A Phase II Study of Cytokine Induced Memory-like NK Cell Adoptive Therapy after Haploidentical Donor Hematopoietic Cell Transplantation

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Protocol Revision History

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SCHEMA

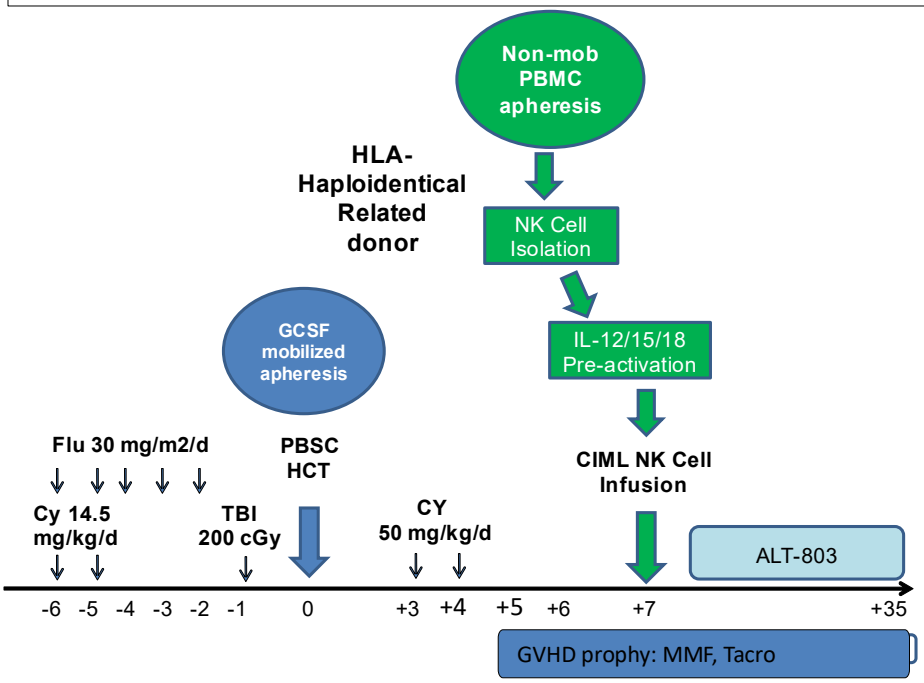
Inclusion Criteria:

- AML patients with refractory disease who have not achieved a complete remission (CR) after two rounds of AML specific induction therapy* (primary induction failure), or relapsed disease after obtaining a complete remission and failed one cycle of re-induction therapy
- Available related haplo-identical HSC and NK cell donor
- Adequate organ function:

Exclusion Criteria:

- Uncontrolled bacterial, fungal, or viral infections, known HIV, active hepatitis B or C
- New progressive pulmonary infiltrates
- Pregnant and / or breastfeeding
- Previous allogeneic HCT

* standard dose 10-day decitabine or 7-day azacitidine therapy will count as one cycle of AML specific induction/re-induction therapy



CIML NK Cell Dose: All NK cell obtained from a leukapheresis (range 0.5 to $10x10^6$/kg)
ALT-803 Dose: 10 mcg/kg SQ every 3 weeks (± 14 days) for a total of 4 doses starting on CIML NK cell infusion day (Day +7).
Donor Stem Cell Graft: Haplo donor will undergo standard G-CSF mobilized PBSC collection via standard 20L leukapheresis with target CD34+ cell collection goal of at least $4x10^6$ /kg.
Donor NK Cell Collection and Generation of CIML NK Cells: Haplo donor will undergo non-mobilized leukapheresis on Day +6. PBMCs will be collected using standard collection techniques. NK cells will be purified from PBMC with CD3 depletion and CD56 positive selection. This NK cell product will be pre-activated for 12-18 hours with a combination of rhIL-12, rhIL-15, and rhIL-18 under GMP conditions. The cells will be washed 2 times prior to infusion on Day +7.

1.0 BACKGROUND AND RATIONALE

1.1 Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is one of the most common hematologic malignancies, with an estimated prevalence of 3.8 cases per 100,000.[1] AML is primarily a disease of older adults, with two-thirds of the new cases occurring in patients > 60 years old. [2] In older AML patients, the 5 year disease-free survival (DFS) is extremely poor at 10-20%.[3,4] These suboptimal outcomes in older AML patients are related to a combination of unfavorable biologic characteristics of the leukemia, comorbidities, poor performance status, and organ dysfunction that limits aggressive treatment options.[5] In addition to age, genetic alterations in the leukemic cells strongly influence the outcomes of AML patients with current treatment regimens. [5] The standard chemotherapy-based treatment regimens for AML patients involve an initial 7+3 (ara-C for 7 days and 3 days of idarubicin or daunorubicin) to achieve a complete remission (CR). While up to 75% of AML patients under the age of 60 years achieve CR following intensive treatment regimens, the CR rates are only in the range of 40-50% in older AML patients with the intensive induction regimens.[5] Similarly, only around 50% of the young and 39% of the elderly AML patients in poor prognostic groups are able to achieve a CR with the current intensive induction regimens.[5] The median overall survival (OS) for patients not achieving a CR is <1 year, even with best palliative treatment. Nearly all AML patients treated only with induction chemotherapy relapse.[6] For younger patients, standard post-induction consolidation chemotherapy includes 3-4 cycles of high dose cytarabine (good or intermediate risk) or an allogeneic stem cell transplant (poor risk or intermediate with a sibling donor). Questions remain about the best approach for consolidation therapy in the older AML patients, which is required for almost all patients to achieve long-term DFS. Unfortunately, many patients who go on to receive consolidation chemotherapy only will ultimately relapse. The prognosis of the patients who relapse after the consolidation therapies is extremely poor, especially if the relapse occurs in first 6 months of the consolidation therapy. Re-induction followed by allogeneic stem cell transplantation (if they are able to achieve a complete remission) is the most common regimen used for refractory or relapsed AML patients. However, many of these patients are not eligible for intensive regimens and only a minority of these patients are able to achieve a complete remission in order to be eligible for allogeneic transplantation.

In addition, the AML patients who are able to achieve morphologic complete remission but continue to have evidence of minimal residual disease (MRD) undergoing allogeneic hematopoietic cell transplantation (allo-HCT) also have extremely poor post-transplant outcomes similar to patients undergoing allo-HCT in active disease. [7] Thus, these patients will also benefit from additional anti-leukemia therapy when MRD is detected.

1.2 AML and Allogeneic Hematopoietic Cell Transplantation

Allo-HCT using a hematopoietic stem cell (HSC) graft from major histocompatibility complex (MHC) matched siblings or MHC matched unrelated donors and a myeloablative conditioning regimen is the most common treatment for refractory

or relapsed AML patients and provides 3-year OS rates of 17-20%, but approaching a 3-year OS rate of 42% in patients with good risk profiles.[8,9] Unfortunately many of our patients with relapsed / refractory disease are not able to tolerate myeloablative conditioning regimens, making them ineligible for potentially curative treatment option.

Many patients (particularly those from a mixed-race or ethnic background) lack an HLA-matched related or unrelated donor (URD). Conservative estimates indicate that 50% of patients with leukemia do not find a suitable URD in a timely manner.[10] The use of a haploidentical donor is a viable alternative for most, as nearly all patients have an available related donor with whom they share a single HLA haplotype.

MHC haploidentical hematopoietic cell transplantation (haplo-HCT) has been performed sparingly, as they were *historically* associated with high incidences of graft failure, severe graft-versus-host disease (GVHD), and excessive transplant related mortality (TRM).[11,12] However, recent studies have shown that outcomes from haplo-HCT using post-transplant cyclophosphamide (PT-Cy) are comparable to non haplo allo-HCT.[13,14,15, 16] Most of the haplo-HCTs using PT-Cy platform have used bone marrow as the source of stem cells. However recent studies of haplo-HCT using T-cell replete peripheral blood as source of stem cells (PBSC) with PT-Cy have shown encouraging outcomes.[13] In the myeloablative setting, a prospective study done by Solomon group showed grade II-IV aGvHD incidence of 30% and cGvHD incidence of 35%. (15) In another study by Bacigalupo et al. including 148 patients with various hematologic malignancies, the rates were 24% and 12% respectively.[14] In non-myeloablative setting, using PBSC, our center has shown a grade II-IV aGvHD rate of 53% at 90 days and cGvHD of 8% at 2 years.[17] While limited data are available, outcomes of non-myeloablative haplo-HCT for patients with AML not in complete remission have poor outcomes. In a clinical trial using RIC based haplo-HCT for rel/ref AML, 9 out of 10 patients relapsed within the first 6 months after their transplant despite having received prophylactic donor lymphocyte infusions (DLIs**).**[18] **I**nstitutional data from WUSM indicate that patients with AML not in remission who undergo non-myeloablative conditioning have 1 year leukemia free survival rates of ≤10% (unpublished).

Outcomes were compared in patients with various hematologic malignancies after haplo-HCT using PBSC with contemporaneous MRDs and MUDs and showed equivalent outcomes.[15], Similarly, in a retrospective study post-transplant outcomes specifically in AML and MDS patients were also similar in haplo-HCT, MUDs, and MRDs.[19]

1.3 NK Cell Immunotherapy for AML

Natural killer (NK) cells are innate lymphoid cells important for host defense against infection and mediate anti-tumor immune responses.[20,21] Traditionally, NK cells have been categorized as innate effectors because they use germline-encoded activating and inhibitory NK and cytokine receptors to orchestrate their rapid proliferative and functional (i.e., IFN-γ production and cytotoxicity) responses.[20,22] These effector functions are governed by a complex balance of activating and inhibitory signals transferred via several classes of receptors, a number of which recognize “self” MHC class I antigens.[23] Self-tolerance is

mediated by inhibitory killer immunoglobulin-like (KIR) and other receptors which transmit signals that interrupt the activation signals upon binding of their cognate class I HLA ligands.[24–26] The loss of inhibitory KIR-ligand expression by infected or malignant targets renders them susceptible to NK cell killing, particularly when the targets also present ligands for activating receptors. This observation was applied by Ruggeri et. al. to allogeneic HCT for leukemia, where they reasoned that lack of inhibitory HLA ligands to KIR expressed on donor NK cells would facilitate donor NK cell recognition of the patient's AML blasts.[27–29] In their initial study, HLA-haploidentical donor NK cell alloreactivity against the patient's AML blasts was associated with long term DFS.[27,29] Further, these studies demonstrated minimal GVHD, suggesting that NK cell alloreactive responses did not cause GVHD. Indeed, studies have suggested that HLA-haploidentical donor NK cells can ameliorate or protect against GVHD.[27,30] Subsequent studies in various HCT contexts have studies the role of KIR genetics and transplant outcomes, adding evidence that NK cell immune response contribute to graft versus leukemia effects.[31,32] Thus, one strategy to "target" or provide triggering specificity of NK cells against AML blasts (without GVHD) is through use of HLA-haploidentical NK cells. Such NK cells can be easily isolated in quantity from a donor leukapheresis, and thus are an attractive cell population to harness for anti-leukemia immunotherapy.

Using such HLA-haploidentical donor NK cells to target AML blasts, several early phase clinical trials tested the safety and preliminary efficacy of adoptive NK cell infusions for patients with hematologic and non-hematologic malignancies.[33,34,35] Patient GVHD or any other major toxicities has not been attributed to the adoptive transferred donor NK cells to date. Importantly, some of these early NK cell-based adoptive studies have reported leukemia clearance and complete remissions attributed to the donor NK cells. Miller et al reported CR induction in 5 out of 19 refractory AML patients.[33] A subsequent report indicated that enhancement of donor NK cell expansion via elimination of competing and inhibitory regulatory T cells resulted in CR induction in 6 of 12 patients.[36] Similarly, in another study none of the AML patients who were adoptively transferred with haplo-identical NK cells relapsed with a median follow-up of 964 days, and the 2-year event-free survival estimate was 100%.[34] These studies provide proof-of-principle that HLA-haploidentical NK cells are safe and have some utility as a cellular effector for leukemia immunotherapy, however, inadequate persistence, expansion, and in vivo anti-leukemic activity of adoptively transferred donor NK cells remain limitations. This clinical protocol seeks to translate a new development in NK cell biology to provide additional anti-leukemia functionality, expansion, and persistence to adoptive transferred HLA-haploidentical NK cells, in the context of a haplo-HCT.

