Masonic Cancer Center, University of Minnesota
Cancer Experimental Therapeutics Initiative (CETI)

Haploidentical Donor Natural Killer (NK) Cell Infusion with Subcutaneous ALT-803 in Adults with Refractory or Relapsed Acute Myelogenous Leukemia (AML)

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Refer to the Procedures Manual for Affiliate Sites for a complete list of study personnel and contact information.

Revision History

Revision #	Version Date	Detail of Changes	Consent change? (Y/N)
	07/24/2016	Original version for CPRC	n/a
	08/24/2016	In response to CPRC stips and Altor's review Other minor edits and clarifications	n/a
	12/21/2016	Original to IRB and FDA Add cytokine release syndrome (CRS) • Section 9.3: Add risk of CRS • Section 12: Add assessment of CRS with updated Targeted toxicity worksheet in appendix V • Section 11.2.2: Add PK sampling; CRP/ferritin sample collection at 4 time points related to CRS Section 12.2: clarify adverse event documentation Section 14.4: change stopping rule timeframe from Day +100 to Day +42 Other edits through-out protocol including Clarify complete remission refers to CR and CRp Clarify disease eligibility Minor clarifications to inclusion/exclusion criteria Update and clarify study SOC and research activities in Section 11 Update ALT-803 pre-med and monitoring to be consistent across protocols Add IND number and Jeffrey Miller as IND sponsor	n/a
1	5/15/2017	In response to FDA comments on other ALT-803 protocols (for consistency between protocols when applicable): • Sections 8.5 and 10.6 – for patients > 100 kg in weight, ALT-803 dose calculation will be capped at 100 kg • Section 9.6 – revise CRS management plan • Section 13.4 – add section regarding regular teleconferences between lead institution and affiliate sites to facilitate communication between sites Additional edits/clarifications from SIV and other reviews: • Cover page and page 2 – remove Emory as an affiliate site • Synopsis and Section 5.2 – make disease eligibility criteria more clear • Sections 6.5 and 11.2 - update donor screen panel to current panel (replace RPR with treponema, add NAT) • Section 8.3 – for product samples to TTL add a 3rd sampling (after depletion but before ALT-803) • Section 8.4 – delete statement regarding patient not evaluable if requires treatment with steroids	No

Revision #	Version Date	Detail of Changes	Consent change? (Y/N)
		• Section 8.5 – clarify that the ALT-803 dose will be based on patient weight at admission/pre-chemo, update ALT-803 pre meds • Sections 11.1and 11.2.2 – Day +14 bm bx is SOC, not research • Sections 12.1 and 12.6 – align definitions for deviations with updated MCC definitions • Section 12.2 – clarify that a new patient injection reaction diary (appendix VI) is to be started for each injection site and that the diary is to continue to be completed for an injection site until it resolves or the form is completed. • Section 14.4. – update to reflect single stopping rule • Update appendices to reflect changes within the protocol Other minor edits and clarifications	
2	1/22/2019	Change in Principal Investigator	Yes

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Protocol Synopsis

Haploidentical Donor Natural Killer (NK) Cell Infusion with Subcutaneous ALT-803 in Adults with Refractory or Relapsed Acute Myelogenous Leukemia (AML)

Study Design:

This is a multi-institutional Simon's optimal two-stage single arm phase II trial of CD3/CD19 depleted, ALT-803 activated, haploidentical donor NK cells and subcutaneous ALT-803 given after lymphodepleting chemotherapy (CY/FLU) for the treatment of refractory or relapsed acute myelogenous leukemia (AML).

The primary objective is to study the potential efficacy of NK cells and ALT-803 to achieve complete remission (CR/CRp) with or without platelet recovery by Day 42 while maintaining safety. CRp is defined as leukemia clearance (< 5% marrow blast and no circulating peripheral blasts) and neutrophil recovery but with incomplete platelet recovery.

The two stage design will enroll 9 patients in stage 1. If 3 or more of the 9 patients achieve CR/CRp by Day 42 post NK cell infusion, an additional 15 patients will be enrolled in stage 2 to obtain a more precise estimate of CR/CRp. If 10 or more out of the total 24 patients show clearance, further investigation will be considered.

Patients are followed for 1 year from the NK cell infusion to determine disease free survival, treatment related mortality, and time to relapse.

Patients achieving a complete remission (CR or CRp) and neutrophil recovery (ANC > 500 cells/μl) for at least 4 weeks will be considered for allogeneic transplant, independent of this study, to prolong remission.

Primary Objective:

To estimate the rate of complete remission (CR/CRp) by Day 42 after the infusion of CD3/CD19 depleted, ALT-803 activated, donor NK cells and subcutaneous ALT-803 given after lymphodepleting chemotherapy (CY/FLU) for the treatment of refractory or relapsed acute myelogenous leukemia (AML)

Secondary Objectives:

- To determine the incidence of in vivo expansion (≥ 100 donor derived NK cells per μL blood) of NK cells by Day +14
- To document ALT-803 associated toxicity when given subcutaneously in this patient population
- To evaluate the safety of the therapy as measured by rate of treatment related mortality (TRM) at 6 months

Correlative Objectives

- To measure the effect of ALT-803 on the function of adoptively transferred NK cells
- To correlate CR/CRp with in vivo donor derived NK cell expansion
- To characterize ALT-803 immunogenicity

Eligible Diseases:

Diagnosis of acute myeloid leukemia (AML) and meets one of the following disease criteria:

Primary induction failure:

- De novo AML - no CR after 2 or more chemotherapy induction attempts
- Secondary AML (from MDS or treatment related): no CR after 1 or more chemotherapy induction attempts

Relapse after chemotherapy: not in CR after 1, 2 or 3 re-induction attempts

- Patients > 60 years of age, the 1 chemotherapy re-induction is not required

Relapse after hematopoietic stem cell transplant:

