Cytokine Induced Memory-like NK Cell Adoptive Therapy for Relapsed AML after Allogeneic Hematopoietic Cell Transplant in Children and Adults

Washington University School of Medicine
Department of Medicine, Division of Oncology
Department of Pediatrics, Division of Hematology-Oncology
660 South Euclid Avenue, Campus Box 8007
St. Louis, MO 63110

Protocol #: 202206197 (201709041)
Version Date: 04/16/2025

Principal Investigator: Amanda Cashen, M.D.
Email: acashen@wustl.edu
Phone: (314) 454-8323

Pediatric Patients Co-PI: Jeffrey Bednarski, M.D., Ph.D.
Email: bednarski_j@wustl.edu
Phone: 314-454-6018

Laboratory PI: Todd Fehniger, M.D., Ph.D.
Email: tfehnige@wustl.edu
Phone: (314) 747-1385

Sub-Investigators: Shalini Shenoy, MD
Robert Hayashi, MD
Andrew Cluster, MD
Geoffrey Uy, M.D
Ravi Vij, M.D
Camille Abboud, M.D
Meagan Jacoby, M.D., Ph.D.
Iskra Pusic, M.D
Armin Ghobadi, M.D
Mark Schroeder, M.D
Keith Stockerl-Goldstein, M.D.
Kristan Augustin, Pharm.D.

Statistician: Feng Gao, Ph.D.

IND#: 17629
IND Sponsor: Amanda Cashen, M.D.
Clinical Trials.gov#: NCT03068819

Cytokine Induced Memory-like NK Cell Adoptive Therapy for Relapsed AML after Allogeneic Hematopoietic Cell Transplant in Children and Adults

Protocol Revision History

Initial Version		09/06/17
Amendment #1	**(not approved)**	04/25/19
Amendment #1	**(approved)**	06/13/19
Amendment #2		03/27/20
Amendment #3		11/17/20
Amendment #4		01/11/21
Amendment #5		08/06/21
Amendment #6		01/18/22
Amendment #7		06/20/22
Amendment #8		09/23/22
Amendment #9		05/26/23
Amendment #10		04/16/25

Table of Contents

Cytokine Induced Memory-like NK Cell Adoptive Therapy for Relapsed AML after Allogeneic Hematopoietic Cell Transplant in Children and Adults

SCHEMA

Inclusion Criteria:

- AML patients relapsed after allogeneic hematopoietic cell transplant (HCT)
- Donor available for recollection (same donor as used for the HCT)
- Adequate organ function

Pilot pediatric/young adult cohort

- ≥1 and <18 years of age

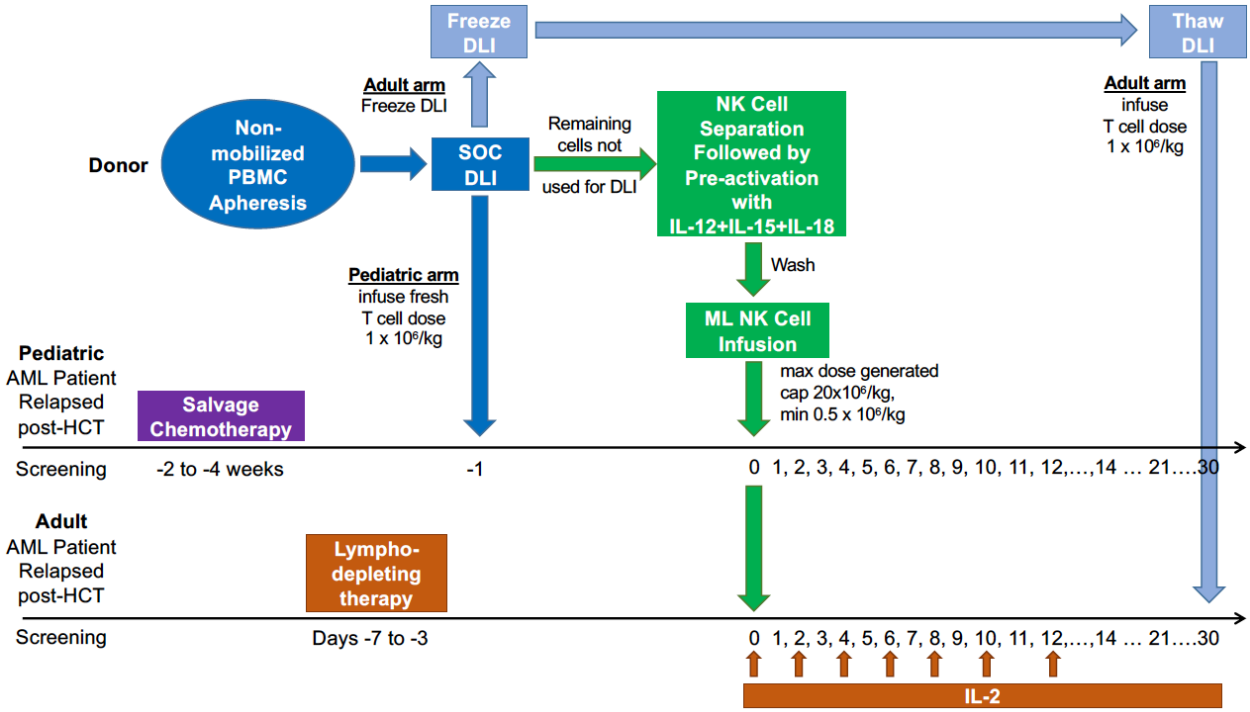
Phase 2 adult cohort

- ≥18 years of age

Exclusion Criteria:

- Active ongoing acute or chronic graft versus host disease (GVHD)
- Uncontrolled bacterial, fungal, or viral infections, known HIV, active hepatitis B or C
- New progressive pulmonary infiltrates
- Pregnant and / or breastfeeding

Schema:



Salvage Chemotherapy:

- Pediatric cohort: Patients will receive standard of care salvage chemotherapy with FLAG (fludarabine, ara-C and G-CSF) or decitabine 2-4 weeks prior to receiving the DLI on day -1 and CIML NK cell infusion on day 0.
- Adult cohort: Patients will receive lymphodepleting therapy with fludarabine and cyclophosphamide on days -7 through -3 prior to receiving CIML NK cell infusion on day 0. DLI will be given on day 30.

Donor Cell Graft: Within 24 hours of planned cellular therapy, matched related or unrelated donor will undergo standard non-mobilized PBSC collection via standard 3x volume leukapheresis. Collection goal is >2 x 10^{10} total nucleated cells.

Donor Lymphocyte (CD3+ T cells) Infusion: Following collection, donor lymphocytes will be immediately infused for pediatric patients and will be frozen for future use for adult patients. Patient will receive donor lymphocyte infusion with 1 x 10^6 CD3+ T cells/kg on day -1 (pediatric) or with T cell dose per standard of care institutional practices and physician discretion on day 30 (adult). Doses for subsequent DLI will be determined by institutional practices.

Donor NK cell separation and CIML NK cell generation: Remaining apheresis product will subsequently be enriched for NK cells by CD3 depletion and CD56 positive selection using Miltenyi's CliniMACS®. The NK cell fraction ($CD3^-CD56^+$ cells) will be activated for 12-18 hours by stimulating the IL-12, IL-15, and IL-18 receptors. Activated NK cells will be infused on day 0. Maximum number of CIML NK cells infused will be capped at 20 x 10^6/kg (minimum: 0.5 x 10^6/kg. Patients receiving <0.5 x 106 CIML NK cells/kg will be followed for safety analysis but will be replaced for efficacy assessment). If an apheresis product contains less than 1 x10^{10} total nuclear cells then NK cells processing will not be done and the product will only be used for SOC DLI. See section 6.4 for details on processing of apheresis product.

IL-2 Dosing (adult cohort only): Low dose rh IL-2 will be administered beginning on Day 0, continuing every other day through Day + 12 (7 doses total).

Additional cycles: A second cycle of protocol therapy may be administered with chemotherapy optional for subsequent cycles, to maintain response, treat residual AML, or in the event of disease progression. This optional second cycle will be considered a re-treatment, with date of second NK cell infusion considered a second Day 0.