1.4 Cytokine-induced Memory-like (CIML) NK Cell Biology

While NK cells have traditionally been considered a member of the innate immune system, paradigm-shifting studies in mice have identified memory-like properties following hapten exposure, virus infection, and combined IL-12, IL-15, and IL-18 cytokine pre-activation.[37–41] Cytokine-induced memory-like NK cells were defined by briefly pre-activating murine NK cells with IL-12, IL-15, and IL-18, followed by adoptive transfer into syngeneic mice. Weeks to months later, memory-like NK cells had proliferated and exhibited enhanced re-stimulation responses to

cytokines or activating receptors.[41,42] This pre-activation approach also resulted in anti-tumor responses to murine NK cell-sensitive cell lines following adoptive transfer in mice.[43] The potential translation of these findings as immunotherapy was established by identification of human IL-12, IL-15, and IL-18-induced memory-like NK cells.[43,44] Key properties of human memory-like NK cells include enhanced proliferation, expression of the high affinity IL-2R$\alpha\beta\gamma$, and increased IFN-γ production following re-stimulation with cytokines or activating receptors.[45] More recently, the Fehniger lab has demonstrated that human CIML NK cells are superior to naïve/control NK cells in their ability to recognize AML blasts and cell lines, kill leukemia targets in vitro, and control leukemia targets in vivo in an NSG mouse model.[46] These findings were promising and recently translated to the clinic in a first-in-human study of CIML NK cells in rel/ref AML. None of the patients treated on this phase I study have had dose limiting toxicity (DLTs) including graft versus host disease (GVHD). Encouragingly 7 out of 11 patients treated with rel/ref AML have had an IWG response. The initial findings from this study were recently published the journal Science Translational Medicine. [47]

1.5 Translating CIML NK cells Adoptive Therapy for Relapsed / Refractory AML

Based on the above in vitro and pre-clinical mouse model findings, we have embarked on a phase I first-in-human clinical trial of CIML NK cells for patients with relapsed / refractory AML (Clinicaltrials.gov # NCT01898793). This trial design used escalating doses (0.5 x 10^6/kg, 1 x 10^6/kg and maximum capped at 10 x 10^6/kg) of haploidentical CIML NK cells. Patients received lymphodepleting chemotherapy (fludarabine plus cyclophosphamide) before their CIML cell infusion, based on previously published adoptive NK cell studies.[33,48] This approach was based on the experience from multiple studies in the past involving the use of lymphokine-activated killer (LAK) cells (prepared by ex vivo stimulation of peripheral blood mononuclear with IL-2) to treat immune-sensitive malignancies like melanoma and renal cell cancer. In our study, we utilized a lymphodepleting regimen consisting of 5 doses of fludarabine at 25mg/m^2 and 2 doses of cyclophosphamide at 60 mg/kg. This regimen is adapted from the one used by Rosenberg's group at NIH and has been associated with successful therapy of refractory melanoma patients with adoptive transfer of cytokine activated tumor infiltrating T cells.

Thus far we have treated 17 patients with relapsed / refractory AML and advanced MDS on this phase 1 study. None of these patients have met criteria for dose limiting toxicity including GVHD. Of 11 evaluable rel/ref AML patients, we have had 7 IWG AML responses. These include 6 patients with complete remissions (CR/CRi). Correlative analyses utilizing donor-specific HLA mAbs allowed tracking of donor memory-like NK cell frequency and function following adoptive transfer. Donor memory-like NK cells were detectable in the blood and bone marrow (BM) of all tested patients with informative HLA and peak in frequency at 7-8 days post-infusion, and contract after 14-21 days as expected following recipient T cell recovery. Memory-like NK cells exhibit significantly increased Ki67%$^+$ as a marker of proliferation at days 3 and 7 post adoptive transfer. Moreover, functional analyses of NK cells at days 7-8 post-transfer reveal increased numbers of donor IFN-γ^+ NK cells following restimulation with K562 leukemia cells in the same blood or BM. Further, response in our patients correlated with BM NK cell frequency.

Thus, human IL-12/15/18-induced memory-like NK cells expand and have enhanced anti-AML function following adoptive transfer in patients, thereby constituting a promising translational innovation for immunotherapy of AML.[46] Here, we propose to add CIML NK cells following haplo-HCT to provide additional graft-versus-leukemia effect in a setting whereby adoptively transferred donor CIML NK cells will not be rejected by reconstituting donor T cells. This may provide an ideal opportunity for these cells to expand, persist, and respond following transfer, compared to the 'window of opportunity' afforded by the isolated donor CIML NK cell infusion approach.

1.6 Study Rationale

1.6.1 Rationale for adding CIML NK cells to the allogeneic transplantation

Allogeneic stem cell transplant can provide durable disease control for some patients with relapsed or high risk AML, but transplant outcomes are poor for patients who have detectable leukemia at the time of transplant. Myeloablative conditioning regimens are generally preferred in patients who can tolerated an intensive transplant approach, but risk of relapse remains high if patients are not in remission before transplant.[7,8]

In recent years, reduced intensity conditioning has facilitated transplantation in patients who are otherwise unable to tolerate more intensive conditioning regimens. Reduced intensity conditioning regimens use modified doses to intensify the immunosuppression and reduce the toxicity of the regimen, thus allowing allo-transplantation to be used in older or sicker populations. This initially safer transplant has allowed allografts to be offered to patients with co-morbid illness, complications of their cancer or their prior cancer therapy who had not been considered eligible for conventional myeloablative transplantation. In recent years there have been several studies showing favorable outcomes using reduced intensity conditioning (RIC) based haplo-HCT in patients with high risk hematologic malignancies including AML.[49,15]. However, risk of relapse after the transplantation is still a major cause of mortality in these patients with relapse rates of >50%, with many relapses occurring early post HCT.[49] In a clinical trial using RIC based haplo-HCT for rel/ref AML, 9 out of 10 patients relapsed within the first 6 months after their transplant despite having received prophylactic donor lymphocyte infusions (DLIs**).**[18] At WUSM only 1 of 6 rel/ref AML patients treated with RIC based haplo-HCT was relapse free at one year after transplantation (un-published data). Thus use of reduced intensity haplo-HCT is unlikely to be successful in providing long-term leukemia free survival in patients with relapsed / refractory AML if haplo-T cells are not provided adequate time to develop and respond to patient AML blasts.

Based on the initial promising results with our ongoing CIML NK cell trial (see Section 1.5 above), we hypothesize that combining the CIML NK cells with a haplo-HCT platform will significantly enhance the graft versus leukemia and therefore potentially provide potentially curative therapy for

these patients with otherwise extremely poor prognosis. Combining same donor CIML NK cells with the HCT platform will also potentially allow these adoptively transferred cells to persist for longer duration as they should not be rejected by donor T cells. The use of CIML NK cells is unlikely to lead to excessive GVHD as previous studies have not been associated with excessive GVHD rates.[33,34,35] Further the patients on this study will receive the standard GVHD prophylaxis using tacrolimus and mycophenolate mofetil (MMF).

1.6.2 Rationale for use of ALT-803 after CIML NK cell infusion

Interleukin-15 (IL-15) is a cytokine and growth factor capable of expanding activated T cells and NK cells. By broad consensus, the NCI Immunotherapy Workshop (2007) ranked IL-15 as the #1 agent with "high potential for immunotherapy."[50] Recombinant human IL-15 (rhIL-15) has a short half-life and therefore needs to be giving as a continuous IV infusion or by daily subcutaneous injections.[51]

ALT-803 is a novel drug developed by ImmunityBio (ImmunityBio, Culver City, CA), to overcome many of the biologic, regulatory, and commercial limitations of unmodified rhIL-15. Under physiologic circumstances, IL-15 and IL-15 receptor-alpha (IL-15Rα) are coordinately expressed by antigen-presenting cells (i.e., monocytes and dendritic cells).[52] To induce a signal, IL-15 bound to IL-15Rα is presented 'in trans' to neighboring NK cells or CD8+ T cells expressing only the IL-2/15$\beta\gamma$ receptor, resulting in multiple signals that trigger survival, proliferation, metabolic fitness, and cytotoxic capacity. At the immunologic synapse, IL15 trans-presentation appears to be a dominant mechanism for IL-15 action in vivo, providing tight physiologic control over the functions of IL-15 under homeostatic conditions and in response to immune stimuli.[53] ALT-803 is a novel recombinant human super-agonist IL-15 complex (i.e., IL-15N72D:IL-15RαSu/IgG1 Fc complex) with a prolonged serum half-life in preclinical animal models. In addition, ALT-803 contains a novel IL-15 mutein with a single substituted amino acid (rhIL-15N72D) that has a 4-fold increase in biologic activity greater than wild-type IL-15 (IL-15 wt).[54] The IL- 15:IL-15R complex increases activity at lower concentrations, and the fusion with Ig-G1 Fc increases serum half-life, providing more ideal pharmacokinetics with prolonged cytokine function.[55]

Because of the availability, ease of delivery (subcutaneous injections), prolonged half-life, and potentially higher efficacy, ALT-803 is currently being studied in several ongoing clinical trials including three studies at our center including in patients that have relapsed after an allogeneic HCT (Clinicatrial.gov # NCT01885897, NCT02099539, NCT02384954). In the study NCT01885897 where ALT-803 is being used in patients relapsed after allo-HCT at 10 mcg/kg SQ, no serious adverse events, including GVHD, have been observed. Preliminary reports have shown favorable effects on NK cells.[56]

Most of the previously published adoptive NK cell protocols (including our ongoing non-transplant CIML NK cell study) have used recombinant human IL-2 (rhIL-2) to support NK cell expansion and persistence in vivo. Since this will also activate patient regulatory T cells[57] and may not optimally expand NK cells, we have changed rhIL-2 to ALT-803 at 10mcg/kg q3weeks (subcutaneous injections) for 4 doses starting at the day of CIML NK cell infusion. We expect a similar adverse event profile as rhIL-2, and potentially improved NK cell expansion and persistence.

1.6.3 Rationale for the NK cell correlative laboratory investigation

Clinical trial administration of adoptively transferred memory-like NK cells provides a unique opportunity to their biology within patients. In order to define CIML NK cell biology in post-transplant setting, we will analyze peripheral blood and bone marrow biopsy samples to assess the persistence, expansion, and duration of anti-leukemia response of the adoptively transferred CIML NK cells. Based on the observations from pre-clinical mouse model and data from our first-in-human trial, we expect the CIML NK cells to persist for a prolonged period of time following adoptive transfer into an MHC-compatible immune environment within these patients, and thus potentially overcome the limited persistence upon NK cell adoptive transfer observed in previous clinical studies. In addition, our first-in-human study provided only a brief 'window of opportunity' for HLA-haploidentical NK cells to expand and respond, before they were eliminated by patient T cells. By administering donor NK cells in the haplo-HCT setting, we expect to see longer term persistence and a prolonged opportunity for the adoptively transferred NK cells to mediate anti-leukemia responses. Mass cytometry (CyTOF2) allows for high throughput analysis of single cells for a large number of parameters, and has recently been used to deeply immunophenotype and track the inhibitory and activating receptor diversity of human NK cells in health or the setting of viral infection.[58,59] In unpublished data CIML and control NK cells are identifiable as discrete populations on CyTOF2 analysis and we will use this technology to distinguish the adoptively transferred memory-like cells from the naïve NK cells generated from the donor-derived stem cell graft. Unique donor or recipient HLA markers will also be used to distinguish donor form patient. In addition, mass cytometry will allow the assessment of expanding NK cells to respond to leukemia targets ex vivo. This should complement flow cytometry studies, and also allow us to further study their function, phenotype, and possible unique features after their adoptive transfer in these patients.

CIML NK cells produce IFN-γ and other pro-inflammatory cytokines, and thus monitoring of serum cytokine concentrations will be included to assess for any systemic cytokine release. ALT-803 levels will also be monitored.

