A phase 1/2 study of cytokine-induced memory-like NK cells in patients with AML or MDS

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Principal Investigator Signature Page

Principal Investigator (printed):

Name of Institution:

PI Signature *Date*

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

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SCHEMA

Eligible Patients

Inclusion Criteria:

- **Phase I:** AML patients with refractory disease who have not achieved a complete remission (CR) after induction therapy (primary induction failure), or relapsed disease after obtaining a complete remission.
  OR
  High-risk (by ELN criteria) AML in complete remission (CR) and has either refused hematopoietic stem cell transplantation OR is currently not eligible for hematopoietic stem cell transplantation OR for whom hematopoietic stem cell transplantation is being reserved for later relapse.
  OR
  MDS with excess blasts (>5%) and progressive disease at any time after initiation of DNA hypomethylator treatment OR failure to achieve complete or partial response or hematological improvement OR intolerance
- **Phase II:** AML patients with refractory disease who have not achieved a CR after induction therapy or relapsed AML Favorable-risk CBF mutated AML and APL patients will be excluded.
- **Pediatric cohort:** AML patients with refractory disease who have not achieved a complete remission (CR) after induction therapy (primary induction failure), or relapsed disease after obtaining a complete remission.
- Age $\geq$ 18 years old (Phase I and II)
- Age 1-17 years old (Pediatric cohort)
- Available related haplo-identical NK cell donor
- Karnofsky performance status $\geq$ 50 %
- Adequate organ function:
  - Total bilirubin $\leq$ 2 mg/dL
  - AST(SGOT)/ALT(SGPT) $\leq$ 3.0 x ULN
  - Creatinine WNL or CrCl $\geq$ 50 mL/min/1.73 m^2
  - Oxygen saturation ≥90% on room air
  - Ejection fraction ≥35%

Exclusion Criteria:

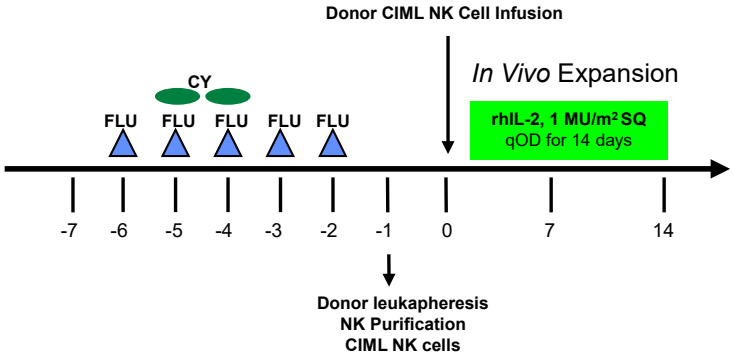
- Patients who relapse after allogeneic transplantation are not eligible.
- Circulating blast count $\geq$30,000/µL (despite hydroxyurea).
- Uncontrolled bacterial or viral infections, or known HIV, Hepatitis B or C infection.
- New progressive pulmonary infiltrates
- Pregnant and/or breastfeeding.
- Additional exclusion for phase II only:
  - Patients who have isolated extramedullary relapse
  - Patients who have been treated with more than one salvage regimen for relapsed or refractory AML

Dose Levels in Phase I:

CIML NK Cell Dose Escalation Schedule	
Dose Level	**CIML NK cell dose**
Level 1 (starting dose)	0.5 x 10^6/kg
Level 2	1.0 x 10^6/kg
Level 3	Maximum NK cells available*

*All CIML NK cells available from the donor leukapheresis, NK cell purification, and CIML process will be infused, up to a maximum of 10x10^6/kg.

Recipient Treatment Plan in Phase I:



Preparative Regimen:
Fludarabine 25 mg/m^2x 5
Cyclophosphamide 60 mg/kg x 2

CIML NK Cell Product:
$CD3^-CD56^+$ selected,
IL-12+IL-15+IL-18 stimulated,
haploidentical donor NK cells

Dose Levels in Lead-in Cohort, Phase II, and Pediatric Cohort:
Maximum tolerated / tested dose (MTD) in phase I will be used in the Lead-in Cohort and phase II part of this study. For Pediatric cohort, all CIML NK cells available will be infused, up to max dose of 10 x 10^6/kg.

Recipient Treatment Plan in Pediatric Cohort: The treatment in the pediatric cohort will follow the plan for Phase I.

Recipient Treatment Plan in Lead-in Cohort and Phase II/ALT-803:
ALT-803 (10 mcg/kg SQ on Days 0, 5) will replace IL-2.. In the Lead-in Cohort three patients will receive maximal tolerated / tested (MTD) dose of the CIML NK cells (derived from phase I) and enrollment on the phase II part of the study will start only if ***none*** of the three patients develop any unexpected grade ≥3 adverse events related to the use of ALT-803 or any of stopping rules listed in Section 5.3.4 as assessed by Day +35. Dose de-escalation plan for ALT-803 is listed in Section 5.3.6.

Recipient Treatment Plan in Phase II/IL-2:
The treatment plan will follow the plan for Phase I.

Donor Cell Collection Plan and Generation of CIML NK Cells:
The haploidentical donor identified by HLA matching of the immediate family members (parents, siblings, and children) will undergo non-mobilized leukapheresis on Day -1. Peripheral blood mononuclear cells (PBMCs) will be collected using standard collection techniques. The apheresis product will be T-cell depleted (CD3 depletion) and CD56 positive selected using Miltenyi

CliniMACS®. This NK cell product will be pre-activated for 12-18 hours with a combination of rhIL-12 (10 ng/mL), rhIL-15 (50 ng/mL), and rhIL-18 (50 ng/mL) under GMP conditions. The cells will be washed 2 times prior to infusion on Day 0.

Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ASH	American Society of Hematology
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BM	Bone marrow
BMT	Bone marrow transplant
BUN	Blood urea nitrogen
CALGB	Cancer and Leukemia Group B
CBC	Complete blood count
CFR	Code of Federal Regulations
CIML NK cells	Cytokine Induced Memory-like NK cells
CNS	Central nervous system
CR	Complete remission
CRc	Cytogenetic complete remission
CRi	Complete remission incomplete
CRm	Morphologic complete remission
CRF	Case report form
CST	Central standard time
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLTs	Dose Limiting Toxicities
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
DSMC	Data Safety Monitoring Committee
EC	Ethics Committee
ECG (or EKG)	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
EE	Efficacy-Evaluable
EFS	Event free survival
FAB	French-American-British classification
FDA	Food and Drug Administration
FISH	fluorescent in situ hybridization
GCP	Good Clinical Practice
G-CSF	Granulocyte colony stimulating factor, filgrastim (Neupogen)
GM-CSF	Granulocyte/macrophage colony stimulating factor, sargramostim, (Leukine, Prokine)
HDACs	Histone deacetylases
HHS	Department of Health and Human Services’
HI	Hematologic improvement

HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
ICH	International Conference on Harmonization
IFN	Interferon
IL	Interleukin
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	Intravenous (i.v.)
IVRS	Interactive Voice Response System
IWG	International Working Group
LDH	Lactate dehydrogenase
LPS	lipopolysaccharide
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDS	Myelodysplastic syndrome
MTD	Maximum tolerated dose
NCCN	National Cancer Center Network
NCI	National Cancer Institute
NIH	National Institutes of Health
NK	Natural killer cell
OHRP	Office of Human Research Protections
ORR	Overall response rate
OS	Overall survival
PB	Peripheral blood
PBMC	Peripheral blood mononuclear cell
PD	Progressive disease
PI	Principal investigator
PR	Partial response (Partial remission)
PSA	Prostate-specific antigen
QASMC	Quality Assurance and Safety Monitoring Committee
RAEB	Refractory anemia with excess blasts
RAEB-t	Refractory anemia with excess blasts in transformation
RARS	Refractory anemia with ringed sideroblasts
RBC	Red blood cell (count)
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RFS	Relapse free survival
RR	Response rate
SAE	Serious adverse event
SCC	Siteman Cancer Center
SCT	Stem cell transplant
SD	Stable disease
TF	Treatment failure
TPP	Therapeutics Product Programme

TRAIL	TNF-related apoptosis-inducing ligand
TSH	Thyroid stimulating hormone
TTP	Time to progression
UPN	Unique patient number
US	Ultrasound
WBC	White blood cell (count)
FCBP	Females of child bearing potential
WHO	World Health Organization

Table of Contents

1.0 BACKGROUND AND RATIONALE

1.1 Acute Myeloid Leukemia (AML) and Myelodysplastic Syndrome (MDS)

Acute myeloid leukemia (AML) is one of the most common hematologic malignancies, with an estimated prevalence of 3.8 cases per 100,000.[1] AML is primarily a disease of older adults, with two-thirds of the new cases occurring in patients ≥ 60 years old.[2] In older AML patients, the 5 year disease-free survival (DFS) is extremely poor at 10-20%.[3,4,5,6] These suboptimal outcomes in older AML patients are related to a combination of unfavorable biologic characteristics of the leukemia, comorbidities, poor performance status, or organ dysfunction that limits aggressive treatment options.[5] In addition to age, genetic alterations in the leukemic cells strongly influence the outcomes of AML patients with current treatment regimens.[5] The standard chemotherapy-based treatment regimens for AML patients involve an initial 7+3 (ara-C for 7 days and 3 days of idarubicin or daunorubicin) to achieve a complete remission (CR). While up to 75% of AML patients under the age of 60 years achieve CR following intensive treatment regimens, the CR rates are only in the range of 40-50% in older AML patients with the intensive induction regimens.[5] Similarly, only around 50% of the young and 39% of the elderly AML patients in poor prognostic groups are able to achieve a CR with the current intensive induction regimens.[5] The median overall survival (OS) for patients not achieving a CR is <1 year, even with best palliative treatment. Nearly all AML patients treated only with induction chemotherapy relapse.[7] For younger patients, standard post-induction consolidation chemotherapy includes 3-4 cycles of high dose cytarabine (good or intermediate risk) or an allogeneic stem cell transplant (poor risk or intermediate with a sibling donor). Questions remain about the best approach for consolidation therapy in the older AML patients, which is required to for long-term DFS. Unfortunately, a fair number of patients who go on to receive consolidation chemotherapy ultimately relapse. The prognosis of the patients who relapse after the consolidation therapies is extremely poor, especially if the relapse occurs in first 6 months of the consolidation therapy. Re-induction followed by allogeneic stem cell transplantation is the most common regimen used for refractory or relapsed AML patients. However, many of these patients are not eligible for such an intensive treatment. For those patients with refractory or relapsed AML ineligible for an allogeneic transplant, no approved or standard treatments are available, and therefore there is an urgent need for the development of novel therapies/treatments for this patient population.

The majority of AML patients who initially attain a CR will eventually relapse.[8] The prognosis of the relapsed AML patients also remains poor unless the AML is defined by core-binding factor translocations.[8] While allogeneic HCT provides a potentially curative option for the relapsed AML patients, the post HCT survival is extremely poor unless the patients are able to achieve complete remission prior to undergoing transplantation.[9,10] Commonly used salvage regimens aimed at achieving a CR in relapsed patients include CLAG (cladribine, cytarabine and G-CSF), MEC (mitoxantrone, etoposide and cytarabine) and FLAG-IDA (fludarabine, cytarabine, G-CSF and idarubicin) with remission rates in the range of 30-35% in patients who relapse ≤18 months after their first CR.[11,12,13,14,15] This underscores an unmet need for developing more effective therapeutic options aimed at achieving better CR rates in relapsed AML patients.

