lung and skin. Blocking CB_1R by selective antagonists attenuated the disease progression in animal models of organ fibrosis. Therefore, CB_1R antagonist identified therapeutic target for multiple forms of fibrosis.

Inducible nitric oxide synthase (iNOS), an enzyme that catalyzes the generation of proinflammatory reactive nitrogen species involved in cell injury, inflammation and oxidative stress, is induced in fibrotic tissues like liver, lung, heart and skin. Recently, dual targeting CB_1R and iNOS inhibition by a single molecule provided improved anti-fibrotic efficacy targeting these proteins alone. Currently, a dual-target CB_1R and iNOS

inhibitor (MRI-1867) is a potential clinical candidate for fibrotic disorders and under clinical development.

5.1.4.2 Objective

The laboratory of Dr. Cinar at NIH would like to explore the status of endocannabinoids / CB_1R system and iNOS in specimens (plasma and skin biopsies) from patients with cGVHD. Identifying overactivity of CB_1R system and iNOS in cGVHD may suggest potential pathologic roles of CB_1R and iNOS in cGVHD. Furthermore, it could identify CB_1R and iNOS as therapeutic targets for cGVHD.

5.1.4.3 Methods

Liquid chromatography and triple quad mass spectrometry (LC-MS/MS). Immunohistochemistry will be performed on plasma (about 200 uL) and frozen and parafilm embedded skin biopsies. De-identified and coded samples will be sent from the Biospecimen Processing Core (BPC) to Dr. Cinar's lab for analysis (shipping address: 5625 Fishers lane room 2S-18 Rockville, MD 20852). Dr. Cinar will not be given or have access to identifiable data or samples from subjects.

5.2 Sample Processing

See Section **15.11**, Appendix K for planned sample processing details/techniques that may be adjusted as needed.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main BPC number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov

The samples will be processed, barcoded, and stored in the BPC until requested by the investigator.

5.3 Sample Storage, Tracking and Disposition

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.3.1 Sample Data Collection

All samples sent to the Biospecimen Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each

sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.3.2 Future Studies

Blood, fluid and tissue specimens collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study, when patients give consent, in the Optional Studies section of the informed consent document, for storage for future use. However, this research may only be done if the risks of the new questions were covered in the consent document. If new risks are associated with the research (e.g. analysis of germ line genetic mutations) protocol amendment will be required and informed consent will be obtained from all research subjects to whom these new studies and risks pertain.

5.3.3 Protocol Completion/Sample Destruction

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested).. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

6 DATA COLLECTION AND EVALUATION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency

and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

6.1 DATA COLLECTION PROCEDURES

- After obtaining Informed Consent, a file will be created in the database with standardized forms. The study database will maintain data for described endpoints in the protocol. The medical record will maintain complete records on each patient including any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records. The study database will serve as the primary source from which all research analyses will be performed.
- Available data on donor characteristics will be entered into the study database. Note: Per NMDP regulations, and to maintain donor confidentiality, unrelated donor source documents will *not* be sent to the NIH. The NMDP will maintain all required source documents in accordance with NMDP policies and procedures.
- Documentation of data verification will be tracked in the study database.
- Please see **Appendix G** for detailed information to be included in the database
- Concurrent medications will not be captured for the duration of the study except for GVHD specific systemic immunosuppressive drugs

6.1.1 Adverse Event (AE) Recording:

Grade 1 adverse events will not be recorded.

If a patient has relapsed disease adverse events will no longer be recorded except grade 5 events and unanticipated problems.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

As the two induction therapy regimens are standard regimens with well-defined toxicity profiles, adverse events will not be collected during induction therapy administered prior to the conditioning chemotherapy except grade 5 events and unanticipated problems. This also includes any laboratory data before Day -7.

Record adverse events from the first study intervention (i.e. D-7) then as directed below.

- Hematologic events (anemia, neutropenia, etc..) are not considered adverse events as they the desired outcome the conditioning chemotherapy
- Grade 2-4 AEs will be recorded up to 14 days after palifermin dose (e.g. day +6 post-transplant) regardless of attribution to IND.
- After day +6 post-transplant, only grade 4 AEs occurring up to 100 days post transplantation and infections noted infection **6.1.2** will be recorded. After 100 days post transplantation only infections per section **6.1.2** will be recorded up to 24 months.
- Any grade 5 AE will be recorded.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event

6.1.2 Infection Recording Requirements

- Any grade 2-4 infection occurring from Day+7 to 24 months will be recorded using the Cordonnier scale only (see Section **15.10**, appendix J for details). These events will not be captured as adverse events unless they meet the requirements mentioned in the adverse events recording section of the protocol:
    - CMV/EBV
        - Results of nasopharyngeal virus panel (Nasal Wash) that may include Influenza A, Influenza B, Parainfluenza 1, 2 or 3; Adenovirus, Respiratory Syncytial Virus (RSV), Rhinovirus, Coronavirus, Meta-pneumovirus, or any other nasopharyngeal virus.
        - Sepsis, Bacteremia, Candidemia, Aspergillus, Varicella Zoster, Viral Encephalitis, Pneumocystis jiroveci, Toxoplasmosis gondii, and pneumonia

* If a patient has relapse disease infection data will no longer be recorded or reported

6.2 RECORD KEEPING

All patients must have signed an Informed Consent, and on-study eligibility checklist will be filled out by the Research RN and faxed to the Central Registration Office (CRO) before patient is entered on the study.

Complete records must be maintained on patients; these will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on the study database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flow-sheets, specialty forms for pathology, radiation, or surgery; adverse event assessment; and off-study summary sheet, including a final assessment by the treating physician.

6.3 GENOMIC DATA SHARING PLAN

Unlinked genomic data will be deposited in public genomic databases in compliance with the NIH Genomic Data Sharing Policy.

Note: The analyses planned will not generate incidental genomic findings; as indicated in Section **5.3.2**, the study will be updated for appropriate consent, management, etc., added in an amendment if the plan should change.

6.4 RESPONSE CRITERIA

6.4.1 Definitions

6.4.1.1 Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with palifermin.

6.4.1.2 Evaluable for Disease Response: All patients will be evaluable for disease response from Day +0 transplant onwards. Subjects will no longer be followed for GVHD endpoint if it is necessary to deviate from original prophylaxis plan (e.g. more rapid tapering of immunosuppression for malignancy relapse, any anti-malignancy therapy, DLI administration)

6.4.2 GVHD Diagnosis/Grading Criteria

Formal acute GVHD assessment will be performed on days +28 (± 7 days), +60 (± 4 days), +100 (+/- 14 days) and +180 (+/- 14 days).

Formal chronic GVHD assessment will be performed on days +28, +60, +100, + 180, +270, +365, yearly thereafter until 5 years.

Acute and chronic graft-versus-host disease (GVHD) will be graded according to NIH cGVHD staging criteria specified in **Appendix C**: Acute Graft Versus Host Disease Evaluation (Worksheet) and **Appendix D** respectively

6.4.3 Malignancy Response Criteria

Please see **Appendix B**.

6.5 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a

copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/hrpp-policy-guidelines/.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found https://irbo.nih.gov/hrpp-policy-guidelines/. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/hrpp-policy-guidelines/.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary..

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NATIONAL MARROW DONOR PROGRAM REPORTING CRITERIA

7.4.1 Adverse Events

7.4.1.1 NMDP shall be notified of serious adverse events at possibly, probably or definitely related to the product

7.4.1.2 Fatal or potentially life-threatening adverse events possibly, probably or definitely related to the product shall be reported to NMDP by close of the next business day following determination of the event

Please contact the NMDP research coordinator

- Data will also be sent to the Center for International Bone Marrow Transplant Research (CIBMTR).

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **8.4.**

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI.

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of Expedited Reporting to CCR

8.4.1 Disease progression

Death and hospitalization that are deemed to be due to disease progression, and not attributable to the intervention will not be reported as an SAE unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

The event, and the assessment that it was caused by disease progression will be documented in the medical records.

8.4.2 Late occurring SAEs

Any serious adverse event that occurs > 30 days post Palifermin unless possibly, probably, or definitely related to IND.

The PI will submit a summary table of all grade 3-5 events, whether or not considered related to the product, every 6 months. The report shall include the number of patients treated in the timeframe, the number of events per AE term per grade which occurred in the 6-month timeframe and in total since the start of the study, attribution, and type/category of serious.

Reports will be submitted to the Center for Cancer Research (CCR) at OSROSafety@mail.nih.gov

The Sponsor might request case summaries for those events if, upon review, the Sponsor determines that an aggregate safety report is required (21CFR312.32(c)(1)(iv)).

8.5 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI.

8.5.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.5.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm for the duration of active study therapy.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until the last dose is administered should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.7 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies,

or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

- The primary objectives of this study are to determine a reasonable safe maximum dose level of palifermin to use in connection with an alloHSCT regimen and to determine in a pilot fashion if adding palifermin to the TMS regimen will result in a modest reduction of the incidence of severe chronic GVHD.
- In the phase 1 portion of the study, we will use a standard 3+3 design to determine maximum dose level of palifermin that could be administered to patients receiving a transplant using the TMS regimen. If 4 different increasing doses are investigated then up to 24 patients may be needed in this portion of the study.
- Once the dose to use has been determined, in the phase II portion of the trial patients will be treated at that dose of palifermin along with the TMS regimen. Patients treated at that dose level in the phase I portion of the trial will be included in the phase II portion of the trial.
- Based upon results from the recent 07C0195 trial, when analyzed by cumulative incidence curve analysis, the probability of having severe chronic GVHD on the TMS regimen was approximately 28% at two years. It would be useful to try to see if the 28% rate of severe cGVHD by 2 years as seen on the prior study could be substantially improved upon. With 27 patients in arm 4 to be evaluated in the phase II (including those who were included in the dose escalation phase), an exact binomial test with a 10% one-sided significance level will have 87.3% power to detect the difference between 28% with severe chronic GVHD at 2 years and 10% on the present trial.
- In practice, in addition to forming the fractions of patients who have not experienced severe cGVHD by 2 years, a Kaplan-Meier curve of the probability of avoiding development of severe chronic GVHD will be constructed, and will have values at key time points such as 12, 24 and 36 months without severe chronic GVHD estimated, along with appropriate 80% and 95% confidence intervals to explore whether the present results exceed those from the prior study with TMS alone, in an informal comparison without formal statistical testing. As death due to any cause is a substantial competing risk, a cumulative incidence curve of development of severe chronic GVHD competing with death due to any cause will also be constructed. This curve can also be compared informally to the corresponding curve from the TMS arm from the 07C0195 study. In addition, as exploratory evaluations, Kaplan-Meier and cumulative incidence curves adjusting for the competing risk of death from any cause may be constructed moderate

and severe chronic GVHD. These curves may also potentially be compared to the corresponding curves from the TMS arm from the 07C0195 study. In addition, curves that include patients treated with fewer than the maximum safe number of doses may be constructed for exploratory purposes.