In the context of allogeneic HCT, long term clinical outcomes have been associated with KIR haplotypes and KIR to KIR-ligand mistmatch.[27,31,60,61] The KIR genotyping of the donor NK cells and the HLA typing of the recipient will be used to analyze any association of KIR or KIR-ligand parameters and clinical outcomes. Moreover, the mass cytometry data will

allow us to track leukemia-reactive NK cell populations based on activating and inhibitory receptor expression, which is like a more accurate representation of NK cell specificity in this context.

Finally, should patients respond and relapse following haplo-HCT plus haplo-NK cell infusion, AML blasts may have escaped NK cell immune surveillance. Additional studies will be performed to identify such immune escape mechanisms, which include mass cytometry assessment of NK cell inhibitor receptors, their ligands, regulatory cell populations. In addition, samples will be collected for sequencing the AML to assess the impact of baseline mutations and clonal architecture on responses and relapse in this setting.

2.0 OBJECTIVES

2.1 Primary Objective

To determine the rate of leukemia free survival (LFS) at 1 year post-transplantation.

2.2 Secondary Objectives

1. To determine the rate of LFS at 3 months post-transplantation.
2. To determine the CR rate at day 28 post-transplantation.
3. To determine the rate of overall survival (OS) at 1-year post-transplantation.
4. To determine the incidence of relapse in patients who are found to be in CR on day 28 marrow post-transplant.

2.3 Exploratory Objectives

1. To determine the incidence of transplant related mortality (TRM) at day 100 post-transplantation.
2. To determine the incidence of transplant related mortality (TRM) at 6 months post-transplantation.
3. To determine the incidence of transplant related mortality (TRM) at 1 year post-transplantation.
4. To determine the time to neutrophil engraftment.
5. To determine the time to platelet engraftment.
6. To determine the engraftment rates at day 100 post-transplant by the donor / recipient STR (single tandem repeat) chimerism and or X/Y FISH in sex mismatched donor-recipient pairs.
7. To determine the incidence and severity of acute GVHD rates.
8. To determine the incidence and severity of chronic GVHD rates.

2.4 Correlative Objectives

1. To evaluate the number, phenotype, and function of memory-like NK cells following adoptive transfer. The maximal number of blood and bone marrow memory-like NK cells will be correlated with clinical endpoints. The maximal

number of functional (e.g., IFN-γ+) CIML NK cells after ex vivo leukemia re-stimulation will be correlated with clinical endpoints.
2. To assess serum cytokine levels, and ALT-803 levels, before and after CIML NK cell infusion.
3. To assess functional responses and gene expression of memory-like NK cells and graft-derived NK cells to leukemia targets.
4. To assess immune reconstitution following haplo-HCT with same donor NK cell infusion.
5. To assess AML blasts and the BM microenvironment pre-therapy and at first relapse to identify mechanisms of immunoevasion and assess association with AML mutations and clonal architecture
6. To determine the impact of KIR genotype and KIR ligand mismatches on the post-transplant outcomes.

3.0 PATIENT SELCTION

3.1 Recipient Eligibility Criteria

3.1.1 Inclusion Criteria

1. Refractory AML without complete remission (CR) after 2 or more cycles of induction therapy (primary induction failure), or AML relapsed after obtaining a CR and failed one or more cycles of re-induction therapy. Standard dose 10-day decitabine (20 mg/m^2 daily IV x 10 days) or 7-day azacitidine (75-100 mg/m^2 daily SC/IV x 7 days) will be considered as one cycle of induction therapy.
2. At least 18 years of age
3. Available HLA-haploidentical donor that meets the criteria in Section 3.2.
4. Patients with known CNS involvement with AML are eligible provided that they have been treated and CSF is clear for at least 2 weeks prior to enrollment into the study. CNS therapy (chemotherapy or radiation) should continue as medically indicated during the study treatment.
5. Karnofsky performance status > 60% (see Appendix A)
6. Adequate organ function as defined below:
   a. Total bilirubin < 2 mg/dl
   b. AST(SGOT)/ALT(SGPT) < 3.0 x IULN
   c. Creatinine within normal institutional limits OR creatinine clearance > 60 mL/min/1.73 m^2 by Cockcroft-Gault Formula (See Appendix B)
   d. Oxygen saturation ≥90% on room air and adjusted DLCO of at least 40%
   e. Ejection fraction ≥40%
7. Able to be off of corticosteroids (10 mg or less of prednisone or equivalent doses of other systemic steroids are allowed) and any other immune suppressive medications beginning on Day -3
8. Women of childbearing potential must have a negative pregnancy test within 28 days prior to study registration. Female and male patients (along with their female partners) must agree to use two forms of acceptable contraception, including one barrier method, during

participation in the study including throughout the initial evaluation period (100 days after CIML NK cell infusion).
9. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.1.2 Exclusion Criteria

1. Relapsed after allogeneic transplantation.
2. Circulating blast count >30,000/μL by morphology or flow cytometry (cyto-reductive therapies including leukapheresis or hydroxyurea are allowed).
3. Uncontrolled bacterial or viral infections, or known HIV, Hepatitis B or C infection.
4. Presence of donor specific antibodies (DSA) with Mean Fluorescence Intensity (MFI) of ≥5000 as assessed by the single antigen bead assay, < 6 weeks prior to starting transplant conditioning
5. Uncontrolled angina, severe uncontrolled ventricular arrhythmias, or EKG suggestive of acute ischemia or active conduction system abnormalities.
6. New or progressive pulmonary infiltrates. Progressive pulmonary infiltrate is defined as an increase of 20% or greater from prior radiologic exam. Radiologic assessment methods may be CT or PA/L x-ray imaging. Infiltrates attributed to infection must be stable or improving after 1 week of appropriate therapy, or 4 weeks for presumed or proven fungal infections to be eligible.
7. Known hypersensitivity to one or more of the study agents
8. Received any investigational drugs within the 14 days prior to the first day of transplant conditioning
9. Pregnant and/or breastfeeding

3.2 Donor Eligibility Criteria

3.2.1 Inclusion Criteria

1. Related donor (parents, sibling, offspring, or offspring of sibling)
2. At least 18 years of age
3. HLA-haploidentical donor/recipient match by molecular typing at the HLA-A, HLA-B and HLA-DRB1 loci.
4. In general good health, and medically able to tolerate leukapheresis required for harvesting the NK cells for this study.
5. Ability to understand and willingness to sign an IRB approved written informed consent document

3.2.2 Exclusion Criteria

1. Positive for hepatitis, HTLV, or HIV infection
2. Pregnant and/or breastfeeding

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of the signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of the appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 STUDY DESIGN

This is a standard phase 2 study powered to demonstrate improvement in the 1-year leukemia free survival (LFS) to 30% from <10% expected in this extremely high-risk patient cohort. We expect to enroll a total 30 patients on this study based on the above-mentioned power calculation.

A formal safety evaluation will be done after every 6th patient enrolled and the trial will be stopped if noted to have unusually higher engraftment failure (acute GVHD rates (>60% any grades or >30% grade III/IV or ≥ 50% severe cGVHD) or engraftment failure rates

(≥15%) or transplant-related mortality (>30%). Please see sections 6.17 and 14.3 on safety and early stopping rules for more detailed information.

6.0 TREATMENT PLAN

6.1 Overall Treatment Plan

The recipient will receive a standard of care conditioning regimen. Following the preparative regimen, graft cell infusion will occur on Day 0. The graft will have been collected in the standard fashion from a haploidentical donor mobilized using G-CSF. Patients will receive post-transplant cyclophosphamide on Days +3 and +4. GvHD prophylaxis with tacrolimus and mycophenolate mofetil (MMF) will start on Day +5. MMF will continue until Day +35 and tacrolimus until Day +180 in the absence of GvHD. G-CSF will start on Day +14 and will continue until neutrophil engraftment as per institutional guidelines. The same donor will undergo non-mobilized large volume (20L) leukapheresis on Day +6, and the NK cell product (CIML dose acceptable range is 0.5 to $10x10^6$/kg) will be infused into the recipient on Day +7. Subcutaneous ALT-803 at a dose of 10mcg/kg will begin approximately 4 hours after the NK cell infusion and will continue q 3 weeks for a total of 4 doses.

6.2 Conditioning Regimen Options

Recipients may receive one of the following standard of care conditioning regimens, as chosen by the treating physician. Dosing adjustments for recipient's weight and creatinine clearance will be per standard of care.

6.2.1 Fludarabine/cyclophosphamide/TBI

Fludarabine on Days -6 to -2, IV, 30 mg/m^2/day, total dose of 150 mg/m^2

Cyclophosphamide on Days -6 to -5, IV, 14.5 mg/kg/day (total dose of 29 mg/kg) given along with mesna.

Total body irradiation (TBI) on Day -1 at a dose of 200 cGy

6.2.2 Fludarabine/fractionated TBI

Fludarabine on Days -6 to -4, IV, 30 mg/m^2/day, total dose of 90 mg/m^2

TBI twice daily for 4 days to total dose of 1200 cGy

6.2.3 Busulfan/fludarabine

Busulfan on Days -6 to -3, IV, 3.2 mg/kg, dose adjusted per standard of care to achieve AUC 73.9-90.3 mg x h/L

Fludarabine on Days -6 to -2, IV, 25 mg/m^2/day, total dose of 125 mg/m^2

6.3 Donor Hematopoietic Cell Collection

G-CSF mobilized peripheral blood stem cells from the chosen related haploidentical donor will be used as a source of stem cells in all the patients. Donors will receive subcutaneous G-CSF from Day -4 until Day 0 and undergo 20L apheresis per institutional guidelines. Target CD34+ cell dose will be at least 4 x10^6/kg-bw with plans for freezing down any extra cells if collection dose exceeds 5 x 10^6/kg-bw.

Two consecutive days for collection are allowed in case of the target CD34+ cell dose being less than the target 4 x10^6/kg-bw from the first day of collection. In the latter event the products from the two days will be infused on the second day of collection per our standard institutional guidelines to provide a uniform post-HCT cyclophosphamide dose schedule. If a donor is unable to mobilize at least 3 x 10^6/kg-bw CD34+ cells over the course of two days of apheresis, the recipient will be removed from study and will not receive the CIML NK cell infusion.

6.4 Donor Cell Infusion

The related donor cells collected as described in Section 6.3 will be infused on Day 0 per institutional guidelines. In the event the stem cell infusion occurs over 2 days, both days will be considered Day 0 for required assessments and timing of subsequent visits.

6.5 Post-Transplant Cyclophosphamide

Post-transplant cyclophosphamide will be administered IV over 120 minutes (±15 minutes) at a dose of 50mg/kg on Day +3 and +4. Administration should follow institutional guidelines.

Cyclophosphamide will be dosed according to the recipient's ideal body weight (see Appendix C), unless actual body weight is less than ideal body weight, in which case actual body weight will be used.

No additional cyclophosphamide dose modifications are allowed.

Mesna will be administered at 20mg/kg IV over 15 minutes BID for 4 doses, initiated immediately prior to each dose of cyclophosphamide and repeated 4 hours after completion of each cyclophosphamide. Administration and dose adjustments per institutional guidelines.

6.6 Donor Leukapheresis for CIML NK Cell Generation

On Day +6 (one day before the planned CIML NK cell infusion), peripheral blood mononuclear cells will be collected by a single standard 20-L apheresis over 4-5 hours from the same haploidentical related donor that provided the HCT graft.

6.7 Cytokine-induced memory-like (CIML) NK Cell Product Infusion

The CIML NK cells will be infused on Day +7 without a filter or pump, by gravity . Flush tubing on completion of the CIML NK cells with normal saline to ensure all of the cells are infused.

Patients should be pre-medicated with acetaminophen 650 mg PO and/or diphenhydramine 25 mg PO/IV within 1 hour before and 4 hours after the NK cell infusion. Demerol 25-50 mg IV may be given for chills/rigors during the NK cell infusion. Intravenous hydration may be administered per institutional guidelines.

Vital signs should be obtained before the CIML NK cell infusion, every 15 minutes during the infusion, and then every 30 minutes x 2. Additional vitals may be done consistent with the institutional guideline or as needed for safety.