Myeloid dysplastic syndromes (MDS) are clonal neoplastic disorders involving one or more myeloid lineage cells. MDS patients without treatment often progress to acute myeloid leukemia and currently hypomethylating agents including azacytidine and decitabine are the only class of medications which have demonstrated some clinical

efficacy in these patients (lenalidomide is effective in a small subset of MDS patients with a specific cytogenetic abnormality, 5q-). However, MDS patients who have failed and/or progressed while on hypomethylating agents have very limited treatment options. Even with hematopoietic cell transplantation, the outcomes are suboptimal in MDS patients with pre-transplant high blast counts, underscoring an urgent need for developing more effective treatment options for these patients. NK cells have activity against myeloid malignant cells and therefore use of CIML NK cells, which have demonstrated even more potent activity against leukemia, are an attractive option to explore in MDS patients who have failed and/or progressed on hypomethylating agents. They would also be an attractive option for MDS patients with high blast percentage and serve as a bridge to hematopoietic cell transplantation.

This study evaluates a novel CIML NK cell therapy for AML or MDS patients in the phase I portion and patients with relapsed AML in the phase II portion.

1.2 Therapeutic Use of HLA-Haploidentical NK Cells

Natural killer (NK) cells are lymphocytes important for host defense against infection and malignant tumor cells.[16,17] 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.[18] Their effector function is 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.[18,19] Self-tolerance is mediated by inhibitory killer immunoglobulin-like (KIR) and other receptors which transmit signals that interrupt the cytotoxic pathway upon binding of their cognate class I HLA ligands. The loss of 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. NK cells, which can be easily isolated in high quantity from donor leukapheresis products, do not cause but may protect against graft vs. host disease (GVHD).[20,21] Thus NK cells are an attractive cell population to exploit for anti-tumor immunotherapy. Using haplo-identical donors to enhance the chances of KIR-ligand mismatch, several clinical studies have been successfully completed to date using NK cells for patients with hematologic and non-hematologic malignancies.[22,23,24] None of these studies have reported GVHD or any other major toxicity from the NK cells transferred adoptively into these patients. Further, some of these early NK cell-based adoptive studies have reported leukemia clearance and complete remissions. Miller et al reported CR induction in 5 out of 19 refractory AML patients.[22] Similarly, in another study none of the AML patients who were adoptively transferred with haplo-identical NK cells relapsed with a median follow-up of 964 days, and the 2-year event-free survival estimate was 100%.[23]

1.3 Study Rationale

1.3.1 Rationale for the use of CIML NK cells

Although the results of the initial studies involving adoptive transfer of the HLA-haploidentical NK cells into patients with AML resulted in CRs, approaches using conventional NK cells appear limited by short duration CRs occurring in a minority of AML patients. We have recently identified cytokine-induced memory-like (CIML) NK cells, which exhibit enhanced responses against leukemia target cells and upon cytokine restimulation[25,26]. Further, murine CIML NK cells exhibit similar

functional properties[25] and have demonstrated markedly enhanced tumor control in a pre-clinical mouse model involving lymphoma and melanoma cell lines.[27] These findings are promising and can be translated to the clinic. We propose using established NK cell adoptive immunotherapy platforms, and in addition an overnight pre-activation with rhIL-12, rhIL-18, and rhIL-15 to generate CIML NK cells. Based on the above in vitro and pre-clinical mouse model findings, we hypothesize that upon adoptive transfer these CIML NK cells will maintain enhanced effector functions and will also have an increased proliferation and more durable persistence in the recipient.

We have also seen an increased expression of CD25 resulting in a high affinity IL-2R$\alpha\beta\gamma$ on the human CIML NK cells (unpublished data). This increased CD25 makes the CIML NK cells amenable to further expansion and activation with low dose IL-2. Early studies utilizing daily IL-2 administration for 14 days found high rates of constitutional symptoms; therefore, every other day administration was adopted and will be used in this study.[22] Further, IL-2 at a dose of $1x10^6$ IU was well tolerated in a recently published study and therefore all the patients in this study will be treated at the same dose in phase I.[20]

Recently, we have performed and published additional pre-clinical studies that support the use of CIML NK cells in AML therapy. This study demonstrated that CIML NK cells have 1) enhanced IFN-γ and TNF responses to primary AML blasts, 2) enhanced expression of granzyme B and killing of leukemia targets, 3) respond to primary AML blasts regardless of inhibitory KIR engagement, 4) improved in vivo control of human leukemia engrafted in to immunodeficient NSG mice, compared to conventional NK cells.[28] In addition, other laboratories have recently shown enhanced anti-tumor control by murine and human IL-12/15/18 pre-activated NK cells.[29]

Based on these properties, we hypothesized that the adoptive transfer of allogeneic CIML NK cells into AML patients would have a potent GvL effect and induce remission in the patients with otherwise extremely poor prognosis. As toxicity of CIML NK infusion may be dose dependent, we proposed to test three dose levels of CIML NK cells: 0.5×10^6 cells/kg, 1.0×10^6 cells/kg, and maximum cell dose (between 2.0×10^6 cells/kg and 10×10^6 cells/kg). The starting dose level was chosen as approximately 1/10 to 1/20 of prior conventional NK cell studies, due to initial caution regarding the potential for cytokine release syndrome (CRS). No CRS has been observed in the dose-escalation cohort.

In the phase II and pediatric patients, we intend to use maximal tolerated or tested (MT/TD) CIML NK cell dose as determined from the phase I part of this study.

1.3.2 Lymphodepleting Preparative Regimen

Most of the current adoptive immunotherapy trials involve a lymphodepleting condition prior to the infusion of the NK cells. [22,21] This is based on the experience from multiple studies in the past involving the use of lymphokine-activated killer (LAK) cells (prepared by ex vivo stimulation of peripheral blood mononuclear with IL-2) to treat immune-sensitive malignancies like melanoma and renal cell cancer. In these studies, limited clinical benefit was observed due to the poor expansion of the adoptively transferred cells. We now understand that there is limited

expansion as host lymphocytes compete with infused cells for access to cytokines and other growth factors critically needed by the adoptively transferred lymphocytes. Based on these observations, successful expansion of the adoptively transferred cell requires adequate prior lymphodepletion in order to 'clear space' for the infused cells. In our study, we chose a lymphodepleting regimen consisting of 5 doses of fludarabine at 25mg/m^2 and 2 doses of cyclophosphamide at 60mg/kg for adults. 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. For the Pediatric cohort, lymphodepleting therapy will be 4 doses of fludarabine at 30 mg/m^2 and 2 doses of cyclophosphamide at 500 mg/m^2. This regimen has been used successfully prior to infusion of chimeric antigen receptor T cells to treat pediatric patients with relapsed leukemia.

1.3.3 Rationale for Replacing IL-2 with ALT-803 in the Phase II Part of the Trial

The rationale for using IL-2 and ALT-803 is to support the donor derived NK cells in vivo after adoptive transfer. Interleukin-15 (IL-15) is a cytokine and growth factor capable of expanding activated T cells and NK cells. By broad consensus, the NCI Immunotherapy Workshop (2007) ranked IL-15 as the #1 agent with "high potential for immunotherapy".[30] Recombinant human IL-15 (rhIL-15) has a short half-life and therefore needs to be giving as a continuous IV infusion or by daily subcutaneous injections.[31] Another potential hurdle in incorporating rhIL-15 into clinical protocols is the limited availability of this cytokine as currently there are no commercial sources of clinical grade IL-15 available.

ALT-803 is a novel drug developed by Altor BioScience Corporation (Altor, Miramar, FL), to overcome many of the biologic, regulatory, and commercial limitations of unmodified rhIL-15. Under physiologic circumstances, IL-15 and IL-15 receptor-alpha (IL-15Rα) are coordinately expressed by antigen-presenting cells (i.e., monocytes and dendritic cells).[32] To induce a signal, IL-15 bound to IL-15Rα is presented 'in trans' to neighboring NK cells or CD8+ T cells expressing only the IL-2/15$\beta\gamma$ receptor, resulting in multiple signals that trigger survival, proliferation, metabolic fitness, and cytotoxic capacity. At the immunologic synapse, IL15 trans-presentation appears to be a dominant mechanism for IL-15 action in vivo, providing tight physiologic control over the functions of IL-15 under homeostatic conditions and in response to immune stimuli.[33] ALT-803 is a novel recombinant human super-agonist IL-15 complex (i.e., IL-15N72D:IL-15RαSu/IgG1 Fc complex) with a prolonged serum half-life in preclinical animal models. In addition, ALT-803 contains a novel IL-15 mutein with a single substituted amino acid (rhIL-15N72D) that has a 4-fold increase in biologic activity greater than wild-type IL-15 (IL-15 wt).[34] The IL- 15:IL-15R complex increases activity at lower concentrations, and the fusion with Ig-G1 Fc increases serum half-life, providing more ideal pharmacokinetics with prolonged cytokine function.[35]

Because of the availability, ease of delivery (regular subcutaneous injections), prolonged half-life and potentially higher efficacy, ALT-803 is currently being studied in several ongoing clinical trials including three studies at our center including in patients that have relapsed after an allogeneic HCT (Clinicatrial.gov #

NCT01885897, NCT02099539, NCT02384954). In the study NCT01885897 where ALT-803 is used in patients relapsed after allo-HCT at 10 mcg/kg SQ, no serious adverse events, including GVHD, have been observed.

Most of the previously published adoptive NK cell protocols (including our ongoing non-transplant CIML NK cell study) have used recombinant human IL-2 (rhIL-2) to support NK cell expansion and persistence in vivo. Most NK cells express the intermediate affinity IL-2Rβ/γc heterodimer that provides the intracellular signaling domains and is activated by intermediate dose IL-2 or intermediate dose IL-15. Low dose IL-2 supports NK cells by binding to the heterotrimeric high affinity IL-2R comprised on IL-2Rα (CD25), IL-2/15β, γc: IL-2Rαβγ. Our pre-clinical data demonstrated that CD25, and hence the IL-2Rαβγ is induced on CIML NK cells, providing the rationale for utilizing LD IL-2 to support CIML NK cell post-infusion. Correlative immunomonitoring has revealed that after in vivo transfer, CD25 is down-regulated within 1-3 days post transfer, indicating that IL-15 would be ideal for ongoing support in patients after CIML transfer. In addition, low dose IL-2 also expands patient CD25+ regulatory T cells. We therefore changed low dose rhIL-2 to ALT-803 at 10mcg/kg on Days 0 and 5 (subcutaneous injections) for 2 doses starting at the day of CIML NK cell infusion. This dose of ALT-803 has been well tolerated by patients who have relapsed following allogeneic HCT.[36] We expect a similar adverse event profile as rhIL-2, and potentially improved NK cell expansion and persistence. IL-2, IL-15, and ALT-803 all signal through the same shared IL-2/15Rβ/γc-chain heterodimer receptor; however, ALT-803 is simply replacing IL-2 in our Lead-in Cohort and phase II part of the protocol to potentially provide better support for the growth and expansion of the adoptively transferred CIML NK cells. ALT-803 will not be used in the Pediatric portion of this trial.

1.3.4 Rationale for Returning to IL-2 in the Phase II Part of the Trial

Based on the data indicating that ALT-803/IL-15 result in more modulation of the NK cells in vivo, we performed a lead in cohort with ALT-803 replacing IL-2 at a dose of 10 mcg/kg SQ administered q5 days starting on the date of NK cell infusion. The first two patients treated in the ALT-803 lead in cohort experienced a set of symptoms consistent with cytokine release syndrome (CRS) including fevers, elevated markers of inflammation between days 10-14 after ML NK cell infusion. Correlative science revealed that recipient CD8 T cells were expanding during this period with an activated phenotype (CD38+HLA-DR+), and their expected allo-rejection was the likely source of the CRS (Figure 1). With the concern that prolonged ALT-803 may enhance recipient CD8 T cell recovery based on the first-in-human ALT-803 trial, we reduced the number of ALT-803 doses from 4 q5 days to 2 q5 days. Despite this change, we continued to see recipient CD8 T cell activation at day 10 post NK cell transfer and reduced the ALT-803 dose to a single dose on the day of infusion. Based on the CD8 T cell activation and expansion, we performed PK analyses and observed ALT-803 serum levels of approximately 1000 pg/mL persisting 5 days after the SQ dose. During this period, no patient (0 of 9) that received ALT-803 support following ML NK cell transfer obtained a CR or CRi, in contrast to 4 of 5 patients with active AML treated with similar number of ML NK cells in the rhIL-2 dose expansion of the Phase 1 part of the study.