1.0 BACKGROUND AND RATIONALE

1.1 Acute Myeloid Leukemia and Allogeneic Hematopoietic Cell Transplantation

Acute myeloid leukemia (AML) is one of the most common hematologic malignancies, with an estimated prevalence of 3.8 cases per 100,000.[1] In older AML patients, the 5 year disease-free survival (DFS) is extremely poor at 10-20%.[2,3] In addition to age, genetic alterations in the leukemic cells strongly influence the outcomes of AML patients with current treatment regimens.[4] The standard chemotherapy-based induction 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). Only around 50% of the young AML patients in poor prognostic groups are able to achieve a CR with the current intensive induction regimens.[4] Since nearly all AML patients treated only with induction chemotherapy relapse[5], patients are subsequently treated with consolidation chemotherapy. 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 is the most common regimen used for refractory or relapsed AML patients.

Allo-HCT using a hematopoietic stem cell (HSC) graft from major histocompatibility complex (MHC) matched siblings or MHC matched unrelated donors is the most curative treatment for patients with AML who are able to achieve a CR. However, relapse remains the most significant cause of treatment failure after allo-HCT occurring in 20-70% of the AML patients. [6,7]

1.2 Donor Lymphocyte Infusion (DLI) for Relapse after Allo-HCT

Donor lymphocyte infusion (DLI) preceded by salvage chemotherapy like FLAG or decitabine are the widely used treatment strategy to treat patients who relapse after allo-HCT.[6,7] However only up to 30% of patients are able to achieve a complete remission and the long term survival continues to be very poor after DLI. Further, acute graft versus host disease occurs in approximately 30-50% of these patients after DLI adding to the morbidity and mortality in these patients.[7]

Because of the poor efficacy of this approach, there is an unmet need to develop novel protocols aimed at providing safer and more effective treatment approaches to the patients who relapse after allo-HCT.

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.[8,9] 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.[8,10] 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.[11] 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.[12–14] 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.[15–17] In their initial study, HLA-haploidentical donor NK cell alloreactivity against the patient's AML blasts was associated with long term DFS.[15,17] 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.[15,18] Subsequent studies in various HCT contexts have investigated the role of KIR genetics and transplant outcomes, adding evidence that NK cell immune response contribute to graft versus leukemia effects.[19,20] 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.[21,22,23] 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.[21] 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.[24] Similarly, in another study none of the AML patients who were adoptively transferred with allogeneic NK cells relapsed with a median follow-up of 964 days, and the 2-year event-free survival estimate was 100%.[22] These studies provide proof-of-principle that allogeneic 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 NK cells, in the context of a DLI in patients who relapse after allo-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.[25–29] 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.[29,30] This pre-activation approach also resulted in anti-tumor responses to murine NK cell-sensitive cell lines following adoptive transfer in mice.[31] 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.[31,32] 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.[33] 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.[34] These findings were promising and recently translated to the clinic in a first-in-human study of CIML NK cells in relapsed/refractory (rel/ref) AML.

In addition to the cocktail of IL-12, IL-15, and IL-18, a soluble fusion protein complex (HCW9201) comprising biologically active human IL-12p70, IL-15/IL-15Rα, and IL-18 proteins has been produced and purified using cGMP processes. The activity of HCW9201 for inducing CIML NK cells has been extensively evaluated and compared to that of the IL-12/IL-15/IL-18 cocktail. Overall, these studies established equivalent or better activation and memory-like NK cell differentiation following short-term (12-18 hour) incubation with HCW9201, compared to the IL-12/IL-15/IL-18 cytokine mixture. These findings include 1) induction of IL-12R-, IL-15R-, and IL-18R-stimulated signaling cascades, 2) increases in human NK cell activation markers (IL-2Rα surface expression; IFN-γ and TNF production), 3) in vitro proliferation, and 4) enhanced metabolism. HCW9201 pre-activation also results in CIML NK cell differentiation following growth in IL-15, as evidenced by 1) acquisition of the CIML NK cell mass cytometry profile, 2) demethylation of the IFN-γ gene promoter, 3) enhanced in vitro re-stimulation responses (IFN-γ production, cytotoxicity) against leukemia targets, and 4) control of K562 leukemia in a human xenograft model. Therefore, these studies directly support the substitution of HCW9201 for the IL-12/IL-15/IL-18 cytokines as an ancillary material (per USP <1043>) for the production of CIML NK cells for adoptive cell therapies.

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

Based on the above in vitro and pre-clinical mouse model findings, we recently completed 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 NK cell infusion, based on previously published adoptive NK cell studies.[21,35] 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 such as melanoma and renal cell cancer. In our study, we utilized a lymphodepleting regimen consisting of 5 doses of fludarabine at 25 mg/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.

We treated 17 patients with relapsed / refractory AML on this phase 1 study. None of these patients met criteria for dose limiting toxicity, including GVHD. Of the 11 evaluable patients 7 had clinical responses, including 6 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. Thus, human IL-12/IL-15/IL-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.[34] *The results from this clinical trial were published in Science Translational Medicine*.[36]

Here, we propose to add CIML NK cells following DLI to provide additional graft-versus-leukemia effect in patients who have relapsed after allo-HCT. 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 combining CIML NK cell infusion with DLI

DLI following salvage chemotherapy is the one of the most widely used treatment approaches in patients who relapse after allo-HCT. However, the CR rates and long-term survival remain very poor in these patients and, therefore, there is an unmet need to develop more effective treatment approaches in patients who relapse after allo-HCT.

Based on the initial promising results with our CIML NK cell trial (see Section 1.5 above), we hypothesize that combining the CIML NK cells with DLI approach will significantly enhance the graft versus leukemia and therefore potentially provide curative therapy for these patients with otherwise extremely poor prognosis. Combining CIML NK cells with the DLI platform will also potentially allow these adoptively transferred cells to persist for longer duration as they should not be rejected by donor T cells as the CIML NK cells are derived from the same donor. The use of CIML NK cells is unlikely to lead to excessive GVHD as previous studies have not been associated with excessive GVHD rates.[21,22,23,36]

1.6.2 Rationale for the NK cell correlative laboratory investigation

Clinical trial administration of adoptively transferred memory-like NK cells provides a unique opportunity to study 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 models 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 allo-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.[37,38] In unpublished data CIML and control NK cells are identifiable as discrete populations on CyTOF2 analysis and we intend to use this technology to distinguish the adoptively transferred cells from the naïve NK cells generated from the donor derived stem cells in the recipient. Unique donor or recipient HLA markers will also be used to distinguish cells from the donor and the 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 NK cell 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.

2.0 OBJECTIVES

2.1 Primary Objectives – Pilot Pediatric/Young Adult Cohort

1. To determine the feasibility of successfully generating CIML NK cells concurrent with SOC donor lymphocyte infusion (DLI) from the original stem cell donor. Feasibility is defined as the ability to generate and successfully infuse CIML NK cells with SOC DLI.
2. To assess the safety of administering CIML NK cells plus DLI in regards to unexpected early mortality, unacceptable GVHD, or prolonged neutropenia.

2.2 Secondary Objectives – Pilot Pediatric/Young Adult Cohort

1. To determine complete remission (CR/CRi) rate at Day 30 after the CIML NK cell infusion using modified IWG criteria
2. To determine the rate of leukemia-free survival (LFS) and overall survival (OS) at 100 days post CIML NK cell infusion
3. To determine the rate of leukemia-free survival (LFS) and overall survival (OS) at 1 year post CIML NK cell infusion
4. To determine the incidence and severity of acute GVHD rates after CIML NK cell infusion
5. To determine the incidence and severity of chronic GVHD rates after CIML NK cell infusion

2.3 Primary Objectives – Phase 2 Adult Cohort

1. To assess the safety of administering CIML NK cells plus DLI in regards to unexpected early mortality, unacceptable GVHD, or prolonged neutropenia
2. To determine the rate of leukemia-free survival (LFS) at 6 months (+/-1 month) post CIML NK cell infusion

2.4 Secondary Objectives – Phase 2 Adult Cohort

1. To determine complete remission (CR/CRi) rate at Day 30 after the CIML NK cell infusion using modified IWG criteria
2. To determine the rate of leukemia-free survival (LFS) and overall survival (OS) at 100 days post CIML NK cell infusion

3. To determine the rate of leukemia-free survival (LFS) and overall survival (OS) at 1 year post CIML NK cell infusion
4. To determine the incidence and severity of acute GVHD after CIML NK cell infusion
5. To determine the incidence and severity of chronic GVHD after CIML NK cell infusion

2.5 Correlative Objectives – Both cohorts

1. To evaluate the number, phenotype, persistence, and function of the CIML NK cells following adoptive transfer. In exploratory analyses, the maximal number of blood and bone marrow CIML 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 functional responses of CIML NK cells to leukemia targets.
3. To assess AML blasts and the BM microenvironment pre-therapy and at first relapse to identify mechanisms of NK cell immunoevasion.
4. To define the KIR genotype of donor NK cells, and in exploratory analyses correlate with clinical endpoints.
5. To define the cytokine profiles associated with CIML NK cell plus DLI administration.
6. To define the immune cell reconstitution following CIML NK cell plus DLI administration.