Patients will be monitored for signs of acute reactions to the allogeneic NK cell infusion, including acute hemolytic reactions (back pain, facial flushing, fever/chills, chest pain, dyspnea, headache, shock), febrile non-hemolytic reactions (fever/chills), allergic reactions (urticaria, wheezing, facial edema), anaphylaxis (signs of autonomic dysregulation, severe dyspnea, pulmonary/laryngeal edema, bronchospasm / laryngospasm, hypotension), and transfusion-related acute lung injury (TRALI). Treatment of these reactions will follow standard institutional practice, and therapy decisions will be made by the treating physician. If severe reactions or anaphylaxis, the CIML NK cell infusion will be discontinued, and medical therapy will include intravenous antihistamines, epinephrine, and corticosteroids, and the use of cardiac-respiratory support measures as needed.

CIML NK cells are expected to produce IFN-γ for 16-24 hours after NK cell infusion. Expected constitutional toxicities from IFN-γ include myalgias, arthralgias, fever, and rigors.

If the CIML NK cell dose is $< 0.5 \times 10^6$ cells/kg then all cells (after quality control testing) should be given and the therapy plan will remain the same. Patients receiving $< 0.5 \times 10^6$ cells/kg will be replaced with an additional patient for the efficacy assessment though will continue to follow them for the safety analysis.

6.8 Administration of ALT-803

ALT-803 will start approximately 4 hours after the CIML NK cell infusion. ALT-803 will be administered at a dose of 10 mcg/kg subcutaneously beginning Day +7 (on the day of CIML NK cell infusion), and then every 21 days (± 14 days) for a total of 4 doses.

Patients should be pre-medicated with acetaminophen 650 mg PO and/or diphenhydramine 25 mg PO/IV within 1 hour before and 4 hours after ALT-803 administration.

Patients should be monitored for ALT-803 related targeted toxicities. Fevers, rash, and myalgias are expected, and should be treated supportively. ALT-803 has been associated with rash around the area of injection which may occur up to 7-10 days after the initial injection. The Wallace ‘rule of nines’ (used to assess burns) will be used to assess the extent of the rash. For localized rashes (involving <25% of the

body surface) the patients will be treated with topical steroids creams (e.g., triamcinolone cream) along with oral Benadryl. For these localized rashes subsequent doses of ALT-803 will not be held. However, if the rash involves ≥30% of the body surface area then in addition to the topical steroid cream and other supportive cares subsequent doses of ALT-803 will be delayed until the rash improves by at least 50%. ALT-803 may be delayed for up to 96 hours due to injection reaction rash.

If the patient experiences a severe acute reaction during the CIML NK cell infusion, ALT-803 should be held until the toxicity resolves to grade 2 or better. ALT-803 may be started if the toxicity resolves to grade to or better within 96 hours of CIML NK cell infusion. Missed doses of ALT-803 will not be made up.

If ALT-803 cannot be started within 96 hours after the CIML NK cell infusion, no ALT-803 will be given and the patient will be replaced for response assessment but will remain on the study to be accounted for in describing the toxicity of the experimental treatment.

6.9 GvHD Prophylaxis

Tacrolimus and mycophenolate mofetil (MMF) will be given in the immediate post-transplant period for GvHD prophylaxis.

Tacrolimus will be given per institutional practices, orally at a dose of 0.03 mg/kg or intravenously at a dose of 0.03 mg/kg or an initial flat dose of 1mg/day starting Day +5. Serum levels of tacrolimus should be drawn 24 hours after the initiation of tacrolimus infusion. Subsequent measurement of serum levels and dose adjustment of tacrolimus to maintain a suggested level of 5-15 ng/mL, should be per the institutional guidelines. Mycophenolate mofetil will be given at a dose of 15 mg/kg TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1g TID, IV or PO). MMF prophylaxis will start Day +5 and discontinue after the last dose on Day +35. Tacrolimus may continue until Day +180 and MMF through Day+35 in the absence of GvHD. Dose adjustments, including dose tapering, may be made according to the judgement of the treating physician.

6.10 G-CSF

Filgrastim will be administered starting on Day +14 to continue through neutrophil engraftment as defined in Section 12.2. Dosing will be determined by institutional guidelines.

6.11 Prohibited Medications

The only prohibited medications are systemic corticosteroids or other immune suppressive medications. These must be avoided beginning on Day -3 and continuing until 30 days after the infusion of the CIML NK cells. However, corticosteroids are allowed if deemed medically necessary and 25 mg of IV hydrocortisone may be administered in a 24-hour time period if medically indicated for a drug or transfusion reaction. Patients are allowed to be treated with high dose

steroids if deemed necessary by the treating physician in life threatening medical complications and/or GVHD.

All medications other than those listed above may be used to treat or prevent toxicity as per institutional guidelines.

Patients will be monitored for signs and symptoms of cytokine release syndrome (CRS). CRS will be assessed and managed per the algorithm published by Lee et al .[62]

6.12 Supportive Care

Patients should receive full supportive care, including but not limited to intravenous fluids, transfusions of blood and blood products, antibiotics, antivirals, antibacterial agents, and antiemetics, when appropriate at the discretion of the treating clinician.

6.13 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, or women who have had a tubal ligation) are required to have a negative urine pregnancy test within 28 days prior to the first dose of the preparative regimen.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and throughout the evaluation period (100 days after the CIML NK cell infusion).

If a patient is suspected to be pregnant, the treatment should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 30 days after the first dose of the preparative regimen, the investigator must be notified in order to facilitate outcome follow-up.

6.14 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

Patients will be removed from the study any of the following reasons:

- Donor unable to mobilize at least 3.0 x 10^6 cells/kg-bw over the course of 1-2 days
- Death

- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

6.15 Retreatment with ALT-803

Patients may be treated with additional doses of ALT-803 if they have persistent AML (including minimal residual disease (MRD) positive) after day 90, or if they have relapsed AML after achieving complete remission. At retreatment, ALT-803 will be given 10 mcg/kg subcutaneously q21 days (+/- 14 days) for up to 4 doses. Administration and monitoring will be per Section 6.8.

6.16 Duration of Follow-up

Following completion of protocol treatment patients will be followed for up to 48 months to assess for OS and LFS. Patients that begin another anti-leukemic therapy (other than donor lymphocyte infusion) will be considered off study and will no longer be followed for AEs. They will be assessed for GvHD for 1 year.

6.17 End of Study Definition

The end of study is defined as any one of the following (whichever occurs first):

- The date of the four-year follow-up
- The date of death
- Lost to follow-up
- Patient withdraws consent

6.18 Study Related Adverse Event Monitoring and Stopping Rules

6.18.1 Stopping Rules

To ensure there is enough follow up time for the engraftment assessment, 35-day interval will be maintained between first 3 treated patients. All patients who receive CIML NK cells will be evaluable for toxicity.

Haploidentical NK cells have been administered to a large number of patients without these events occurring; however, there exists theoretical risks for CIML NK cells that will be monitored. The following additional stopping events are defined to assess for these later events (potentially related to CIML cells), which will be assessed while the patient remains on study.

1. Engraftment failure (ANC < 500/μL by Day +35 in the absence of disease recurrence)

2. Any Grade and Grade III-IV acute GVHD by 6 months
3. Extensive chronic GVHD by 1 year
4. Excessive mortality at 8 months

If any of the late stopping events listed above occurs (while the patients are on study and have not received additional AML therapy/ transplant) at an estimated frequency of ≥15% for engraftment failure >60% any grade acute GvHD ≥ 30% grade III-IV acute GvHD (as assessed by NIH consensus criteria), ≥ 50% severe chronic GVHD, or transplant-related mortality >30% accrual will be suspended and reviewed for safety of continuation. See exact guidelines below, in Section 14.3, statistical design of stopping rules.

6.18.2 Toxicity and Response Evaluations

All patients who receive CIML NK cells are evaluable for toxicity. Patients are monitored from CIML NK cell infusion until the last scheduled subject contact at 48 months after the last injection of ALT-803, and until all toxicities are resolved or stabilized. All patients who receive a CIML NK cell infusion and at least one dose of ALT-803, and who undergo a Day 28 bone marrow biopsy for response assessment, are evaluable for disease response.

6.18.3 Treatment Failure/Progressive Disease

CIML NK cells may require weeks to fully clear AML blasts, unlike traditional cytotoxic chemotherapy. Patients who fail to clear AML blasts at the Day 28 (+/- 3 days) bone marrow assessment or with persistent circulating blasts at Day 28 may be considered a treatment failure/progressive disease and may be started on other anti-leukemia therapies at the discretion of the treating physician. Patients may continue on protocol at the discretion of the treating physician, which is recommended if marked reduction of AML is observed.

Donor lymphocyte infusion(s) (DLI) may be given to patients who have persistent AML (including minimal residual disease (MRD) positive) after day 60 or relapsed AML at any time after achieving complete remission. DLIs should be administered as per standard of care doses and procedures. A CD34 boost may be administered at any time as indicated for treatment of poor engraftment. CD34 boost should be administered as per standard of care doses and procedures.

Patients who receive anti-leukemia therapy other than DLIs or ALT-803 after Day 28 (+/- 3 days) will be considered off study and will only be assessed for GvHD for 1 year. However, clinical study correlative science samples may be collected for patients per protocol, after alternative AML therapy has been administered.

7.0 PHARMACEUTICAL INFORMATION

7.1 Fludarabine (Fludara)

7.1.1 Description

The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono-β-D-arabino-furanosyl) (2-fluoro-ara-AMP). The molecular formula of fludarabine phosphate is $C_{10}H_{13}FN_5O_7P$. Its molecular weight is 365.2

7.1.2 Dose Forms and Strengths

Fludarabine is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2.

Fludarabine is supplied in a clear glass single dose vial (6mL capacity) and packaged in a single dose vial carton in a shelf pack of five.

7.1.3 Storage and Stability

Store under refrigeration, between 2º-8ºC (36º-46ºF).

7.1.4 Availability

Commercially available by prescription

7.1.5 Preparation

Preparation should follow institutional guidelines.

7.1.6 Administration

Please see Sections 6.2.

7.1.7 Warnings and Precautions

Life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia/thrombocytopenic purpura (ITP), Evan's syndrome, and acquired hemophilia have been reported to occur in patients receiving fludarabine.

Serious and sometimes fatal infections, including opportunistic infections and reactivations of latent viral infections such as VZV (Herpes zoster), Epstein-Barr virus and JC virus (progressive multifocal leukoencephalopathy) have been reported in patients treated with fludarabine.

Objective weakness, agitation, confusion, seizures, visual disturbances, optic neuritis, optic neuropathy, blindness and coma have occurred in CLL patients treated with fludarabine at the recommended dose.

High doses of fludarabine have been associated with an irreversible central nervous system toxicity characterized by delayed blindness, coma and death. High doses are also associated with severe thrombocytopenia and neutropenia due to bone marrow suppression. There is no known specific antidote for fludarabine overdosage.

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

7.2 Cyclophosphamide

7.2.1 Description

Cyclophosphamide is a white crystalline powder with the molecular formula C7H15Cl2N2P·H2O and a molecular weight of 279.1. The chemical name for cyclophosphamide is 2-[bis (2-chloroethyl) amino] tetrahydro-2H-1, 3, 2-oxazaphosphorine 2-oxide monohydrate.

7.2.2 Mechanism of Action

Cyclophosphamide is classed as an alkylating agent of the nitrogen mustard type. An activated form of cyclophosphamide, phosphoramide mustard, alkylates or binds with many intracellular molecular structures, including nucleic acids. Its cytotoxic action is primarily due to cross-linking of strands of DNA and RNA, as well as to inhibition of protein synthesis.

7.2.3 Dose Forms and Strengths

Cyclophosphamide is commercially available for parenteral injection as 100 mg, 200 mg, 500 mg, 1 g, and 2 g vials

7.2.4 Storage and Stability

Unopened vials of cyclophosphamide are stable until the date indicated on the package when stored at or below 25°C (77°F).

7.2.5 Availability

Commercially available by prescription

7.2.6 Preparation

Preparation should follow institutional guidelines.