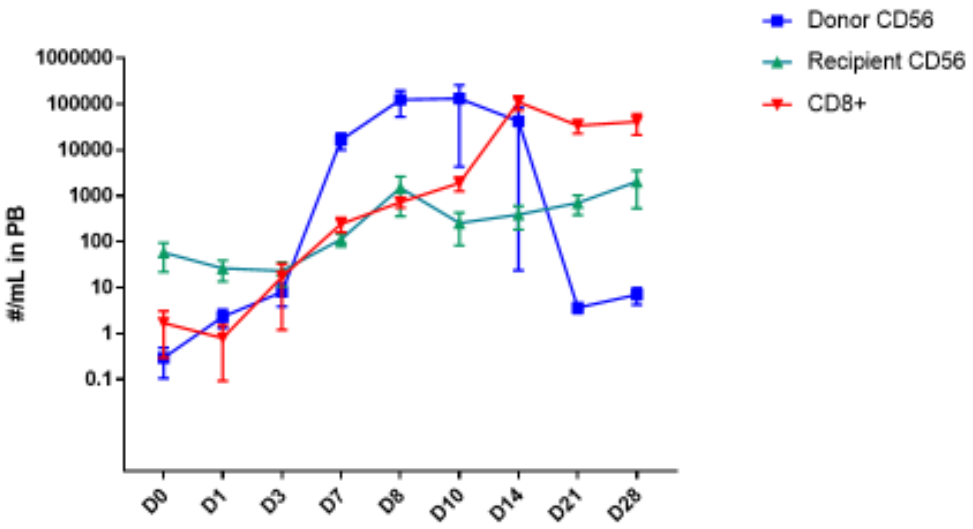


Figure 1. A. Summary data demonstrating donor CD8+ T cells expand over 10-14 days with ALT-803 support, with elimination of donor ML NK cells. The number of each lymphocyte subset is shown, with early expansion in CD56+ NK cells, and then rapid decline between days 10-21, inversely correlated with recipient T cell numbers. This pattern is suggestive of recipient CD8 T cells expanding and rejecting allogeneic donor ML NK cells. Summary data is shown including ALT-803 ML NK cell cohort patients.

To test our hypothesis that ALT-803 was promoting recipient allo-rejection of donor NK cells and thereby limiting the NK cell's anti-leukemia efficacy, we performed in vitro experiments. Peripheral blood mononuclear cells (PBMC) were stimulated in a mixed lymphocyte reaction (MLR) in the presence of IL-2 (standard control conditions) or with IL-2 plus ALT-803. The stimulator cells were ML NK cells from an allogeneic source, to mimic the immune responses occurring within patients on the trial. This revealed that the presence of ALT-803 resulted in more rapid and robust CD8 T cell proliferation in this MLR, supporting the idea that ALT-803 was enhancing CD8 T cell responses to ML NK cells. We also used the day 11 stimulated PBMC as effectors in a cytotoxicity assay, with the targets being the stimulating ML NK cells, and observed selective, MHC Class I-dependent killing of the allogeneic ML NK cells, only in the presence of ALT-803 (Figure 2). Concurrently, data from an independent allogeneic NK cell adoptive therapy trial being performed at the University of Minnesota showed a similar lack of efficacy (0 of 10 patients achieving a CR or CRi, compared to 30% CR/CRi rate when rhIL-2 was used following the NK cell infusion).

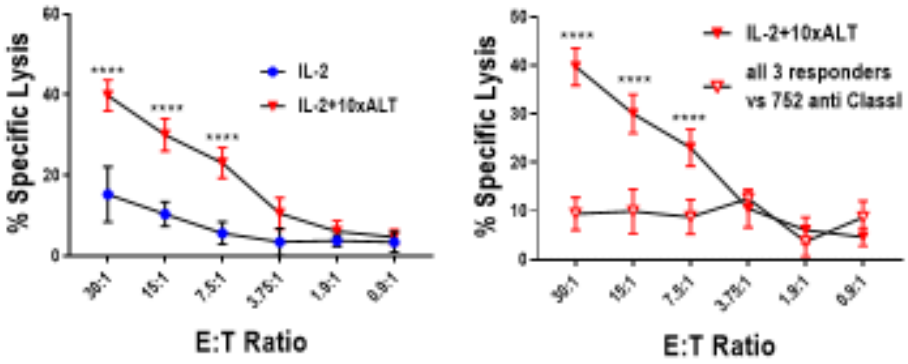


Figure 2. CD8 T cells kill allogeneic ML NK cells in the presence of ALT-803. PBMC were stimulated in a mixed lymphocyte reaction against irradiated 752 donor ML NK cells in the absence (IL-2) or presence (IL-2xALT) of ALT-803. After 11 days, PBMC was used as an effector against 752 ML NK cells and cytotoxicity was measured. Allogeneic ML NK cells were killed by effector PBMC only in the presence of ALT-803. In the same experiment, anti-MHC Class I mAbs were used to block the recognition of target allogeneic ML NK cells by CD8 T cells. Anti-Class I mAbs eliminated the killing, indicating that the effector population killing ML NK cells was CD8 T cells. Representative of 2 independent experiments with N=6 MLR effectors.

Based on the evidence of increased CD8 T cell activation, the in vitro data indicating that ALT-803 promoted recipient CD8 T cell expansion and killing of donor ML NK cells, and the lack of clinical responses using ALT-803, the lead in cohort was closed, and a decision was made to return to rhIL-2 support, mimicking the cytokine support utilized in the phase 2 portion of the trial. Since this finding of IL-15 receptor agonists promoting recipient CD8 T cell responses against allogeneic donor NK cells can be generalized to any allogeneic cell therapy, we will report this ALT-803 support cohort to the immunotherapy community immediately. The Phase 2 portion of the ML NK cell trial will proceed, with the safe and established rhIL-2 support from the phase 1 dose escalation.

1.4 Correlative Studies Background

Data from in vitro experiments, the xenograft pre-clinical mouse model, and from the initial 9 patients treated in phase I show enhanced cytokine (IFN-γ)[26] and degranulation response of the CIML NK cells in response to K562 leukemia targets.[28] In addition, we have seen more robust proliferative response of the CIML NK cells in vivo. In order to confirm these findings upon their adoptive transfer into larger numbers of AML patients, we plan to analyze peripheral blood and bone marrow biopsy samples to assess the persistence, expansion, and sustained anti-leukemia response of the adoptively transferred CIML NK cells. Recent advances in mass cytometry (CyTOF) have made it possible to perform detailed phenotypic and functional analysis of the immune cells including NK cells.[37] Our initial experience using CyTOF has revealed a unique multidimensional phenotype of CIML NK cells and we hope to be able to utilize this

information to better characterize the CIML NK cells especially after adoptive transfer into patients.[28]

IL-12/15/18 pre-activated NK cells actively 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. In the context of allogeneic SCT, maximum clinical benefit from NK cells has been previously reported in the patients with KIR-ligand mistmatch.[22,20] The KIR typing of the donor NK cells and HLA typing of the recipient will be done to see whether there is a beneficial effect of KIR to KIR ligand mismatch after the adoptive transferred of haplo-identical CIML NK cells. Several studies have also shown a favorable effect of donor KIR B haplotype and KIR B haplotype content scoring on the risk of relapse after matched donor SCT.[38,39] All donors in the study will have KIR typing performed along with assessment of KIR B haplotype score to assess for any relationship with the responses in the patients treated on this study.

While mechanisms whereby AML cells may evade or resist NK cell surveillance have been described, few studies have evaluated mechanisms following NK cell adoptive immunotherapy. Through identification of barriers to effective memory-like NK cell leukemia response, future clinical strategies may be developed to overcome these resistance mechanisms. Here, we will also use mass cytometry and candidate approaches to investigate NK cell resistance mechanisms, including CIML NK cell inhibitory and activating receptors, their ligands on AML blasts and in the BM microenvironment, and populations of regulatory cells present in the BM microenvironment.

Some of the major advances in understanding AML biology including the possible mechanisms for relapse and clonal progression have come from next generational sequencing studies.[40,41] We will collect samples to perform exome sequencing of AML blasts prior to therapy, during remissions, and after relapse post CIML NK cell infusion. Furthermore, samples for immune gene expression profiling (using RNAseq) will be collected to help further characterize the AML microenvironment pre-therapy, at the time of remission, and at the time of relapse.

2.0 OBJECTIVES

2.1 Primary Objective

Phase I: To determine the maximal tolerated or tested dose (MT/TD) of CIML NK cells administered to AML and MDS patients.

Phase II: To determine the complete remission rate (CR/CRi) in patients with relapsed or refractory AML following CIML NK therapy.

Pediatric Cohort: To determine the safety of CIML NK cells administered to pediatric AML patients.

2.2 Secondary Objectives

1. To determine the safety of the CIML-NK cells adoptively transferred into AML and MDS patients (phases I and II).

2. To determine the response of CIML NK cell therapy in AML and MDS patients (phases I,II, and pediatric).
3. To determine the duration of remission, time to progression, disease-free survival, and overall survival of AML and MDS patients treated with CIML NK cells (phases I, II, and pediatric).

2.3 Correlative Objectives

1. To evaluate the number, phenotype, KIR mismatch status, and function of CIML NK cells following adoptive transfer, as well as serum cytokines (phase I).
2. To define the percentage and number of CIML NK cells present in the peripheral blood and correlate with CR/CRi rate.
3. To define the percentage and number of peripheral blood IFN-γ^+ CIML NK cells after ex vivo leukemia re-stimulation and correlate with CR/CRi rate.
4. To determine the phenotype, proliferation, and function of in vivo expanded CIML NK cells in the PB and BM utilizing mass cytometry.
5. To define potential CIML NK cell resistance factors, and the AML microenvironment pre-therapy, after CIML NK cell therapy, and at the time of relapse using mass cytometry and immune profiles (RNAseq).
6. To define the changes in recurrent somatic mutations in AML pre-therapy, at day 30 after CIML using next-generation sequencing.

3.0 PATIENT SELECTION

3.1 Inclusion Criteria

1. Diagnosis requirement for phase I patients:
   Refractory AML without complete remission (CR) after induction therapy (primary induction failure) or relapsed AML after obtaining a CR.

   OR

   High-risk AML (by ELN criteria; See Appendix C) in complete remission (CR) and has either refused hematopoietic stem cell transplantation OR is currently not eligible for hematopoietic stem cell transplantation OR for whom hematopoietic stem cell transplantation is being reserved for later relapse. This is inclusive of patients with minimal residual disease evidenced by cytogenetics, molecular testing, and/or flow cytometry.

   OR

   Myelodysplastic syndrome (MDS) with excess blasts (>5%) and progressive disease (see section 12.4) at any time after initiation of DNA hypomethylator treatment during the past 2 years, OR failure to achieve complete or partial response or hematological improvement (see section 12.4) after at least six cycles of azacytidine or four cycles of decitabine administered during the past 2 years, OR intolerance to azacytidine or decitabine. MDS patients with isolated 5q- abnormalities that meet these criteria after lenalidomide therapy and DNA hypomethylator therapy are also eligible.