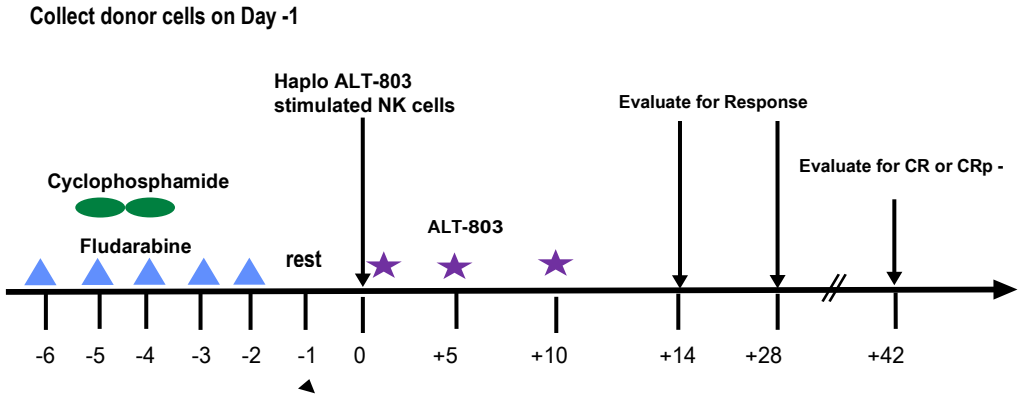
- Relapse must have occurred > 18 months after transplant
- No re-induction attempts required and no more than 1 re-induction attempt is allowed.

Notes:

1) For hypomethylating agents (i.e. decitabine, azacititdine) to count as an induction/re-induction attempt, the patient must have completed a minimum of 3 monthly cycles
2) For targeting agents (i.e. sorafenib) to count as an induction/re-induction attempt, the patient must have completed a minimum of 1 month without attaining CR
3) 7+3 followed by 5+2 counts as TWO induction attempts
4) Use of hydroxyurea is permitted to control blasts until Day -3 per Section 8.7
5) A history of AML related CNS involvement is allowed if CSF analysis is negative on 2 test dates at least 2 weeks apart prior to study treatment. The use of ongoing CNS maintenance therapy is allowed while on study

Key Inclusion Criteria:	• age ≥18, but ≤ 70 years • Karnofsky performance status ≥ 60% • available HLA-haploidentical donor (aged 12 to 75 years) with donor/recipient match based on a minimum of intermediate resolution DNA based Class I typing of the A and B locus (at least 2/4 class I allele) • adequate liver, renal, pulmonary and cardiac function • ability to be off prednisone and other immunosuppressive drugs for at least 3 days prior to the NK cell infusion • voluntary written consent
Key Exclusion Criteria:	• acute leukemia of ambiguous lineage • pregnant or breastfeeding • new or progressive pulmonary infiltrates • active autoimmune disease requiring systemic immunosuppressive therapy • history of severe asthma currently on chronic systemic medications (mild asthma requiring inhaled steroids only is eligible)
Accrual Objective:	Stage 1: 9 patients Stage 2: 15 patients Targeted yearly enrollment - 8 patients

Study Schema



Lymphodepleting Chemotherapy:

▲ Fludarabine 25 mg/m^2 x 5 days start Day -6

⬬ Cyclophosphamide 60 mg/kg x 2 days on Day -5 and -4

ALT-803 Stimulated Donor NK Cells:

The apheresis product (collected Day -1) will be enriched for NK cells with the large-scale CliniMacs® device (Miltenyi) by depletion of CD3$^+$ cells to remove T-lymphocytes and depletion of CD19$^+$ cells to remove B-lymphocytes. The NK cell enriched product will be stimulated by overnight incubation with 36.1 ng/mL ALT-803 under GMP conditions and infused on Day 0.

ALT-803 to Facilitate NK Cell Survival and Expansion:

★ ALT-803 at 10 mcg/kg subcutaneously (SC) with the 1st dose administered on Day 0 (no sooner than 4 hours post NK cells), Day +5 and Day +10 for 3 doses total

1 Objectives

1.1 Primary Objective

To estimate the rate of complete remission with or without platelet recovery (CR/CRp) by Day 42 after the infusion of CD3/CD19 depleted, ALT-803 stimulated, donor NK cells and subcutaneous ALT-803 given after a non-myeloablative preparative regimen for the treatment of refractory or relapsed acute myelogenous leukemia (AML)

1.2 Secondary Objectives

- To determine the incidence of in vivo expansion (≥ 100 donor derived NK cells per µL blood) of NK cells by Day +14
- To document ALT-803 associated toxicity when given in this patient population
- To evaluate the safety of the therapy as measured by rate of treatment related mortality (TRM) at 6 months

1.3 Correlative Objectives

- To measure the effect of ALT-803 on the function of adoptively transferred NK cells
- To correlate CR/CRp with in vivo donor derived NK cell expansion
- To characterize ALT-803 immunogenicity

2 Background and Significance

2.1 Human Natural Killer (NK) Cells as Mediators of Antitumor Therapy

We and others have focused on the role of NK cells in tumor suppression. Human NK cells are a subset of peripheral blood lymphocytes defined by the expression of CD56 or CD16 and the absence of the T-cell receptor (CD3) [1-10]. A number of studies suggest that NK cells may have a role in tumor surveillance. Cell lines susceptible to NK lysis are designated “NK sensitive” targets. The prototype target is the hematopoietic cell line K562. Following incubation of NK cells with various cytokines, in particular IL-2, NK cells acquire the ability to lyse a broad array of fresh and cultured tumor targets not normally sensitive to NK lysis (NK resistant targets) including the Raji and Colo cell lines.