3.0 PATIENT SELCTION

3.1 Recipient Eligibility Criteria

3.1.1 Inclusion Criteria

1. Relapsed AML after HLA-matched or HLA-mismatched related or unrelated allogeneic hematopoietic cell transplant
2. **For pilot pediatric/young adult patient cohort:** ≥1 and <18 years of age
3. **For phase 2 adult patient cohort**: ≥18 years of age
4. Available original donor (same donor as used for the initial stem cell transplant) that is willing and eligible for non-mobilized collection (refer to Section 3.2)
5. 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.
6. Karnofsky performance status > 60 % (see Appendix A)
7. 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
8. Not currently requiring systemic corticosteroid therapy (10 mg or less of prednisone or equivalent doses of other systemic steroids are allowed) or any other immune suppressive medications
9. 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).
10. 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. Acute or chronic GVHD with ongoing active systemic treatment.
2. Circulating blast count >10,000/μL by morphology or flow cytometry (cyto-reductive therapies, including salvage chemotherapy, is encouraged prior to study enrollment).
3. Uncontrolled bacterial or viral infections, or known HIV, Hepatitis B, or Hepatitis C infection.
4. Uncontrolled angina, severe uncontrolled ventricular arrhythmias, or EKG suggestive of acute ischemia or active conduction system abnormalities.
5. New or progressive pulmonary infiltrates concerning for new or uncontrolled infectious process.
6. Known hypersensitivity to one or more of the study agents
7. Received any investigational drugs within the 14 days prior to CIML NK cell infusion date
8. Pregnant and/or breastfeeding

3.2 Donor Eligibility Criteria

3.2.1 Inclusion Criteria

1. At least 2 years of age
2. Donor weight must be at least 15 kg
3. Same donor as used for the allo-HCT
4. In general good health, and medically able to tolerate leukapheresis
5. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative)
6. For donors < 18 years, eligible to undergo standard of care leukapheresis for DLI

3.2.2 Exclusion Criteria

1. Active hepatitis, positive for HTLV, or HIV on donor viral screen
2. Pregnant

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

5.1 Pilot Pediatric/Young Adult Cohort

This part of the study is aimed at determining the feasibility and safety of generating CIML NK cell product for infusion after SOC DLI to enhance graft versus leukemia effect in pediatric and young adult patients. The goal is to enroll a total of 24 patients to complete this part of the study.

As a pilot study, a formal safety evaluation will be done after every 3rd patient enrolled, and the trial will be suspended based on unacceptable early mortality or GVHD (see Section 6.14.1).

5.2 Adult Cohort

This part of the study will include a statistically independent cohort of adult patients in a 2-stage design to determine the safety and efficacy of CIML NK cell therapy with SOC DLI. The goal is to enroll approximately 1 - 2 patients per month.

Patient safety will be continuously monitored and the trial will be suspended based on unacceptable early mortality or GVHD (see Section 6.14.1).

6.0 TREATMENT PLAN

6.1 Overall Treatment Plan

Pediatric cohort: The recipient will receive standard of care salvage chemotherapy consisting of fludarabine, cytarabine, and G-CSF (FLAG) to be started 2 to 4 weeks prior to the CIML NK cell infusion. 5-day decitabine is an acceptable alternative for FLAG, and another standard of care salvage chemotherapy regimen, if clinically appropriate and approved by the study PI, may be used.

Adult cohort: The recipient will receive lymphodepleting chemotherapy with fludarabine and cyclophosphamide beginning on day -7.

Both pediatric and adult cohorts: The donor will undergo non-mobilized leukapheresis on Day -2 or -1. Standard of care DLI will be given either fresh on day -1 (1×10^6 CD3+ cells/kg, pediatric cohort) or frozen for administration on day 30 (dose per physician discretion, adult cohort). Additionally, 9×10^6 CD3+ cells/kg will be reserved for potential future DLIs. If the original apheresis product contains $>1 \times 10^{10}$ total nuclear cells, the remaining cells will then be used for generation of CIML NK cells, which will be infused on Day 0. See Section 6.4 for details on processing of apheresis product.

A second cycle of therapy may be administered > 30 days after the administration of the first course of protocol therapy to maintain response or to treat persistent/relapsed AML, if a patient continues to meet the inclusion/exclusion criteria. Chemotherapy may be omitted before a second infusion of DLI and CIML NK cells. In the setting of GVHD following the first cycle of therapy, the T cell DLI may be omitted, and ML NK cells administered. The date of the second NK cell infusion will be considered a second Day 0. Patients who receive a second NK cell infusion will have protocol assessments (including correlative samples) performed on the same schedule as the initial NK cell infusion (refer to Section 9) with the exception of bone marrow biopsies, which will be performed only as clinically indicated to assess response. If CIML NK cells are not available for a second infusion (e.g., due to donor availability), a DLI alone may be given.

6.2 Salvage Chemotherapy (Pediatric cohort)

6.2.1 Salvage therapy with FLAG

FLAG will be given standard of care per institutional guidelines.

6.2.2 Salvage Therapy with Decitabine

Decitabine will be given standard of care per institutional guidelines.

6.3 Lymphodepleting Chemotherapy with Flu/cy (Adult cohort)

Patients will receive a lymphodepleting regimen consisting of 5 doses of fludarabine at $25 mg/m^2$ and 2 doses of cyclophosphamide at 60mg/kg as follows:

Day	Therapy
-7	Fludarabine
-6	Fludarabine Cyclophosphamide
-5	Fludarabine Cyclophosphamide
-4	Fludarabine
-3	Fludarabine

Dose and/or schedule adjustments consistent with the standard of care may be made on an individual patient basis as needed for safety.

Patients should receive allopurinol daily, unless contraindicated, beginning day -6 prior to chemotherapy start and continuing until day +7 or as clinically indicated. Additional tumor lysis prophylaxis or treatment (IVF, rasburicase, etc.) may be administered as clinically indicated.

Patients should receive standard antiviral and antibacterial prophylaxis as per institutional guidelines.

6.3.1 Fludarabine

Fludarabine is administered at a dose of 25 mg/m2 as a one-hour IV infusion once a day for 5 doses beginning on Day -7. Administration should follow institutional guidelines. Cladribine 5 mg/m2 once a day for 5 doses beginning on Day -7 may be substituted for fludarabine, administerd per institutional guidelines.

6.3.2 Cyclophosphamide

Cyclophosphamide is administered at a dose of 60 mg/kg as a 2-hour IV infusion on Days -6 and -5. Administration should follow institutional guidelines.

6.4 Donor Leukapheresis for DLI and CIML NK Cell Generation

On Day -2 or -1, peripheral blood mononuclear cells will be collected by a standard apheresis over 4-5 hours (with a target volume of at least 20 L for patients ≥ 18 years of age) from the same donor that provided the HCT graft. For related local donors, apheresis will occur on Day -1. For unrelated donors, apheresis will occur on Day -2 or -1, with the goal of processing the product into T cell and NK cells on Day -1. The dose of the DLI will be $1x10^6$ CD3+/kg the first cycle in the pediatric cohort, and follow institutional practices for the adult cohort and for subsequent cycles in the pediatric cohort.