Diagnosis requirement for phase II patients:
Refractory AML without CR after induction therapy (primary induction failure) or relapsed AML after obtaining a CR. Favorable-risk core binding factor (CBF) mutated AML and acute promyelocytic leukemia (APL) will be excluded.

Diagnosis requirement for pediatric cohort patients:
Refractory AML without complete remission (CR) after induction therapy (primary induction failure) or relapsed AML after obtaining a CR.

2. Age requirement for phase I and phase II patients: At least 18 years of age.
3. Age requirement for pediatric cohort: 1-17 years of age.

4. Available HLA-haploidentical donor that meets the following criteria:

   a. Related donor (parent, sibling, offspring, or offspring of sibling)
   b. At least 18 years of age
   c. HLA-haploidentical donor/recipient match by at least Class I serologic typing at the A&B locus.
   d. In general good health, and medically able to tolerate leukapheresis required for harvesting the NK cells for this study.
   e. Negative for hepatitis, HTLV, and HIV on donor viral screen
   f. Not pregnant
   g. Voluntary written consent to participate in this study

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/Lansky performance status $\geq$ 50 % (see Appendix A).

7. Adequate organ function as defined below:
   a. Total bilirubin $\leq$ 2 mg/dL
   b. AST(SGOT)/ALT(SGPT) $\leq$ 3.0 x ULN
   c. Creatinine within normal institutional limits OR creatinine clearance $\geq$ 50 mL/min/1.73 m^2 by Cockcroft-Gault Formula (adults) or Schwartz formula (pediatric cohort) (See Appendix B)
   d. Oxygen saturation ≥90% on room air
   e. Ejection fraction ≥35%

8. Able to be off corticosteroids and any other immune suppressive medications beginning on Day -3 and continuing until 30 days after the infusion of the CIML NK cells. However, use of low-level corticosteroids is permitted if deemed medically necessary. Low-level corticosteroid use is defined as 10mg or less of prednisone (or equivalent for other steroids) per day.

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 and throughout the DLT evaluation period.

10. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. Relapsed after allogeneic transplantation.

2. Isolated extramedullary relapse (phase II only).

3. More than one course of salvage chemotherapy for primary induction failure or AML relapsing after CR1 (phase II only).

4. Circulating blast count ≥30,000/μL by morphology or flow cytometry (cytoreductive therapies including leukapheresis or hydroxyurea are allowed).

5. Uncontrolled bacterial or viral infections, or known HIV, Hepatitis B or C infection.

6. Uncontrolled angina, severe uncontrolled ventricular arrhythmias, or EKG suggestive of acute ischemia or active conduction system abnormalities.

7. New progressive pulmonary infiltrates on screening chest x-ray or chest CT scan that have not been evaluated with bronchoscopy. Infiltrates attributed to infection must be stable/ improving after 1 week of appropriate therapy (4 weeks for presumed or proven fungal infections).

8. Known hypersensitivity to one or more of the study agents.

9. Received any investigational drugs within the 14 days prior to the first dose of fludarabine.

10. Pregnant and/or breastfeeding.

3.3 Inclusion of Women and Minorities

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

4.0 REGISTRATION PROCEDURES

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

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

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

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials Core Protocol Procedures for Secondary Sites* packet at least one business day prior to registering patient:

1. Your name and contact information (telephone number, fax number, and email address)
2. Your site PI's name, the registering MD's name, and your institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Current approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Overall Treatment Plan

5.1.1 Phase I Treatment Plan

The recipient will begin a lymphodepleting preparative regimen of fludarabine and cyclophosphamide on Day -6. The haploidentical donor identified by HLA matching of the immediate family members will undergo non-mobilized large volume (20-L) leukapheresis on Day -1, and the NK cell product will be infused into the recipient on Day 0. Subcutaneous IL-2 will begin approximately 2-4 hours after infusion and will continue every other day through Day 12 for a total of 7 doses. Dose escalation will proceed as described in Section 5.3.3. The interval between enrollments of consecutive subjects within the first treatment level 1 will be 35

days, to allow a complete assessment of safety events within the DLT assessment period, based on the safety profile of prior NK cell therapy studies. Subsequently, dose levels 2 and 3 will not require a staggered enrollment of consecutive subjects. However, to escalate to the next dose level, all patients enrolled at the lower dose level must complete their 35-day DLT assessment.

5.1.2 Lead-in Cohort and Phase II/ALT-803 Treatment Plan

The recipient will begin a lymphodepleting preparative regimen of fludarabine and cyclophosphamide on Day -6. The haploidentical donor identified by HLA matching of the immediate family members will undergo non-mobilized large volume (20-L) leukapheresis on Day -1, and the NK cell product will be infused into the recipient on Day 0. Subcutaneous ALT-803 (10 mcg/kg) will begin approximately 4 hours after the infusion and will continue for a total of 2 doses (Days 0 and 5).

ALT-803 dose modifications are listed in Section 5.3.6.

5.1.3 Phase II/IL-2 Treatment Plan

The recipient will begin a lymphodepleting preparative regimen of fludarabine and cyclophosphamide on Day -6. The haploidentical donor identified by HLA matching of the immediate family members will undergo non-mobilized large volume (20-L) leukapheresis on Day -1, and the NK cell product will be infused into the recipient on Day 0. Subcutaneous IL-2 will begin approximately 2-4 hours after infusion and will continue every other day through Day 12 for a total of 7 doses.

5.1.4 Pediatric Cohort

The recipient will begin a lymphodepleting preparative regimen of fludarabine and cyclophosphamide on Day -6. The haploidentical donor identified by HLA matching of the immediate family members will undergo non-mobilized large volume (20-L) leukapheresis on Day -1, and the NK cell product will be infused into the recipient on Day 0. CIML NK cells will be infused at maximum cell dose of 10 x 106/kg. Subcutaneous IL-2 will begin approximately 2-4 hours after infusion and will continue every other day through Day 12 for a total of 7 doses.

5.1.5 Dose Escalation of CIML NK Cells (Phase I)

CIML NK Cell Dose Escalation Schedule	
Dose Level	**CIML NK cell dose**
Level 1 (starting dose)	0.5 x 10^6/kg
Level 2	1.0 x 10^6/kg
Level 3	Maximum NK cell number/kg*

*All CIML NK cells available from the donor leukapheresis, NK cell purification, and CIML process will be infused, up to a maximum of 10x10^6/kg.

The median number of CIML NK cells collected from each donor is expected to be 5.0 x 10^6 cells/kg to 10 x 10^6 cells/kg. If less than the targeted CIML NK cell numbers are

available (≥2.0 x 10^6 cells/kg for dose level 3), then all cells (after quality control testing) should be given and the therapy plan will remain the same. These patients will be considered not evaluable for DLT assessment at the intended dose level, and they will be replaced with an additional patient at the intended dose level. Any patient that receives CIML NK cells will be included in the safety analyses at the dose level received. If more than the targeted number of CIML NK cells are available, then the patient will only receive the cell dose assigned, and all additional cells will be used for correlative studies.

Dose escalation will not occur until all patients in the cohort have completed the DLT evaluation (35 days) and the Principal Investigator has been able to review all toxicities. Dose escalations will proceed until the MTD has been reached or until dose level 3 has been completed.

After determining the maximal tolerated / tested dose (MT/TD) in phase I, the phase II portion of the study will be initiated using the MTD CIML NK cell dose.

5.2 Definition of MTD, DLT, Dose Escalation Criteria, and Evaluability

5.2.1 Definition of Maximum Tolerated or Tested Dose (MT/TD)

The maximum tolerated dose of the CIML NK cells is defined as the dose level immediately below the dose level at which 2 patients of a cohort (of 2 to 6 patients) experience dose-limiting toxicity or the maximum dose if ≤ 1 patient suffers a DLT at the maximum dose. If these criteria are met at the maximum proposed dose, this will be defined as the maximum tested dose.

5.2.2 Dose Limiting Toxicities (DLTs)

Non-Hematologic dose limiting toxicities (DLT) are defined as any CTCAE grade 3 or higher non-hematologic adverse event considered possibly, probably, or definitely related to CIML NK cell infusion. Non-clinically significant metabolic adverse events (abnormalities in serum sodium, potassium, chloride, magnesium, calcium, phosphorous, blood urea nitrogen, and glucose that last less than 48 hours that respond to appropriate medical therapy) will not be considered DLTs regardless of CTCAE grade. The observation period for DLTs is 35 days following the CIML NK cells.

Hematologic dose limiting toxicity is defined as failure to recover hematopoiesis (ANC $\underline{\geq}$ 500/μL) by Day 35 post-CIML NK cell infusion. Hematologic AEs related to persistent disease/disease relapse or other causes will not be considered DLTs. A standard bone marrow biopsy will be performed at Day 28 post-infusion, which will determine if persistent disease/relapse is the cause of persistent bone marrow suppression. Patients with clinical progression of their AML or MDS after CIML NK cell infusion may receive cytoreductive therapy (e.g., hydroxyurea, cytarabine) to control their disease and remain evaluable for DLTs throughout the DLT period. Any AE that is probably or definitely related to cytoreductive therapy would not be considered a DLT.

Since GVHD and late onset bone marrow suppression may be related to CIML NK cells, additional stopping rules are included for safety (see Section 5.3.4).

5.2.3 Dose Escalation Criteria

Dose escalations in the phase I part will proceed as follows:

Number of Patients with a DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥ 2	Dose escalation will be stopped. Three additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. If 0 of these 3 patients experience DLT, proceed to the next dose level. If 1 or more of this group suffer DLT, then dose escalation is stopped. Three additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤ 1 out of 6 at highest dose level below the maximally administered dose or the maximally administered dose if < 2 patients suffer a DLT at this dose level	This is generally the recommended phase II dose. At least 6 patients must be entered at the recommended phase II dose prior to its opening.

5.2.4 Additional Stopping Rules

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

1. Development of neutrophil suppression (ANC $\underline{<}$ 500/μL) between Day 35 and Day 100 in the absence of disease recurrence or other causes.
2. Grade II-IV acute GVHD involving the liver, skin, or GI tract within 100 days after the NK cell infusion even in any of the patients treated on study
3. Extensive chronic GVHD involving any of the organ systems and occurring in any of the patients after infusion of the CIML NK cells.

If any of the late stopping events listed above occurs (while the patients are on study and have not received additional AML therapy) at an estimated frequency of ≥25% (separate events), accrual will be suspended and reviewed for safety of continuation.

5.2.5 Toxicity and Response Evaluations

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment through 100 days after the conclusion of treatment or death for toxicity evaluation.

All patients who receive a CIML NK cell infusion and at least 4 doses of IL-2 or 1 dose of ALT-803, and who undergo a Day 28 bone marrow biopsy for response assessment, are evaluable for disease response.

5.2.6 ALT-803 Dose Modifications and Stopping Rules for Toxicity

If one or more of the three patients treated in the Lead-in Cohort develops any unexpected grade ≥3 adverse events at least possibly related to the use of ALT-803 or any of the stopping rules (listed above in Section 5.3.4) is met, the dose of ALT-803 will be de-escalated to 6 mcg/kg and three patients will be treated at that dose. The study will be suspended if any of the three patients in the lead-in cohort treated with de-escalated dose (6 mcg/kg) ALT-803 develop any unexpected grade ≥3 adverse events at least possibly related to the use of ALT-803 and / or any of the stopping rules is met. Otherwise, enrollment to the phase II part of the study will be initiated. The dose of ALT-803 used in phase II will be 2 doses at 10 mcg/kg if de-escalation has not been required or 2 doses at 6mcg/kg if de-escalation was required. Furthermore, the ALT-803 dose will be de-escalated to 6mcg/kg if any of the patients treated on the phase II part of the study develop any unexpected grade ≥3 adverse events at least possibly related to the use of ALT-803 or any of stopping rules (listed above in Section 5.3.4) is met. The study will be suspended if any of the patients treated at the de-escalated dose in phase II develop any of these criteria.