2.2 NK Cell-Based Immunotherapy for Advanced Malignancies

In a number of tumor types generally considered unresponsive to chemotherapy, a series of well-publicized trials at the National Cancer Institute have documented anti-tumor effects in patients using so-called adoptive

immunotherapy [11]. This approach involves harvesting mononuclear cells from patients via lymphapheresis, expansion of the cells ex vivo using high concentrations of the lymphokine Interleukin-2 (IL-2), and administration of the expanded and IL-2 activated cells (lymphokine-activated killer, or LAK, cells) to the patient along with IL-2. This approach produced approximately 20% complete and partial responses in initial trials at the NCI, with responses occurring primarily in melanoma, renal cell cancers, and NHL [12]. The MHC-unrestricted cytolytic activity of LAK cells is mediated predominantly by cells with the NK phenotype [13]. A randomized trial performed at the NCI [11] suggests that a portion of the antitumor activity is mediated by exogenous LAK cells, and a portion by the high-dose IL-2 which is apparently required to maintain high levels of cytolytic activity [14]. Our recent study demonstrated that adoptively transferred NK cells could expand in vivo, and that induction of remission in 5 of 19 poor-prognosis AML patients was associated with NK expansion and KIR ligand-mismatch donors [15].

2.3 rhIL-15

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[16]." Based on preclinical non-human primate and early phase clinical trial data, including those at the University of Minnesota, IL-15 regimens can unquestionably be designed to prospectively and reproducibly increase T-cell and NK-cell counts.

The NCI Biological Resource Branch has manufactured E. coli-expressed recombinant human IL-15 (rhIL-15), and this is the first IL-15 product tested here at the University of Minnesota. Systemic administration of NCI rhIL-15 by daily intravenous (IV) bolus has been shown to increase the number of circulating CD8+ T and NK cells, but the cytokine has a very short half-life. We have established the MTD for NCI-manufactured rhIL-15 using IV and subcutaneous dosing. Although safer with subcutaneous administration allowing more drug delivery, we identified limitations with the rhIL-15 in clinical testing. Specifically, high levels of free rhIL-15 decrease circulating IL-15Rα, acting as a negative feedback signal to reduce further IL-15 trans-presentation. This is the rationale in this trial for using IL-15/IL-15Rα-Fc (ALT-803), an IL-15 product that physiologically trans-presents IL-15.

2.4 IL-15/IL-15Rα-Fc (ALT-803)

This trial will evaluate an alternative IL-15 construct designed to have a prolonged serum half-life. The novel IL-15 immunoconjugate, ALT-803, was

developed by our collaborator, Altor BioScience Corporation (Altor, Miramar, FL), to overcome some of the biologic, regulatory, and commercial limitations of monomeric NCI rhIL-15. Under natural circumstances, IL-15 and IL-15 Receptor-alpha (IL-15R) are coordinately expressed by antigen-presenting cells (i.e., monocytes and dendritic cells) [17]. During signaling by the IL-15 pathway, IL-15 bound to IL-15R is presented in trans to neighboring NK or CD8+ T cells expressing only the IL-2R receptor. 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 [18]. ALT-803 is a soluble complex consisting of two protein subunits of a human IL-15 variant[19] associated with high affinity to a dimeric human IL-15 receptor α (IL-15Rα) sushi domain/human IgG1 Fc fusion protein [20].

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)11. The human IL-15Rα sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the human IL-15 receptor α subunit (IL-15Rα) (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. ALT-803 has a prolonged serum half-life in preclinical animal models and has a 4-fold increase in biologic activity greater than wild-type IL-15 (IL-15 wt) [19].

2.5 ALT-803 by Subcutaneous Injection

In August 2016, Altor prepared a Safety Summary of ALT-803 based on several ongoing early stage trials for various cancer indications (unverified/unaudited data). At the time 107 patients had received at least 1 dose of ALT-803, of which 35 were by subcutaneous injection and 26 of those patients treated at the University of Minnesota. Dosing ranged from 1 mcg/kg to 20 mcg/kg with the majority treated at 6 mcg/kg (n=16) and 10 mcg/kg (n=12). A summary of ALT-803 by route and dose is found in Section 10.8. The Altor clinical update concurred with our experience that subcutaneous dosing was generally well tolerated with an injection site related skin rash as the most notable event, often quite widespread; however none were dose limiting. Typically the rash resolved by 7 days post-injection. Other effects included fever, fatigue, and changes in blood pressure.

3 Study Rationale

Patients with poor prognosis acute myelogenous leukemia (AML) cannot be cured with standard therapy. This protocol is based on previous University of Minnesota protocols testing adoptive transfer of NK cells to treat patients with advanced leukemia who failed standard therapies. Allogeneic NK cells, which are not suppressed through inhibitory receptor recognition of "self" class I MHC molecules expressed on cancer cells, may effectively treat these leukemias. We have extensive experience using adoptive transfer of haploidentical NK cells and IL-2 following lymphodepleting chemotherapy (cyclophosphamide and fludarabine) and find that 30-50% can achieve a remission and proceed to best donor transplant. IL-2 was administered to the patient after NK cell infusion in an attempt to expand them in vivo.

Although these results are an improvement over standard salvage therapy for patients with refractory leukemia (expected CR rate of 10%), they suggest that adoptive cell therapy in its current form requires additional anti-tumor activity [21]. The limitations of our prior approaches include the lack of in vivo NK cell expansion in most possibly due to the stimulation of Treg by IL-2. IL-15 in contrast to IL-2, does not support the expansion of regulatory T cells (Tregs). We now know that IL-15 will overcome these limitations and successfully expand NK cells in vivo perhaps leading to safer preparative regimens and greater clinical efficacy. Thus we will test adoptive transfer of haploidentical NK cells with the IL-15 super-agonist ALT-803 in patients with refractory AML.