7.2.7 Administration

Please see Section 6.2.

7.2.8 Warnings and Precaution

To reduce the risk of hemorrhagic cystitis, Mesna and adequate hydration should be administered per institutional guidelines

7.3 Busulfan

7.3.1 Description

Busulfan is a bifunctional alkylating agent known chemically as 1,4-butanediol, dimethanesulfonate. The molecular formula of busulfan is CH3SO2O(CH2)4OSO2CH3 with a molecular weight of 246 g/mole.

7.3.2 Mechanism of Action

Busulfan is a bifunctional alkylating agent in which two labile methanesulfonate groups are attached to opposite ends of a four-carbon alkyl chain. In aqueous media, busulfan hydrolyzes to release the methanesulfonate groups. This produces reactive carbonium ions that can alkylate DNA. DNA damage is thought to be responsible for much of the cytotoxicity of busulfan.

7.3.3 Dose Forms and Strengths

Busulfan Injection (busulfan) is supplied as a clear, colorless, sterile, solution in 10 mL single-dose vials for intravenous administration upon dilution. Each vial contains 60 mg of busulfan in N,N-dimethylacetamide (DMA), 3.3 mL and Polyethylene Glycol 400, NF 6.7 mL. The solubility of busulfan in water is 0.1 g per L and the pH of Busulfan Injection diluted to approximately 0.5 mg per mL busulfan in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP as recommended for infusion reflects the pH of the diluent used and ranges from 3.4 to 3.9.

7.3.4 Storage and Stability

Busulfan Injection diluted in 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP is stable at room temperature (25°C) for up to 8 hours but the infusion must be completed within that time.Busulfan Injection diluted in 0.9% Sodium Chloride Injection, USP is stable at refrigerated conditions (2°C to 8°C) for up to 12 hours but the infusion must be completed within that time.Busulfan Injection is a cytotoxic drug. Follow applicable special handling and disposal procedures. Store refrigerated between 2° and 8°C (36° and 46°F). Discard unused portion.

7.3.5 Availability

Commercially available by prescription

7.3.6 Preparation

Preparation should follow institutional guidelines.

7.3.7 Administration

Please see Sections 6.2.

7.3.8 Warnings and Precautions

The most frequent serious consequence of treatment with Busulfan Injection at the recommended dose and schedule is prolonged myelosuppression, occurring in all patients (100%). Severe granulocytopenia, thrombocytopenia, anemia, or any combination thereof may develop. Hematopoietic progenitor cell transplantation is required to prevent potentially fatal complications of the prolonged myelosuppression. Monitor complete blood counts, including white blood cell differentials, and quantitative platelet counts daily during treatment and until engraftment is demonstrated. Absolute neutrophil counts dropped below 0.5 x 10^9/L at a median of 4 days post-transplant in 100% of patients treated in the Busulfan Injection clinical trial. The absolute neutrophil count recovered at a median of 13 days following allogeneic transplantation when prophylactic filgrastim was used in the majority of patients. Thrombocytopenia (less than 25,000/mm^3 or requiring platelet transfusion) occurred at a median of 5 to 6 days in 98% of patients. Anemia (hemoglobin less than 8.0 g/dL) occurred in 69% of patients. Use antibiotic therapy and platelet and red blood cell support when medically indicated.

Seizures: Initiate anticonvulsant prophylactic therapy prior totreatment with Busulfan Injection. Monitor patients with history of seizure disorder, head trauma or receiving epileptogenicdrugs.

Hepatic Veno-Occlusive Disease (HVOD): Increased risk ofdeveloping HVOD at AUC greater than 1,500 µM•min. Monitor serum transaminases, alkaline phosphatase and bilirubin daily.

Embryo-fetal Toxicity: Can cause fetal harm. Advise of potential risk to a fetus and use of effective contraception.

Cardiac tamponade has been reported in pediatric patients with thalassemia who received high doses of oral busulfan andcyclophosphamide. Abdominal pain and vomiting preceded the tamponade in most patients.

7.4 Total Body Irradiation

7.4.1 Mechanism of Action

Total body irradiation (TBI) works via direct toxicity from radiation induced damage to target cells and systems.

7.4.2 Dose Forms and Strengths

TBI is administered under the guidance of radiation oncologists. The maximally tolerated dose of TBI is approximately 15 Gy. TBI-based regimens typically fractionate the radiation and administer the total dose over several days, typically four, which helps decrease toxicity and increase tolerability.

7.4.3 Warnings and Precautions

Neutropenia may occur that requires dose adjustments

Risk of developing infections, including opportunistic infections is increased. These infections include, but are not limited to: BK virus (associated with polyoma virus-associated nephropathy [PVAN]) and JC virus (associated with progressive multifocal leukoencephalopathy [PML]); may result in serious adverse effects. Immunosuppression increases the risk for CMV viremia and/or CMV disease.

Use can be associated with an increased risk of the development of lymphoma or other malignancies.

7.4.4 Administration

Please see Sections 6.2.

7.5 CIML NK Cells

7.5.1 Preparation of CIML NK Cells and Product Release Criteria

Cell product processing will be performed at the Siteman Cancer Center Biological Therapy Core and supervised by Dr. Fehniger. The apheresis product will undergo NK cell purification with sequential depletion of CD3+ T cells and positive selection of CD56+ (CD3-/CD56+) NK cells.

The purified NK cells will be cultured for 12-18 hours in X-VIVO 15 media containing rhIL-12 (10ng/ml), rhIL-15 (50 ng/ml) and rhIL-18 (50 ng/ml). After incubation, the cells will be washed at least 2 times in a cytokine-free media solution to remove the cytokines. After the last wash 5 mL of the cell solution will be used to determine the final NK cell numbers prior to infusion. All CIML NK cells available from the donor leukapheresis, NK cell purification, and CIML processing will be infused, up to a maximum of $10.0x10^6$/kg. The median number of CIML NK cells collected from each donor is expected to be 5.0×10^6cells/kg to 10.0×10^6cells/kg based on data from the first-in-human clinical trial. If the CIML NK cell dose is $< 0.5 \times 10^6$ cells/kg then all cells (after quality control testing) should be given and the therapy plan will remain the same. These patients will be replaced with an additional patient for the efficacy assessment though will continue to follow them for the safety analysis. If more than 10×10^6/kg of CIML NK cells are available and additional cells will be stored for research and correlative studies.

As part of the lot release criteria, the T cell (CD3+) cell dose in the infusion product will be less than 3 x 10^5 CD3+/kg of the recipient.

CIML NK cell Product Lot Release		
Assay	**Test method**	**Value**
Viability	7-AAD, flow cytometry	≥70%
NK cell (CD56+CD3-)	flow cytometry	≥70%
T cell (CD3+)	flow cytometry	<3.0 x10^5 CD3+ cells/kg
Endotoxin	LAL method	< 5.0 EU/kg.
Gram stain	Clinical micro	No organisms

Any questions related to the NK cell product generation or infusion, including lot release criteria and approval, may be addressed by the laboratory or clinical PIs of the protocol.

7.6 ALT-803

ALT-803, a recombinant human superagonist IL-15 complex, is the working name of the drug under investigation. Its active ingredient is ALT-803 and its pharmacologic class is as a targeted anticancer immunotherapeutic.

ALT-803 has been referred to as IL-15N72D:IL-15RαSu/IgG1 Fc complex in various preclinical study reports, publications, and other related documents. No other names exist for this product, as it is a novel investigational biologic.

7.6.1 Formulation and Composition

The biological drug product, ALT-803, is formulated in a phosphate buffered saline solution. The drug substance is produced by a recombinant mammalian cell line and is manufactured without the use of animal derived components. The vialed quantitative composition of ALT- 803 is listed in the table below.

7.6.2 Quantitative Composition of ALT-803

PBS Formulation: Sodium Chloride (USP) 8.18 g/L; Sodium Phosphate Dibasic (USP) 2.68 g/L; Potassium Phosphate Monobasic (NF) 1.36 g/L pH 7.4.

7.6.3 Structural Formula

ALT-803 is a soluble complex consisting of 2 protein subunits of a human IL-15 variant associated with high affinity to a dimeric IL-15R sushi domain/human IgG1 Fc fusion protein. The IL-15 variant is a 114 aa polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D).6. The human IL-15R sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the IL-15R subunit (aa 1-65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). Aside from the N72D substitution, all of the

protein sequences are human. Based on the amino acid sequence of the subunits, calculated molecular weight of the complex comprising 2 IL-15N72D polypeptides and a disulfide linked homodimeric IL-15RαSu/IgG1 Fc protein is 92.4 kDa. Each IL-15N72D polypeptide has a calculated molecular weight of approximately 12.8 kDa and the IL-15RαSu/IgG1 Fc fusion protein has a calculated molecular weight of approximately 33.4 kDA.

Component	Concentration	Amount/Vial
ALT-803	1 mg/mL	1.2 mg
Phosphate Buffered Saline (PBS)	QS	1.2 mL

Both the IL-15N72D and IL-15RαSu/IgG1 Fc proteins are glycosylated resulting in an apparent molecular weight of ALT-803 as approximately 114 kDa by size exclusion chromatography. The isoelectric point (pI) determined for ALT-803 range from approximately 5.6 to 6.5. Thus, the fusion protein is negatively charged at pH 7. The calculated molar extinction coefficient at A280 for ALT-803 is 116,540 M-1, or 1.26 OD280 for a 1 mg/mL solution of ALT-801, or one OD280 is equivalent to 0.79 mg/mL solution of ALT-803.

7.6.4 Storage and Handling

Study medication is provided in a 2 mL vial containing 1.2 mL of ALT-803 at a concentration of 1 mg/mL. Vials are packaged in cartons and shipped to the clinical site. Study medication must be maintained at a temperature between 2°C and 8°C.

7.6.5 Stability

Stability studies are ongoing and will be continued throughout the clinical study. Based on previous lots, the study drug is expected to be stable for at least 2 years. The site will be periodically updated on the stability of the drug and will be immediately informed if there is evidence that the drug no longer meets its stability specifications.

7.6.6 Agent Ordering and Agent Accountability

ALT-803 is produced in the USA by ImmunityBio, Culver City, CA. After manufacturing, the product is stored at ImmunityBio for clinical supply, packaging, and labeling. The label indicates the product name, strength, manufacturing date, and the study requirement information. ALT-803 will be shipped from ImmunityBio

7.6.7 Preparation and Administration

ALT-803 dose calculation will be based on actual body weight. The calculated amount of ALT-803 will be drawn into a syringe for subcutaneous injection. The stock concentration is 1 mg/ml. Doses will be drawn directly into the syringe for injection. If the total subcutaneous dose

is greater than 1.5 mL, the dose will be divided into 2-3 subcutaneous injections as needed.

8.0 CORRELATIVE STUDIES

8.1 Sample Collection

Sixty mL of peripheral blood in sodium heparin (green top) tubes will be collected at the following time points:

- Screening
- Day +3 prior to the cyclophosphamide
- Day +7 prior to the CIML NK cell infusion
- Day +10
- Day +14
- Day +21
- Day +28
- Day +35
- Day +42
- Day +60
- Day +70
- Day +84
- Day +100
- 6 months after transplant
- 9 months after transplant
- 12 months after transplant
- 18 months after transplant
- 2 years after transplant

Ten mL of peripheral blood in serum (red top) tube(s) will be collected at the following time points:

- Screening
- Day +3 prior to the cyclophosphamide
- Day +7 prior to the CIML NK cell infusion
- Day +7 1 hour (+/- 30 minutes) after CIML NK cell infusion
- Day +7 4-6 hours after CIML NK cell infusion
- Day +8 (24 hours +/- 4 hours after CIML NK cell infusion)
- Day +9
- Day +10
- Day +14
- Day +21
- Day +28
- Day +35
- Day +42
- Day +60
- Day +70
- Day +84
- Day +100

- 6 months after transplant
- 9 months after transplant
- 12 months after transplant
- 18 months after transplant
- 2 years after transplant

Additional samples may also be collected at key clinical events, including suspected relapse, infection, suspected onset of GVHD. Peripheral blood (sodium heparin and red top) sample collection days will allow a +/- 3 day window following Day+10 samples. The intensified serum sample collection around Day+7 (CIML NK cell infusion) will be utilized to monitor for CRS-associated cytokines. Additional serum samples may be drawn at the time of clinically identified CRS, in addition to the time points listed above. For the BM aspirate at Day+14, a similar window of +/- 3 days will be allowed to facilitate timely laboratory processing. Any change from the original sample collection day should be reviewed with the laboratory PI or PI's staff (see contact information in Section 8.2) to facilitate timely sample processing.