ALT-803 has been associated with rash around the area of injection, which may occur up to 7-10 days after the initial injection. For localized rashes (involving <25% of the body surface), subsequent doses of ALT-803 will not be held. However, if the rash involves ≥50% of the body surface area, subsequent doses of ALT-803 will be delayed until the rash improves significantly (as determined by the treating physician) in addition to supportive care measures (as described in Section 6.6.1). ALT-803 may be delayed for up to 96 hours due to injection reaction rash. If > 96-hour delay is required, the dose will be skipped.

Delay the scheduled study treatment one week each for up to 2 weeks for the following events until a recovery is sufficient to resume study treatment; otherwise, discontinue study treatment:

- Elevated post-treatment creatinine to such that calculated GFR is < 40 mL/min
- Febrile neutropenia
- Thrombocytopenia with bleeding or anemia
- ALC ≥ 50,000/μL or total WBC ≥ 60,000/μL
- Systolic hypotension < grade 3 that does not correct with bolus fluids over 6 hours

Skipped doses of ALT-803 will not be made up.

Discontinue treatment with ALT-803 if a patient experiences any grade 3 event that is not clearly unrelated to ALT-803 administration that does not resolve to grade 1 or lower within a week despite the use of medical intervention or any grade 4 event with the following exceptions:

- Grade 3 or 4 lymphopenia, leukopenia, or neutropenia that recovers within 14 days
- Grade 3 or 4 thrombocytopenia or anemia that recovers within 14 days
- Nausea or vomiting that is controllable with antiemetics within 72 hours
- Hypotension of grade 3 that persists for < 4 hours and does not require hospitalization (unless admission is precautionary)

Additional events that require ALT-803 discontinuation are:

- Arrhythmia of any kind
- ALC ≥ 50,000/μL sustained for 14 days
- WBC ≥ 60,000/μL sustained for 14 days
- Idiosyncratic drug reaction that prevents further administration of treatment
- Grade 2 allergic reaction with bronchospasm or any grade 3 or 4 allergic / infusion-related reaction

5.2.7 ALT-803 Dose Modifications for Biologic Outcomes

In the lead-in cohort of patients treated with the Phase II schedule of ALT-803, correlatives studies revealed shorter persistence of donor CIML NK cells than was observed in Phase I patients who were treated with low dose rhIL-2. This coincided with increased activation of recipient CD8+ T cells, suggesting that multiple doses of ALT-803 may be enhancing donor NK cell rejection. Such a decrement in CIML NK cell exposure is expected to decrease the efficacy of the study treatment, provided by the anti-leukemia activity of the donor CIML NK cells. Based on recent data of the PK of ALT-803 administered SQ, we expect that the multiple doses of ALT-803 persist into the early recipient T cell recovery period, and promote early recipient T cell activation. Thus, ALT-803's impact on recipient T cells is probably responsible for the observed decline in donor CIML NK cell activity in the lead in portion of the phase 2 cohort.

In order to improve persistence and anti-leukemia "window of opportunity" for the donor CIML NK cells, the dosing of ALT-803 will be decreased to one dose on day 0. . If CIML NK cell persistence appear limited or early recipient T cell activation occurs on the modified ALT-803 schedule, compared to the rhIL-2 dose level 3 of the phase 1, ALT-803 treatment will be discontinued, and Phase II patients will instead be treated with IL-2 after day 0, per the Phase I schedule.

6.0 STUDY TREATMENT

6.1 Lymphodepleting Preparative Regimen

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

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

	Cyclophosphamide
-3	Fludarabine
-2	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.

6.1.1 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 -6. Administration should follow institutional guidelines.

6.1.2 Cyclophosphamide

Cyclophosphamide is administered at a dose of 60 mg/kg as a 2-hour IV infusion on Days -5 and -4. Administration should follow institutional guidelines.

6.2 Donor Leukapheresis

On Day -1 (one day before the planned NK cell infusion), peripheral blood cells will be collected by a single apheresis from the haploidentical related donor. The collection should take place over 5 hours.[42] The apheresis procedure will be done as per standard institutional procedures (which may include placement of a central line if necessary). If the goal minimum NK cell dose will not be met based on the initial assessment of the leukapheresis product, a second collection/procedure may be performed. The donor's central line will remain placed until it is confirmed that a second collection is not required.

6.3 Preparation of CIML NK Cells and Product Release Criteria

Cell product processing is performed at the Siteman Cancer Center Biological Therapy Core. The apheresis product will undergo NK cell purification with the CliniMACS® device (Miltenyi) by sequential depletion of $CD3^+$ T cells and positive selection of $CD56^+$ ($CD3^-$/$CD56^+$) NK cells.

The purified NK cells are cultured for 12-18 hours in X-VIVO 15 media containing rhIL-12 (10 ng/mL), rhIL-15 (50 ng/mL) and rhIL-18 (50 ng/mL). After incubation, the cells are washed two times in a cytokine-free media solution to remove the cytokines. After the last wash, the final resuspension will be used to determine the final NK cell numbers prior to infusion.

The appropriate predetermined NK cell dose ($0.5x10^6$/kg, $1x10^6$/kg, or Max NK capped at $10x10^6$/kg) will be used for infusion in the phase I part of the study. The final T cell ($CD3^+$) cell dose in the infusion product has to be less than 3×10^5 $CD3^+$/kg of the recipient.

For phase II and pediatric cohort, MT/TD CIML NK cell dose will be infused into the relapsed patients (max dose is 10×10^6/kg).

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 $x10^5$ T cells/kg
Endotoxin	LAL method	< 5.0 EU/kg
Gram stain	Clinical micro	No organisms

6.4 CIML NK Cell Product Infusion

The CIML NK cells are infused on Day 0 without a filter or pump, slowly by gravity over at least 15 minutes. Flush tubing on completion of the CIML NK cells with normal saline to ensure all of the cells are infused.

Patients are 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 the NK cell infusion. Demerol may be given for chills/rigors during the NK cell infusion. Intravenous hydration may be administered per institutional guidelines.

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

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

CIML NK cells are expected to produce IFN-γ for 16-24 hours after NK cell infusion. Expected constitutional toxicities from IFN-γ include myalgias, arthralgias, fever, and rigors. IFN-γ therapy has been safely used in patients with idiopathic pulmonary fibrosis and chronic granulomatous disease (CGD)[43,44]. The most common side effects seen in those patients were fevers, headache, chills, rash, vomiting, and diarrhea.

6.5 Interleukin-2 (IL-2) – Phase I, Phase II/IL-2, and Pediatric Cohorts

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.

6.5.1 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.

6.5.2 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.

6.6 ALT-803 – Lead-in Cohort and Phase II/ALT-803 ONLY

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

If ALT-803 cannot be started within 96 hours after the CIML NK cell infusion, no ALT-803 will be given and the patient will be replaced for statistical analysis of the primary objective.

6.6.1 ALT-803 Administration

ALT-803 will be administered subcutaneously at the dose and schedule determined during the lead-in (please refer to Section 5.3.6).

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

Patients should be monitored for ALT-803 related targeted toxicities. Fevers, rash, and myalgias are expected, and should be treated supportively.

ALT-803 has been associated with rash around the area of injection, which may occur up to 7-10 days after the initial injection. For localized rashes (involving <25% of the body surface) the patients will be treated with topical steroids creams (e.g., triamcinolone cream) along with oral Benadryl. For these localized rashes subsequent doses of ALT-803 will not be held. However, if the rash involves ≥50% of the body surface area then in addition to the topical steroid cream and other supportive cares subsequent doses of ALT-803 will be delayed until the rash improves significantly (as determined by the treating physician). ALT-803 may be delayed for up to 96 hours due to injection reaction rash.

6.7 Vascular Leak Syndrome

The administration of allogeneic NK cells and IL-2 administration has not been associated with vascular leak syndrome in previous published studies. Nevertheless, patients will be monitored for weight gain (by weights at least 3 times per week) and pulmonary edema during IL-2 administration.

6.8 Prolonged Marrow Suppression

Cyclophosphamide and fludarabine are expected to cause transient marrow suppression (less than 2 weeks). CIML NK cells infusion may induce bone marrow suppression at later time points, and therefore, as a precaution, patients' hematologic recovery will be assessed through day 100 (see Sections 5.3.2 and 5.3.4).

6.9 Treatment Failure/Progressive Disease

CIML NK cells may require days to weeks to clear AML blasts, unlike traditional cytotoxic chemotherapy. The presence of AML blasts at Day 14 would not constitute a reason for removal from the protocol unless deemed necessary by the treating physician. Cytoreductive therapy (e.g., hydroxyurea, cytarabine) may be used to control disease during the DLT period as described in Section 5.3.2. at the discretion of the treating physician. Patients who fail to clear AML blasts at the Day 28 bone marrow assessment or with persistent circulating blasts at Day 28 would be considered a treatment failure/progressive disease and should be started on other anti-leukemia therapies at the discretion of the treating physician. Patients who receive other anti-leukemia therapy after

the DLT period will be considered off study and no future follow up visits are required other than annual survival visits.

6.10 Allogeneic Transplantation

Patients who are found to be in remission or who have cleared the AML blasts on Day 28 marrow are allowed to proceed to an allogeneic transplant at the discretion of the treating physician. Patients who proceed to allogeneic transplant will be considered off study and no future follow up visits are required other than annual survival visits. The patients' medical records will be reviewed to collect data on relapse and survival.

6.11 Prohibited Medications

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

G-CSF or GM-CSF are not to be routinely used in the first two weeks after the CIML NK cell infusion but are allowed if deemed medically necessary by the treating physician.

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

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 fludarabine.

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 DLT evaluation period.

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 fludarabine, 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.

In the absence of treatment delays due to adverse events, treatment may continue till the last dose of IL-2 or ALT-803 or until one of the following criteria applies:

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

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

6.14 Duration of Follow-up

After completion of the CIML NK cell therapy, patients will be followed in the clinic for up to 12 months. After 12 months, patients will be followed annually for survival and relapse. Patients who proceed to other anti-leukemic therapy or allogeneic transplant will be considered off study and no future follow up visits are required other than annual survival visits. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7.0 PHARMACEUTICAL INFORMATION

7.1 Fludarabine

7.1.1 Description

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

7.1.2 Dose Forms and Strengths

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

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

7.1.3 Storage and Stability

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

7.1.4 Availability

Commercially available by prescription

7.1.5 Preparation

Preparation should follow institutional guidelines.

7.1.6 Administration

Please see Section 6.1.1.

7.1.7 Warnings and Precautions

- Life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia/thrombocytopenic purpura (ITP), Evan's syndrome, and acquired hemophilia have been reported to occur in patients receiving fludarabine
- Serious, and sometimes fatal infections, including opportunistic infections and reactivations of latent viral infections such as VZV (Herpes zoster), Epstein-Barr virus and JC virus (progressive multifocal leukoencephalopathy)) have been reported in patients treated with fludarabine.
- Objective weakness, agitation, confusion, seizures, visual disturbances, optic neuritis, optic neuropathy, blindness and coma have occurred in CLL patients treated with fludarabine at the recommended dose.
- High doses of fludarabine have been associated with an irreversible central nervous system toxicity characterized by delayed blindness, coma and death. High doses are also associated with severe thrombocytopenia and neutropenia due to bone marrow suppression. There is no known specific antidote for fludarabine overdose.
- The use of fludarabine in combination with pentostatin is not recommended due to the risk of severe pulmonary toxicity.
- Pregnancy Category D

7.2 Cyclophosphamide

7.2.1 Description

Cyclophosphamide is a white crystalline powder with the molecular formula $C_7H_{15}Cl_2N_2P \cdot H_2O$ and a molecular weight of 279.1. The chemical name for cyclophosphamide is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate.