4 Study Design

This is a multi-institutional, Simon's optimal two-stage single arm phase II trial of CD3/CD19 depleted, ALT-803 activated, donor NK cells and subcutaneous ALT-803 given after lymphodepleting chemotherapy (CY/FLU) for the treatment of refractory or relapsed acute myelogenous leukemia (AML). The primary objective is to study the potential efficacy of NK cells and subsequent ALT-803 dosing to achieve complete remission (CR) with or without platelet recovery (CRp) by Day 42 while maintaining safety. CR is defined as ≤5% blasts in the bone marrow with recovery of neutrophils and platelets. CRp is defined as leukemia clearance (< 5% marrow blast and no circulating peripheral blasts) and neutrophil recovery but with incomplete platelet recovery.

The two stage design will enroll 9 patients in stage 1. If 3 or more of the 9 patients show leukemic clearance by Day 42 post NK cell infusion, an additional 15 patients will be enrolled in stage 2 to obtain a more precise estimate of CR/CRp. If 10 or

more out of the total 24 patients show clearance, further investigation will be considered.

The CD3/CD19 depleted, ALT-803 stimulated donor NK cells are infused on Day 0, after a non-myeloablative preparative regimen of fludarabine/ cyclophosphamide. Subcutaneous ALT-803 is given on Day 0 (no sooner than 4 hours after the NK cell infusion), Day +5 and Day +10 for a total of 3 doses.

Patients are followed for 1 year from the NK cell infusion for disease free survival (DFS), treatment related mortality (TRM), and incidence of relapse.

Patients achieving a complete remission (CR or CRp) with neutrophil recovery (ANC > 500 cells/µl) for at least 4 weeks will be considered for allogeneic transplant, independent of this study, to prolong remission. Follow-up will continue for 1 year from the NK cell infusion.

5 Patient Selection

Study entry is open to patients 18 - 70 years of age regardless of gender, race, or ethnic background. While there will be every effort to seek out and include minority patients, the patient population is expected to be no different than that of other high risk acute myeloid leukemia studies at the University of Minnesota and the other participating institutions.

Inclusion Criteria:

5.1 ≥18 but ≤ 70 years of age

5.2 Diagnosis of acute myeloid leukemia (AML) and meets one of the following disease criteria:

- Primary induction failure:
  - De novo AML - no CR after 2 or more chemotherapy induction attempts
  - Secondary AML (from MDS or treatment related) - no CR after 1 or more chemotherapy induction attempts
- Relapse after chemotherapy: not in CR after 1, 2, or 3 re-induction attempts
  - Patients > 60 years of age, the 1 cycle of chemotherapy is not required
- Relapse after hematopoietic stem cell transplant:
  - Relapse must have occurred > 18 months after transplant
  - No re-induction required and no more than 1 re-induction attempt is allowed.

Notes:

1) For hypomethylating agents (i.e. decitabine, azacititdine) to count as an induction/re-induction attempt, the patient must have completed a minimum of 3 monthly cycles
2) For targeting agents (i.e. sorafenib) to count as an induction/re-induction attempt, the patient must have completed a minimum of 1 month without attaining CR
3) 7+3 followed by 5+2 counts as TWO induction attempts
4) Use of hydroxyurea is permitted to control blasts until Day -3 per Section 8.7
5) A history of AML related CNS involvement is allowed if CSF analysis is negative on 2 test dates at least 2 weeks apart prior to study treatment. The use of ongoing CNS maintenance therapy is allowed while on study.

5.3 HLA-haploidentical related donor (aged 12 to 75 years) with donor/recipient match based on a minimum of intermediate resolution DNA based Class I typing of the A and B locus (at least 2/4 class I allele)

5.4 Karnofsky Performance Status ≥ 60% (appendix III)

5.5 Adequate organ function within 14 days of study registration (28 days for pulmonary and cardiac) defined as:

- Creatinine: ≤ 2.0 mg/dL
- Hepatic: AST and ALT < 3 x upper limit of institutional normal
- Pulmonary Function: oxygen saturation ≥ 90% on room air; PFT's required only if symptomatic or prior known impairment - must have pulmonary function >50% corrected DLCO and FEV1.
- Cardiac Function: LVEF ≥ 40% by echocardiography, MUGA or cardiac MRI, no uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities

5.6 Able to be off prednisone or other systemic immunosuppressive medications for at least 3 days prior to NK cell infusion (excluding preparative regimen pre-medications)

5.7 Sexually active females of child bearing potential and males with partners of child bearing potential must agree to use effective contraception during therapy and for 4 months after completion of therapy

5.8 Voluntary written consent prior to the performance of any research related procedures

Exclusion Criteria:

5.9 Acute leukemias of ambiguous lineage

5.10 Pregnant or breastfeeding - The agents used in this study include those that fall under Pregnancy Category D - have known teratogenic potential. Women of child bearing potential must have a negative pregnancy test at screening

5.11 Active autoimmune disease requiring systemic immunosuppressive therapy

5.12 History of severe asthma and currently on systemic chronic medications (mild asthma requiring inhaled steroids only is eligible)

5.13 New or progressive pulmonary infiltrates on screening chest X-ray or chest CT scan unless cleared for study by Pulmonary. Infiltrates attributed to infection must be stable/improving (with associated clinical improvement) after 1 week of appropriate therapy (4 weeks for presumed or documented fungal infections).

5.14 Uncontrolled bacterial, fungal or viral infections including HIV-1/2 or active hepatitis C/B - chronic asymptomatic viral hepatitis is allowed

5.15 Received any investigational agent within the 14 days before the start of study treatment (1st dose of fludarabine)

5.16 Prior ALT-803

6 Donor Selection

Donor selection will be in compliance with 21 CFR 1271. Donor assessment and consent process will occur in the apheresis center or similar donor facility.

6.1 HLA-haploidentical related donor (aged 12 to 75 years) with donor/recipient match based on a minimum of intermediate resolution DNA based Class I typing of the A and B locus (at least 2/4 class I allele) - It is recognized individual institutions may have differing donor age guidelines. This is

acceptable as long as no donor is younger than 12 years or older than 75 years.