***For individuals under the age of 18, participants must already be scheduled to undergo leukapheresis**

6.5 Separation of the Apheresis Product into DLI and NK Cell Fractions

A standard apheresis product contains ≥ 2 x 10^{10} total nucleated cells that are ≥ 50% CD3+

T cells. Thus, a standard apheresis product is expected to contain 1 x 10^{10} CD3+ T cells. A 100 kg patient (note: most patients will be smaller) would require 1 x 10^8 total CD3+ cells to provide the targeted CD3+ T cell dose (1 x 10^6 cells/kg). Our institutional SOC is to reserve additional aliquots for potential future DLIs, if needed. Product is reserved at: 1 x 10^6 cells/kg, 3 x 10^6 cells/kg and 5 x 10^6 cells/kg. Including the original infusion, a total of 10 x 10^6 CD3+ T cells will be used/saved for DLI. This quantity of cells will be available in ≤ 10% of the apheresis product. Sufficient remaining apheresis product (approximately 90% of collection) will be available for CIML NK cell generation.

If the collected apheresis product contains < 1 x 10^{10} total nucleated cells, then NK cell separation will not be performed. In this case, patients will only receive the SOC DLI. These patients will be followed for safety evaluations but will be replaced by an additional patient for evaluation of efficacy.

6.6 Standard of Care Donor Lymphocyte Infusion ($CD3^+$ T Cell Infusion)

A DLI containing donor derived T cells will be infused to the patient. Donor lymphocytes will be washed, and then processed per standard institutional DLI practices. In the pediatric cohort, the DLI will be administered fresh on Day -1 (up to 24 hours after apheresis). In the adult cohort, the DLI will be viably cryopreserved and then thawed per standard institutional practice and administerd on day 30. Extra cells will be viably cryopreserved using standard institutional DLI practices for possible future DLIs (see Section 6.4).

These donor lymphocytes will be infused without a filter or pump, slowly by gravity per standard of care. On completion of the cell infusion, the tubing will be flushed with normal saline to ensure all of the cells are infused.

Patients age 18 and older 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 DLI. Demerol 25-50 mg IV may be given for chills/rigors during the DLI. Patients aged 1-17 will receive acetaminophen 15 mg/kg, diphenhydramine 1 mg/kg, and Demerol 0.5 mg/kg IV as needed for chills/rigors during the DLI. Intravenous hydration may be administered per institutional guidelines.

6.7 CIML NK Cell Generation and Infusion

The process for CIML NK cell generation and infusion will be the same for both the first and optional second cycle of treatment. Each day of infusion will be considered Day 0.

NK cells from the remaining apheresis product will be purified using Miltenyi's CliniMACS® with CD3 depletion followed by CD56 positive selection to generate $CD3^-CD56^+$ NK cells.

The NK cell fraction ($CD3^-CD56^+$ cells) will be activated for 12-18 hours by stimulating the IL-12, IL-15, and IL-18 receptors for 12-18 hours. The cells will be washed 2 times prior to infusion on Day 0. The process of NK cell selection, activation and infusion follows the manufacture protocol in the phase I study in adults,[36] as described in the CMC section of the IND.

The CIML NK cells (dose: max capped at 20 x 10^6/kg, minimum dose allowed is 0.5 x

10^6/kg) will be infused on Day 0 without a filter or pump, by gravity. On completion of the CIML NK cell infusion, the tubing will be flushed with normal saline to ensure all of the cells are infused.

Patients age 18 and older should be pre-medicated with acetaminophen 650 mg PO and/or diphenhydramine 25 mg PO/IV within 1 hour before and 4 hours after cell infusion. Demerol 25-50 mg IV may be given for chills/rigors during cell infusion. Patients aged 1-17 will receive acetaminophen 15 mg/kg, diphenhydramine 1 mg/kg, and Demerol 0.5 mg/kg IV as needed for chills/rigors during 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 for 1 hour. Additional vitals may be performed 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.

6.8 IL-2 Administration (Adult cohort only)

IL-2 will start approximately 4 hours after the CIML NK cell infusion.

If the patient experienced a severe acute reaction during the CIML NK cell infusion, IL-2 should be held until the toxicity resolves to grade 2 or better. IL-2 may be started if the toxicity resolves to grade two or better within 72 hours of CIML NK cell infusion. Missed doses of IL-2 will not be made up.

If IL-2 cannot be started within 72 hours after the CIML NK cell infusion, no IL-2 will be given and the patient will be replaced for statistical analysis.

6.8.1 IL-2 administration

IL-2 will be administered subcutaneously at a dose of 1 million units/m^2 every other day from Day 0 to Day +12 (7 doses total). IL-2 will be dosed using actual body weight, with BSA capped at 2.2m^2 to ensure that only one vial of IL-2 is used per patient.

Patients should be pre-medicated with acetaminophen 650 mg PO (15 mg/kg with max of 650 mg PO for pediatric patients) and/or diphenhydramine 25 mg PO/IV (1 mg/kg with max of 25 mg for pediatric patients) within 1 hour before and 4 hours after IL-2 administration.

Patients should be monitored for IL-2 related targeted toxicities. Fevers, rash, and myalgias are expected, and should be treated supportively.

6.8.2 IL-2 Dose Modifications

The development of any grade 3 or higher adverse event possibly, probably or definitely related to IL-2 will result in a modification of the planned IL-2 treatment.

Grade 3 toxicity: if the toxicity resolves to grade 2 or better within 48 hours, IL-2 can be resumed at a reduced dose (0.5 million units/m^2). If the toxicity persists beyond 48 hours, or worsens or recurs after re-challenging with reduced dose, IL-2 will be permanently discontinued.

Grade 4 toxicity: permanently discontinue IL-2.

Additionally, if a patient cannot tolerate full dose IL-2 due to fevers, rash, constitutional symptoms, etc. that are possibly, probably or definitely related to IL-2 but do not meet grade 3 or 4 criteria, a dose decrease to 0.5 million units/m^2 is allowed at the discretion of the investigator.

6.9 G-CSF

Filgrastim (Neupogen) may be administered after infusion of CIML NK cells, at the discretion of the treating physician. Dosing will be determined by institutional guidelines.

6.10 Prohibited Medications

Systemic corticosteroids are strongly discouraged (10 mg or less of prednisone or equivalent doses of other steroids are allowed). In addition, other immune suppressive medications used to treat GVHD are strongly discouraged for up to 2 weeks prior to starting the salvage chemotherapy. Immunosuppression should be stopped prior to salvage chemotherapy administration, and not administered until 30 days after the infusion of the CIML NK cells. However, corticosteroids are allowed if deemed medically necessary and IV hydrocortisone (25 mg in patients ≥ 18 years old; 1 mg/kg/dose in patients aged 1 to ≤ 17 years) 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.

6.11 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.

6.12 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.

6.13 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 for any of the following reasons:

- 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

6.14 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 only be followed for GVHD and survival.

6.15 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

6.16 Study Related Adverse Event Monitoring and Stopping Rules

6.16.1 Stopping Rules

To ensure adequate follow up time for adverse event assessment, a 35-day interval will be maintained between first 3 treated patients of the pilot study cohort. All patients who receive CIML NK cells will be evaluable for toxicity.

Allogeneic NK cells have been administered to a large number of patients without early mortality, GVHD, or unexplained prolonged cytopenias; however, there exists theoretical risks for CIML NK cells that will be monitored. The following stopping events are defined to assess for these events (potentially related to CIML cells), which will be assessed while the patient remains on study.

Early mortality.
Patients will be continually assessed for unexpected early mortality (as assessed at Day +30 and Day +100 after first CIML NK cell infusion), associated with the study treatment. The expected rate of early mortality is 20% and the maximum allowable rate is 45%. The study will be suspended for review if early mortality EXCEEDS 1 in the first 2, or 2 of the first 4, or 3 of the first 8 or 4 of the first 11, 5 of first 12, 7 of 16, 8 of 19, 9 of 23, 10 of 26, 11 of 29, or 12 observed before 33 patients enrolled.

GVHD.
GVHD is an expected event following donor DLI in this clinical setting, and CIML NK cells are not expected to induce GVHD.