7.2.2 Mechanism of Action

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

7.2.3 Dose Forms and Strengths

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

7.2.4 Storage and Stability

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

7.2.5 Availability

Commercially available by prescription

7.2.6 Preparation

Preparation should follow institutional guidelines.

7.2.7 Administration

Please see Section 6.1.2.

7.2.8 Warnings and Precautions

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

7.3 Interleukin-2

7.3.1 Mechanism of Action

- Systemic: Aldesleukin has been shown to possess the biological activity of human native interleukin-2. In vitro studies performed on human cell lines demonstrate the immunoregulatory properties of aldesleukin, including:
  - Enhancement of lymphocyte mitogenesis and stimulation of long-term growth of human interleukin-2 dependent cell lines;
  - Enhancement of lymphocyte toxicity;
  - Induction of killer cell (lymphokine-activated killer [LAK] cells and natural killer [NK] cells) activity; and
  - Induction of interferon-gamma production.
- The in vivo administration of aldesleukin in select murine tumor models and in the clinic produces multiple immunological effects in a dose-dependent manner. These effects include activation of cellular immunity with profound lymphocytosis, eosinophilia, and thrombocytopenia, and the production of cytokines, including tumor necrosis factor, interleukin-1 and gamma interferon. In vivo experiments in murine tumor models have shown inhibition of tumor growth. However, the exact mechanism by which aldesleukin mediates its antitumor activity in animals and humans is unknown.
- Aldesleukin causes a capillary leak syndrome (CLS) as a result of increased

capillary permeability, leading to extravasation of plasma proteins and fluid into the extravascular space and contributing to loss of vascular resistance. Interleukin-2 has been reported to reversibly decrease serum cholesterol concentrations. Interleukin-2 has been reported to transiently decrease serum testosterone and dihydroepiandrosterone concentrations and to transiently increase plasma estradiol concentrations. It has also been reported to transiently increase adrenal secretion of ACTH and cortisol.

7.3.2 Dose Forms and Strengths

Lyophilized vials containing 22 million units

7.3.3 Availability

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

7.3.4 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.

Any IL-2 that is not utilized for injection into a patient will be stored at 2-8°C and provided to the Fehniger Lab for use in correlative studies.

7.3.5 Administration

Please see Section 6.5.

7.3.6 Warnings and Precautions

- patients with a history of cardiac disease exhibiting a normal thallium stress test
- patients with a history of pulmonary disease exhibiting normal pulmonary function tests
- severe hypotension (due to capillary leak syndrome)
- impaired neutrophil function (reduced chemotaxis)
- increased risk of disseminated infection
- kidney and liver function impairment (avoid concurrent administration with nephrotoxic or hepatotoxic drugs)
- mental status changes
- exacerbation of preexisting autoimmune diseases or initial presentation of autoimmune and inflammatory disorders
- may increase the risk of allograft rejection in transplant patients (due to enhancement of cellular immune function)
- cardiac, pulmonary, renal, hepatic, or central nervous system impairment

- patients with a history of seizures
- Pregnancy Category C

Most toxicities have been described at high doses of IL-2 often used in the treatment of patients with renal cancer and metastatic melanoma. To decrease the chances of these side effects we have chosen a low dose of 1 million units/m^2, which has been given safely and without major adverse events.[23,45] This dose is below the MTD established in a pediatric study of subcutaneous IL-2[46]. In addition, the combination of IL-2 plus CIML NK cells may have a risk of capillary leak syndrome (CLS).

7.4 ALT-803

ALT-803 is a recombinant human super-agonist IL-15 complex. Its active ingredient is ALT-803 and its pharmacologic class is as a targeted anticancer immunotherapeutic. ALT-803 has been referred to as IL-15N72D:IL-15RαSu/IgG1 Fc complex in various preclinical study reports, publications, and other related documents.

7.4.1 Formulation and Composition

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

7.4.2 Quantitative Composition of ALT-803

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

7.4.3 Structural Formula

ALT-803 is a soluble complex consisting of 2 protein subunits of a human IL-15 variant associated with high affinity to a dimeric IL-15R sushi domain/human IgG1 Fc fusion protein. The IL-15 variant is a 114 aa polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D).6. The human IL-15R sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the IL-15R subunit (aa 1-65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). Aside from the N72D substitution, all of the protein sequences are human. Based on the amino acid sequence of the subunits, calculated molecular weight of the complex comprising 2 IL- 15N72D polypeptides and a disulfide linked homodimeric IL-15RαSu/IgG1 Fc protein is 92.4 kDa. Each IL-15N72D polypeptide has a calculated molecular weight of approximately 12.8 kDa and the IL-15RαSu/IgG1 Fc fusion protein has a calculated molecular weight of approximately 33.4 kDA.

Component	Concentration	Amount/Vial
ALT-803	2 mg/mL	0.6 mg

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

7.4.4 Storage and Handling

Study medication is provided in a 2 mL vial containing 0.6 mL of ALT-803 at a concentration of 2 mg/mL. Vials are packaged in cartons and shipped to the clinical site. Study medication must be maintained at a temperature between 2°C and 8°C.

7.4.5 Stability

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

7.4.6 Agent Ordering and Agent Accountability

ALT-803 is produced in the USA by Altor Biosciences Corporation, Miramar, FL. After manufacturing, the product is stored at Altor Biosciences Corporation for clinical supply, packaging, and labeling. The label indicates the product name, strength, manufacturing date, and the study requirement information. ALT-803 will be shipped from Altor Biosciences Corporation.

7.4.7 Preparation and Administration

ALT-803 dose calculation will be based on actual body weight. The calculated amount of ALT-803 will be drawn into a syringe for subcutaneous injection. The stock concentration is 2 mg/mL; withdraw the appropriate volume of ALT-803 for the calculated dose into a syringe. If the total subcutaneous dose is greater than 1.5 mL, the dose will be divided into 2-3 subcutaneous injections as needed.

Please follow the formula below to determine the volume of ALT-803 needed for a subcutaneous injection:

ALT-803 Volume Needed = (_____ µg/kg x _____ kg) ÷ 2000 µg/mL (ALT-803 vial concentration)

7.4.8 Toxicity

ALT-803 can cause many side effects, which may be similar to the side effects of interleukin-2 (IL-2), which has been used for more than 20 years.

Most likely (greater than	Less likely (3% to 10% - 1	Rarely (< 3% - 1 in 30

10% - 1 in 10 patients)	in 30 to 1 in 10 patients)	patients)
• Weight gain with swelling of hands and feet due to fluid retention • Feeling tired or short of breath due to a low red blood count (anemia) • Increase in blood pressure • Flu-like symptoms such as fever, chills, shaking, headache, stiffness, aching muscles and joints • Increased risk of infection due to a low white blood count • Increased risk of bruising and bleeding due to a low platelet count	• Heart problems - causing low blood pressure, dizziness, chest pain or changes in heart rhythm (heart beat) • Pain and redness at the injection site • Changes in liver and kidney function as detected on routine blood tests • Cough and shortness of breath • Mouth sores • Confusion, sleepiness and depression especially in older persons or persons with a history of depression	• Allergic reaction • Temporary thinning of hair

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. For patients aged ≥ 18 years, 60 mL of PB will be collected. For patients aged 1-17 years, 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 sodium heparin (green top) tube(s) at the following time points:
    - Screening*
    - Day 0 prior to the CIML NK cell infusion
    - Day 1
    - Day 3
    - Day 7
    - Day 8
    - Day 10
    - Day 14
    - Day 21
    - Day 28
    - Day 42
    - Day 60
    - Day 100
    - 6 months
    - 9 months
    - 12 months

- At disease relapse

*An additional ten mL of peripheral blood in sodium heparin (green top) tube(s) will be collected from patients ≥ 18 years at screening.

- Ten mL of peripheral blood in serum (red top) tube(s) will be collected at the following time points:
  - Screening
  - Day 0 prior to the CIML NK cell infusion
  - Day 1
  - Day 3
  - Day 7
  - Day 8
  - Day 10
  - Day 14
  - Day 21
  - Day 28
  - Day 42
  - Day 60
  - Day 100
  - 6 months
  - 9 months
  - 12 months
  - At disease relapse

- Additional peripheral blood samples may be collected at key clinical events, including suspected relapse, infection, or cytokine release syndrome.

- Ten mL of bone marrow (2-5 mL for patients age 1-17) in sodium heparin (green top) tube(s) will be collected at the following time points:
  - Screening
  - Day 8
  - Day 14
  - Day 28
  - Between Day 42 and Day 60
  - Day 100
  - At disease relapse

- Additional samples for correlative studies (donor and apheresis product)
  - 30 mL of peripheral blood from donor in sodium heparin (green and pink top) tube(s) at screening
  - 1 mL of leukapheresis product prior to NK cell purification
  - 5 x 10^6 NK cells post MACS purification
  - 5 x 10^6 CIML NK cells prior to washing
  - Any additional CIML NK cells not administered

8.2 Sample Handling

Specimens will be transported at room temperature immediately to the Fehniger Lab, Monday-Friday, 8AM-5PM. 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 samples will be submitted once the final NK cell product aliquot is obtained.

Attention: Fehniger Lab / Michelle Becker-Hapak or Tim Schappe
WU-Oncology
Southwest Tower Building Room 724/733
500 South Kingshighway
St. Louis, MO 63110
Clinical Correlates Lab: (314) 273-0153
Research Lab: (314) 747-1547
Secondary: (314) 747-1385 / Tertiary: (314) 510-2397 (pager)
Fax: (314) 362-9333
tfehnige@wustl.edu, tschappe@wustl.edu, mbecker-hapak@wustl.edu, acashen@wustl.edu

8.3 Sample Processing

For some assays, cells will be isolated and used immediately. In other assays, samples will be isolated, cryopreserved and stored in the Fehniger Lab (6th floor southwest tower building) in liquid nitrogen or -70^{o}C freezers for future batch analysis. 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

1. HLA antibody identification
2. Assessment of donor cell chimerism
3. Lymphocyte subset and monocyte number
4. NK cell percentage, number and immunophenotype
5. NK cell function (degranulation, cytotoxicity, cytokine-production, proliferation, survival) in response to cytokine stimulation, NK cell receptor ligation, or leukemia cell triggering (including patient AML blasts).
6. KIR genotype of donor
7. Serum cytokine measurements (e.g., IFN-γ, TNF, GM-CSF)
8. Whole genome and/or exome and/or RNA sequencing of BM or PB samples to define recurrent somatic mutations, clonal architecture, and gene expression profiles.
9. Deep phenotyping of the BM or PB via mass spectrometry (CyTOF) to determine the potential markers of CIML NK cell response and/or resistance mechanisms.
10. CyTOF analysis of the marrow prior to treatment, Day 30, and on relapse after CIML NK cell infusion to better help understand the interaction between AML blasts and marrow microenvironment potentially contributing to the disease response and/or progression after CIML NK cell infusion.

9.0 STUDY CALENDAR

Scheduled evaluations up to Day 35 may be performed +/-3 days from the targeted date; assessments to be performed after Day 35 may be done +/-7 days of the targeted date.