- This trial is of limited size and intends to establish if a trend toward improvement in severe chronic GVHD incidence may occur by the addition of palifermin. Should these results appear promising, the palifermin regimen studied in this trial may be explored in a subsequent more definitive, multi-institutional study.
- The following are stopping rules for patients included in the phase II portion (including 3 to 6 at the MTD from the dose escalation phase) based on observing non-relapse mortality (NRM) rates which are inconsistent with those identified on 07C0195:
    - If anytime within the first 15 patients who are included in the phase II portion, there are 5 or more patients who have experienced NRM within 100 days of PBSC, no further patients will be accrued to this trial as soon as this is able to be determined. This is because the TMS arm of 07C0195 has a 100 day NRM cumulative incidence of 10.8%, and 5/15 has a lower one-sided 90% confidence interval of 17.2%, which exceeds the rate experienced on the 07C0195 trial. 4/15 has a lower one-sided 90% CI of 12.2%, and thus this could be potentially interpretable as being essentially marginally consistent with that of the prior trial and not sufficiently worse to mandate early stopping.
    - Additionally, if anytime within the first 15 patients who are included in the phase II portion, there are 6 or more patients who have experienced NRM within 6 months of PBSC, no further patients will be accrued to this trial as soon as this is able to be determined. This is because the TMS arm of 07C0195 has a 6 month NRM cumulative incidence of 16.2%, and 6/15 has a lower one-sided 90% confidence interval of 22.6%, which exceeds the rate experienced on the 07C0195 trial. 5/15 has a lower one-sided 90% CI of 17.2%, and thus this could be potentially interpretable as being essentially marginally consistent with that of the prior trial and not sufficiently worse to mandate early stopping.
    - Finally, if anytime within the first 15 patients who are included in the phase II portion, there are 7 or more patients who have experienced NRM within 12 months of PBSC, then no further patients will be accrued to this trial as soon as this is able to be determined. This is because the TMS arm of 07C0195 has a 12 month NRM cumulative incidence of 19.0%, and 7/15 has a lower one-sided 90% confidence interval of 28.2%, which exceeds the rate experienced on the 07C0195 trial. 6/15 has a lower one-sided 90% CI of 22.6% and thus this could be potentially interpretable as being essentially marginally consistent with that of the prior trial and not sufficiently worse to mandate early stopping.
    - Assuming accrual of 10 patients per year, and assuming that up to 18 patients are included **only** in the phase I portion and 27 are included in the phase II portion (including 3-6 patients on both the phase I and II portions),

the accrual period for the entire trial is expected to be 4-4.5 years. In order to allow for non-evaluable patients, the accrual ceiling will be set at 50 patients.

- In addition to toxicity, chronic GVHD and immunological endpoints, the study may report analysis for additional transplant outcomes supported by the data, including: Overall survival, failure-free survival, non-relapse mortality (death in clinical remission), relapse-related mortality (death without clinical remission), GVHD related mortality, rate of achieving remission, malignancy relapse (recurrence after remission)/progression, incidence of severe infections, engraftment of neutrophils and platelets, graft failure, incidence of starting steroids and steroids administration AUC, rate of starting secondary therapy for acute and chronic GVHD separately, time to permanent (>6 months) interruption of immunosuppression, malignancy remission and cGVHD severity at last yearly follow-up. It will also be of interest to compare the fraction of patients with grade II-IV aGVHD among patients included in the phase II portion of the present trial to that of patients treated on the TMS arm of 07C0195. An analysis based on 39 patients from the TMS arm of 07C0195 showed that 21/39 (53.8%) of patients experienced grade II-IV aGVHD. With 27 patients on the present trial, and 39 from the prior trial, a Fisher's Exact Test with a 10% one-sided sided significance level will have 84.3% power to detect the difference between a 24% rate of grade II-IV aGVHD on the present study 54% on the TMS arm of 07C0195. (**Appendix A** and **Appendix B** show definitions of hematopoietic engraftment and disease responses).
- Acute and chronic GVHD incidence will be reported in 2 ways:
  - Total incidence across trial independently of other interventions,
  - No longer followed for GVHD endpoint if it is necessary to deviate from original prophylaxis plan (e.g. more rapid tapering of immunosuppression for malignancy relapse, any anti-malignancy therapy, DLI administration).

11 HUMAN SUBJECTS PROTECTION

11.1 RATIONALE FOR SUBJECT SELECTION

Lymphoid malignancies affect all races and sexes. However, in some instances males are more likely than females to be affected, and this will be reflected in the sex distribution of this study. For example, while NHL has a higher incidence in African Americans from the mid-to-late teens to mid-50s, the incidence in white individuals exceeds African Americans beginning at age 55. Among women, Hispanics of all races have the second highest incidence of NHL after whites.

This trial will use a convenience sample of participants from subjects being screened at the NCI. Therefore, targeted selection by race or sex for equal distribution will not be feasible. Should trends in racial, ethnic or sex differences become evident, additional studies may be designed or this trial be amended to further explore those differences.

Patients with high-risk hematologic malignancies will be the subjects for this study. Allogeneic HSCT represents a potentially curative treatment for patients with the

disease characteristics selected for inclusion. The expected survival of such patients with conventional chemotherapy is approximately one year or shorter. Eligibility in this protocol is limited to adults (age ≥ 18 years).

11.1.1 Participation of Children

Children will not be enrolled in this study. Immune reconstitution is different between children and adults, which is an exploratory endpoint of this study.

11.1.2 Unrelated Donors

The National Marrow Donor Program (NMDP) IRB will be responsible for the review and continuing oversight of protocol procedures that relate only to NMDP unrelated donors. These donors will not be enrolled on this protocol.

11.1.3 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **11.2**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see section **11.3.1** for consent procedure.

11.2 Risk/Benefit Assessment

11.2.1 Benefits

There is a potential benefit to the transplant recipient, as demonstrated by the published literature documenting long-term disease-free survival for patients with high-risk hematologic malignancies following allogeneic HSCT. This carefully defined patient population is a reasonable one in which to explore strategies to improve the results of allogeneic HSCT from unrelated donors, which historically has achieved prolonged remission and survival for some patients but has been associated with significant morbidity and high transplant-related mortality. The strategies chosen in this protocol extend our previous work to develop a platform for consistent engraftment and immune reconstitution using unrelated donor products.

11.2.2 Risks

11.2.2.1 Radiation Risks

This research study involves exposure to radiation from up to:

- 6 CT scans of the chest in order to determine changes in the thymus measurements
- 1 DEXA scan

- 1 MUGA scan (if performed instead of echocardiogram)

Note: The 6 CT scans of the chest will be replaced with a CT scan of neck/chest/abdomen/pelvis in lymphoma patients (neck may be omitted in follow-up if no disease).

The amount of radiation exposure received from these procedures is equal to approximately 1.43 rem (or 8.27 rem in lymphoma patients). These levels of radiation may be associated with an increased risk of cancer.

11.2.2.2 Transplant risks

The potential risks to the transplant recipient have been carefully considered and are felt to be potentially less than would otherwise occur with myeloablative allogeneic HSCT. The risks graft rejection, acute and chronic GVHD, infection, and relapse will be present, as discussed previously. Other risks related to drugs used in this study are found in section **0** and in the informed consent document.

11.2.2.3 Procedure Risks

Stem Cell Infusion: The donor cells will be infused fresh or frozen with a chemical called DMSO to protect them from the effects of freezing. Patients receiving thawed cells often develop side effects from the DMSO. DMSO side effects may include fever and allergic reactions, such as skin rash, itching, difficulty breathing, and low blood pressure. These reactions are usually mild and temporary, and they can be easily treated with IV fluids and medications.

Bone Marrow Aspiration and Biopsy: This procedure usually causes only mild pain for a short time at the biopsy site. Very rarely, bleeding or an infection may occur at the biopsy site.

Blood Draws: Side effects of blood draws include pain and bruising in the area where the needle was placed, lightheadedness and rarely, fainting. When a large amount of blood is drawn, your red blood cell count may drop causing anemia. Anemia can cause a lack of energy and other symptoms. Transfusions of red blood cells are sometimes needed to treat anemia.

Central Venous Catheter: Side effects of placing a central venous line in your chest wall include bleeding, bruising, blood clots, or pain in the area of insertion. This line will be placed by physicians with experience in this procedure. These physicians will discuss the above risks at the time of the line insertion. Rarely, placement of a central venous catheter can result in a collapsed lung. If a collapsed lung occurs, it may require hospitalization and temporary insertion of a plastic tube in your chest to re-expand the lung.

Echocardiogram: Other than possibly experiencing some minor skin irritation from the electrodes there are no anticipated risks related to complete the electrocardiogram and/or the echocardiogram.

Pulmonary Function Tests: PFTs are safe for most participants; however, some may experience dizziness, shortness of breath and fainting. In rare PFTs may lead to a collapsed lung. In participants with asthma, PFTs may precipitate an asthma attack.

Lumbar Puncture (Spinal Tap): Though lumbar punctures are generally recognized as safe, some risks may include pain or bleeding at the site of needle insertion (the low back), infection, and headache.

Skin Biopsies: Whenever possible, we will perform biopsies on covered areas of the body. There may be minor bleeding right after the procedure and this can easily be controlled by applying pressure on the spot for a few minutes. Rarely, a bruise might form and this usually heals on its own. Sometimes a small infection may occur at the biopsy site. This can usually be treated with topical antibiotics. On the very rare occasion that a larger or deeper infection occurs, oral antibiotics may be needed for 7-10 days. An infection can be recognized by redness, soreness, and pus at the site. It generally starts 2 days or more after the procedure and does not clear up in another couple of days. These biopsy/excision sites generally heal very well, leaving red, white, dark or skin-colored flat scars. Sometimes, the scar that forms may be a bit thicker than usual. Rarely, a keloid (large, painful or itchy scar) may form. Keloids are more likely to form on the chin, earlobes, chest and upper backs of Blacks and Asians between adolescence and the 30's.

Oral Mucosa Biopsy: There may be minor bleeding, bruising, numbness and slight swelling. There is also the possibility of infection. Mouth sores have occurred in some patients, but this is uncommon.

11.3 Informed Consent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with local policy, including HRPP policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. For the optional biopsies, the patient will consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When

required, witness signature will be obtained similarly as described for the investigator and participant..

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

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11.3.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **11.1.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **11.3.**

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigators, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements

- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

12.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NCI CCR.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

13.1 CYCLOPHOSPHAMIDE (CTX, CYTOXAN)

13.1.1 Source

Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.

13.1.2 Preparation

Cyclophosphamide will be reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.

13.1.3 Storage and Stability

The vials are stored at room temperature. Following reconstitution as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.

13.1.4 Administration

The cyclophosphamide used in this regimen will be mixed in 100 ml 0.9% sodium chloride, Inj., and given as an IVPB over 30 minutes in the induction regimen. In the preparative regimen it is given over two hours. Patients will be instructed to drink an adequate amount of fluids and empty their bladders frequently during cyclophosphamide administration.

13.1.5 Toxicities

Please refer to the package insert for a complete listing of all toxicities.

- Nausea and vomiting - variable; symptomatically improved with standard anti-emetics and/or benzodiazepines [e.g., lorazepam].
- Water retention – cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hours after IV administration, necessitating frequent accurate assessment [q 1-2 hours] of intake, urine output and urine specific gravity. This effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide.
- Cardiomyopathy - cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving the dose of cyclophosphamide used in this protocol. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see section **2**, patient eligibility]. Congestive failure is managed according to standard medical therapeutics.
- Hemorrhagic cystitis – this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites. Although sub-clinical hematuria is not uncommon at this dose level, clinically significant hematuria or serious hemorrhage can usually be avoided by maintaining a high urine volume and frequent voidings and the administration of Mesna. Diuresis is maintained for 24 hours after completion of last dose by parenteral infusions of 0.9% Sodium Chloride with potassium chloride. Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to ensure this diuresis. Continuous bladder irrigation may be used for control of significant hematuria.
- Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

13.1.6 Hydration Guidelines

All patients should receive 0.9%NS at the following volumes (based on cyclophosphamide dose levels) and rates with half the specified volume given before starting cyclophosphamide administration and half the volume given after completion of the cyclophosphamide administration.