If a patient is retreated with ALT-803 per Section 6.15, sixty mL of peripheral blood in sodium heparin (green top) tubes will be collected pre-dose on Day 1, on Day 8, and on each day of ALT-803 administration thereafter.

2-5 mL of bone marrow aspirate in green top (sodium heparin) tube(s) will be collected at the following time points:

- Screening
- Day +14
- Day +28
- Day +100
- At any clinically indicated BM biopsy/aspirate collection

Additional samples for correlative studies (donor and apheresis product):

- 20 mL of peripheral blood from donor in sodium heparin (green top) at screening
- 20 x 10^6 MNCs from the first (stem cell graft) leukapheresis product
- 20 x 10^6 MNCs from leukapheresis product prior to NK cell purification
- 20 x 10^6 NK cells after purification, prior to cytokine activation
- 20 x 10^6 cytokine pre-activated NK cells prior to washing
- Any additional cytokine pre-activated NK cells not administered

8.2 Sample Handling

All samples are preferably drawn between 7-9AM, for early AM delivery to Fehniger lab.

Specimens will be transported at room temperature immediately to the Fehniger Lab, Monday-Friday, for receipt between 8AM-4PM. Please notify the lab and/or Dr. Fehniger prior to submission. Sample collections that are outside those hours should be directly discussed with Dr. Fehniger at least 72 hours in advance. The CIML NK cell processing and final product samples will be received from the NK cell product generation team.

Attention: Fehniger Lab / Tim Schappe / Michelle Becker-Hapak
WU-Oncology 6th floor Southwest Tower Building, Room 634
4940 Parkview Place
St. Louis, MO 63110
Lab: (314) 362-1547 / (314) 747-1385 / Pager: (314) 510-2397
Fax: (314) 362-9333
tfehnige@wustl.edu / tschappe@wustl.edu mbecker-hapak@wustl.edu

8.3 Sample Processing

Some samples will be immediately utilized in correlative assays. In order to allow for batch assessments, samples will be isolated, cryopreserved and stored in the Fehniger Lab (6th floor southwest tower building) in liquid nitrogen or -70 degree C freezers. No PHI is included on the sample vials. These samples may be stored for future research as new scientific findings arise in the field.

8.4 Planned Correlative Studies

- Anti-HLA antibody identification (differentiate patient and donor NK cells)
- Assessment of donor cell chimerism (molecular testing and cell based assays)
- Immune cell frequencies and numbers (flow or mass cytometry)
- NK cell frequency, number, phenotype, proliferation (flow or mass cytometry)
- NK cell function in response to cytokine stimulation, NK cell receptor ligation, or leukemia cell triggering (including autologous leukemia blasts).
- NK cell gene expression / transcriptome / epigenome profiling
- KIR genotype of donor and recipient
- AML immunoevasion assessments (flow or mass cytometry, genome sequencing of baseline and relapse samples, including non-malignant tissue)
- Serum cytokine and ALT-803 measurements. For CRS monitoring, we will follow implicated or potentially related cytokines (e.g. IFNγ, TNFα, IL-6, IL-10, IL-13, CRP, IL-2Rα, IL-1Rα, IL-8, IL-12, IL-15) at the following time points relative to the CIML NK cell dose: pre-infusion; 1, 4-6, and 24 hours; at the time of CRS if this occurs, and Days 2, 4, 7, 14, 21, and 28 following the CIML NK cell infusion, as listed in Section 8.1.

9.0 STUDY CALENDAR

Scheduled evaluations up to Day +35 may be performed +/-3 days from the targeted date; evaluations following Day +35 may be performed +/-7 days of the targeted date; evaluations following Day +100 may be performed +/-30 days of the targeted date.

	Screening (within 28 days of registration)	Day -6	Day 0	Day +14	Day +21	Day +28	Day +35	Day +42	Day +60	Day +70	Day +84	Day +100	6, 9 and 12 mos[2]	18, 24, 36, and 48 mos
Consent	X													
Medical history	X	X	X	X	X	X	X	X	X			X	X	X
Physical exam w/ VS	X[4]	Daily[1]			X	X	X	X	X			X	X	X[7]
Weight	X	At least 3 times a week[1]												
Height	X													
Karnofsky PS	X											X	X	
HLA typing (donor and recipient)	X													
Viral panel (donor and recipient)[3]	X													
CMV (donor and recipient)	X													
CBC, diff, plt	X	Daily[1]			X	X	X	X	X			X	X	X[15]
BUN, creat, glucose, Na, K, Cl	X	Daily[1]			X	X	X	X	X			X	X	
AST, ALT, bili, alk phos	X	Weekly[1] and on Days +1,+2			X	X	X	X	X			X	X	
Donor Specific Antibodies (recipient)	X													
Chimerism testing[12]	X					X						X	X[13]	
Correlative studies[5]	X	See Section 8.0			X	X	X	X	X	X	X	X	X	X[14]
Pregnancy test[6]	X													
BM aspirate and core	X			X		X[11]						X[11]	X[13]	
Chest x-ray or chest CT[8]	X													
PFT[8]	X													

EKG[8]	X													
Echo or MUGA[8]	X													
Acute GvHD assessment[9]				X	X	X	X	X	X			X	X	
Chronic GvHD assessment[10]												X	X	

1 – Until neutrophil recovery or as clinically appropriate; CMP instead of BMP on Wednesdays and Sundays, and on Days +1 and +2
2 – After 12 months formal follow-up ends, however, patients will be followed annually thereafter for survival
3 – Donor- HTLV, HIV and hepatitis panel; Recipient- HIV and hepatitis panel
4 – 0_2 Saturation on room air required at screening
5 – See section 8.0 for required samples
6 – For women of childbearing potential only
7 – A telephone call to assess OS and LFS can be made in lieu of a physical exam
8 – May have occurred within 90 days of registration
9 – See Appendix D
10 – See Appendix E
11 – MRD testing required if in morphologic CR
12 – X/Y FISH for sex-mismatch donor-recipients but otherwise must be STR
13 – 6 and 12 months only
14 – 18 and 24 months only
15 – CBC only at 18 and 24 months

10.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix F for definitions and Appendix G for a grid of reporting timelines.

All adverse events will be collected from CIML NK cell infusion until 100 days after CIML NK cell infusion and 30 days after last injection of ALT-803. Adverse events of interest will be collected until the last scheduled subject contact at 48 months after the last injection of ALT-803. Adverse events of interest are graft failure, any grade acute graft versus host disease, and extensive chronic graft versus host disease. If a patient is retreated with ALT-803 (per Section 6.17.4), adverse events possibly, probably, or definitely related to ALT-803 will be collected until 30 days after the last dose of ALT-803. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

- Baseline adverse events, which shall be recorded on the medical history CRF

Refer to the data submission schedule in Section 11 for instructions on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in Section 10.1

10.1 Sponsor-Investigator Reporting Requirements

10.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

10.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Investigator (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

10.1.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR**

HRPO/QASMC. It is the responsibility of the Washington University Sponsor-Investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix F for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix F) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix F) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:
    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
    - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
    - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within **15 calendar days** after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report

must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

10.2 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 10.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

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11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
Demographics On-Study Form Treatment History Form	Baseline
Haplo-HSCT Form	Day 0
CIML NK Infusion Form	Day 7
ALT-803 Dosing Form	Day 7 Day 28 Day 49 Day 70
Chimerism Form	Day 28 Day 100 6 Months 12 Months
Response Form	Baseline Day 28 Day 100 6 Months 9 Months 12 Months 24 Months 36 Months

	48 Months Premature Discontinuation
Acute GvHD Form	Day 14 Day 21 Day 28 Day 42 Day 60 Day 100 6 Months 9 Months 12 Months Any time aGvHD is suspected
Chronic GvHD Form	Day 100 6 Months 9 Months 12 Months Any time cGvHD is suspected
Correlative Studies Form	Baseline Day 3 Day 7 Day 10 Day 14 Day 21 Day 28 Day 35 Day 42 Day 60 Day 70 Day 84 Day 100 6 Months 9 Months 12 Months 18 Months 24 Months
Correlative Studies Form – Donor	Day 7
Survival	6 Months 9 Months 12 Months 24 Months 36 Months 48 Months Premature Discontinuation
Adverse Events Form	As outlined in section 10.5

12.0 EFFICACY ASSESSMENT

12.1 Response

Response will be assessed according to the criteria from the IWG as stated below.

12.1.1 Complete Remission (CR)

Morphologically leukemia free state (i.e. bone marrow with <5% blasts by morphologic criteria and no blasts with Auer rods, no evidence of

extramedullary leukemia) and absolute neutrophil count ≥1000 /µL and platelets ≥100,000 /µL. Patient must be independent of transfusions

12.1.2 Complete Remission with Incomplete Blood Count Recovery (CRi)

All of the above criteria for CR must be met, except that absolute neutrophils <1000 /µL or platelets <100,000 /µL in the blood.

12.1.3 Partial remission (PR)

All of the criteria for CR must be met, except that leukemic blasts in the bone marrow may range from 5 to 25% as long as the count has decreased by at least 50% from pre-study treatment, or <5% blasts in the presence of Auer rods or abnormal morphology.

12.1.4 Treatment Failure (TF)

Patient survives > 28 days from the CIML NK cell infusion with persistent leukemia in the last peripheral blood smear or bone marrow (>25% blasts), or with persistent extramedullary disease, but without further clinical deterioration due to leukemia or increase of blast population in the bone marrow or peripheral blood.

12.1.5 Progressive Disease (PD)

Patient survives 28 days from the CIML NK infusion with increase of blast population in the bone marrow or peripheral blood by >10% or aggravation or new development of extramedullary disease or further deterioration or death due to leukemia.

12.1.6 Morphologic Leukemia Free State

Bone marrow with <5% blasts by morphologic criteria, no blasts with Auer rods, and no evidence of extramedullary leukemia.

12.2 Definitions for Safety and Efficacy Assessments

12.2.1 Neutrophil Engraftment

Time to neutrophil engraftment is measured by determining the first of 3 consecutive measurements of neutrophil count ≥ 500/µl following conditioning regimen-induced nadir.

12.2.2 Platelet Engraftment

Time to platelet engraftment is measured by determining the first of 3 consecutive measurements of platelet count ≥ 20,000/µl without platelet transfusion support for 7 days.

12.2.3 Primary Graft Failure

Failure of neutrophil engraftment by Day +35.

12.2.4 Secondary Graft Failure

Primary engraftment followed by a drop in the neutrophil count to less than 500/µl for more than 3 consecutive days without any apparent cause such as drugs or opportunistic infection.

12.2.5 Full Donor Chimerism

Greater than or equal to 95% donor cells within the bone marrow.

12.2.6 Acute GVHD

Acute GVHD rate and severity for the first 100 days after PBSC infusion will be assessed based on the Minnesota-Center for International Blood and Marrow Transplant Research (CIBMTR) GVHD grading system (Appendix D). All grades of severity of acute GVHD will be collected.

12.2.7 Steroid Refractory Acute GVHD

Steroid refractory acute GvHD will be defined as no improvement in GVHD within 7 days of starting high-dose systemic steroids (≥ 2 mg/kg of intravenous methylprednisolone or equivalent doses of other steroids) or progression of GvHD while on 2 mg/kg of intravenous methylprednisolone (or equivalent dose of other steroids).

12.2.8 Chronic GVHD

Incidence and severity of chronic GVHD will be assessed based on the NIH consensus criteria and global severity scoring system. Attempts should be made to confirm the diagnosis pathologically by biopsy of target organ(s).