	Screening (within 28 days of registration)	Day 0	Day +1	Day +3	Day +7	Day +8	Day +10	Day +14	Day +21	Day +28	Day +35	Day +42	Day +60	Day +100	6, 9 and 12 months[2]	Disease Relapse
Consent	X															
Medical History	X															
Physical Exam w/ vital signs	X[5]		Daily[1]						X	X	X	X	X	X	X	X
Weight	X		At least 3 times a week[1]													
Height	X															
Karnofsky PS	X[11]													X	X	
HLA typing[9]	X															
Viral panel[4]	X															
CBC, diff, plt	X		Daily[1]						X	X	X	X	X	X	X	X
BUN, creat, glucose, Na, K, Cl	X		Daily[1]						X	X	X	X	X	X	X	X
AST, ALT, bili, alk phos	X		Weekly[1]						X	X	X	X	X	X	X	X
Correlative Studies[6]	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
Pregnancy test[7]	X															
Bone Marrow Biopsy	X[11]					X[10]		X		X		X[3]		X		X
Chest x-ray or chest CT scan	X[11]															
PFT	X[11, 12]															
EKG	X[11]															
Echo or MUGA	X[11]															
AE assessment			AEs will be collected from Day 0 to Day +35; however, bone marrow suppression (ANC ≤ 500/µL) and AEs of GVHD involving the liver, skin, or GI tract will be recorded to Day 100.													

1 – Until neutrophil recovery or as clinically appropriate
2 – After 12 months' formal follow-up ends, however, patients will be followed annually thereafter for disease-free survival and overall survival
3 – Perform at a clinically appropriate time for the patient somewhere between days 42 and 60 visit
4 – Donor- HTLV, HIV, CMV, and hepatitis panel; Recipient- HIV, CMV, and hepatitis panel
5 – 0^2 Saturation on room air required at screening
6 – See section 8.0 for required samples
7 – For women of childbearing potential only
8 – If donor cells are present at Day 14, recheck at subsequent visits until they are not detectable on two serial assessments.
9 – On recipient and donor
10 – For correlative studies only
11 – Recipient only12—Record O2 saturation by pulse ox if patient is too young for PFTs

10.0 REGULATORY AND REPORTING REQUIREMENTS

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

Adverse events will be tracked from the time of CIML NK cell infusion start through 35 days following the infusion. Additionally, bone marrow suppression (ANC < 500/μL) and AEs of GVHD involving the liver, skin, or GI tract will be recorded to 100 days following the infusion. All adverse events must be recorded on the toxicity tracking case report form (CRF) with the exception of:

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

Refer to the data submission schedule in Section 12 for instructions on the collection of AEs in the EDC.

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

10.1 Sponsor-Investigator Reporting Requirements

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

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

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

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

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

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

10.1.3 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements**. PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University principal investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix D) 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 D) no later than **15 calendar days** it is determined that the information qualifies for reporting. Report an adverse event (Appendix D) 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 7.1 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 AND SAFETY MONITORING

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

For phase I dose escalation studies, the Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy (phase I studies only if efficacy is objective of the protocol)
- Measures of efficacy – provide a summary of tissue samples collected by site, tissue expected to obtain, % of tissue received, and % of tissue missing
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by cohorts with the number of dose-limiting toxicities indicated
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

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

12.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed within 60 days of the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form Medical History Form Treatment History Form	Prior to starting treatment
Safety Labs	Baseline Day 0 Day 14 Day 28 Day 60 Day 100 6 Months 9 Months 12 Months
CIML NK Cell Infusion	Day 0
IL-2 Dosing Form (phase I and pediatric cohorts)	7x between Day 1 and Day 12
ALT-803 Dosing Form (phase II)	Day 0 and Day 5
Bone Marrow or Response form	Baseline Day 14 Day 28 Day 60 Day 100 6 Months 9 Months 12 Months Disease relapse Premature discontinuation
DLT Evaluation	Day 0
Additional Stopping Rules	Day 0
Correlative Studies Form	At the time points described in Section 8.0
Survival	6 Months 9 Months 12 Months Annually after 12 Months Premature Discontinuation
Adverse Events Form	Ongoing
MedWatch Form	See Section 10.0 for reporting requirements

12.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 10.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Medical History Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac disorders: cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

13.0 EFFICACY ASSESSMENT

The primary objective of the study is to determine the safety and MTD of the adoptively transferred CIML NK cells into patients with AML or MDS. However, the secondary objectives include assessing for the efficacy of the CIML NK cells to induce remission and positively affect the survival of these patients.

13.1 Percentage of Myeloid Blasts in the Bone Marrow

Bone marrow examination will be performed according to the study calendar in Section 9.0.

13.2 Blood Counts

Blood counts including a differential will be done at all visits. In patients with blood counts below the limits of normal pre-treatment, improvement in blood counts during therapy may indicate a benefit for the patient.

13.3 Response for AML patients

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

13.3.1 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

13.3.2 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.

13.3.3 Partial remission (PR)

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

13.3.4 Stable Disease (SD)

Stable disease (SD) requires the following:

Failure to achieve at least a PR at Day 14 but with no evidence of progression and still pending blood count recovery.

13.3.5 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.

13.3.6 Progressive Disease (PD)

Patient survives until the final dose of IL-2 or ALT-803 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.

13.4 Response for MDS patients – Phase I ONLY

13.4.1 Complete Remission (CR)

Complete remission (CR) requires all of the following:
Bone marrow:

- ≤ 5% myeloblasts with normal maturation of all cell lines.
  Persistent dysplasia will be noted

Peripheral blood:

- Hgb ≥11 g/dL
- Platelets ≥ 100 x 10^9/L
- Neutrophils ≥ 1.0 x 10^9/L
- Blasts 0%

13.4.2 Marrow CR (mCR)

Marrow CR requires the following:
Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment
Peripheral blood: if hematologic improvement responses, they will be noted in addition to marrow CR

13.4.3 Partial Remission (PR)

Partial remission (PR) requires the following (absolute values must last at least 2 months):
All the CR criteria (if abnormal before treatment), except:
Bone marrow blasts decreased by ≥ 50% over pretreatment but still >5%.Cellularity and morphology are not relevant.

13.4.4 Stable Disease (SD)

Stable disease (SD) requires the following:
Failure to achieve at least a PR, but with no evidence of progression for at least 2 months.

13.4.5 Progressive Disease (PD)

Progressive disease (PD) requires any of the following:

- Less than 5% blasts at baseline: ≥ 50% increase in blasts to > 5% blasts
- 5%-10% blasts at baseline: ≥ 50% increase to > 10% blasts
- 10%-20% blasts at baseline: ≥ 50% increase to > 20% blasts
- 20%-30% blasts at baseline: ≥ 50% increase to > 30% blasts
- At least 50% decrement from maximum remission/response in granulocytes or platelets
- Reduction in Hgb by ≥ 2 g/dL
- New or worsened transfusion dependence not related to study drug toxicity

13.4.6 Relapse after CR or PR

Relapse after CR or PR requires a previous CR or PR as defined above and subsequently development of one of the following:

- Return to pretreatment bone marrow blast percentage
- Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets
- Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence

13.4.7 Addition Definitions

Hematologic Improvement (HI):
Erythroid response requires all of the following (only required if pretreatment Hgb <11 g/dL):

- Hgb increase by ≥ 1.5 g/dL
- Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation

Platelet response requires one of the following (only required if pretreatment platelets < 100 x 10^9/L):

- Absolute increase of ≥ 30 x 10^9/L (for patients starting with > 20 x 10^9/L platelets)
- Increase from < 20 x 10^9/L to > 20 x 10^9/L and absolute increase ≥ 100% (for patients starting with < 20 x 10^9/L)

Neutrophil response requires the following (only required if pretreatment ANC <1.0 x 10^9/L):
At least 100% increase and an absolute increase > 0.5 x 10^9/L

RBC Transfusion Dependence and Independence:
RBC transfusion independence is defined as:

- Hemoglobin > 90 g/L (9.0 g/dL)
- Receipt of ≤ 1 RBC transfusion during the previous 8 weeks (56 days)

RBC transfusion dependence is defined as:
- Hemoglobin ≤ 90 g/L (9.0 g/dL)
- Receipt of ≥ 2 RBC transfusions during the previous 8 weeks (56 days).

Cytogenetic Response:
Complete: Disappearance of the chromosomal abnormality without appearance of new ones
Partial: At least 50% reduction of the chromosomal abnormality

Disease Transformation:
Transformation to AML with ≥20% blasts on bone marrow aspirate or core biopsy.

13.5 Duration of remission (DOR)

DOR is defined only for patients who achieve a CR, mCR ,or PR, and is measured from the first date of attaining CR, mCR, or PR until the date of disease progression or death.

13.6 Time to Progression (TTP)

TTP is defined as the time from date of first dose of fludarabine until evidence of disease progression.

13.7 Disease-Free Survival (DFS)

DFS is defined as the time from the day CR, mCR, or CRi is documented until disease progression or death.

13.8 Overall Survival (OS)

OS is defined from the date of first dose of fludarabine on this study until death.

14.0 AUDITING

As coordinating center of this trial, Washington University (via the Quality Assurance and Safety Monitoring Committee (QASMC) will monitor each participating site to ensure that all protocol requirements are being met; that applicable federal regulations are being followed; and that best practices for patient safety and data collection are being followed per protocol. Participating sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if

during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Items to be evaluated include:

- Subject screening and enrollment
- Reporting of adverse events
- Maintenance of HIPAA compliance
- Completeness of regulatory documentation
- Completeness of participant documentation
- Acquisition of informed consent
- IRB documentation
- Issues of protocol adherence

Additional details regarding the auditing policies and procedures can be found at https://siteman.wustl.edu/wp-content/uploads/2015/10/QASMC-Policies-and-Procedures-03.31.2015.pdf

15.0 STATISTICAL CONSIDERATIONS

This is a phase I/II study aimed at establishing the maximum tolerated or tested dose of donor CIML NK cells (phase I) in relapsed/refractory AML and MDS patients, and preliminary assessment of efficacy (phase II) in relapsed AML patients.

15.1 Statistical Design and Sample Size

15.1.1 Phase I Design and Sample Size

This is a phase 1 clinical trial with the primary objective of establishing the maximum tolerated dose of CIML NK cells in AML or MDS patients. We propose to use a standard 3+3 dose escalation design and include 3 dose levels of CIML NK cells to define the MT/TD of CIML NK cells. Dose escalation will be carried out as described in Section 5.3.3. We expect to enroll 6-18 patients depending on DLTs encountered. At least 6 patients will be treated at the phase I MT/TD before opening the phase II portion of the study.

15.1.2 Phase II Design and Sample Size

A lead-in cohort of eight patients with relapsed or refractory AML was treated with ALT-803 (Phase II/ALT-803) to establish the safety and immune effects of that treatment (See section 1.3.4).

The phase II/IL-2 portion will enroll patients with relapsed or refractory AML. Based on reports in large numbers of AML patients,[11,12,13,14] the expected CR rate with standard salvage chemotherapy is 34% in patients that relapse within 18 months of obtaining CR1. Patients who fail to achieve CR1 have a similar CR rate to salvage chemotherapy. We will use a clinically interesting goal of 54% CR/CRi (20% increase over historical control). In a Simon two-stage optimal design with power=0.8 and significance level=0.05 will require 46 patients with evaluable response as described in section 5.3.5. We will treat 18 patients in the

first stage, and will continue to stage 2 if 8 or more CR/CRi are observed. In the second stage an additional 28 patients will be treated, and study will be considered positive if 21 or more CR/CRi are observed in the 46 total patients treated. If the true CR rate is 34%, the probability of correct early stopping is 0.75 with this design.

15.1.3 Pediatric Cohort Design and Sample Size

The primary objective of the Pediatric Cohort is to establish the safety of CIML NK cells in pediatric patients. 10 patients will be treated at the phase I MTD.