Cyclophosphamide Dosage Levels	Fluid Volume and Administration Rate
1 & 2	1000 mL 0.9%NS @ 300 – 500 mL/h
Levels 3, 4, & 5	2000 mL 0.9%NS @ 300 – 500 mL/h
Levels ≥6	2500 mL 0.9%NS @ 300 – 500 mL/h

13.2 MESNA (SODIUM 2-MERCAPOETHANESULFONATE, MESNUM, MESNEX)

13.2.1 Supply

Mesna will be will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a 100mg/ml solution.

13.2.2 Preparation

Dilute up to 20 mg Mesna/mL fluid in D5W or 0.9% sodium chloride, Inj. Mesna should be started concurrently with the cyclophosphamide in the preparative regimen. Mesna will be given at 1200 mg/m^2 in 500 ml by continuous IV infusion over 24 hour infusion for four doses (days -6, -5, -4, and -3).

13.2.3 Storage and Stability

Intact ampules are stored at room temperature. Diluted solutions (1 to 20 mg/dl) are physically and chemically stable for at least 24 hours under refrigeration. Mesna is chemically stable at room temperature for 48-72 hours in D5W, 48-72 hour in D5W/0.45% sodium chloride injection, or 24 hours in 0.9% sodium chloride injection.

13.2.4 Administration

To be administered intravenously as continuous infusion.

13.2.5 Toxicities

Nausea, vomiting, diarrhea. Please refer to the package insert for a complete listing of all toxicities.

13.3 FILGRASTIM (G-CSF, NEUPOGEN®)

13.3.1 Supply

Commercially available as filgrastim injection in a concentration of 300μg/ml in 1ml (300μg) and 1.6ml (480μg) vials.

13.3.2 Preparation

For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water to a concentration greater than 5μg/ml. Dilution of filgrastim to a final concentration of less than 5μg/ml is not recommended at any time. Do not dilute with saline at any time;

product may precipitate. Filgrastim diluted to concentrations between 5 and 15μg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. The dose may be "rounded down" to within 10% of patient's calculated dose to use the drug cost-effectively.

13.3.3 Storage and Stability

Filgrastim for injection should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking.

13.3.4 Administration

Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 15 to 30 minutes.

13.3.5 Toxicities

Medullary bone or skeletal pain is the most commonly reported toxicity. In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. Four cases of splenic rupture have been reported in healthy donors when given filgrastim or other myeloid growth factors for peripheral blood stem cell mobilization; 1 of these cases resulted in fatality. Five additional cases of splenic rupture have been reported in cancer patients undergoing chemotherapy or peripheral blood stem cell mobilization; splenic rupture may have contributed to deaths in 2 of these cases. One additional death due to splenic rupture after filgrastim therapy was reported to the manufacturer without additional information. According to the manufacturer, the reporting rate for splenic rupture with filgrastim is less than 1 in 486,000. Please refer to the package insert for a complete listing of all toxicities.

13.4 FLUDARABINE

13.4.1 Supply

Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludarabine is stored at room temperature.

13.4.2 Preparation

Fludarabine should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7–8.5. Fludarabine will be diluted in 100 to 125ml of either 5% dextrose in water or 0.9% sodium chloride, and infused IV over 30 minutes.

13.4.3 Storage and Stability

Reconstituted fludarabine is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the

prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.

13.4.4 Administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

13.4.5 Toxicities

Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, auto-immune hemolytic anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m^2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agents. Please refer to the package insert for a complete listing of all toxicities.

13.5 ETOPOSIDE, DOXORUBICIN, AND VINCRISTINE (IN EPOCH)

13.5.1 Supply

Etoposide, doxorubicin, and vincristine will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

13.5.2 Preparation

For this study, etoposide, doxorubicin, and vincristine comprising a daily dose (a 24-hour supply) will be diluted in 0.9% Sodium Chloride Injection, USP (0.9%NS). Product containers will be replaced every 24 hours to complete the planned duration of infusional treatment. Product volumes will be determined by the amount of etoposide present in a 24-hour supply of medication. For daily etoposide doses ≤130 mg, admixtures will be diluted in approximately 500 mL 0.9%NS. For daily etoposide doses >130 mg, admixtures will be diluted in approximately 1000 mL 0.9%NS.

Etoposide + doxorubicin + vincristine admixtures will be administered by continuous IV infusion over 96 hours with a suitable rate controller pump via a central venous access device.

All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine, *PLUS* 40 mL overfill (excess) fluid and a

proportional amount of drug to compensate for volume lost in parenteral product containers and administration set tubing**.**

Etoposide Dose	Volume of Fluid Containing a Daily Dose	Volume of Overfill (fluid + drug)	Total Volume in the Product (including overfill)
≤ 130 mg	528 mL	40 mL	568 mL
> 130 mg	1056 mL	40 mL	1096 mL

Before dispensing 3-in-1 admixtures, Pharmacy staff will:

[1] Purge all air from the drug product container,

[2] Attach an administration set appropriate for use with a portable pump,

[3] The set will be primed close to its distal tip, and

[4] The set will be capped with a Luer-locking cap.

Pre-printed product labeling will identify the ‘Total Volume To Infuse’ and the ‘Volume of Overfill (fluid + drug)’.

Bags will be exchanged daily for four consecutive days to complete a 96-hour drug infusion (unless treatment is interrupted or discontinued due to un-anticipated events).

13.5.3 Administration

Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver one of two fixed volumes at one of two corresponding fixed rates based on the amount of etoposide and fluid that is ordered (see the table, below).

Etoposide Dose	Total Volume to Infuse per 24 hours	Volume of Overfill (drug-containing fluid)*	Administration Rate
≤ 130 mg	528 mL	40 mL	22 mL/hour
>130 mg	1056 mL	40 mL	44 mL/hour

DO NOT attempt to infuse the overfill

At the end of an infusion, some residual fluid is expected because overfill (excess fluid and drug) was added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.

Example at right: The amount of fluid remaining in a bag after completing a 24-hour infusion (1056 mL delivered).

13.5.4 Storage and Stability

Stability studies conducted by the Pharmaceutical Development Section, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, USP (0.9%NS) at concentrations, respectively, of 1, 25, and 125 mcg/mL; 1.4, 35, and 175 mcg/mL; 2, 50, and 250 mcg/mL; and 2.8, 70, 350 mcg/mL are stable for at least 36 hours at room temperature when protected from light. Also, admixtures containing vincristine, doxorubicin, and etoposide concentrations of 1.6, 40, and 200 mcg/mL are stable for at least 30 hours at 32°C. [63].

13.5.5 Toxicities

Please refer to the package inserts for a complete listing of all toxicities.

- Etoposide: nausea, vomiting, stomatitis, diarrhea, thrombocytopenia, neutropenia, and alopecia. Secondary AML has been associated with etoposide. Bradycardia and hypotension are sometimes observed with etoposide administration.
- Doxorubicin: Cardiotoxicity is particularly noted after cumulative doses of greater than 550 mg/m^2. Other toxicities include myelosuppression, nausea, vomiting, stomatitis, diarrhea, and alopecia. Skin infiltration of doxorubicin causes tissue necrosis.
- Vincristine causes neurological toxicities such as paresthesia, jaw pain, ataxia, foot-drop, cranial nerve palsies, paralytic ileus, constipation, abdominal pain, and loss of deep tendon reflexes. It is also a vesicant, and occasionally causes alopecia and myelosuppression.

13.6 PREDNISONE

13.6.1 Supply

Prednisone will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources. It is commercially available in a large number of oral dosage strengths including pills and liquid formulations.

13.6.2 Storage and Stability

Prednisone tablets should be stored in the container provided away from heat. The product labeling bears the manufacturer's expiration dating for stability. Tablets should be stored in well-closed containers at temperatures between 15-30^{o}C.

13.6.3 Administration

Prednisone will be given on days 1-5 of EPOCH induction therapy. Prednisone utilization may be simplified by using only 20- and 50-mg tablets to produce individual doses and by stratifying prednisone doses by a patient's body surface area (BSA), according to the chart below. These are recommendations and not requirements.

BSA (m^2)	Each Dose
1.25 – 1.49	80 mg
1.5 – 1.83	100 mg
1.84 – 2.16	120 mg
2.17 – 2.41	140 mg
2.42 – 2.6	150 mg
2.61 – 2.69	160 mg
2.7 – 3	170 mg

In patients unable to tolerate oral medication, methylprednisolone can be substituted at an equivalent dosage, diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water or 0.9% sodium chloride and infused over 15 minutes.

13.6.4 Toxicities

Prednisone frequently causes immunosuppression, muscle wasting, fluid retention, proximal muscle weakness, glucose intolerance, thinning of skin, redistribution of body fat, Cushingoid facies, and propensity to gastrointestinal ulceration. To reduce gastrointestinal side effects, prednisone should be taken with food. Please refer to the package insert for a complete listing of all toxicities.

13.7 RITUXIMAB (RITUXAN)

13.7.1 Supply

The NIH Clinical Center Pharmacy Dept. will purchase rituximab from commercial sources. Rituximab is provided in pharmaceutical grade glass vials containing 10 mL

(100 mg) or 50 mL (500 mg) at a concentration of 10 mg of protein per milliliter. Please refer to the FDA-approved package insert for rituximab for product information, extensive preparation instructions, and a comprehensive list of adverse events.

13.7.2 Preparation

Rituximab will be diluted to a final volume of 0.9% Sodium Chloride or 5% Dextrose Injection to prepare a standard product with concentration of 2 mg/ml. Caution should be taken during the preparation of the drug, as shaking can cause aggregation and precipitation of the antibody

13.7.3 Storage and Stability

Rituximab for clinical use should be stored in a secure refrigerator at 2 to 8 degrees C. After dilution, rituximab is stable at 2-8 degrees C (36-46 degrees F) for 24 hours and at room temperature for an additional 12 hours.

13.7.4 Administration

During rituximab infusion, a patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored according to the standard of care. Medications readily available for the emergency management of anaphylactoid reactions should include: epinephrine (1:1000, 1 mg/mL) for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment.

Prophylaxis against hypersensitivity and infusion-related reactions associated with rituximab will include acetaminophen 650 mg and diphenhydramine hydrochloride 50-100 mg administered 30 to 60 minutes prior to starting rituximab. Patients will also receive their first dose of prednisone 60 mg/m^2 (or a glucocorticoid equivalent dose of an alternative steroid) at least 60 minutes before rituximab treatment commences.

Rituximab will be administered as an intravenous infusion at 375 mg/m^2 on day 1 of each cycle of EPOCH, immediately prior to starting etoposide + doxorubicin + vincristine administration. Rituximab infusions will be administered to patients primarily in an outpatient clinic setting.

First dose:

The initial dose rate at the time of the first rituximab infusion should be 50mg/hour (25 mL/hr) for the first 30 minutes. If no toxicity is seen, the dose rate may be escalated gradually in 50 mg/hour (25 mL/h) increments at 30 minute intervals) to a maximum of 400 mg/hour (maximum rate = 200 mL/h).

Second and Subsequent Doses (select the appropriate administration timing):

90-minute Administration

If the first dose of rituximab was well tolerated, subsequent doses may be administered over 90 minutes with 20% of the total dose given in the first 30 minutes, and remaining 80% of the total dose administered over the subsequent 60 minutes; e.g.:

Two-Step Rate Escalation	Volume to administer (X mL)

1st portion (0 – 30 minutes)	$\frac{\text{Total Dose (mg)}}{2} \times 0.2 = \text{X mL (over 30 min)}$
2nd portion (30 – 90 minutes)	$\frac{\text{Total Dose (mg)}}{2} \times 0.8 = \text{X mL (over 60 min)}$

Special Note: The 90-minute infusion scheme is not recommended for patients with clinically significant cardiovascular disease or high circulating lymphocyte counts (≥5000/mcL).