6.2 Body weight of at least 40 kilograms

6.3 In general good health as determined by the medical provider

6.4 Adequate organ function defined as:

- Hematologic: hemoglobin, WBC, platelet within 10% of upper and lower limit of normal range of test (gender based for hemoglobin)
- Hepatic: ALT < 2 x upper limit of normal,
- Renal: serum creatinine < 1.8 mg/dl

6.5 Performance of a donor infectious disease screen panel including CMV Antibody, Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Antibody, HIV 1/2 Antibody, HTLVA 1/2 Antibody, Treponema and Trypanosoma Cruzi (T. Cruzi) plus HBV, HCV, WNV, HIV by nucleic acid testing (NAT);or per current standard institutional donor screen – must be negative for HIV and active hepatitis B

6.6 Not pregnant - females of childbearing potential must have a negative pregnancy test within 7 days of apheresis

6.7 Voluntary written consent (and assent if donor < 18 years of age) prior to the performance of any research related procedure

7 Patient/Donor Registration

To be eligible for registration to this study, the patient must meet each inclusion criteria listed and none of the exclusion on the eligibility checklist based on the eligibility assessment documented in the patient's medical record.

The eligibility checklist for both the patient and donor will be completed at the time of study registration.

7.1 Registration with the University of Minnesota Clinical Trials Office

Upon completion of the screening evaluation, eligibility checklist and obtaining consent, the site study coordinator or designee will register the patient. The donor also is registered in OnCore. Complete registration information is found in the study's Procedures Manual for Affiliate Sites.

Affiliate sites only: At the time of registration, the signed consent will be uploaded into OnCore as an attachment under the appropriate record (patient or donor).

Affiliates are responsible for fulfilling any local registration requirements.

7.2 Patients Who Do Not Begin Study Treatment

If a patient is registered to the study and is later found not able to begin study treatment (beginning with the 1st dose of fludarabine), the patient will be removed from study and treated at the physician's discretion. The study staff will update OnCore of the patient's non-treatment status (off study). The reason for removal from study prior to starting study treatment will be clearly indicated in OnCore. The patient will be replaced to complete enrollment.

8 Treatment Plan

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, antimicrobials, etc.).

Patients are to receive allopurinol 300 mg PO daily, unless known allergy, beginning prior to chemotherapy start and continuing until Day +14 or as clinically indicated.

Study Day	Therapy	Protocol Section
-6	Fludarabine 25 mg/m^2: 1 hour infusion	8.1
-5	Fludarabine 25 mg/m^2: 1 hour infusion Cyclophosphamide 60 mg/kg: 2 hour infusion	8.1
-4	Fludarabine 25 mg/m^2: 1 hour infusion Cyclophosphamide 60 mg/kg: 2 hour infusion	8.1
-3	Fludarabine 25 mg/m^2: 1 hour infusion	8.1
-2	Fludarabine 25 mg/m^2: 1 hour infusion	8.1
-1	Rest day for patient Donor apheresis and cell product processing	8.1 8.2, 8.3
0	NK cell infusion ALT-803 10 mcg/kg SQ no sooner than 4 hours after the NK cell infusion	8.4 8.5
+5	ALT-803 10 mcg/kg SQ	8.5
+10	ALT-803 10 mcg/kg SQ	8.5

8.1 Preparative Regimen (Day -5 through Day -2)

The administration of the preparative regimen will follow the institutional dosing guidelines. Dose and/or schedule adjustments consistent with the standard of care may be made on an individual patient basis as needed for safety.

Patients are to receive allopurinol 300 mg PO daily, unless known allergy, beginning prior to chemotherapy start and continuing until Day +14 or as clinically indicated.

Fludarabine is administered as a 1 hour intravenous infusion per institutional guidelines once a day for 5 doses beginning on Day -6.

Cyclophosphamide 60 mg/kg will be administered as a 2 hour intravenous infusion on Day –5 and Day -4.

Cyclophosphamide dosing is calculated based on ABW (Actual Body Weight) unless ABW is >150% of the IBW (Ideal Body Weight). Then the dose should be computed using adjusted body weight.

Ideal body weight is calculated using 50kg + [2.3kg x (height in inches – 60)] for men; 45.5kg + [2.3kg x (height in inches – 60)] for women.

Adjusted body weight = IBW + 0.5(ABW-IBW).

Cyclophosphamide associated hydration will be given according to recommended institutional standards (recommend starting 12 hours prior to cyclophosphamide).

Mesna is given as five divided doses (totaling the daily cyclophosphamide dose in mg) with the 1st dose prior to the cyclophosphamide infusion, then at 3, 6, 9, and 12 hours later.

8.2 Donor Leukapheresis (Day -1)

The donor will be scheduled for the morning before the day of the planned NK cell infusion to undergo leukapheresis. Peripheral blood mononuclear cells (PBMCs) will be collected by a single apheresis over 5 hours as described by McKenna, et al[22]. At the time of the apheresis, three 9 or 10 ml green top tubes will be collected for research per Section 11.2.1.

8.3 Preparation of Allogeneic NK Cells / Product Release Criteria

The apheresis product will be T cell-depleted (CD3-) and B cell-depleted (CD19-) using the Miltenyi Biotec CliniMACS® Cell Selection System and CD3 and CD19 MicroBeads and reagent (Miltenyi Biotec, Auburn, CA). The NK cell enriched product will be activated by overnight stimulation with 36.1 ng/mL ALT-803 under GMP conditions in the treating institution cell processing facility. ALT-803 at 36.1 ng/mL is the equivalent of NCI IL-15 at 10 ng/ml based on the chemical conversions. ALT-803 is a bigger molecule requiring and as a result a 3.6 fold more is needed to give the same amount of IL-15 tested previously.