The expected rate of grade IV acute GVHD is 20% and the maximum allowable rate is 45%. The study will be suspended for review if grade IV GVHD EXCEEDS 1 in the first 2, or 2 of the first 4, or 3 of the first 8 or 4 of the first 11, 5 of first 12, 7 of 16, 8 of 19, 9 of 23, 10 of 26, 11 of 29, or 12 observed before 33 patients enrolled or 5 in the total of 12 patients.

Prolonged cytopenias:
Cytopenias of multiple lineages are expected following salvage chemotherapy and T cell DLI.

If any patient has persistent neutropenia at 8 weeks post CIML NK cell infusion (ANC < 500/μL persisting for > 2 weeks), patients would be evaluated with a BM biopsy to assess for AML recurrence vs. GVHD vs. loss of donor chimerism. If cytopenias were not explained by these or other causes, and possibly related to

CIML NK cells, the study would be suspended and reviewed for safety of continuation.

6.16.2 Toxicity and Response Evaluations

All patients who receive CIML NK cells are evaluable for toxicity. Patients are monitored for 100 days after each CIML NK cell infusion for toxicity. All patients who receive a CIML NK cell infusion and who undergo a Day 28 bone marrow biopsy for response assessment (after the first NK cell infusion) are evaluable for disease response.

6.16.3 Treatment Failure/Progressive Disease

CIML NK cells and T 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 or current AML at Day 28 may receive a second cycle of protocol therapy. This second cycle of protocol therapy will be considered a re-treatment, with the date of the second NK cell infusion being a second Day 0.

Patients may continue on protocol at the discretion of the treating physician, which is recommended if reduction of AML is observed. Treatment failure/progressive disease patients will be started on other anti-leukemia therapies at the discretion of the treating physician. Patients who receive other anti-leukemia therapy after Day 28 (+/- 3 days), other than therapy that enhances graft-versus-leukemia, will be considered off study and no future follow up visits are required after Day +100 other than survival visits and follow-up for GVHD.

7.0 PHARMACEUTICAL INFORMATION

7.1 CIML NK Cells

7.1.1 Preparation of CIML NK Cells and Product Release Criteria

Cell product processing will be performed at the BJH Clinical Cell Therapy Laboratory and the Siteman Cancer Center Biological Therapy Core Facility supervised by the laboratory PI. Apheresis product remaining (after removal of cells for SOC DLI) will undergo 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 as described in the CMC for IND 17629. This includes stimulation of NK cells via the IL-12, IL-15, and IL-18 receptors After incubation, the cells will be washed at least 2 times to remove the IL-12, IL-15, and IL-18 stimulating protein(s). All activated NK cells available from the donor leukapheresis, NK cell purification, and CIML processing will be infused, up to a maximum of $20.0x10^6$/kg. If the NK cell dose is less < 0.5×10^6 cells/kg then all cells (after quality control testing) should be given and the therapy plan will remain the same, as per Section 6.0. If more than 20×10^6/kg of CIML NK cells are available, then the additional cells will be stored for research and correlative studies.

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 clinical or laboratory PIs of the study.

7.2 IL-2

7.2.1 Dose Forms and Strengths

Lyophilized vials containing 22 million units

7.2.2 Availability

IL-2 will be provided to patients free of charge by the study. The PI will obtain IL-2 from commercial sources.

7.2.3 Preparation

Reconstitute each IL-2 vial with 1.2mL sterile water for injection. Prepare diluent of 5% Dextrose with 0.1% albumin by adding 0.1mL of 25% albumin to 24.9mL D5W. Add 1.2mL of the 0.1% albumin in 5% dextrose diluent to the previously reconstituted IL-2 vial to yield a final concentration of 9 MIU/mL. Individual doses should be drawn into 1mL syringes and kept refrigerated at 2-8°C. Prepared syringes should be used within 14 days of vial reconstitution.

8.0 CORRELATIVE STUDIES

8.1 Sample Collection

The volume of PB collected in sodium heparin tubes for immunomonitoring will depend upon the patient age and weight, since this study includes both adult and pediatric patients. For patients aged 18 or older, 60 mL of PB will be collected. For patients aged 1-17, the following volumes will be collected based on weight:

- <20 kg, 10 mL
- 20-60 kg, 30mL
- >60 kg, 60 mL

The appropriate amount of peripheral blood (as described above) will be collected in in 10mL sodium heparin (green top) tubes at the following time points:

- Screening
- Day -3 (±2 days) (but prior to the T cell DLI for pediatric patients)
- Day 0 (but before the CIML NK cell infusion)
- Day +7 (±2 days)
- Day +14 (±3 days)
- Day +28 (±3 days)
- Day +60 (±7 days)
- Day +100 (±15 days)
- 6 months (±1 month)
- 9 months (±1 month)
- 12 months (±1 month)

Patients who undergo a second NK cell infusion will have blood drawn at all of the time points listed above relative to the second Day 0 with the exception of screening.

An additional 10 mL of peripheral blood in serum (red top) tube(s) will be collected at the following time points:

- Screening
- Day -3 (±2 days) but prior to the T cell DLI
- Day 0 (but before the CIML NK cell infusion)
- Day +7 (±2 days)
- Day +14 (±3 days)
- Day +28 (±3 days)
- Day +60 (±7 days)
- Day +100 (±15 days)
- 6 months (±1 month)
- 9 months (±1 months)
- 12 months (±1 months)

Patients who undergo a second NK cell infusion will have blood drawn at all of the time points listed above relative to the second Day 0 with the exception of screening.

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

- Screening
- Day +7 (±2 days)
- Day +14 (±3 days)
- Day +28 (±3 days)
- Day +60 (±7 days, adult cohort only)
- Day +100 (±15 days)
- 6 months (±1 months) (adult cohort)
- At any clinically indicated BM biopsy/aspirate collection

Patients who undergo a second NK cell infusion will NOT have bone marrow collected at any of the time points listed above relative to the second Day 0. Bone marrow will be collected for research purposes only at any clinically indicated 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

    - For patients aged 1-17, the following volumes will be collected based on weight:
        - <20 kg, 10 mL
        - 20-60 kg, 30 mL
        - >60 kg, 60 mL
- 20 x 10^6 MNCs from the leukapheresis product
- 20 x 10^6 T cells after step one selection (i.e., CD3 depletion)
- 20 x 10^6 cytokine or HCW9201 pre-activated NK cells prior to washing
- Any additional cytokine or HCW9201 pre-activated NK cells not administered

8.2 Sample Handling

All samples should be 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
WU-Oncology 6th floor Southwest Tower Building, Room 634
425 South Euclid
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

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°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

- 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 or HCW9201 stimulation, NK cell receptor ligation, or leukemia cell triggering (including autologous leukemia blasts).
- NK cell gene expression / transcriptome 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 cytokines: Cytokines (IFNγ, TNFα, IL-6, IL-10, IL-13, CRP, IL-2Rα, IL-1Rα, IL-8, IL-12, IL-15) will be measured time points mentioned above in the section 8.1 (for the serum sample collection)

9.0 STUDY CALENDAR

Scheduled evaluations up to Day +7 may be performed ±2 days from the targeted date; evaluations following Day +7 may be performed ±3 days from the targeted date; evaluations following Day +28 may be performed ±7 days of the targeted date; evaluations following Day +60 may be performed ±15 days of the targeted date; evaluations following Day +100 may be performed ±1 month of the targeted date.

Patients who receive the optional second CIML NK cell infusion will undergo the protocol assessments described below, with the day of second NK cell infusion being considered a second Day 0. This includes all safety labs, AE monitoring, and sample collection for correlative research with the exception of bone marrow aspirate and core, which will be performed only as clinically indicated to assess response.