Standard Administration for Second & Subsequent Infusions

Patients who tolerate initial treatment without experiencing infusion-related adverse effects but for whom the 90-minute infusion scheme during subsequent treatments is considered inappropriate, may receive subsequent rituximab doses at the Standard Rate for Subsequent Infusions, which is as follows:

Begin at an initial rate of 100 mg/hour (50 mL/h) for 30 minutes. If administration is well tolerated, the administration rate may be escalated gradually in 100-mg/hour (50-mL/h) at 30-minute intervals to a maximum rate of 400 mg/hour (maximum rate = 200 mL/h).

CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

13.7.5 Toxicities

The most serious adverse reactions caused by rituximab include infusion reactions, tumor lysis syndrome, mucocutaneous reactions, hypersensitivity reactions, cardiac arrhythmias and angina, and renal failure. Please refer to the package insert for a complete listing of all toxicities.

- *Fatal and Severe Infusion Reaction*s: Deaths within 24 hours of rituximab infusion have been reported. Approximately 80% of fatal infusion reactions occurred in association with the first infusion. Severe reactions typically occurred during the first infusion with time to onset of 30 to 120 minutes. Signs and symptoms of severe infusion reactions may include hypotension, angioedema, hypoxia or bronchospasm, and may require interruption of rituximab administration. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. In the reported cases, the following factors were more frequently associated with fatal outcomes: female sex, pulmonary infiltrates, and chronic lymphocytic leukemia or mantle cell lymphoma.
- *Management of severe infusion reactions:* The rituximab infusion should be interrupted for severe reactions and supportive care measures instituted as medically indicated (e.g., intravenous fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen). In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Patients requiring close monitoring during first and all subsequent infusions include those with pre-existing cardiac and pulmonary conditions, those with prior clinically significant cardiopulmonary adverse events and those with high numbers of circulating malignant cells (= 25,000/mm^3) with or without evidence of high tumor burden.

- *Tumor Lysis Syndrome (TLS)*: Acute renal failure requiring dialysis with instances of fatal outcome has been reported in the setting of TLS following treatment with rituximab. Rapid reduction in tumor volume followed by acute renal failure, hyperkalemia, hypocalcemia, hyperuricemia, or hyperphosphatemia, have been reported within 12 to 24 hours after the first rituximab infusion. Rare instances of fatal outcome have been reported in the setting of TLS following treatment with rituximab. The risks of TLS appear to be greater in patients with high numbers of circulating malignant cells (= 25,000/mm^3) or high tumor burden. Prophylaxis for TLS should be considered for patients at high risk. Correction of electrolyte abnormalities, monitoring of renal function and fluid balance, and administration of supportive care, including dialysis, should be initiated as indicated. Following complete resolution of the complications of TLS, rituximab has been tolerated when re-administered in conjunction with prophylactic therapy for TLS in a limited number of cases.
- *Severe Mucocutaneous Reactions*: Mucocutaneous reactions, some with fatal outcome, have been reported in patients treated with rituximab. These reports include paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the patient's underlying malignancy), Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1 to 13 weeks following rituximab exposure. Patients experiencing a severe mucocutaneous reaction should not receive any further infusions and seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The safety of re-administration of RITUXAN to patients with any of these mucocutaneous reactions has not been determined.
- Infusion reactions and lymphopenia are the most commonly occurring adverse reactions. Mild to moderate infusion reactions consisting of fever and chills/rigors occur in the majority of patients during the first rituximab infusion. Other frequent infusion reaction symptoms included nausea, pruritus, angioedema, asthenia, hypotension, headache, bronchospasm, throat irritation, rhinitis, urticaria, rash, vomiting, myalgia, dizziness, and hypertension. These reactions generally occur within 30 to 120 minutes of beginning the first infusion, and resolved with slowing or interruption of the rituximab infusion and with supportive care (diphenhydramine, acetaminophen, IV saline, and vasopressors). In an analysis of data from 356 patients with relapsed or refractory, low-grade NHL who received 4 (N = 319) or 8 (N = 37) weekly infusions of rituximab, the incidence of infusion reactions was highest during the first infusion (77%) and decreased with each subsequent infusion (30% with fourth infusion and 14% with eighth infusion).

13.8 CYTARABINE (CYTOSINE ARABINOSIDE, CYTOSAR, ARA-C)

13.8.1 Supply

Cytarabine will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as un-reconstituted lyophilized powder (100 mg,

500 mg, 1 g, and 2 g vials). Cytarabine for injection is available for subcutaneous, intravenous, and intrathecal administration.

13.8.2 Preparation

To prepare high dose (2 gm/m^2) cytarabine intravenous infusions, each vial of drug should be reconstituted with sterile water for injection USP (containing no preservative) with the diluent volumes listed below. Note that the manufacturers recommended diluent for cytarabine is bacteriostatic water for injection containing benzyl alcohol. For preparation of intrathecal injection doses and for high dose intravenous dosing regimens, ONLY a preservative-free diluent should be used. The recommended volumes for reconstitution include the following:

Vial Size	Volume of Diluent	Concentration
100 mg	5 ml	20 mg/ml
500 mg	10 ml	50 mg/ml
1 g	10 ml	100 mg/ml
2 g	20 ml	100 mg/ml

- The drug will be further diluted in 500 ml of 5% Dextrose Injection or 0.9% Sodium Chloride Injection prior to administration.

13.8.3 Storage and Stability

Vials should be stored at room temperature (25°C or 77°F); excursions permitted to 15-30° C (59-86°F). [See USP Controlled Room Temperature.] Once reconstituted with sterile water for injection, USP the reconstituted vial should be used within 24 hours. After final dilution with 0.9% Sodium Chloride Injection or 5% Dextrose Injection, the prepared dose should be used within 24 hours if stored at room temperature or 48 hours if refrigerated.

13.8.4 Administration

Begin each cytarabine dose 3.5 hours after completion of the preceding fludarabine dose. Intravenous doses should be infused over at least 2 hours.

13.8.5 Toxicity

Acute dose-limiting toxicity with IV administration consists of severe leukopenia and thrombocytopenia. Nausea and vomiting may be dose limiting at higher doses. Other adverse reactions include diarrhea, immunosuppression, anorexia, stomatitis, oral ulceration, flu-like syndrome, fever, hepatic dysfunction, and alopecia. At high doses, as in this protocol, keratoconjunctivitis, dermatitis, and central nervous system toxicity (e.g., ataxia, somnolence, coma, dysarthria) may occur. Occasionally, the CNS impairment is not fully reversible. Renal impairment will enhance toxicity. In the event that signs of CNS toxicity occur, the cytarabine will be interrupted and the M.D. notified. No further cytarabine will be administered if there is CNS toxicity (any grade) deemed related to cytarabine. Please refer to the package insert for a complete listing of all toxicities.

Conjunctivitis Prophylaxis: Corticosteroid ophthalmic drops will be administered 2 drops to each eye every 6 hours starting prior to first dose and continuing until 24 hours after the last dose of cytarabine has been completed.

13.9 METHOTREXATE (METHOTREXATE SODIUM, MTX, NSC-740)

13.9.1 Supply

Methotrexate will be obtained commercially and is supplied as a 25 mg/ml preservative-free isotonic solution for injection.

13.9.2 Preparation

The desired dose will be diluted in 5% dextrose in water or 0.9% sodium chloride.

13.9.3 Storage and Stability

Methotrexate should be stored at room temperature and protected from light. Once the prepared dose is diluted for administration, the solution is stable for 24 hours refrigerated or at room temperature when protected from light.

13.9.4 Administration

Methotrexate will be given as IV infusion over 15 minutes.

13.9.5 Toxicities

The toxicity associated with methotrexate primarily involves the gastrointestinal tract. Severe mucositis can occur, particularly in patients who have received an intensive preparative regimen. Other gastrointestinal symptoms include nausea, vomiting and diarrhea. Transient elevations in serum transaminases have been seen. The myelosuppression associated with methotrexate often results in delayed engraftment. Folinic acid can effectively circumvent the enzymatic block produced by methotrexate. Please refer to the package insert for a complete listing of all toxicities.

13.10 SIROLIMUS (RAPAMYCIN, RAPAMUNE®, WYETH-AYERST LABORATORIES)

13.10.1 Supply

For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

13.10.2 Storage and Stability

Oral tablets should be stored 20-25 C° (68-77 F°). Oral solution should be refrigerated (2-8 C° or 36-46 F°).

13.10.3 Administration

Because food, in particular fatty foods, can decrease the absorption of sirolimus, it is suggested the tablets should be administered consistently between meals.

13.10.4 Toxicities

Sirolimus induces immune suppression, which has been associated with opportunistic infection and an increased rate of malignancy, particularly skin cancer.

Some individuals may develop hypersensitivity to sirolimus. May cause an increase in cholesterol and triglycerides, which may be associated with pancreatitis. With long-term administration, may result in impaired renal function. Metabolism is via the cytochrome p450 pathway, and as such, co-administration of voriconazole is prohibited with sirolimus can result in prolonged blood levels of sirolimus. As such, if the administration of voriconazole is required the dose of sirolimus will be reduced by 90% and close attention must be given relative to serum sirolimus levels and associated toxicities [98]. Please refer to the package insert for a complete listing of all toxicities.

13.11 TACROLIMUS (KF-506, PROGRAF®)

13.11.1 Supply

Tacrolimus will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Tacrolimus is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL for administration by intravenous infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v.

13.11.2 Preparation

For parenteral doses, tacrolimus injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use. Parenteral doses of tacrolimus will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing.

13.11.3 Storage and Stability

Capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light.

13.11.4 Administration

Tacrolimus will be administered as a continuous intravenous infusion over 24 hours or given orally in divided doses every 12 hours.

13.11.5 Toxicities

Tacrolimus can cause neurotoxicity and nephrotoxicity, particularly when used in high doses. Mild to severe hyperkalemia has been observed following tacrolimus administration. Serum potassium levels should be monitored and potassium-sparing diuretics should not be used during tacrolimus therapy. Neurotoxicity, including tremor, headache, and other changes in motor function, mental status, and sensory function have been reported with tacrolimus administration. Tremor and headache have been associated with high whole-blood concentrations of tacrolimus and may respond to dosage adjustment. Seizures have occurred in adult and pediatric patients receiving tacrolimus. Coma and delirium also have been associated with high plasma concentrations of tacrolimus. The most common adverse reactions reported with tacrolimus administration include infection, tremor, hypertension, abnormal renal function, constipation, diarrhea, headache, abdominal pain and insomnia. Less common adverse reactions reported with tacrolimus administration include anorexia, cholangitis,

jaundice, diarrhea, dyspepsia, hepatitis, ALT (SGPT) increased, AST (SGOT) increased, abnormal ECG, angina pectoris, arrhythmia, acute kidney failure, acidosis, alkaline phosphatase increased, alkalosis, bicarbonate decreased, bilirubinemia, BUN increased, dehydration, edema, hypercalcemia, , hypocalcemia, hypercholesterolemia, hyperlipemia, hyperphosphatemia, hyperuricemiahypoglycemia, hypomagnesemia, hyponatremia, hypophosphatemia, hypoproteinemia, lactic dehydrogenase increase, peripheral edema, weight gain, Cushing's syndrome, diabetes mellitus, anemia, allergic reaction, and photosensitivity reaction. Please refer to the package insert for a complete listing of all toxicities.