Refer to the chemistry, manufacturing and control (CMC) section of the Investigational New Drug (IND) application for additional preparation information and lot release criteria.

Cell Product Samples to MCC Translational Therapy Lab

Samples of the cell product from enrolling sites will be sent to the Masonic Cancer Center's Translational Therapy Lab (TTL) for cell function and phenotype studies being performed under a separate protocol. The following samples will be collected:

1) the apheresis unit before processing (100 x 10^6 cells)
2) the product after depletion, but prior to addition of ALT-803 (10 x 10^6 cells)
3) the final NK cell product (50 x 10^6 cells) pulled prior to calculating lot release

Both samples will be sent as a single shipment on Day 0 for overnight delivery to the Masonic Cancer Center's Translational Therapy Lab (TTL). Refer to the Laboratory Manual for shipping information.

8.4 Allogeneic $CD3^-$ $CD19^-$ Selected NK Cell Infusion (Day 0)

Pre-medications: Patients should be pre-medicated with acetaminophen 650 mg PO and/or diphenhydramine 25 mg PO before and 4 hours after the NK cell infusion. Corticosteroids should not be used.

Infusion guidelines: The NK cells will be infused without a filter or pump, slowly by gravity over at least 15 minutes, but no more than one hour.

If less than 1.5 x 10^7 MNC/kg are available, then all cells (after quality control testing) should be given and the therapy plan will remain the same. Outcomes

will not be included in the primary analysis for these patients and they will be replaced.

Monitoring: Patients will be monitored for adverse effects of the NK cell infusion such as rash, acute allergic reaction, bronchospasm, respiratory distress, and acute vascular leak syndrome. If severe acute reactions occur (defined as CTCAE v 4.0 grade 4 - life-threatening consequences; urgent intervention indicated), the infusion will be stopped.

Hydration will be administered per institutional guidelines. Any elevation of daily metabolic monitoring will be treated with more aggressive hydration, while being attentive to fluid overload.

Corticosteroids are to be avoided Day -3 through Day +14 (during the period of in vivo NK cell expansion) except for pre-medications for the preparative regimen. If absolutely necessary, steroids may be given as clinically indicated but aiming for a cumulative dose of systemic steroids ≤ 100 mg of hydrocortisone per day during this window.

8.5 ALT803 (Day 0, +5, and +10)

ALT-803 10 mcg/kg will be administered subcutaneously on Day 0 (no sooner than 4 hours after the NK cell infusion), Day +5 and Day +10.

ALT-803 dosing will be based on a weight obtained at admission/pre-chemotherapy.. For patients > 100 kilograms weight, the ALT-803 dose will be calculated using a weight capped at 100 kg. The dose will be re-calculated in subsequent doses in the event of a 10% or greater weight change from the baseline weight.

Injection sites should be rotated per institutional guidelines and each injection site separated by at least 1 inch.

The 1st dose of ALT-803 will be given no sooner than 4 hours after the NK cell infusion in the absence of a grade 4 NK cell infusion related toxicity. If the patient experiences grade 4 infusion related toxicity during the NK cell infusion and it resolves to grade 2 or better within the 48 hours, ALT-803 may be started late with all planned doses to be given; however the administration of remaining 2 doses will be shifted to maintain a minimum of 4 days between dosing.

If ALT-803 cannot be started within 48 hours after the NK cell infusion, no ALT-803 will be given and the patient will be replaced.

A window of +2 days for ALT-803 dosing is allowed in the event of scheduling issues or patient status; however all injections must be separated by a minimum of 4 days rest and be completed by Day +14. Refer to "Dose Hold" below.

Monitoring: Patients will be observed for a minimum of 2 hours after the 1st dose for immediate adverse events. Vital signs (heart rate, blood pressure, respiration, temperature, and oxygen saturation) will be documented prior to each ALT-803 injection and then at 30, 60, and 120 minutes with a ± 20 minute window for each time point. If the first dose is well tolerated after 2 hours of monitoring, subsequent doses may be administered with 30 minute post-dose monitoring.

Pre medications: Give acetaminophen 650 mg PO and diphenhydramine 25 mg PO prior to and 4 hours after each ALT-803 dose. On Day 0, if acetaminophen post dose for NK cell infusion was given within the last 4 hours, do not give an additional dose prior to the 1st dose of ALT-803.

Dose modification: A one-time dose decrease to 6 mcg/kg is allowed if recurrent constitutional symptoms seem related to ALT-803 dosing. Patients unable to tolerate the reduced dose will be discontinued from further ALT-803. Re-escalation of the dose is not permitted.

Dose hold: A 48 hour window is permitted for the 1st dose as described above. The 2nd or 3rd dose of ALT-803 may be held on the day of a planned dose for either of the following situations:

- the patient has a fever of > 101°F (38.3 °C)
- if in the opinion of the treating physician, holding would be of benefit to the patient

The following guidelines must be followed: 1) A minimum 4 day rest period is required between doses and 2) any dose not given by Day +14 will be skipped.

8.6 Supportive Care

Throughout the study, the investigator may prescribe any concomitant medications or treatment deemed necessary to provide adequate supportive care. Supportive care may include antibiotics, anti-fungals, analgesics, transfusions, growth factors.

8.7 General Concomitant Medication Guidelines

Concurrent chemotherapies (except hydroxyurea) with ALT-803 are prohibited. Hydroxyurea may be used to control blast count until Day -3.

Administration of glucocorticoids is discouraged during the ALT-803 treatment period as the use of systemic steroid medications may result in loss of therapeutic effects of the study drug. Sustained use of steroids or steroid use to treat related toxicity will be an indication to stop ALT-803. Transient use is permitted as needed per standard of care. Topical steroid cream is permitted.