12.2.9 Recurrence/Relapse

This is defined as reappearance of blasts in the blood or the finding of ≥ 5% blasts in the bone marrow, not attributable to any other cause after a prior CR (CR, CRc, or CRi). If there are no blasts in the peripheral blood and 5-20% blasts in the bone marrow, bone marrow biopsy should be repeated in > 1 week to confirm relapse.

12.2.10 Adverse Events

Adverse events will be assessed and graded according to NCI Common Toxicity Criteria version 4 as outlined in Section 10.1

12.2.11 Evaluable for Toxicity

All subjects will be evaluable for toxicity from the time of their CIML NK cell infusion.

12.2.12 Treatment Related Mortality

Death that results from a transplant procedure related complication (e.g. infection, organ failure, hemorrhage, GVHD) rather than from relapse of the underlying disease or an unrelated cause.

12.3 Event-Free Survival (EFS)

EFS is defined as the time from day 0 until failure to engraft, treatment failure, disease progression/relapse, or death from any cause (whichever occurs first).

12.4 Overall Survival (OS)

OS is defined as the time from the date of Day 0 until death from any cause.

12.5 Non-Relapse Mortality (NRM)

NRM is defined as any death that is not due to relapse, regardless of the time frame.

12.6 Leukemia Free Survival (LFS)

LFS is defined as the time from achievement of CR to the time of relapse, death in remission, or last follow-up.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason

- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities and separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

14.0 STATISTICAL CONSIDERATIONS

This is a phase II study with the primary endpoint of leukemia free survival rate (LFS) at 1-year post-transplant. Additional endpoints are listed in Section 2.

14.1 Study Design and Sample Size Calculation

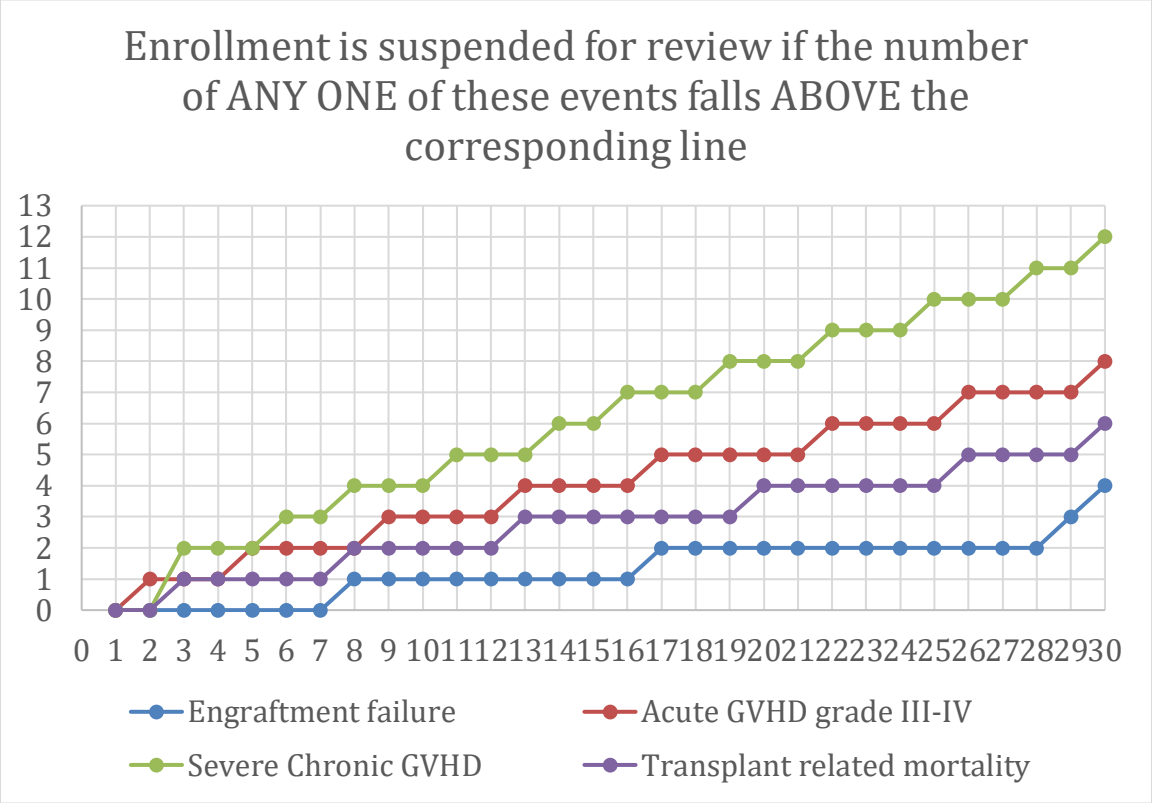
A Fleming one stage design will be used to determine efficacy with respect to the primary endpoint. One-year LFS with current standard of care is ~10% (as outlined above), and a 20% increase is considered of clinical interest. A total of 30 patients evaluable for 1-year LFS will provide power=.90 at a one-sided .05 significance level to identify an increase of 20% from 10% to 30% 1-year LFS.

14.2 Final Data Analysis Plan

The primary endpoint of this study is to determine 1-year LFS in patients with relapsed/refractory AML receiving a standard of care conditioning regimen followed byhaploidentical donor transplantation and same-donor memory-like NK cell infusion. AEs/SAEs will be summarized by patient, type and grade as defined by the CTCAE v4.03. The secondary end points of CR rate at Day 28 post-transplantation will be reported with 95% confidence intervals. Median time treatment failure, overall survival, and disease free survival will be estimated by Kaplan-Meier curves. For binary endpoints such as toxicity, summary statistics including proportions and their 95% confidence interval will be reported with 95% confidence intervals. Frequencies and incidence of serious adverse events will be tabulated by patient, type and grade with 95% confidence intervals. SAS 9.4/SAS-STAT 14.2 or higher (SAS Institute, Cary, NC, USA) will be used for all the analyses.

14.3 Safety and Toxicity Monitoring and Stopping Rules

The primary adverse events of interest are engraftment failure (expected rate 5%, maximum allowable 15%), grade III-IV acute GVHD (expected rate 15%, maximum allowable 30%), severe chronic GVHD (expected rate 25%, maximum allowable 50%) and transplant related mortality (expected rate 10%, maximum allowable 30%). Four continuous toxicity monitoring rules will be used, each with significance level .10 and power .90. Power is higher and significance level lower in order to increase the probability of correctly identifying excess toxicity at the expense of possible incorrect early stopping. Stopping rules are described in the plot and tables below:



# Patients	Engraftment failure	Acute GVHD grade III-IV	Severe Chronic GVHD	Transplant related mortality
1	0	0	0	0
2	0	1	0	0
3	0	1	2	1
4	0	1	2	1
5	0	2	2	1
6	0	2	3	1
7	0	2	3	1

8	1	2	4	2
9	1	3	4	2
10	1	3	4	2
11	1	3	5	2
12	1	3	5	2
13	1	4	5	3
14	1	4	6	3
15	1	4	6	3
16	1	4	7	3
17	2	5	7	3
18	2	5	7	3
19	2	5	8	3
20	2	5	8	4
21	2	5	8	4
22	2	6	9	4
23	2	6	9	4
24	2	6	9	4
25	2	6	10	4
26	2	7	10	5
27	2	7	10	5
28	2	7	11	5
29	3	7	11	5
30	4	8	12	6

The maximum number of participants experiencing events allowed before suspension, probability of incorrect early stopping with low toxicity and probability of correct early stopping with high toxicity are tabulated below. 'Low' and 'high' toxicity are the expected and maximum allowable values for each adverse event:

	Maximum number of events allowed	Probability of incorrect stopping with low toxicity	Probability of correct early stopping with high toxicity
Engraftment failure	4	0.10	0.71
Acute GVHD grades III-IV	8	0.10	0.69
Severe chronic GVHD	12	0.10	0.88
Transplant related mortality	6	0.10	0.90

14.3.1 Additional Stopping Rule for Patient Mortality

The expected death rate in patients with active AML undergoing non-myeloablative transplantation has historically been high, with a 2 year PFS and OS of approximately 10%.[18] Due to this high expected mortality rate, deaths will be monitored at 2 time points, 8 months (the approximate median survival time) and the end of study. Since the expected overall

survival at 2 years is approximately 10% and expected median survival time is about 8 months, the expected death rate is about 50%, or 15 deaths among 30 patients, at 8 months and 90%, or 27 deaths among 30 patients, at 2 years. Given an overall survival rate of 90% at 2 years, the probability of >18 deaths at 8 months is .10 (1 chance in 10). At 2 years the probability of >28 deaths is .18 (1 in 5). The study will be suspended for review due to excess mortality if more than 18 deaths are observed during the first 8 months on study or if the 28^{th} death is observed before the study is completed.

14.4 Replacement of Patients

If less than the targeted CIML NK cell numbers (at least 0.5 x 10^6/kg) are available for infusion, then all CIML NK cells (after quality control testing) will be given and the patient will continue with protocol therapy. These patients will be evaluated for AEs, and reported in the intent to treat analyses. However, these patients will be replaced to ensure adequate numbers of patients are treated to assess a treated cohort. If a patient for unexpected reasons is unable to receive CIML NK cells, they will not be included in the safety analyses.

14.5 Analysis Plan for Secondary and Correlative Endpoints

Correlative endpoints will be compared before and after treatment using paired t-tests, nonparametric Wilcoxon signed rank tests or clustered Poisson models, depending on the observed distributions.

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APPENDIX A: Karnofsky Performance Status

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

APPENDIX B: Cockcroft-Gault Formula

$$eC_{Cr} = \frac{(140 \text{ - Age}) \ \times \ \text{Mass (in kilograms)} \ \times \ [0.85 \ if \ Female]}{72 \ \times \ \text{Serum Creatinine (in mg/dL)}}$$

APPENDIX C: Ideal Body Weight and Adjusted Ideal Body Weight

Ideal Body Weight (IBW):
Males IBW = 50kg + 2.3 kg/inch over 5 feet
Females IBW = 45.5kg + 2.3 kg/inch over 5 feet

Adjusted Body Weight (ABW):
ABW = IBW + 0.4(actual weight - IBW)

APPENDIX D: MINNESOTA-CIBMTR STAGING AND GRADING FOR ACUTE GvHD

Acute GVHD Staging

	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin (% BSA)	No rash	< 25%	25%-50%	> 50%	Generalized erythroderma with bullae
Gut (diarrhea, mL/day)	< 500	> 500	> 1000	> 1500	Severe abdominal pain +/- ileus
Upper GI		Persistent, severe nausea			
Liver (bilirubin, mg/dL)	≤ 2	2.1-3	3.1-6	6.1-15	> 15

BSA = body surface area; GI = gastrointestinal.