13.12 PALIFERMIN (KEPIVANCE®, SOBI PHARMACEUTICALS) IND # 124506

13.12.1 Supply

Palifermin is supplied by the manufacturer, Sobi Pharmaceuticals, for the purposes of this study to the NIH pharmacy. Palifermin is packaged as a lyophilized powder with 6.25 mg/vial.

13.12.2 Preparation

Palifermin is reconstituted using 1.2 mL sterile water followed by gentle agitation for 1-3 minutes.

13.12.3 Storage and stability

Palifermin is stable as a lyophilized powder at room temperature until the expiration date provided by the manufacturer. After reconstitution, palifermin is stable at room temperature for up to 1 hour or can be kept at 2-8 degrees C up to 24 hours before injection.

13.12.4 Administration

See section **3.3.1.1**

13.12.5 Toxicities

The purpose of this study is to determine toxicities at this dosage and schedule. At lower doses, Palifermin led to edema, pain, fever, mouth or tongue thickness, arthralgia, erythematous rash, pruritis, dysguesia, dysthesia, paresthesia, and elevated lipase/amylase. Please refer to the package insert for a complete listing of all toxicities.

13.13 PERIPHERAL BLOOD STEM CELLS

PBSC Collection of matched unrelated donors will be performed according to NMDP Standards, which adhere to the strictest interpretation of AABB and FDA standards. (See **Appendix E**)

13.13.1 PBSC Products will be received by NIH DTM. Products will be processed according to standard DTM operating procedures.

- Targeted cell infusion dose is greater than or equal to 7 x 10^6 CD34+ cells/kg recipient weight
- If recipient BMI (Body Mass Index) is 35 or less, use actual body weight

- If recipient BMI is greater than 35, use practical body weight as calculated by Cell Processing Services (CPS). See form DTM-Form-5086 in Appendix I
- If targeted cell dose is exceeded, excess cells may be cryopreserved according to CD3+ cell content, in escalating doses of:
  - 1×10^6 CD3+/kg
  - 5×10^6 CD3+/kg
  - 1×10^7 CD3+/kg
  - 5×10^7 CD3+/kg
- When collected cells dose is 5-7 $\times 10^6$ CD34+ cells/kg recipient weight, on case by case basis DLI aliquots can be stored only after consultation and permission of the PI or LAI.

13.13.2 Upon arrival at NCI, the following tests will be performed on blood samples accompanying the stem cell product:

- ABO compatibility testing (per AABB Standards). If an ABO incompatibility exists between the donor and patient, the graft will be processed in the cell processing laboratory, according to standard DTM operating procedures.
- Hepatitis A, Adenovirus, EBV, and Toxoplasma screens will be performed upon receipt of the PBSC product if additional donor blood is available at the time of collection. The results of these tests do not affect eligibility.
- PCR test of DNA mini-satellite regions for future determination of chimerism

13.13.3 Storage and stability

Cells may be processed, cryopreserved, and stored in liquid nitrogen until the day of transplant upon delivery to the NIH Department of Transfusion Medicine (DTM). In the event that cryopreservation is required, special permission will be obtained from NMDP.

13.13.4 Toxicities

Death occurs in between 15% and 40% of subjects two years after stem cell transplant. Subjects may also experience complications that include graft versus host disease, graft rejection, veno-occlusive disease and late transplant complications that can affect any organ in the body. Rarely, patients may develop a second cancer such as lymphoma, leukemia, lung cancer or other tumors.

14 REFERENCES

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15 APPENDICES

15.1 APPENDIX A: DEFINITIONS / GLOSSARY

- Neutrophil Recovery: Designated by the first of 3 consecutive days with an ANC above 500/mm^3.
- Platelet Recovery: Designated by the first of 7 days where the platelet count remains above 20,000/mm^3 without transfusion support.
- Lymphocyte Recovery: Designated by the first of 3 consecutive days with ALC above 500 /mm^3.
- Sustained Donor Engraftment: Neutrophil recovery associated with complete donor chimerism at day 100. Any patient who dies before day 28 will not be evaluated for engraftment but will be evaluable for palifermin.
- Primary Graft Failure: Failure to achieve sustained donor engraftment defined as ANC >0.5x10^9/L not reached by day +28 after SCT. .
- Secondary Graft Failure: Documented sustained donor engraftment as defined above followed by: 1: severe neutropenia (ANC<500/mm^3) and bone marrow biopsy revealing a cellularity of less than 25% or 2: absence of donor cells in the marrow or blood as demonstrated by a chimerism assay without subsequent improvement occurring either spontaneously or after growth factor treatment. Improvement is defined as ANC >500mm^3 consistently. Severe neutropenia with marrow cellularity > 25% is not secondary graft failure.
- Aplasia: Less than 5% marrow cellularity as measured on bone marrow biopsy.

15.2 APPENDIX B: MALIGNANCY RESPONSE ASSESSMENT

The malignancy response assessments will be done per the CIBMTR post-transplant criteria and may be viewed at: https://www.cibmtr.org/manuals/fim/1/en/topic/none .

15.3 APPENDIX C: ACUTE GRAFT VERSUS HOST DISEASE EVALUATION (WORKSHEET)

- Acute GVHD is diagnosed at any time after transplant before or after day 100 providing there are no concurrent diagnostic signs of chronic GVHD

Glucksberg Scoring System:

Stage	Skin	Liver	Gut
1	Rash < 25% BSA	Total bilirubin 2-2.9 mg/dl	Diarrhea 500-1000ml/d
2	Rash 25-50% BSA	Total bilirubin 3-6 mg/dl	Diarrhea 1000-1500ml/d
3	Generalized erythoderma	Total bilirubin 6.1-15 mg/dl	Diarrhea >1500ml/d
4	Desquamation and bullae	Total bilirubin > 15 mg/dl	Diarrhea >2000ml/d & Pain +/- ileus

*BSA = body surface area; use "rule of nines" or burn chart to determine extent of rash

- Isolated upper GI symptoms need a biopsy confirmation
- Elevation of transaminase without elevated bilirubin is not graded as acute GVHD (a note should say GVHD if liver biopsy proven)

Overall GVHD grading according to Keystone consensus:

Grade	Skin	Liver	Gut	PS**
0 (none)	0	0	0	0
I	+ to ++	0	0	0
II	+ to +++	+	+	+
III	++ to +++	++ to +++	++ to +++	++
IV	+ +to ++++	+ +to ++++	+ +to ++++	+++

*The nominal stage is reduced by 1 if organ is simultaneously and unequivocally affected by a complication other than GVHD
**PS is not used in routine practice acute GVHD grading
[+]Gut stage 4 has frequent poor outcome and is commonly designated as grade IV especially if more severe symptoms and deterioration in performance status
[++]Designation as grade III would be appropriate for only scattered bullae

Acute GVHD data collection: **(Patient Label)**

(present or absent, date of diagnosis, stage at diagnosis, and max stage during first 6 months)

Date of evaluation	Timepoint (D28, etc.)	Classic Vs. Late Acute	*Stage*	**Glucksberg Scoring System**			
				Skin	**Liver**	**GI**	**Grade***
			1	*Rash < 25% BSA*	*Total bilirubin 2-2.9 mg/d*	*Diarrhea 500-1000ml/d*	
			2	*Rash 25-50% BSA*	*Total bilirubin 3-6 mg/dl*	*Diarrhea 1000-1500ml/d*	
			3	*Generalized erythoderma*	*Total bilirubin 6.1-15 mg/dl*	*Diarrhea >1500ml/d*	
			4	*Desquamation and bullae*	*Total bilirubin > 15 mg/dl*	*Diarrhea >2000ml/d &Pain +/- ileus*	

- Date of initial diagnosis of acute GVHD: _____
- Grade of acute GvHD at time of initial diagnosis: _____
- Organ involvement at time of initial diagnosis (1= liver only, 2 = skin only, 3 = GI only, 4 = liver + skin, 5 = liver + GI, 6 = skin + GI, 7 = liver + skin + GI) : _____
- Max aGvHD grade in first 6 months: _____

*Grade	Skin	Liver	Gut
0	0	0	0
I	+ to ++	0	0
II	+ to +++	+	+
III	++ to +++	++ to +++	++ to +++
IV	++ to ++++	++ to ++++	++ to ++++

LIP Signature_____ Page ____ of _____

Patient Name & MRN

Acute GVHD data collection: **(Patient Label)**

(present or absent, date of diagnosis, stage at diagnosis, and max stage during first 6 months)

Date of Diagnosis (MM/DD/YYYY)	Timpoint -D28 -D60 -D100	1.Classic Vs. 2.Late Acute	Stage	Glucksberg Scoring System			Grade*
				Skin	Liver	GI	
			1	*Rash < 25% BSA*	*Total bilirubin 2-2.9 mg/d*	*Diarrhea 500-1000ml/d*	
			2	*Rash 25-50% BSA*	*Total bilirubin 3-6 mg/dl*	*Diarrhea 1000-1500ml/d*	
			3	*Generalized erythoderma*	*Total bilirubin 6.1-15 mg/dl*	*Diarrhea >1500ml/d*	
			4	*Desquamation and bullae*	*Total bilirubin > 15 mg/dl*	*Diarrhea >2000ml/d &Pain +/- ileus*	

Evaluation of aGVHD diagnosis at 6 months:

- Grade of acute GvHD at time of initial diagnosis: _____
- Organ involvement at time of initial diagnosis (1= liver only, 2 = skin only, 3 = GI only, 4 = liver + skin, 5 = liver + GI, 6 = skin + GI, 7 = liver + skin + GI) : _____
- Max aGvHD grade in first 6 months: _____

*Grade	Skin	Liver	Gut	PS
0	0	0	0	0
I	+ to ++	0	0	0
II	+ to +++	+	+	+
III	++ to +++	++ to +++	++ to +++	++
IV	++ to ++++	++ to ++++	++ to ++++	+++

15.4 APPENDIX D: CHRONIC GRAFT VERSUS HOST DISEASE EVALUATION (WORKSHEET)

NIH 2014 Organ Scoring of Chronic GVHD

	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† **SCORE % BSA** ☐ *GVHD features to be scored by BSA:* **Check all that applies:** ☐ Maculopapular rash/erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1-18% BSA	☐ 19-50% BSA	☐ >50% BSA
SKIN FEATURES SCORE:	☐ No sclerotic features		☐ **S**uperficial sclerotic features "not hidebound" (able to pinch)	**Check all that applies:** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration

Other skin GVHD features (NOT scored by BSA)
Check all that applies:
☐ Hyperpigmentation
☐ Hypopigmentation
☐ Poikiloderma
☐ Severe or generalized pruritus
☐ Hair involvement
☐ Nail involvement
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

MOUTH *Lichen planus-like features present:* ☐ **Yes** ☐ **No**	☐ 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

☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

NIH Organ Scoring of Chronic GVHD (continued)

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
EYES *Keratoconjunctivitis sicca (KCS) confirmed by Ophthalmologist:* ☐ **Yes** ☐ **No**	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤ 3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops > 3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
GI Tract ***Check all that applies:*** ☐ Esophageal web/ proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss* ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss* (<5%)	☐ Symptoms associated with mild to moderate weight loss* (5-15%)	☐ Symptoms associated with significant weight loss* >15%, requires nutritional supplement for most calorie needs or esophageal dilation
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
LIVER	☐ Normal total bilirubin and ALT < 2 x NUL	☐ Normal total bilirubin and ALT ≥2 x NUL	☐ Elevated total Bilirubin but ≤3 mg/dL NUL	☐ Elevated total bilirubin > 3 x NUL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*				
LUNGS** **Symptoms score**:	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring 0_2)
Lung obstructive function score: FEV1 ☐	☐ FEV1≥80%	☐ FEV1 60-79	☐ FEV1 40-59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed				
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