	Screening[14]	Day -3	Day 0	Day 7	Day +14	Day +21	Day +28	Day +35	Day +42	Day +60	Day +100	6, 9, 12, and 18 months[2]	24, 36, and 48 months
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/ Lansky PS	X										X	X	X
Viral panel (donor and recipient)[3]	X												
CBC, diff, plt	X	Daily[1]				X	X	X	X	X	X	X[15]	
BUN, creat, glucose, Na, K, Cl	X	Daily[1]				X	X	X	X	X	X	X[15]	
Bilirubin, AST, ALT, ALP	X	Weekly[1] and on Days +1,+2				X	X	X	X	X	X	X[15]	
Chimerism testing[12]	X						X				X		
Correlative studies[5]	X	See Section 8.0					X			X	X	X[15]	
Pregnancy test[6]	X												
BM aspirate and core	X			X[11]	X		X			X[17]	X	X[17]	
Chest x-ray or chest CT[8]	X												
PFT[8,16]	X												
ECG[8]	X												
Echo or MUGA[8]	X												
Acute GvHD assessment[9]	X				X	X	X	X	X	X	X	X[13]	
Chronic GvHD assessment[10]	X										X	X[15]	

1 – CBC is run daily from Day -3 to Day +14, until neutrophil engraftment, or as clinically appropriate. BMP is run daily or as clinically appropriate from Day -3 to Day +14 with the following exception: CMP is run on Wednesdays, Sundays, Day +1, and Day +2. Otherwise CMP is run weekly.

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 – O_2 Saturation on room air required at screening for recipient only
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 – Day 7 aspirate only (research only)
12 – X/Y FISH for sex-mismatch donor-recipients but otherwise must be STR
13 – Month 6 only
14 – Within 28 days of registration
15 – Through Month 12 only
16 – If PFTs are not performed: O_2 sats > 88% at the time of enrollment or chest X ray that indicates no evidence of active infection or a condition that markedly alters lung function as determined by the study chair and local institutional PI.
17 – Adult cohort only, BM aspirate and core at day 60 and Month 6

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.

Adverse events will be tracked from start of treatment through 100 days after the last CIML NK cell infusion, and until all toxicities are resolved or stabilized. From 100 days to 48 months, the last scheduled subject contact, targeted collection of grade 3 or 4 AEs related to study therapy that encompass hematologic AEs, infection, or GVHD will collected. Unless a SUSAR (Serious Unexpected Serious Adverse Reaction), these may be submitted with the Annual Report. 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 and surgical history CRF

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

Reporting requirements for the 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 Sponsor-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 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.

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 Medical and Surgical History Form Treatment History Form	Baseline
CD3+ T cell DLI Form	Day -1 Optional Day -1 (minors) Day 30 (for adults)
CIML NK Infusion Form	Day 0 Optional Day 0
Chimerism Form	Screening Day 28 Day 100 Optional Day 28 Optional Day 100
CBC Form	Screening Day -3 Day 0 Day 7 Day 14 Day 21 Day 28 Day 35 Day 42 Day 50 Day 60 Day 100 Optional Day -3 Optional Day 0

	Optional Day 7 Optional Day 14 Optional Day 21 Optional Day 28 Optional Day 35 Optional Day 42 Optional Day 50 Optional Day 60 Optional Day 100
Response Form	Screening Day 14 Day 28 Day 100 6 Months 9 Months 12 Months 24 Months 36 Months 48 Months Optional Day 14 Optional Day 28 Optional Day 100
Acute GvHD Form	Screening Day 14 Day 21 Day 28 Day 35 Day 42 Day 60 Day 100 6 Months Optional Day 14 Optional Day 21 Optional Day 28 Optional Day 35 Optional Day 42 Optional Day 60 Optional Day 100
Chronic GvHD Form	Screening Day 100 6 Months 9 Months 12 Months Optional Day 100
Correlative Studies Form	Screening Day -3 Day 0 Day 7 Day 14 Day 28 Day 60 Day 100 6 months 9 months 12 months Optional Day -3 Optional Day 0

	Optional Day 7 Optional Day 14 Optional Day 28 Optional Day 60 Optional Day 100
Donor Correlatives Form	Screening
Follow-Up Form	6 Months 9 Months 12 Months 24 Months 36 Months 48 Months
Death Form	Time of death
Adverse Events Form	Ongoing

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 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.

12.1.4 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.

12.2 Definitions for Safety and Efficacy Assessments

12.2.1 Neutrophil Recovery

Time to neutrophil engraftment is measured by determining the first measurement of neutrophil count ≥ 500/μL of three consecutive measurements following chemotherapy-induced nadir.

12.2.2 Platelet Recovery

Time to platelet engraftment is measured by determining the first measurement of platelet count ≥ 50,000/μL without platelet transfusion support for 7 days.

12.2.3 Acute GVHD

Incidence and severity of acute GVHD will be assessed based on the Minnesota and CIBMTR grading scale. Attempts should be made to confirm the diagnosis pathologically by biopsy of target organ(s).

12.2.4 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).

12.2.5 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).

12.2.6 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, pr 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.

12.2.7 Adverse Events

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

12.2.8 Evaluable for Toxicity

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

12.3 Response Review

At the end of the study all responses will be reviewed by an expert independent of the study.

12.4 Event-Free Survival (EFS)

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

12.5 Overall Survival (OS)

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

12.6 Leukemia Free Survival (LFS)

LFS is defined as the time from achievement of CR/CRi 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. The first report is required either 30 days after the enrollment of the 5th participant (if sooner than 6 months after study activation) or 6 months after study activation (provided at least one patient has been enrolled; if zero patients have been enrolled at the 6-month mark, the first report will be required one year after accrual opens provided at least one patient has been enrolled).

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:

- Study demographic information (local protocol number, protocol title, list of primary study team members, study sites, primary and secondary sponsors, IND/IDE status, date of most recent QA audit, and study status and history (including activation and suspension dates)
- Accrual information, including study-wide target accrual and actual accrual, anticipated and/or actual accrual end date, and accrual by year by site (if applicable)
- Subject status information presented in both cumulative format (total number of subjects who consented, enrolled, screen failed, started intervention, discontinued intervention, went off study, expired) and current format (number of subjects in screening, on intervention, in follow-up, or off study at time of report)
- Protocol objectives and the number of participants who are evaluable for each objective
- History of study (including summaries of substantive amendments, accrual suspensions and reasons, protocol exceptions, errors, and breaches of confidentiality)
- Summary of exceptions, noncompliance reports, and unanticipated problems reported to the IRB
- Early stopping rules and data describing whether the stopping rules have been met
- Separate SAE and worst grade toxicity tables, each separated by cohort
- Participant-level response and survival data by cohort
- Summary of specimen collection (percentage of participants who have had specimens collected at each required time point)
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety of participants or the ethics of the study

Standardized DSM report tables are generated out of OnCore.

14.0 STATISTICAL CONSIDERATIONS

14.1 Pediatric/Young Adult Patient Cohort

14.1.1 Study Design

This cohort represents a single institution pilot study with the primary objective of establishing the feasibility of generating and infusing CIML NK cells after standard of care DLI in AML patients who are ≥1 and <18 years of age and have relapsed after allogeneic hematopoietic cell transplant. This data will be used to demonstrate feasibility and support the development of a larger phase II study with the objective of evaluating preliminary evidence of efficacy and safety.

14.1.2 Primary Endpoints

“Generation and infusion” will be considered successful if doses above the minimum can be delivered in at least 18 of 24 patients. Target and minimum CIML doses are: maximum capped at $20x10^6$/kg with a minimum dose of $0.5x10^6$/kg

The proposed treatment will be considered safe if 24 patients complete treatment without study suspension and review due to excess early mortality, excess acute or chronic GVHD or prolonged cytopenia.

14.1.3 Secondary Endpoints

Secondary endpoints are the CR/CRi rate at day 30, leukemia-free and overall survival at day 100 and 1 year (as defined in section 2.6), and the incidence and grade or extent of acute and chronic GVHD.

14.2 Phase 2 Adult Patient Cohort

14.2.1 Study Design

For the adult cohort, an independent Simon two-stage design will be used to demonstrate improvement in the 6-month leukemia free survival to ≥40% from ≤20% expected with the use of DLI post-HCT (based on Washington University’s experience using salvage DLI therapy in a similar patient cohort). The trial will enroll 18 patients in the first stage and will continue to the second stage if at least 5 patients are leukemia free 6 months after CIML NK cell infusion. An additional 15 patients will be enrolled to bring the total to 33 patients. The trial will be considered successful if at least 11 of the 33 patients remain leukemia free 6 months after CIML NK cell infusion. The design has power = .80 at a .05 significance level, including both stages. The probability is .72 of correct early stopping if the true 6-month LFS rate is 20%.