Acute GVHD Grading, MN-CIBMTR Criteria

Grade	Skin	Liver	Lower GI	Upper GI
Minnesota				
I	1-2	0	0	0
II	3	1	1	1
III	-	2-4	2-3	-
IV	4	-	4	-
CIBMTR				
A	1	0	0	0
B	2	1-2	1-2	1
C	3	3	3	-
D	4	4	4	-

APPENDIX E: Chronic GvHD Assessment

SKIN (Patient History and Exam)

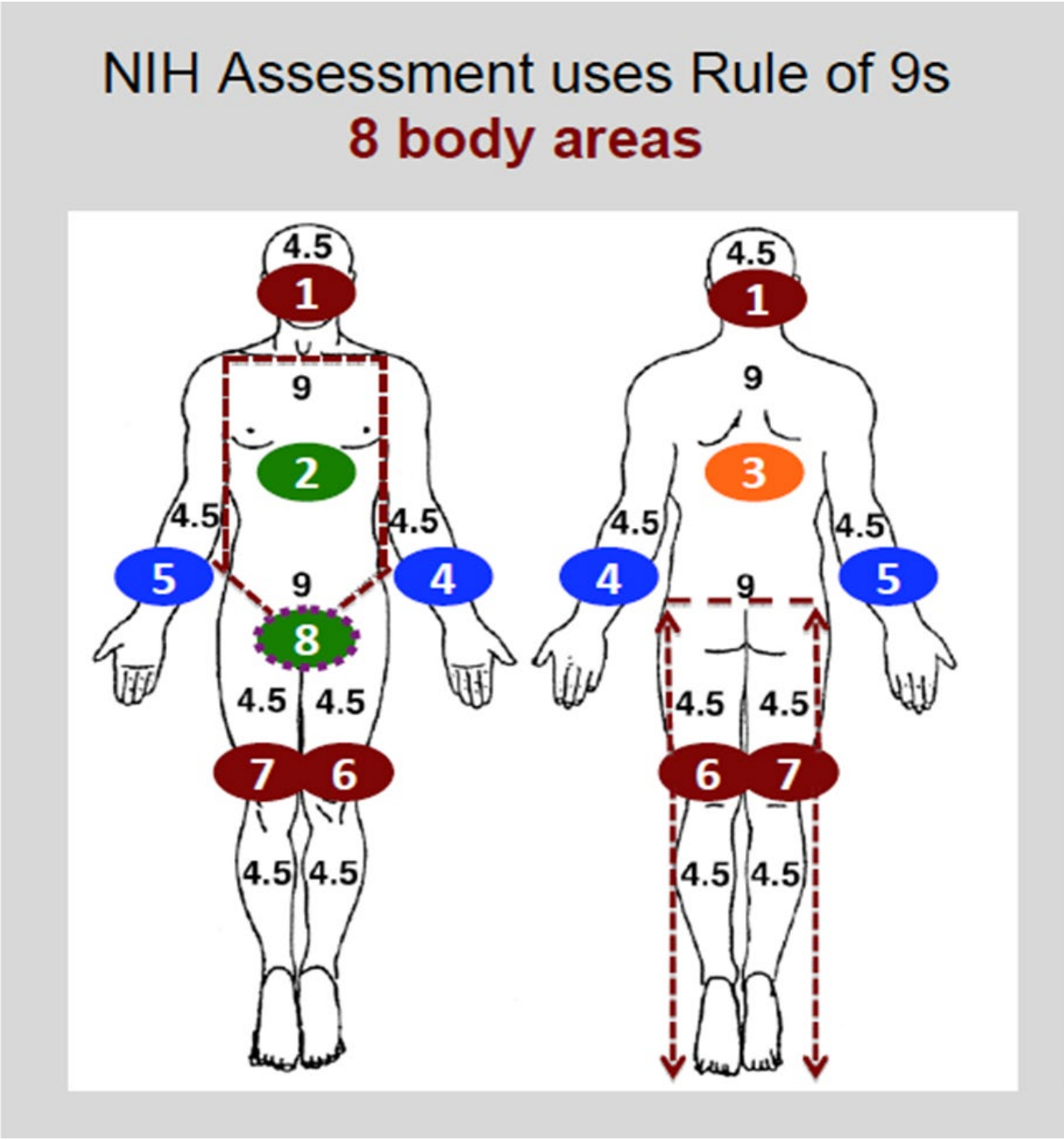
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
Skin Score	No Symptoms	< 18% BSA with disease signs but **NO** sclerotic features	19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch	>50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus

NIH item that scores maximum severity based on:

→ Percent body surface area involved by erythema ***OR***
→ Degree of sclerotic features ***OR***
→ Impaired mobility ***OR***
→ Ulceration ***OR***
→ Severe pruritus

Maculopapular rash ☐	☐	Erythroderma
Lichen planus-like features ☐	☐	Poikiloderma
Papulosquamous lesions or ichthyosis ☐	☐	Sclerotic features
Hyperpigmentation ☐	☐	Pruritus
Hypopigmentation ☐	☐	Hair Involvement
Keratosis pilaris ☐	☐	Nail Involvement
Erythema	☐	% BSA involved: _____



***% BSA Reference**

CGvHD signs and symptoms seen with NAILS, SCALP, BODY HAIR
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

Check all that apply:

Distinctive (seen in Chronic GvHD, but insufficient alone to establish diagnosis of Chronic GvHD)

Dystrophy ☐
Longitudinal ridging, splitting, or brittle features ☐
Onycholysis ☐
Pterygium unguis ☐
Nail loss (usually symmetric; affects most nails) ☐
New onset of scarring or non-scarring scalp alopecia (after recovery from chemoradiotherapy) ☐

Scaling, papulosquamous lesions ☐

Other features/common (seen with both Acute and Chronic GvHD)

Thinning scalp hair, typically patchy, course or dull (not explained by endocrine or other causes) ☐
Premature gray hair ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD
_____
_____
_____
____

CGvHD signs and symptoms seen with MOUTH
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
Oral Score	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

Oral Exam
Check all that apply:

Diagnostic (sufficient to establish Chronic GvHD)
Lichen-type features ☐
Hyperkeratotic plaques ☐
Restriction of mouth opening from sclerosis ☐

Distinctive (seen in Chronic GvHD, but insufficient alone to establish diagnosis of Chronic GvHD)
Xerostomia ☐
Mucocele ☐
Mucosal atrophy ☐
Pseudomembrane ☐
Ulcers ☐

Other features/common (seen with both Acute and Chronic GvHD)
Gingivitis ☐
Mucositis ☐
Erythema ☐
Pain ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD

_____
_____
_____
____

CGvHD signs and symptoms seen with EYES
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
Eye Score	No Symptoms	Mild dry eye symptoms not affecting ADL (requiring eye drops <3x per day) OR asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring eye drops >3x per day or punctal plugs) without vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyewear to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision cause by keratoconjunctivitis sicca

Nuances for a patient using drops < 3 x per day:
- But wearing a Boston Scleral lens → Score 3
- But had punctal plugs placed 7 days → Score 2
- But had plugs placed a month ago →Score 1

Check all that apply:

Distinctive (seen in Chronic GvHD, but insufficient alone to establish diagnosis of Chronic GvHD)
New onset dry, gritty, or painful eyes ☐
Cicatricial conjunctivitis ☐
Keratoconjunctivitis sicca ☐
Confluent areas of punctate keratopathy ☐

Other features/common (seen with both Acute and Chronic GvHD)
Photophobia ☐
Periorbital hyperpigmentation ☐
Blepharitis (erythema of the eyelids with edema) ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD
_____
_____
_____
____

CGvHD signs and symptoms seen with GI TRACT
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
GI Tract Score	No Symptoms	Symptoms such as dysphagia, anorexia, nausea, abdominal pain or diarrhea without significant weight loss (<5%)	Symptoms 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

Check all that apply:

Diagnostic (sufficient to establish Chronic GvHD)
Esophageal web ☐
Strictures or stenosis in the upper to mid third of the esophagus ☐

Other features/common (seen with both Acute and Chronic GvHD)
Exocrine – pancreatic insufficiency ☐
Anorexia ☐
Nausea ☐
Vomiting ☐
Diarrhea ☐
Weight loss ☐
Failure to thrive (infants and children) ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD
_____
_____
_____
____

CGvHD signs and symptoms seen with LIVER

Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

Liver Score	0	1	2	3
	Normal LFT	Normal Bilirubin, with ALT ≥ 5 x ULN or AP ≥ 3 x ULN	Elevated bilirubin ≤ 3 mg/dL or ALT > 5 ULN	Elevated bilirubin > 3 mg/dL

Check all that apply:

Other features/common (seen with both Acute and Chronic GvHD)

Total Bilirubin, alkaline phosphatase
>2x upper limit of normal ☐
ALT or AST > 2 x upper limit of normal ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD

_____
_____
_____
____

CGvHD signs and symptoms seen with LUNGS
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
Lung Score	No Symptoms FEV1 > 80% **OR** LFS = 2	Mild symptoms (shortness of breath after climbing 1 flight of steps) FEV1 60-79% **OR** LFS 3-5	Moderate symptoms (shortness of breath after walking on flat ground) FEV1 40-59% **OR** LFS 6-9	Severe symptoms (shortness at rest requiring O_2) FEV1 ≥39% **OR** LFS 10-12

FEV1:_____ Not done☐ NA☐
DLCO: _____ Not done☐ NA☐
LFS: _____ Not done☐ NA☐

Check all that apply:

Diagnostic (sufficient to establish Chronic GvHD)
Bronchiolitis obliterans diagnosed with lung biopsy ☐

Distinctive (seen in Chronic GvHD, but insufficient alone to establish diagnosis of Chronic GvHD)
Bronchiolitis obliterans diagnosed with PFTs and radiology ☐

Other features/common (seen with both Acute and Chronic GvHD)
Bronchiolitis obliterans with organizing pneumonia (BOOP) ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD
_____
_____
_____
____

CGvHD signs and symptoms seen with MUSCLE, FASCIA, JOINTS
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
Muscles, Fascia & Joints Score	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 to ADL	Contractures **WITH** significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirt, dress self- etc.)

Check all that apply:

Diagnostic (sufficient to establish Chronic GvHD)

Fasciitis ☐
Joint stiffness or contracture secondary to sclerosis ☐

Distinctive (seen in Chronic GvHD, but insufficient alone to establish diagnosis of Chronic GvHD)
Myositis or polymyositis ☐

Other features/common (seen with both Acute and Chronic GvHD)
Edema ☐
Muscle cramps ☐
Arthralgia or arthritis ☐

Please describe:
Patient timeline, extent of finding, duration/rapidity of onset, relationship to immunosuppression and likely attributable to GvHD

_____
_____
_____
____

CGvHD signs and symptoms seen with GENITALIA
Score 0 ☐ Score 1 ☐ Score 2 ☐ Score 3 ☐

	0	1	2	3
Genitalia Score	No Symptoms	Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	Symptomatic with moderate signs on exam **AND** with mild dyspareunia **OR** with discomfort with gynecologic exam	Symptomatic **WITH** advanced sign (stricture, labial, agglutination, or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

Check all that apply:

Diagnostic (sufficient to establish Chronic GvHD)
Lichen planus-like features ☐
Vaginal scarring or stenosis ☐

Distinctive (seen in Chronic GvHD, but insufficient alone to establish diagnosis of Chronic GvHD)
Erosion ☐
Fissures ☐
Ulcers ☐

Other Indicators	None	Mild	Moderate	Severe	Not Assessed
Ascites (serositis)					
Myasthenia Gravis					
Polymyositis					
Platelets less than 100,000/ l					
Pericardial effusion					
Nephrotic syndrome					
Cardiomyopat hy					
Cardiac conduction defects					
Progressive onset					
Pleural Effusion(s)					
Peripheral Neuropathy					
Eosinophilia >500ul					
Coronary artery involvement					

Other, specify: _____

Does the patient have chronic GvHD? **Yes** ☐ **No** ☐
If yes, specify Severity: Mild ☐ Moderate ☐ Severe ☐

NIH CGvHD Global Severity Category reflects overall disability

SEVERITY	ORGAN SCORE	NO. OF ORGANS
Mild	**All 1 (0 for Lung)**	**1-2**
Moderate	**All 1 (0 for Lung)**	**3 or more**
	At least one 2 (1 for Lung)	**1-2**
Severe	**At least one 3 (2 for Lung)**	**1 or more**

Were there additional exams performed? Yes ☐ No ☐
If yes, check all that apply:

Procedure	Date	Result
☐Biopsy		
☐CT Scan		
☐MRI		
☐Photo		
☐Schirmer Eye Test		
☐ PFT		
☐ LFT		
☐Ultrasound/Echocardiogram		
☐X-Ray		
☐Other:_____		

APPENDIX F: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient 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.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death

- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

APPENDIX G: Reporting Timelines

Expedited Reporting Timelines

Event	HRPO	QASMC	FDA
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment	
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.		
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.		
Protocol exception	Approval must be obtained prior to implementing the change		
Clinically important increase in the rate of a serious			Report no later than 15 calendar days after it is determined that the information qualifies for reporting

Event	HRPO	QASMC	FDA
suspected adverse reaction of that list in the protocol or IB			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.		
Breach of confidentiality	Within 10 working days.		
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.		

Routine Reporting Timelines			
Event	**HRPO**	**QASMC**	**FDA**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.
Minor deviation	Report summary information at the time of continuing review.		
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.		
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.		