NIH Organ scoring of chronic GVHD (continued)

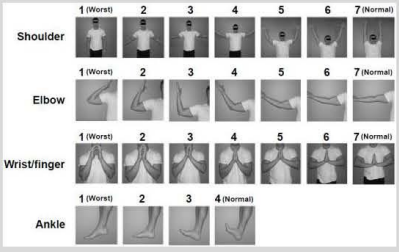
	SCORE 0	SCORE 1	SCORE 2	SCORE 3
JOINTS AND FASCIA P-ROM score *(see below)* Shoulder (1-7):___ Elbow (1-7): ____ Wrist/finger (1-7):___ Ankle (1-4):___	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM *AND* significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GENITAL TRACT (*See Supplemental table*†) **Check all that applies** ☐ Not examined *Currently sexually active* ☐ Yes ☐ No	☐ No signs	☐ Mild signs‡ and females with or without discomfort on exam	☐ Moderate signs‡ and may have signs* of discomfort on exam	☐ Severe signs‡ with o without symptoms
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to its severity (0-3) based on its functional impact where applicable none – 0,mild -1, moderate -2, severe – 3)

☐ Ascites (serositis)___	☐ Myasthenia Gravis___	
☐ Pericardial Effusion___	☐ Peripheral Neuropathy___	☐ Eosinophilia > 500µl___
☐ Pleural Effusion(s)___	☐ Polymyositis___	☐ Platelets <100,000/µl ___
☐ Nephrotic syndrome___	☐ Weight loss* without GI symptoms	☐ Others (specify):_____

Overall GVHD Severity *(Opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic Range of Motion (P-ROM)



† Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score, OR if superficial sclerotic features are present (Score 2), but there is impaired mobility or ulceration (Score 3), the higher level should be used for the final skin scoring.

* Weight loss within 3 months.

**Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

Abbreviations: ECOG (Eastern Cooperative Oncology Group), KPS (Karnofsky Performance Status), LPS (Lansky Performance Status); BSA (body surface area); ADL (activities of daily living); LFTs (liver function tests); AP (alkaline phosphatase); ALT (alanine aminotransferase); NUL (normal upper limit).

‡ To be completed by specialist or trained medical providers (see Supplemental Table).

Supplement – Genital Tract Chronic Graft-versus-Host Assessment and Scoring Form

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

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
GENITAL TRACT (male or female)	☐ No signs	☐ Mild signs and females may have symptoms* WITH discomfort on exam	☐ Moderate signs and may have symptoms* with discomfort on exam	☐ Severe signs with o without symptoms *

Currently sexually active:
☐ Yes ☐ No

Check all signs that applies:

☐ Lichen planus-like features
☐ Lichen sclerosis-like features
☐ Vaginal scarring (female)
☐ Clitoral/labial agglutination (female)
☐ Labial resorption (female)
☐ Erosions
☐ Fissures
☐ Ulcers
☐ Phimosis (male)
☐ Urethral meatus scarring/ stenosis (male)

☐ *Abnormality present but NOT thought to represent GVHD (specify cause):*_____
☐ *Abnormality thought to represent GVHD PLUS other causes(specify cause):*_____

**Genital symptoms are not specific to cGVHD and can represent premature gonadal failure or genital tract infection.*

If a gynecologist is unavailable, external examination may be performed to determine "discomfort on exam" as follows:

a) Spread the labia majora to inspect the vulva for the above signs. Touch the vestibular gland openings (Skene's and Bartholin's), labia minora and majora gently with a qtip. Vulvar pain elicited by the gentle touch of a qtip is classified as discomfort on examination. Palpate the vaginal walls with a single digit to detect bands, shortening, narrowing or other signs of vaginal scarring.

b) If the woman is sexually active, determine whether qtip palpation or gentle palpation of scarred ridges elicits pain similar to that which the woman experiences during intercourse.

Female genitalia: Severity of signs:
1) Mild (any of the following); erythema on vulvar mucosal surfaces, vulvar lichen-planus or vulvar lichen-sclerosis
2) Moderate (any of the following); erosive inflammatory changes of the vulvar mucosa, fissures in vulvar folds
3) Severe (any of the following); labial fusion, clitoral hood agglutination, fibrinous vaginal adhesions, circumferential fibrous vaginal banding, vaginal shortening, synechia, dense sclerotic changes, and complete vaginal stenosis

Male genitalia: Diagnostic features include lichen planus-like or lichen sclerosis-like features and phymosis or urethral scarring or stenosis. Severity of signs: **Mild** – lichen planus-like feature; **Moderate** – lichen sclerosis-like feature or moderate erythema; **Severe** – phimosis or urethral/meatal scarring

Biopsy obtained: ☐ **Yes** ☐ **No** **Site biopsied:**_____**GVHD confirmed by histology:** ☐ **Yes** ☐ **No**

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

Completed by (spell out name):_____ **Date form completed:** _____

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 percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

Global staging (Mild, Moderate, Severe)

If 1-2 Grade 1=Mild

If at least 1 Grade 3=severe

Anything between=Moderate

EXCEPT if Lung Grade 1=moderate Lung Grade 2=severe

15.5 APPENDIX E: NMDP STANDARD PROTOCOL, 22ND

- A copy of the NMDP Standard Protocol may be viewed at:

  http://bethematch.org/About/Who_We_Are/NMDP_Network/Draft_NMDP_Standards_22.aspx

15.6 APPENDIX F: IMMUNIZATIONS

- Immunizations: All recipients will receive immunizations after transplant, according to the following table: (from: http://intranet.cc.nih.gov/bmt/clinicalcare/pdf/Table_II.pdf)

15.7 APPENDIX G: VARIABLES/GUIDANCE FOR DATA COLLECTION IN THE STUDY DATABASE

Patient (recipient) characteristics at protocol entry (if available)

- Sex (male, female)
- Age at enrollment (number)
- Ethnicity (white, black, American Indian/Alaskan native, Asian/Pacific islander, Hispanic, other)
- Karnofsky performance status
- CMV serology status (positive or negative)
- EMV serology status (positive or negative)
- ABO (A, B, AB, O)
- Diagnosis (B-chronic lymphocytic leukemia (B-CLL), T-chronic lymphocytic leukemia (T-CLL), T -prolymphocytic leukemia (T-PLL), B-prolymphocytic leukemia (B-PLL), Hodgkin lymphoma, Follicular lymphoma, marginal zone lymphoma, Burkitt lymphoma or lymphoblastic lymphoma, diffuse large b-cell lymphoma, follicular large cell lymphoma, peripheral T-cell lymphoma, mantle cell lymphoma, anaplastic large cell lymphoma, cutaneous T-cell lymphoma, multiple myeloma, acute myelogenous leukemia, acute lymphocytic leukemia, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelogenous leukemia, NK cell neoplasms, hypatosplenic T-cell lymphoma, enteropathy associated T-cell lymphoma).
- Date of diagnosis (month, year)
- Kahl relapse risk (low, intermediate, high)
- Sorror score (number)
- Chemosensitivity status: chemosensitive (CR or PR to their most recent chemotherapy regimen), chemoresistant (SD or PD to their most recent prior chemotherapy regimen), or not evaluable [55]
- Number of prior treatment regimens (number) [not number of cycles]
- History of prior autologous transplantation (yes/no)
- History of prior allogeneic transplantation (yes/no)
- Malignancy disease status at time of enrollment: CR (Complete Response), MRD (Minimal Residual Disease) or not CR (not Complete Response).
  -IF Minimal residual disease (MRD) present at time of enrollment , what was the method of detection of MRD: flow, cytogenetics, molecular/PCR, monoclonal protein spike, kappa/lambda ratio, other.
- CD3, CD4, CD8, CD 19, CD56, count at enrollment

Donor characteristics (if available)

- Sex (m/f)
- If female, parity (Number of pregnancies)
- Age at stem cell donation
- Ethnicity (white, black, American Indian/Alaskan native, Asian/pacific islander, Hispanic, other)
- CMV serology status (positive or negative)
- EBV serology status (positive or negative)
- ABO type (A, B, AB, O)
- ABO mismatch with recipient (0 = none, 1 = major, 2 = minor, 3 = bidirectional)

Data collection post-induction and prior to transplantation (if available):

- Number of EPOCH cycles pre-transplant (number)
- Number of FLAG cycles pre-transplant (number)

- CD3, CD4, CD8, CD 19, CD56 count post-induction therapy and at Day -7
- Date of palifermin administration
- Dose of palifermin given (mcg/kg)
- Thymus measurements determined by CT scan of chest at baseline (cm^3, size grade (0-5), density grade (1-5).

Transplantation infusion variables (if available)

- PBSC infusion date /Day 0
- Infused CD3+ cell dose per kg (*10^6)
- Infused CD34+ cell dose kg (*10^6)

Post-transplantation variables (if available)

- Thymus measurements (cm^3, size grade (0-5) and density grade (1-5) determined via CT scan chest) post-transplant at day +28(+/- 4 days), +180 days (+/- 14 days), +365 days (+/- 30 days), 24 months (+/- 2 months) and 36 months (+/- 2 months)
- Date of neutrophil recovery (Designated by the first of 3 consecutive days with an ANC above 500/mm^3) and # of days post-transplant that this occurred
- Date of platelet recovery (Designated by the first of 7 days where the platelet count remains above 20,000/mm^3 without transfusion support), and # of days post-transplant that this occurred
- Date of lymphocyte recovery (designated by first of 3 consecutive days with ALC above 500/mm^3), and # of days post-transplant that this occurred.
- Date of sustained donor engraftment (Neutrophil recovery associated with complete donor chimerism (defined as myeloid and lymphoid % donor >=95%) at day 100.)
- Graft failure at Day 28 evaluation (yes, no)
- DLI administered? (yes, no)
- Date of first DLI administered
- Reason for DLI administration (mixed chimerism or disease)
- Disease response assessment at Day +28, Day +100, Day +180, +12 months, 2 years, 3 years, 4 years, 5 year visits per CIBMTR viewed at: https://www.cibmtr.org/manuals/fim/1/en/topic/none
- Date of first disease relapse (progressive malignancy after prior remission)
- Date of first disease progression (progressive existing malignancy)
- Date of remission (applicable only if disease present at time of transplant)
- Date of death
- Cause of death: relapse vs. non-relapse mortality (NRM) (definition of relapse mortality: at the time of death, primary disease is not in clinical remission. Non-relapse mortality: patient in clinical remission at time of death.
- If non-relapse mortality, primary cause of death: a. GVHD, b. infection, c. organ failure, d. other – list.
- Second malignancy (including EBV associated lymphoproliferative disease, excluding non-melanoma skin cancer): yes or no – list.
- Secondary malignancy information: date of diagnosis.
- Date off protocol (no more data collected), reason a. death, b. other - list

Post-transplantation infection data (if available)

- See Section **8.4** and **Appendix J** for detail.