15.2 Final Data Analysis Plan

15.2.1 Phase I Analysis Plan

The primary objective for phase I of this study is to investigate the MTD of CIML NK cell infusion. The MTD will be reported as defined by DLTs in Section 5.3.2. DLTs and other AEs/SAEs will be summarized by patient, type and grade as defined by the CTCAE v4.0.

15.2.2 Phase II Analysis Plan

The primary objective of the phase II is to determine CR/CRi rates using MTD of CIML NK cells determined in the phase I part of the study. Descriptive statistics will be used to summarize baseline patient characteristics (proportion and confidence interval, mean ± SD or minimum, Q1, median Q3, maximum and IQR). Adverse events will be tabulated by patient, type and severity. Complete remission rate and complete cytogenetic remission rate will be calculated with 95% exact binomial confidence intervals. Kaplan Meier estimates will be used to describe the median time to hematologic recovery (neutrophils and platelets), duration of remission, event-free survival, relapse-free survival and overall survival using 95% confidence intervals.

15.3.3 Pediatric Cohort Analysis Plan

The primary objective of the pediatric cohort is to assess safety of CIML NK cell infusion. AEs/SAEs will be summarized by patient, type and grade as defined by the CTCAE v4.0. No pediatric patient will be treated until the previous pediatric patient has completed the treatment period (through Day 14), to allow adequate monitoring of Grade ≥ 3 toxicities before the next patient is treated.

15.2.3 Correlative Endpoints

Linear mixed models will be used to cluster multiple observations per subject or over time, making multiplicity adjusted comparisons of percent and number of CIML NK cells or IFN-γ^+ cells and B content scores by CR/CRi, and intensity of IFN-γ, CD107a or TNF in control or memory-like and licensed or unlicensed cells KIR matched or unmatched cells. Preliminary data indicate these are Gaussian on the original or a transformed (usually log) scale; otherwise, multiplicity adjusted Wilcoxon signed rank tests will be used for paired comparisons. FDR multiplicity adjustment will be carried out within the set of analyses addressing each

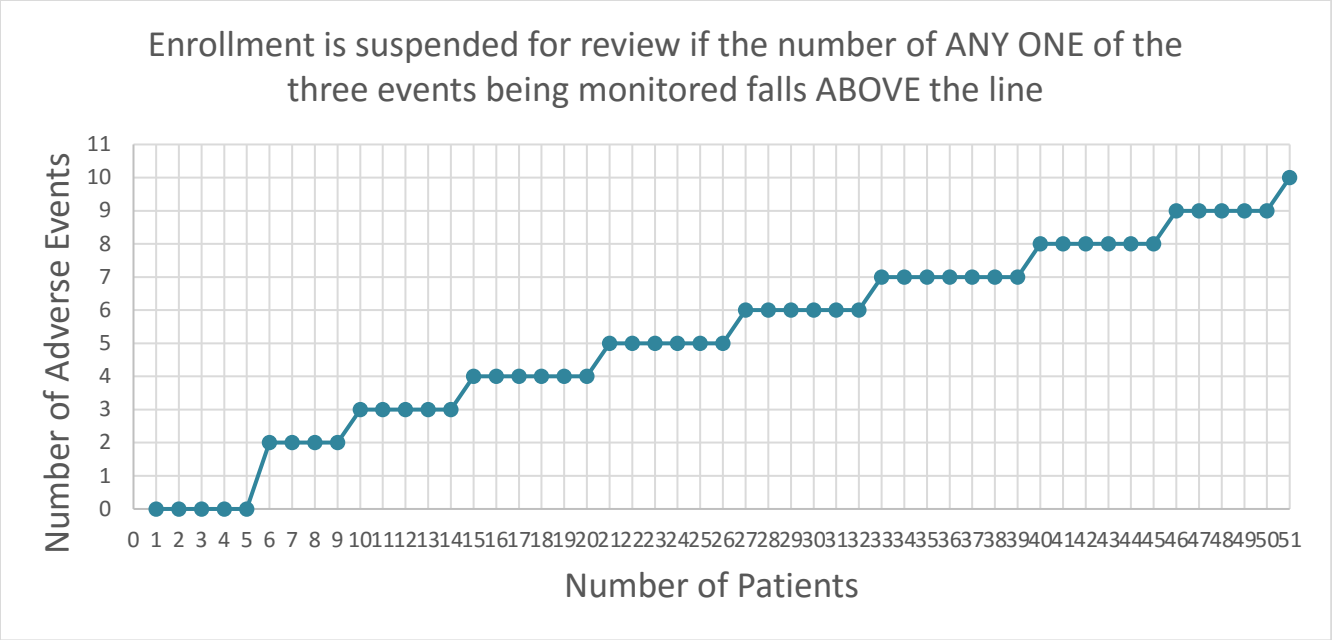
correlative endpoint. Data from 46 patients will provide the following power and significance level to detect the stated mean difference and effect sizes that are seen in preliminary data: **Defining percentages and number of total CIML NK cells, and those that are IFN-γ^+ after paired-patient AML restimulation, and correlation with CR/CRi:** .80 at α=.025, effect size~1.0, mean difference ~2.1 for log # CIML NK or IFN-γ^+ cells, 4.9 for % CIML NK cells, 6.4 for % IFN-γ^+ cells; **Defining the donor KIR genotype and correlation with CR/CRi:** .86 at α=.05 for KIR B content score, effect size=.79, mean difference~1.0; **Determine the phenotype, proliferation, and function of in vivo expanded CIML NK cells in the PB and BM utilizing mass cytometry, and integrate with known KIR to KIR-ligand interactions:** .81 at α=.0083, effect size=.50, IFN-γ^+ mean paired difference=10, %CD107a$^+$ mean paired difference=6, %TNF$^+$ mean paired difference 3; **Define CIML NK cell phenotypic and functional markers associated with elimination of AML in vivo using Citrus.** These analyses involve descriptive (SAM) and predictive (PAM or lasso) modeling. Preliminary data from 19 markers in 7 patients indicate power of .80 at α=.0005 to detect a difference between CR/CRi and <CR/CRi in 5 markers (26%). These 5 have a joint FNR=0% and FPR=20%, suggesting that Citrus will be able to form clusters distinguished by response. **Elucidate additional inhibitory receptor checkpoints on CIML NK cells that limit responses to primary AML** and **Changes in recipient Tregs or MDSCs associated with AML relapse or resistance to CIML NK cell therapy:** Longitudinal models will be used to quantify frequency of inhibitory receptor ligands at 5 time points in PB and BM as predictors of CIML NK cell number and associated AML blast killing rate or of recipient Tregs or MDSCs as predictors of lower CIML NK numbers. Preliminary data indicate these are Gaussian on the original or a transformed (usually log) scale; otherwise, multiplicity adjusted Wilcoxon signed rank tests will be used for paired comparisons. FDR multiplicity adjustment will be carried out. Longitudinal data are not yet available, but preliminary data show correlations between log NK # at peak expansion, providing for data from 46 patients at α=.01 power ≥ .82 to detect correlations ≤ -.48, values observed in PB with %LAG3+ or %PD1+. Correlations between log NK # and %Treg, and log #Treg/μL vary from -.48 to -.87 and can be detected with similar power.

15.3 Toxicity Monitoring

Patients will be monitored for adverse events as listed in Section 5.3.4. The following events will be used to determine the stopping rules with an expected rate of 10% and a maximum allowable rate of 25%.

- Bone marrow suppression between days 35 and 100
- Grade II-IV acute GVHD within 100 days
- Extensive chronic GVHD in any organ system

All accrued patients are included in the toxicity monitoring rule (N=57) because all may be evaluable for toxicity. A single continuous toxicity monitoring rule will be used for each of these three events because the expected and maximum allowable rates are the same.



	Suspend for review if ANY ONE event rate reaches:		
# Patients	# Bone marrow suppression	# Acute GHVD grade 2-4	# Extensive chronic GVHD
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	0	0	0
6	2	2	2
7	2	2	2
8	2	2	2
9	2	2	2
10	3	3	3
11	3	3	3
12	3	3	3
13	3	3	3
14	3	3	3
15	4	4	4
16	4	4	4
17	4	4	4
18	4	4	4
19	4	4	4
20	4	4	4
21	5	5	5
22	5	5	5

23	5	5	5
24	5	5	5
25	5	5	5
26	5	5	5
27	6	6	6
28	6	6	6
29	6	6	6
30	6	6	6
31	6	6	6
32	6	6	6
33	7	7	7
34	7	7	7
35	7	7	7
36	7	7	7
37	7	7	7
38	7	7	7
39	7	7	7
40	8	8	8
41	8	8	8
42	8	8	8
43	8	8	8
44	8	8	8
45	8	8	8
46	9	9	9
47	9	9	9
48	9	9	9
49	9	9	9
50	9	9	9
51	10	10	10

For each application of the monitoring rule has a probability of .837 to correctly identify an excessive adverse event rate and a probability of .052 of incorrectly identifying an excessive rate. The total number of adverse events of any one kind is 10.

15.4 Replacement of Patients

For the phase I portion, if less than the targeted CIML NK cell numbers are available for the dose level enrolled (actual CIML NK cell dose administered < 80% of goal CIML NK cell dose), then all CIML NK cells (after quality control testing) will be given and the patient will continue with protocol therapy. These patients will be considered not evaluable for the primary analysis at the dose level intended, but will be evaluated as part of the analyses at the lower dose level received. These patients will be replaced for the intended CIML NK cell dose level. If a patient for unexpected reasons is unable to receive CIML NK cells, they will not be included in the safety analyses.

For the phase II portion, if the MT/TD is the max NK cell dose, the goal dose will be $0.5x10^6$/kg to $10x10^6$/kg, administering all IL-12/15/18 pre-activated NK cells available up to $10x10^6$/kg. Since complete remissions were observed in dose level 1, the lower goal limit will be inclusive of this initial dose. If $<0.5x10^6$/kg are administered, the patient will be replaced for statistical analysis of the primary objective.

16.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted. Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

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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: Calucation of creatinine clearance

Cockcroft-Gault Formula

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

Schwartz equation

CrCl (ml/min/1.73m2)=[length (cm) x k] /serum creatinine

k = 0.45 for infants 1-52 weeks old
k = 0.55 for children 1-13 year old
k = 0.55 for adolescent females 13-18 years old
k = 0.7 for adolescent males 13-18 years old

APPENDIX C: European Leukemia Net Risk Classification for AML

High-risk is defined as Adverse, intermediate-I and intermediate-II risk groups.

Adverse-Risk: Inv(3)(q21q26.2) or (t3;3)(q21q26.2); RPN1-EVI1 t(6;9)(p23;q34)l DEK-NUP214 t(v;11)(v;q23); MLL rearranged -5 or del(5q); -7; abnl(17p); complex karyotype*

Intermediate-II: t(9;11)(p22;q23); MLLT3-MLL; Cytogenetic abnormalities not classified as favorable or adverse

Intermediate-I: Mutated NPM1 and FLT3-ITD (normal karyotype); Wild-type NPM1 and FLT3-ITD (normal karyotype); Wild-type NPM1 without FLT3-ITD (normal karyotype)

Favorable: t(8;21)q22;q22) RUNX1-RUNX1T1; inv(16)p13.1q22) or t(16;16)(p13.1q22); CBFB-MYH11; Mutated NPM1 without FLT3-ITD (normal karyotype); Mutated CEBPA (normal karyotype).

***Three or more chromosome abnormalities in the absence of one or more of the following, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3);**

APPENDIX D: 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 E: 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		

Event	HRPO	QASMC	FDA
	working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.		
Breach of confidentiality	Within 10 working days.		
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.		

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

Incarceration	working day. Otherwise, report at the time of continuing review. 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.