14.2.2 Primary Endpoints

The primary objectives of the study are to 1) assess the safety of administering CIML NK cells plus DLI in regards to unexpected early mortality, unacceptable GVHD, or prolonged neutropenia and 2) determine the rate of leukemia-free survival (LFS) at 6 months post CIML NK cell infusion.

14.2.3 Secondary Endpoints

Secondary endpoints are the CR/CRi rate at Day 30, leukemia-free and overall survival at Day 100 and 1 year (as defined in section 2.6), and the incidence and grade or extent of acute and chronic GVHD.

14.3 Correlative Endpoints – Both Cohorts

CYTOF2 and flow cytometry will be used to quantify the maximal number of blood and bone marrow CIML NK cells, the maximal number of functional (e.g., IFN-γ+) CIML NK cells after ex vivo leukemia re-stimulation, functional responses of CIML NK cells to leukemia targets, number of AML blasts and other characteristics of the BM microenvironment pre-therapy and at first relapse, and the KIR genotype of donor NK cells. Additional analyses will explore the association of these values with CR/CRi rate at day 30, leukemia-free and overall survival at day 100, month 6, and 1 year, and the incidence and grade or extent of acute and chronic GVHD. Further high dimensional single cell data generated by CYTOF2 and other flow cytometry studies will be carried out to characterize cytokine profiles and to define the extent and nature of T cell reconstitution associated with CIML NK cell plus T cell DLI administration.

14.4 Analysis Plan – Both Cohorts

AEs/SAEs will be summarized by patient, type and grade as defined by the CTCAE v4.0. Rates will be described as proportions with exact binomial confidence intervals. CR/CRi rate at day 30 also will be described as a proportion with exact binomial confidence interval. Kaplan-Meier models will be used to estimate LFS and OS at day 100, month 6, and 1 year with exact pointwise confidence intervals. Acute and chronic GVHD will be described as proportions among all evaluable patients and by grade or extent with exact binomial confidence intervals. The association of response rate, time-specific LFS and OS and acute or chronic GVHD with continuous correlative endpoints (e.g., maximal number of blood and bone marrow CIML NK cells, maximal number of functional (e.g., IFN-γ+) CIML NK cells after ex vivo leukemia re-stimulation, functional responses of CIML NK cells to leukemia targets, number of AML blasts and other characteristics of the BM microenvironment pre-therapy and at first relapse) will be explored using parametric or nonparametric ANOVA, depending on the distribution of fully continuous endpoints, and ordinal tests for trend for ordered scales with unequal intervals. High dimensional single cell data from CYTOF2 will be used to characterize cytokine profiles and T cell reconstitution. The analysis will be primarily visual, clustering and building trees using PCA or random forest-based tree building software such as SPADE or ViSNE.

Analyses will be carried out in SAS 9.4/STAT 14.1 or R 3.3.2 or higher.

14.5 Study Power

For the pediatric/young adult patient cohort, slow enrollment from a comparatively small study population is anticipated. All analyses are considered exploratory, and no formal sample size calculation has been carried out.

For the adult cohort, the Simon two-stage design has power = .80 at a .05 significance level, including both stages. Up to 33 patients may be enrolled with an anticipated enrollment rate of 1-2 patients per month.

14.6 Toxicity and Adverse Event Monitoring

Toxicities to be monitored for early stopping rules are death on or before day +30, acute GVHD grade IV and prolonged neutropenia as described in Section 6.14.1. Each case of prolonged neutropenia will cause the study to be suspended for review. Two continuous toxicity monitoring rules will be used to monitor early mortality and acute GVHD grade IV.

In both cases the expected rate is 20% and the maximum allowable rate is 45%. The study will be suspended for review if each type of toxicity EXCEEDS 1 in the first 2, or 2 of the first 4, or 3 of the first 8 or 4 of the first 11, 5 of first 12, 7 of 16, 8 of 19, 9 of 23, 10 of 26, 11 of 29, or 12 observed before 33 patients enrolled. In each case the significance level is .10 and power = .90. Each of these rules has probability = .10 of stopping when the true rate is 20% (incorrect early stopping) and probability = .67 of stopping if the true rate is as high as 45% (correct early stopping). The maximum number of early deaths or cases of acute GVHD grade IV that can occur without suspension for review is 5 in 12 patients and 12 in 33 patients.

15.0 REFERENCES

1. Estey E, Döhner H. Acute myeloid leukaemia. *Lancet*. 2006;368(9550):1894–907.
2. Farag SS, Archer KJ, Mrózek K, et al. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Blood*. 2006;108(1):63–73.
3. Estey E. Acute myeloid leukemia and myelodysplastic syndromes in older patients. *J Clin Oncol*. 2007;25(14):1908–15.
4. Mrózek K, Marcucci G, Nicolet D, et al. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. *J. Clin. Oncol.* 2012;30(36):4515–23.
5. Cassileth PA, Harrington DP, Hines JD, et al. Maintenance chemotherapy prolongs remission duration in adult acute nonlymphocytic leukemia. *J. Clin. Oncol.* 1988;6(4):583–7.
6. Ghobadi A, Choi J, Fiala MA, et al. Phase I study of azacitidine following donor lymphocyte infusion for relapsed acute myeloid leukemia post allogeneic stem cell transplantation. *Leuk. Res.* 2016;49:1–6.
7. Savani BN, Mielke S, Reddy N, et al. Management of relapse after allo-SCT for AML and the role of second transplantation. *Bone Marrow Transplant.* 2009;44(12):769–777.
8. Caligiuri M a. Human natural killer cells. *Blood*. 2008;112(3):461–9.
9. Pahl J, Cerwenka A. Tricking the balance: NK cells in anti-cancer immunity. *Immunobiology*. 2015;10.1016:/j.imbio.2015.07.012 [Epub ahead of print].
10. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. *Nat Immunol*. 2008;9(5):503–10.
11. Lanier LL. Up on the tightrope: natural killer cell activation and inhibition. *Nat Immunol*. 2008;9(5):495–502.
12. Jonsson HA, Yokoyama WM. Natural killer cell tolerance licensing and other mechanisms. *Adv Immunol*. 2009;101(8):27–79.
13. Joncker NT, Raulet DH. Regulation of NK cell responsiveness to achieve self-tolerance and maximal responses to diseased target cells. *Immunol Rev*. 2008;224:85–97.
14. Orr MT, Lanier LL. Natural killer cell education and tolerance. *Cell*. 2010;142(6):847–56.
15. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science*. 2002;295(5562):2097–100.
16. Farag SS, Fehniger T a, Ruggeri L, Velardi A, Caligiuri M a. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. *Blood*. 2002;100(6):1935–47.
17. Velardi A, Ruggeri L, Mancusi A, Aversa F, Christiansen FT. Natural killer cell allorecognition of missing self in allogeneic hematopoietic transplantation: a tool for immunotherapy of leukemia. *Curr Opin Immunol*. 2009;21(5):525–30.
18. Olson JA, Leveson-Gower DB, Gill S, et al. NK cells mediate reduction of GVHD by inhibiting activated, alloreactive T cells while retaining GVT effects. *Blood*. 2010;115(21):4293–301.
19. Cooley S, Trachtenberg E, Bergemann TL, et al. Donors with group B KIR haplotypes improve relapse-free survival after unrelated hematopoietic cell transplantation for acute myelogenous leukemia. *Blood*. 2009;113(3):726–32.
20. Venstrom JM, Pittari G, Gooley T a, et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *N Engl J Med*. 2012;367(9):805–16.
21. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in