Acute GVHD data collection

- Acute GvHD date of diagnosis
- aGVHD type at diagnosis: classic versus late acute (if late: a. persistent b. recurrent or c. late onset)
- Grade of acute GvHD and organ stages (skin, liver, GI) at time of initial diagnosis, and at each timepoint evaluation at Day +28, Day +100 and Day +180.
- Organ involvement at time of initial diagnosis (1= liver only, 2 = skin only, 3 = GI only, 4 = liver + skin, 5 = liver + GI, 6 = skin + GI, 7 = liver + skin + GI) and at each time point evaluation at Day +28, Day +100 and Day +180.
- Max aGVHD grade in first 6 months
- Acute GVHD skin (max stage during first 6 months)
- Acute GVHD liver (max stage during first 6 months)
- Acute GVHD GI (max stage during first 6 months)
- Date starting systemic steroids for front line acute GVHD therapy
- Date stopping systemic steroids for acute GVHD (for at least 2 weeks)
- Date starting second line systemic therapy for steroid failure (list agent)
- Date stopping all non-steroid immunosuppressive systemic therapy (>4 weeks)

Chronic GVHD data collection

- Date of cGVHD diagnosis
- Patient off systemic steroids for 2 months at time of diagnosis of cGvHD (yes, no)
- Patient off non-steroid systemic immunosuppression or therapy for 2 months at time of diagnosis of cGVHD (yes, no)
- Onset of chronic GVHD: quiescent (>2 weeks no acute GVHD symptoms), progressive, de novo
- NIH global score (mild vs. moderate vs. severe) at time of diagnosis and protocol evaluation time points at Day +28, Day +100, Day +180, Day +270, 1 year, 2 year, 3 year, 4 year and 5 year.
- Organ specific cGVHD NIH scores (0-3): at time date of initial diagnosis and protocol evaluation time points (skin, oral, eyes, GI, liver, lung, joints/fascia, GU)
- Type of systemic immune therapy used to treat cGVHD and starting and stopping dates: steroids, tacrolimus, sirolimus, cyclosporine, mycophenolate mofetil, etanercept, rituximab, prednisone, ECP, anti-thymocyte globulin, cyclophosphamide, daclizumab, thalidomide, pomalidomide, IL-2, other-list
- Date off all systemic cGvHD therapy (including steroids) for >3 months
- Date off systemic steroids for >6 months
- Steroid dose (prednisone equivalent) at each protocol visit
- Patient weight (kg) at each protocol visit

Ancillary post-transplant biology studies

- CD3, CD4, CD8, CD19, C56 count: at +14, 28, 60, 100, 180, 270, 365 days, 18 and 24 months.

- Peripheral blood lymphoid, myeloid and whole blood chimerism on Days +14, 28, 60, 100 and 180
- Bone marrow total chimerism on Day +28, 100, 180 and 12 months Immunoglobulin levels IgG, IgM, IgA, at day 28, 60, 100, 180, 270, 365, 18 and 24 months and then yearly until 5 year follow-up.

Note: Please see sections **6.1 and 8** for what Adverse events that are to be collected in the study database.

15.8 APPENDIX H: STUDY CALENDAR

	Screening[1]	Baseline/ Pre-Induction[1]	Induction therapy	RSH Phase	Within 7 days pre-palifermin	KGF Admin (Day -7)	Day 0 (+/- 1)	Day +7 (+/- 3)	Day +14 (+/- 7)	Day +28[16] (+/- 7)	Day +60 (+/- 7)	Day +100 (+/- 14)	Day +180 (+/- 14)	Day +270 (+/- 14)	1 Yr (+/- 30)	1.5 Yrs (+/- 30)	2, 3, 4, 5 Yrs (+/- 30)
H&P/HSCT timepoint note & Karnofsky Status	X				X		X			X	X	X	X	X	X	X	X
Risk of relapse documentation (Kahl scale), Comorbidity, and Index (HCT-CL)	X																
Chemosensitivity		X															
STR chimerism Profile	X[2]																
Anti-HAV, HBsAg, anti-HBs, anti-HBc, anti-HCV, anti-HIV	X																
T. Cruzi, HTLV I/II, HSV, Toxoplasma[18], Varicella, West Nile, Syphilis		X															
PPD or QuantiFERON Gold		X															
CMV/EBV[9]		X					Weekly until Day +180, then as clinically indicated.										
Tacrolimus and Sirolimus levels[9]							Tacrolimus and Sirolimus start on D-3. Tacrolimus and Sirolimus trough levels to be drawn at least 2 times per week and/or upon symptoms or alterations in renal function. Trough levels may be monitored more frequently if clinically indicated. Sirolimus trough level will also be drawn on D-2 (one day after loading dose)										
U/A & UPC (UPC at screening only)	X												X		X		X
24 hr Urine CrCl (CG formula)	X																
Urine or Serum HCG in female of childbearing potential	X	X[6]	X		X												
ECG	X																
Echo or MUGA scan	X											X[3]					
PFTs	X											X	X	X	X	X	X
Dexa Scan															X		X[3]
CT chest w/o IV contrast for thymus volume measurement				X[8]						X			X		X		X[8] (24m & 36m)
CT NCAP (or other imaging, such as PET/MRI, per PI discretion as clinically indicated)	X[2]			X[3]						X[3]		X[3]	X[3]		X[3]		X[3]
MM work up[4]	X[2]			X[2]						X[3]		X[3]	X[3]		X[3]		X[3]
BM aspirate and biopsy[14] (flow, cytogenetics, molecular studies, research samples to Figg's lab)	X[2]		X[2]							X[3]		X[3]	X[3]		X[3]		X[3]
Optional Lumbar Puncture , IT MTX Administration, and CSF	X	X								X[3]		X	X		X		X

	Screening[1]	Baseline/ Pre-Induction[1]	Induction therapy	RSH Phase	Within 7 days pre-palifermin	KGF Admin (Day -7)	Day 0 (+/- 1)	Day +7 (+/- 3)	Day +14 (+/- 7)	Day +28[16] (+/- 7)	Day +60 (+/- 7)	Day +100 (+/- 14)	Day +180 (+/- 14)	Day +270 (+/- 14)	1 Yr (+/- 30)	1.5 Yrs (+/- 30)	2, 3, 4, 5 Yrs (+/- 30)
Analysis (Patient & Disease Specific)[15]																	
Peripheral blood flow	X			X[3]						X[3]		X[3]	X[3]		X[3]		X[3]
BM chimerism[5]										X		X	X		X[3]		X[3]
Whole blood & WBC chimerism[5]									X	X	X	X	X		X[3]	X[3]	X[3]
ABO type & screen	X					q4 days during transplant hospitalization then as clinically indicated											
CBC with differential[9]	X	X[6]	X[6]	X	x	Daily during transplant hospitalization, then as clinically indicate. If minor ABO mismatch, twice daily from Day+4 until Day+14, then as clinically indicated											
Acute care, hepatic, & mineral panel[9]	X	X[6]	X[6]	X	x	Daily during transplant hospitalization then as clinically indicated											
PT/PTT	X	X				Weekly during transplant hospitalization, then as clinically indicated											
Amylase/lipase		X			x	Daily during transplant hospitalization until Day +6. Then, twice weekly until Day +28 or more often if clinically indicated. After Day +28, weekly until Day +100. Then at each timepoint.						X	X	X	X	X	X
TBNK		X	X[7]						X	X	X	X	X	X	X	X	X
Serum Immunoglobulins		X								X	X	X	X	X	X	X	X
Serum ferritin		X													X		X[3]
TSH, T3, T4 (Thyroxine, free)		X													X		X
Gonadal Assessment -Females: FSH/LH, estrogen Males: Testosterone (free & total)	X														X		X[3]
Research blood, Section 5.1.1 Additional research samples to Figg's Lab[11]		1 SST, 5 CPT & 5 ACD				1 SST & 1 GTT	1 SST & 1 GTT	1 SST & 5 CPT	1 SST & 5 CPT	1 SST & 5 CPT	1 SST & 5 CPT	1 SST & 5 CPT	1 SST, 5 CPT & 5 ACD	1 SST & 5 CPT	1 SST, 5 CPT & 5 ACD	1 SST & 5 CPT	1 SST, 5 CPT & 5 ACD @ 24m
Social Work Consult		X															
Nutrition Assessment		X															
Dental Consult and/or collection		X[13]			X[12]						X[12]		X[12]		X[13]		X[3]
Hepatology Consult in patients with chronic active hepatitis B and/or Hepatitis C	X	X															
Dermatology Consult for research collection and new diagnosis											X		X				X[3]
Ophthalmology Consult		X													X[2]		X[2]
GI Consult					X												
GYN Consult (pre or peri-menopausal women)		X													X		
NIH Advanced Directives Form[10]		X															

	Screening[1]	Baseline/ Pre-Induction[1]	Induction therapy	RSH Phase	Within 7 days pre-palifermin	KGF Admin (Day -7)	Day 0 (+/- 1)	Day +7 (+/- 3)	Day +14 (+/- 7)	Day +28[16] (+/- 7)	Day +60 (+/- 7)	Day +100 (+/- 14)	Day +180 (+/- 14)	Day +270 (+/- 14)	1 Yr (+/- 30)	1.5 Yrs (+/- 30)	2, 3, 4, 5 Yrs (+/- 30)
Acute GVHD Assessment										X		X	X				
Chronic GVHD Assessment										X		X	X	X	X	X	X
GGT[17]			X	X	X	X	X	X	X	X	X	X					

1. In addition to baseline/pre-induction tests listed, all screening tests should be repeated if done > 45 days prior to start date of induction therapy
2. Must be done at least 30 days before the start of induction or based on PI Discretion
3. Per Investigator discretion/clinically indicated
4. MM workup – skeletal survey; total serum protein, serum and urine (24 hour) protein electrophoresis with immunofixation, CRP, immunoglobulin free light chains, beta 2 microglobulin
5. Recipient bone marrow chimerism will be measured on days 28, 100 and 180. Lymphoid and myeloid subset chimerism will also be measured at these points if donor chimerism was < 95% on the previous study. Chimerism also measured as clinically indicated
6. Must be performed within 48 hours of pre-induction therapy
7. TBNK should be done at the end of each induction cycle, at least 48 hours after the cycle's last dose of filgrastim and within three days before the next scheduled cycle. This is done in order to determine CD4 count, which helps determine whether to continue administering additional cycles of induction chemotherapy.
8. CT scan of the chest without IV contrast is done at 24 months and 36 months. After the scan at 36 months, no additional scans are done. This does not need to be performed separately if CT obtained for other reasons is obtained and can be used to collect this data.
9. Of note, for all lab tests done between day +100 and Day +180 (namely CBC, acute care panel, mineral panel, hepatic panel, CMV, EBV, tacrolimus and sirolimus levels) these labs can be obtained at patient's home institution since beyond Day +100, patients are usually stable enough to go home and no longer require all lab testing to be obtained at NIH. The frequency of the labs will be at the discretion of the patient's home physician.
10. As indicated in section **11.1.3**, all subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.
11. An additional 50 ml (6 R&G CPT) will be sent to Figg's Lab at the onset of acute and/or chronic GVHD, prior to the start of systemic GVHD treatment, whenever clinically possible, as per protocol section **5.1.1.2.1**.
12. This is to collect the research dental sample only
13. This is a dental consult only
14. Confirm with the PI if cytogenetics and molecular studies are needed.
15. Confirm with the PI for appropriateness and the need for concurrent intrathecal chemotherapy administration. This will only be completed after protocol consent is signed.
16. This visit is considered the 30 day post-Palifermin safety visit.
17. GGT twice a week through day 100 and then as clinically indicated
18. Toxoplasmosis weekly on positive patients during inpatient hospitalization, then as clinically needed.