Beta-blockers and other anti-hypertensives may potentiate the hypotension and extra caution should be used during ALT-803 regimen treatment period. It is recognized, that ALT-803 treatment, like IL-2 and IL-15, may be associated with low blood pressure especially if the patient is not well hydrated.

8.8 Duration of Study Participation/Opportunity for Allogeneic Transplant

Patients will be followed for disease response (including those who go to transplant) until disease progression/relapse, then for survival only to a maximum of 12 months from the NK cell infusion unless:

- consent is withdrawn
- patient is discharged to hospice and/or not returning to the study site for follow-up – in these cases, follow-up information including date and cause of death may be obtained, as available from other sources
- the patient is unevaluable - if a patient is not evaluable, he/she will be followed only until the resolution or stabilization of treatment related toxicity
- new anti-cancer treatment is started

Formal follow-up ends at 12 months; however for the purposes of IND annual reporting, the date and cause of death will be recorded in OnCore upon knowledge in the follow-up tab.

Patients achieving a complete remission (CR or CRp) may be eligible for subsequent allogeneic transplant. The transplant will be done independent of this study. Patients would continue to be followed for survival through 12 months from the NK cell infusion.

9 Management of Selected Expected Toxicities Associated with NK Cell Infusion and ALT-803

See appendix IV for expected toxicities of the preparative regimen and Section 10.8 for the expected toxicities of ALT-803.

9.1 Tumor Lysis Syndrome

Tumor lysis syndrome is a possible risk associated with therapy of active leukemia. All patients (except those with known allergy) are to receive allopurinol 300 mg every day beginning before chemotherapy and continuing until Day +14 or as clinically appropriate.

9.2 Acute Allergic Reaction Secondary to the Infusion of Allogeneic NK Cells

Although infusion of donor lymphocytes has not been associated with acute allergic reactions, patients will be closely watched for the occurrence of hypotension, dyspnea and angioedema during and immediately after the infusion.

The NK cell infusion will be stopped if severe acute reactions occurs (defined as CTCAE grade 4 - life-threatening consequences; urgent intervention indicated). Refer to NCI Common Terminology Criteria for Adverse Events v4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

9.3 Injection Site Reactions/Skin Rash in Association with ALT-803

Based on current experience, localized skin rashes are common with subcutaneous administration. If a rash occurs and the rash area surrounding the ALT-803 injection site is > 6 cm and symptomatic (painful and/or itchy), it may be treated (at the discretion of the treating physician) with topical 0.05% clobetasol propionate (i.e. 0.05% Cormax) or 0.1% triamcinolone (i.e., Kenalog) cream. Diphenhydramine may be administered pre- (25-50 mg TID orally) and post-dosing (25-50 mg TID orally x 2 days) of ALT-803 at the discretion of the treating physician. Diphenhydramine should be eliminated if not tolerated.

9.4 Hypotension (systolic blood pressure < 90 mm Hg)

ALT-803 dosing should be held for hypotension (defined as systolic blood pressure less than 90 mm Hg) if in the presence of any clinically significant symptoms (in the opinion of the treating physician), until the systolic blood pressure reading is stable. If mild dehydration is suspected, an IV fluid bolus may be used per standard of care.

9.5 Vascular Leak Syndrome

Neither administration of allogeneic NK cells nor autologous IL-2 activated NK infusions have been associated with vascular leak syndrome in our previous experience. Nevertheless, patients will be monitored for weight gain (by weights at least 3 times per week) and pulmonary edema during and after the ALT-803 administration.

9.6 Cytokine Release Syndrome (CRS) or CRS-Like Symptoms

Patients will be monitored for clinical signs and symptoms of CRS through routine daily patient assessments and via the Targeted Expected Toxicities Worksheet (appendix V). Refer to Section 12 for the revised CRS grading system that will be used for this study.

While CRS is a clearly defined syndrome in T cell therapy, it is not known to occur to the same extent in NK cell therapies and may have other manifestations or may evolve differently. We have seen immune activation syndromes with other IL-15 products that include fever, CNS toxicities, and rash. CRS will be graded using the revised grading system by Lee et al.[23]

CRS Revised Grading System (replaces CTCAE v4 CRS grading)	
Grade	**Toxicity Description**
Grade 1	Symptoms are not life threatening and require symptomatic treatment only, e.g., fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Symptoms require and respond to moderate intervention - Oxygen requirement < 40% or Hypotension responsive to fluids or low dose of one vasopressor or Grade 2 organ toxicity
Grade 3	Grade 3 Symptoms require and respond to aggressive intervention - Oxygen requirement ≥40% or Hypotension requiring high dose or multiple vasopressors or Grade 3 organ toxicity or grade 4 transaminitis
Grade 4	Life-threatening symptoms - Requirement for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
Grade 5	Death

Grades 2-4 refer to CTCAE v4.0 grading.

The above will be used for CRS grading in conjunction with CTCAE v 4 for other toxicity grading.

Most patients will already be in the hospital. However, if CRS is suspected after ALT-803 is completed and within 30 days of NK cell infusion, management will be as follows below.

If symptoms occur, CRP, IL-6 and ferritin levels will be assessed. In our experience, having at least 2 of 3 elevations of CPR > 150, IL-6 > 150, or ferritin > 5000 could be consistent with CRS if in the context of persistent grade $\geq$2

fever and other associated symptoms (CRS or neurologic). Of the few patients we have suspected of CRS during treatment with NK cells and IL-15 in the past, all had this degree of fever.

1) CRS grade 1, continue current setting management
2) CRS grade 1 for > 24 hours or CRS grade ≥2, admit to hospital (if not already inpatient)
3) Start methylprednisolone at 100 mg/kg (or equivalent) IV per day for grade 1-2 CRS for 72 hours or earlier if grade ≥2 neuro symptoms.
4) Tocilizumab should be administered if grade ≥3 CRS or grade 1-2 symptoms not responding to steroids after 72 hours (and without other cause of fever identified) or if any upgrade in severity (grade ≥3 CRS or neurologic or other per CTCAE v4).