vivo expansion of human haploidentical NK cells in patients with cancer. *Blood*. 2005;105(8):3051–7.
22. Rubnitz JE, Inaba H, Ribeiro RC, et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. *J. Clin. Oncol.* 2010;28(6):955–959.
23. Curti A, Ruggeri L, D'Addio A, et al. Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high-risk acute myeloid leukemia patients. *Blood*. 2011;118(12):3273–9.
24. Bachanova V, Cooley S, Defor TE, et al. Clearance of acute myeloid leukemia by haploidentical natural killer cells is improved using IL-2 diphtheria toxin fusion protein. *Blood*. 2014;123(25):3855–63.
25. Min-Oo G, Kamimura Y, Hendricks DW, Nabekura T, Lanier LL. Natural killer cells: walking three paths down memory lane. *Trends Immunol*. 2013;34(6):251–8.
26. Rölle A, Pollmann J, Cerwenka A. Memory of Infections: An Emerging Role for Natural Killer Cells. *PLoS Pathog.* 2013;9(9):1–3.
27. O'Leary JG, Goodarzi M, Drayton DL, Yu H, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol*. 2006;7(5):507–16.
28. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature*. 2009;457(7229):557–61.
29. Cooper MA, Elliott JM, Keyel PA, et al. Cytokine-induced memory-like natural killer cells. *Proc Natl Acad Sci USA*. 2009;106(6):1915–9.
30. Keppel M, Yang L, Cooper M. Murine NK cell intrinsic cytokine-induced memory-like responses are maintained following homeostatic proliferation. *J Immumol*. 2013;190(9):4754–62.
31. Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. *J Exp Med*. 2012;209(13):2351–2365.
32. Romee R, Schneider SE, Leong JW, et al. Cytokine activation induces human memory-like NK cells. *Blood*. 2012;120(24):4751–4760.
33. Berrien-Elliott MM, Wagner JA, Fehniger TA. Human Cytokine-Induced Memory-Like Natural Killer Cells. *J. Innate Immun.* 2015;7(6):563–71.
34. Romee R, Maximillian R, Berrien-Elliott MM, et al. Human Cytokine-Induced Memory-like NK Cells Exhibit in Vivo Anti-Leukemia Activity in Xenografted NSG Mice and in Patients with Acute Myeloid Leukemia (AML). *Blood*. 2015;126(23):101.
35. Dudley ME, Yang JC, Sherry R, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol*. 2008;26(32):5233–9.
36. Romee R, Rosario M, Berrien-Elliott MM, et al. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. *Sci. Transl. Med.* 2016;8(357):357:doi: 10.1126/scitranslmed.aaf2341.
37. Horowitz A, Strauss-Albee DM, Leipold M, et al. Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry. *Sci. Transl. Med.* 2013;5(208):208ra145.
38. Strauss-Albee DM, Fukuyama J, Liang EC, et al. Human NK cell repertoire diversity reflects immune experience and correlates with viral susceptibility. *Sci. Transl. Med.* 2015;7(297ra115):1–11.

APPENDIX A: Karnofsky / Lansky Performance Status

Table 1. Karnofsky/Lansky Scale

Karnofsky Scale (recipient age ≥ 16 years)		Lansky Scale (recipient age <16 years)	
Able to carry on normal activity; no special care is needed		**Able to carry on normal activity; no special care is needed**	
100	Normal, no complaints, no evidence of disease	**100**	Fully active
90	Able to carry on normal activity	**90**	Minor restriction in physically strenuous play
80	Normal activity with effort	**80**	Restricted in strenuous play, tires more easily, otherwise active
Unable to work, able to live at home cares for most personal needs, a varying amount of assistance is needed		**Mild to moderate restriction**	
70	Cares for self, unable to carry on normal activity or to do active work	**70**	Both greater restrictions of, and less time spent in active play
60	Requires occasional assistance but is able to care for most needs	**60**	Ambulatory up to 50% of time, limited active play with assistance/supervision
50	Requires considerable assistance and frequent medical care	**50**	Considerable assistance required for any active play, fully able to engage in quiet play
Unable to care for self, requires equivalent of institutional or hospital care, disease may be progressing rapidly		**Moderate to severe restriction**	
40	Disabled, requires special care and assistance	**40**	Able to initiate quite activities
30	Severely disabled, hospitalization indicated, although death not imminent	**30**	Needs considerable assistance for quiet activity
20	Very sick, hospitalization necessary	**20**	Limited to very passive activity initiated by others (e.g., TV)
10	Moribund, fatal process progressing rapidly	**10**	Completely disabled, not even passive play

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: Acute GvHD Assessment – CIBMTR Grading Scale

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

CHRONIC GRAFT-VERSUS-HOST DISEASE (GVHD) ASSESSMENT AND SCORING FORM

Name: _____ Date of birth: _____ Assessment Date _____

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG; KPS or LPS 100%	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Sclerotic features ☐ Keratosis pilaris ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Erythroderma ☐ Hair involvement ☐ Nail involvement ☐ Poikiloderma ☐ Pruritus	☐ No BSA involved ☐ No sclerotic features	☐ 1%-18% BSA involved	☐ 19%-50% BSA involved ☐ Superficial sclerotic features (pinchable)	☐ >50% BSA involved ☐ Deep sclerosis, hidebound, impaired mobility, ulceration
☐ *Abnormality present but NOT thought to represent GVHD*			**List other causes:** _____	
MOUTH Lichen planus-like features present ☐ Yes ☐ No	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms **with** disease signs with partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitations of oral intake
☐ *Abnormality present but NOT thought to represent GVHD*			**List other causes:** _____	
EYES Keratoconjunctivitis sicca confirm by optho ☐ Yes ☐ No	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3x per day or punctual plugs) **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) OR unable to work because of ocular symptoms OR loss of vision caused by keratoconjunctivitis sicca
☐ *Abnormality present but NOT thought to represent GVHD*			**List other causes:** _____	
GI TRACT	☐ No symptoms	☐ Symptoms such as nausea, vomiting, anorexia, dysphagia, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%) or moderate diarrahea	☐ Symptoms associated with significant weight loss >15% requires nutritional supplement for most caloric needs or esophageal dilation or severe diarrahea interfering with daily living
☐ *Abnormality present but NOT thought to represent GVHD*			**List other causes:** _____	
LIVER	☐ 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
☐ *Abnormality present but NOT thought to represent GVHD*			**List other causes:** _____	
LUNGS ☐ PFTS not done	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground	☐ Severe symptoms (shortness of breath at rest; requiring O_2
FEV1 ☐	☐ FEV1 ≥ 80%	☐ FEV1 60-79%	☐ FEV1 40-59%	☐ FEV1 ≤39%
☐ *Abnormality present but NOT thought to represent GVHD*			**List other causes:** _____	

NAME:

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

JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM **AND** significant limitations of ADL (unable to tie shoes, button shirts, dress self, etc.)

☐ *Abnormality present but NOT thought to represent GVHD* **List other causes:** _____

GENITAL TRACT	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

☐ *Abnormality present but NOT thought to represent GVHD* **List other causes:** _____

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

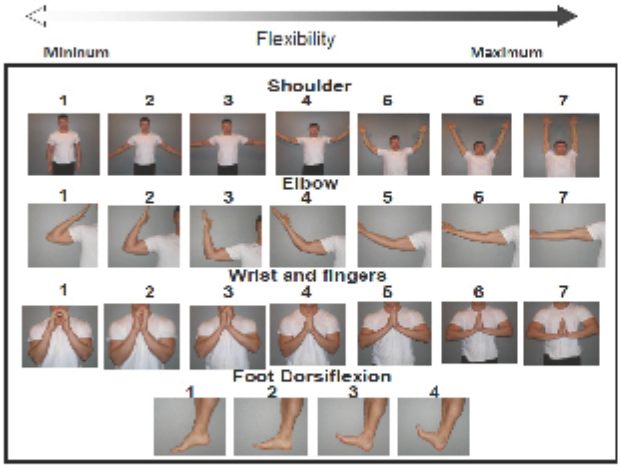
☐ Weight loss	☐ Bronchiolitis obliterans	☐ Bronchiolitis obliterans with organizing pneumonia	
☐ Platelets < 100K	☐ Pericardial Effusion	☐ Pleural Effusion(s)	☐ Ascites (serositis)
☐ Nephrotic syndrome	☐ Peripheral Neuropathy	☐ Myasthenius Gravis	☐ Polymyositis
☐ Malabsorption			
☐ Eosinophilia >500/microliter	☐ Other: _____		

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

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

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

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



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

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 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 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 suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting
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		

Expedited Reporting Timelines			
Event	**HRPO**	**QASMC**	**FDA**
	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.		