15.9 APPENDIX I: PROCESSING CHECKLIST: DTM-FORM-5086

Protocol #: Date: Product Type: DIN: (apply sticker on receipt)

Recipient: Diagnosis:	Donor:

Recipient ABO/Rh: _____ / _____	Donor ABO/Rh: _____ / _____
Recipient Antibody Screen: ☐ NEG or ☐ POS	Donor Antibody Screen: ☐ NEG or ☐ POS
ABO Interpretation by MD: ☐ Autologous donation-recipient ABO/Rh, antibody screen and ABO interpretation are not applicable ☐ No ABO or other red cell incompatibility ☐ Major incompatibility with donor RBCs ☐ Minor incompatibility with donor plasma	Donor TTD & RPR Interpretation: ☐ Results Within Normal Limits (WNL) for Anti-HBc, Anti-HCV, Anti-HIV-1/2, Anti-HTLV-I/II, HBsAg, HIV-1/HCV/HBV NAT, West Nile NAT, RPR and T. Cruzi ☐ CMV test resulted ☐ Results NOT WNL or incomplete (see CRIS report) ☐ Result >30 days (see CRIS report)
Comments:	MD review: Storage of product is ☐ routine ☐ vapor ☐ physically quarantine product due to _____

MD Review: ☐ **Process per Protocol** **or** ☐ **Process per Med Exception**

PBW adult recipient: ___kg **or** **Actual weight pediatric recipient:____kg**

Height in CRIS: _____ cm Date: _____ **Weight in CRIS _____kg Date: _____**

Ideal Body Weight (IBW) and Practical Body Weight (PBW) Calculations or actual weight is used

☐ **Male: (0.91 x _____) - 88 = _____kg IBW** ☐ **Female: (0.91 x _____) - 92.5 = _____kg IBW**

height(cm) **height(cm)**

(_____+ _____) = _____kg = _____kg PBW
Actual IBW 2

% Change in PBW if 2nd or 3rd Collection: PBW current – PBW former X100 = _____
PBW former

☐ **If % change is ≤15 then former PBW is used for calculations _____ kg**
☐ **If % change is >15 then current PBW is used for calculations _____ kg**
Note: % Change in PBW not calculated if 1st collection

Submitted by:_____Date: _____

Service Coordinator Review: _____ / _____	Processing Tech Review: _____ / _____
☐ PSI or Medical Exception reviewed CRIS Processing order # _____ ☐ Recipient and Donor in StemLab	☐ DIN label affixed to form; clerical check of DIN, recipient and donor information, product type on label performed ☐ CRIS Processing order reviewed ☐ CRIS order # verified; entered in StemLab; COMPLETED

Comments:
MD Approved by: _____ Date: _____ **MD Approved by: _____ Date: _____**

Record Reviewed by: _____ Date: _____

15.10 APPENDIX J: INFECTIONS GRADED BY THE CORDONNIER SCALE.

- Certain infection related adverse events (will also be graded by the Cordonnier scale from Day+7 to 24 months post-transplant. This will include the following information: Date of diagnosis of new infection, Affected organ system (if applicable), and the type of infection to be graded. These include:

    - Date of 1st CMV reactivation (defined as CMV > 250 copies/ml or ≥ 3.08 Log $_{10}$ IU/ml by peripheral blood PCR)
    - Date of 1st CMV viremia (defined by viral loads ≥1000 copies/mL (4.12 Log $_{10}$ IU/ml), two consecutive rising values 250-1000 copies/mL (3.08 – 4.12 Log $_{10}$ IU/ml), or in persons with any positive CMV PCR result suspected of having CMV disease)
    - Date of initiating treatment for CMV viremia
    - Date of 1st EBV activation (EBV infection was defined as ≥1000 copies/mL or ≥ 2.70 Log $_{10}$ IU/ml by peripheral blood PCR)
    - Date of 1st nasopharyngeal viral infection (nasal wash)
    - Type of nasopharyngeal viral infection: influenza A, influenza B, parainfluenza 1, 2, and 3, adenovirus, respiratory syncytial virus (RSV), rhinovirus, coronavirus, metapneumovirus, or any other nasopharyngeal virus.
    - bacteremia with severe sepsis or deep organ involvement, and type of bacteria
    - candidemia (≥1 positive blood culture) or candida deep tissue infection, type of candida
    - proven or probable Aspergillus pneumonia
    - severe varicella zoster infection
    - viral encephalitis, and type of virus
    - cytomegalovirus (CMV) organ involvement
    - *Pneumocystis jiroveci* pneumonia
    - *Toxoplasmosis gondii* with organ involvement
    - Any pneumonia with PaO_2 ≤65 mmHg, or
    - Any sepsis syndrome requiring transfer to the intensive care unit (type of bacteria)

Cordonnier Grade (see

Table **3**). [64]

Table 3 Classification of the severity of infections in three grades, specifically designed for the GREFIG study 4 according to the data found in the literature in 1998.

TABLE 1. Classification of the severity of infections in three grades, specifically designed for the GREFIG study (*4*) according to the data found in the literature in 1998

Type of infection/ severity grade	Grade 1	Grade 2	Grade 3	Reference
Bacterial infections	Bacterial focus treated in outpatients (urinary tract infection, angina), excluding any form of bronchopneumonia	Bacteremia without severe sepsis Bacterial focus requiring inpatient management	Bacteremia with severe sepsis Complex bacteremia (deep organ involvement)	(*7*)
Fungal infections	Superficial candida infection	Deep candida infection without candidemia	Candidemia (≥1 positive blood culture) with either sepsis or deep focus	(*8–10*)
		Candidemia without severe sepsis and without focus Aspergillus sinusitis without bone involvement	Proven or probable Aspergillus pneumonia	
Viral infections	Mucous HSV infection	VZV infection	Severe VZV infection (coagulopathy or organ involvement) Any viral encephalitis	(*11–15*)
	Asymptomatic CMV infection	Symptomatic CMV infection (fever)	CMV pneumonia or CMV gut disease	
Parasitic infections		Toxoplasmosis infection without organ involvement	*Pneumocystis jiroveci* pneumonia (regardless of PaO_2 level) CNS or other organ toxoplasmosis	(*16–19*)
Nonmicrobiologically defined infections	Fever of unknown origin (FUO) in neutropenic patients receiving broad spectrum antibiotics	Pneumonia or broncho-pneumonia with PaO_2 >65 mmHg	Any acute pneumonia with PaO_2 ≤65 mmHg	(*14, 20, 21*)
	Clinically documented infection not requiring inpatient management	Any infectious symptoms requiring inpatient management (except FUO in neutropenic patients)	Any sepsis syndrome requiring transfer to Intensive Care Unit	

15.11 APPENDIX K: SAMPLE PROCESSING

Bone marrow

1. Transfer bone marrow from heparinized syringe or GTT to 15-mL conical tube
2. Spin for bone marrow supernatant if required by patient protocol (1300g for 10 min) and distribute 1 mL into 2 vials each, freeze @ -80C (record volume if less than 1 mL)
3. Dilute bone marrow to 10 mL with DPBS without CA and Mg
4. Layer diluted bone marrow on top of 4 mL of LSM in a new tube
5. Spin 30 min @ minimum 400g, room temperature, slow brake
6. Collect cell layer above LSM layer and transfer to 50-mL conical
7. Follow counting and washing procedure
8. After determining correct vials for cells, follow freezing protocol

Saliva

1. Weigh tube to determine saliva volume in grams (subtract weight of tube from total weight
2. Aliquot 1mL of saliva into microfuge tubes labeled pellet (decrease tube number if low volume)
3. Spin saliva for 5min at 10,000x
4. Transfer supernatant to new microfuge tubes labeled supernatant
5. Resuspend pellet tubes in 300ul of 0.5M EDTA
6. Freeze pellets and supernatants at -80 C

Microbiome Swabs

1. Place 1 mL of RNAlater in tubes for the swabs
2. The swabs will be taken and put into the tubes and sealed
3. Freeze these tubes directly into -80 C

Sputum

1. Sterilize forceps in ethanol
2. Pre-weigh 50mL conical
3. Empty sputum onto petri dish and separate the plug from the saliva using forceps
4. Transfer plugs to petri dish lid and make small circular movements to condense them
5. Transfer the plugs to the weighed conical and record sputum weight
6. Add 8 volumes x sputum weighty of DPBS without CA and Mg
7. Disperse sputum gently
8. Vortex 15sec
9. Place tube in a bag of ice and rock on bench rocker for 15min
10. Centrifuge 790g for 10min @ 4 C, brake off
11. Take half of the added DPBS volume and divide into 4 aliquots for freezing at -80 C

Biopsies

1. Have a Styrofoam box with dry ice in the bottom
2. Place a metal can of 2-methylbutane in the box
3. Bring OCT and trays, RNAlater filled tubes, pen for labeling
4. Fill OCT tray for fragment to be placed in and flash freeze in the 2-methylbutane
5. Fragments SNAP frozen can be flash frozen in 2-methylbutane as well
6. Store RNAlater tubes at 4 C overnight before freezing at -80 C
7. Wrap frozen OCT in foil and freeze at -80 C

PDCMF Sample Processing SOP

Purpose and Scope

Isolation, collection, and storage of Peripheral Blood Mononuclear Cells, plasma, serum, bone marrow supernatant and cells, biopsies, cell products, and stool, from clinical trial patient research samples, for cryopreservation, and flow cytometry.

PBMC and CPT plasma

1. Weigh CPT tubes for balancing in centrifuge
2. Spin tubes for 30 min @ 1800g, room temperature, slow brake
3. Collect 1 mL plasma per vial and store @ -80C
4. Collect PBMC layer from top of polymer layer, combining into a 50-mL conical
5. Follow counting and washing procedure
6. After determining correct vials for cells, follow freezing protocol

Counting Cells

1. After placing PBMC layer or bone marrow cells into 50-mL conical, balance tubes and spin for 10 min, 1500 rpm, room temperature
2. Aspirate supernatant
3. Resuspend in 1 mL of DPBS without Ca and Mg
4. Add 10 ul of cell suspension to 90 ul ACK lysis buffer in one well of a 96 well plate
5. Creates 1:10 dilution
6. Add 10 ul of 1:10 dilution to 10 ul of Trypan Blue in another well
7. Creates 1:20 dilution
8. Aliquot dyed cells (20 ul total) on Cellometer slide (use circles)
9. Count the cells in the Cellometer with dilution indicated (20)
10. After counting, dilute samples again to 10 mL with DPBS
11. Spin 10 min at 1500 rpm, room temperature to pellet
12. Aspirate supernatant and resume with freezing protocol

Freezing Cells

1. Resuspend in a 1:1 ratio of chilled Freeze Mix #1 and Freeze Mix #2
    - Resuspend in Freeze Mix #1, then add Freeze Mix #2 dropwise, mix -> final 1 mL per vial
    - Store resuspended cells in aliquots as specified by protocol
2. Aliquot into vials
3. Place vials in a chilled, but not frozen, cryochamber and put in the -80°C freezer overnight
4. Transfer vials to storage boxes (-80°C freezer or liquid nitrogen) and place jars in hall refrigerator to chill to 4° for next use
5. Replace isopropanol in bottle after 5 uses (once a week)

Plasma from GTT-heparin

1. Set aside aliquots for FACS (500uL)
2. Balance and spin GTT for 10 min at 1300g, room temperature
3. Take 1 mL aliquots of top plasma layer and freeze @ -80 C

Serum

1. Make sure blood was drawn at least an hour prior to centrifugation for clotting to occur
2. Balance and spin RTT or SST for 10 min at 1300g, room temperature
3. Make sure granulocytes have gone through the polymer and then take 1 mL aliquots of the top serum layer and freeze @ -80 C