Participating centers must have emergency access (same day) to tocilizumab to take part in this trial.

9.7 Prolonged Marrow Suppression

Pancytopenia will occur with this regimen and should be treated as follows:

Anemia and Thrombocytopenia are expected in all patients, and they will receive standard supportive transfusion care according to transfusion committee guidelines or as modified based on clinical parameters. Prolonged anemia or thrombocytopenia are not unexpected will not count towards toxicity.

Neutropenia:

- Days –7 to +14: In the absence of severe, uncontrolled infection, G-CSF or granulocytes should be avoided. If the clinical situation warrants growth factor use, ALT-803 administration will be stopped and the patient replaced.
- At Day +14: If the ANC is <500/mm^3, G-CSF will be initiated

10 ALT-803 Formulation, Supply, and Potential Toxicity

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

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

10.1 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.

Quantitative Composition of ALT-803

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

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.

10.2 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). 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. 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-803, or one OD280 is equivalent to 0.79 mg/mL solution of ALT-803.

10.3 Storage and Handling

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

10.4 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 over 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.

10.5 Agent Ordering and Agent Accountability

ALT-803 is produced in the USA for Altor BioScience Corporation, Miramar, FL. After manufacturing, the product is stored at Altor BioScience 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 by cold chain from Altor BioScience Corporation to each participating site. (See the Procedures Manual for Affiliate Sites for instructions on how to order ALT-803.)

10.6 Study Drug Preparation and Administration

ALT-803 dose calculation will be based on actual body weight. For patients > 100 kilograms weight, the ALT-803 dose will be calculated using a weight capped at 100 kg. The calculated amount of ALT-803 will be drawn into a syringe for subcutaneous injection. The stock concentration is 1 mg/ml. Doses will be drawn directly into the syringe for injection. If the total subcutaneous dose is greater than 1.5 mL, the dose will be divided into 2-3 subcutaneous injections as needed.

10.7 Agent Inventory Records

The investigator, or a responsible party designated by the investigator (e.g. institutional investigational pharmacy), must maintain a record of the inventory and disposition of all agents received from Altor BioScience Corporation using the Study Agent Drug Accountability Record. (See the Study Procedures Manual for the form to use and instructions on how to complete it.)

10.8 Toxicity

As of August 2016, 107 patients were treated with ALT-803. A summary of exposure by route and dose level is presented below.

Summary of Exposure, by route and dose level

Intravenous:	
Dose	N
< 1 µg/kg IV	17
3 µg/kg IV	12
6 µg/kg IV	13
10 µg/kg IV	6
Total	48

Subcutaneous:	
Dose	N
1 µg/kg SQ	2
3 µg/kg SQ	0
6 µg/kg SQ	16
10 µg/kg SQ	12
15 µg/kg SQ	4
20 µg/kg SQ	1
Total	35

Intravesical:	
Dose	N
100 µg	3
200 µg	3
400 µg	18
Total	24

The most common side effects seen in studies with subcutaneous (under the skin) injections have been change in blood pressure (increase or decrease), fever, fatigue and injection site reaction, and skin rash, which at times has been widespread. These localized skins reactions are common (occurring in more than 50% of patients).

The study drug 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.

Risks of ALT-803		
Most likely (greater than 10% - 1 in 10 patients)	**Less likely (3% to 10% - 1 in 30 to 1 in 10 patients)**	**Rarely (< 3% - 1 in 30 patients)**
• pain and redness at the injection site • 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 or decrease 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 • skin rash	• heart problems - causing low blood pressure, dizziness, chest pain or changes in heart rhythm (heart beat) • 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

Risks of ALT-803		
Most likely (greater than 10% - 1 in 10 patients)	**Less likely (3% to 10% - 1 in 30 to 1 in 10 patients)**	**Rarely (< 3% - 1 in 30 patients)**
• weakness, headache, dizziness • vomiting, nausea, loss of appetite • reduced levels of electrolytes as detected by routine blood tests		

11 Clinical Evaluations and Procedures

Scheduled evaluations up to Day 28 may be performed +/-3 days from the targeted date; assessments to be performed after Day 28 may be done +/-7 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

11.1 Required Clinical Care Evaluations

	Screening Within 30 days of study registration[1]	During preparative regimen until ANC> 500[2]	Day 0	Day +5	Day +7	Day +10	Day +14	Day +21	Day +28	Day +42	Day +60	Day +100	6 and 12 months[8]
Consent	X												
Screening Assessment	X												
Medical History	X												
Physical Exam	X	daily							X	X	X	X	X
Weight	X	3 x week											
Height	X												
Vitals and pulse oximetry	X		X	X		X							
Performance Status	X								X	X		X	X
Toxicity Assessment	X		X	X	X	X	X	X	X	X	X	X	X
HLA typing	X												
Recipient viral panel	X												
CBC, diff, plt	X	daily	X		X		X	X	X	X	X	X	X
Basic metabolic panel (BMP) or equivalent[3]		daily (unless CMP is done)											
Comprehensive metabolic panel (CMP) or equiivalent[4]	X	weekly	X		X		X	X	X	X	X	X	X
Urine or serum pregnancy test for WOCBP[5]	X												
BM biopsy and aspirate[6]	X						X		X	X		X	X
Disease staging	X						X		X	X		X	X
CXR or chest CT scan	X												
Pulmonary Function Tests[7]	X												
EKG	X												
Echocardiogram, MUGA, or Cardiac MRI	X												

1 –within 14 days for labs required for eligibility per Section 5.5, consent and HLA typing exempt from 30 day limit

2 – or as clinically indicated

3 –basic metabolic panel consists of BUN, creatinine, calcium, glucose, lytes (CO2, Cl, Na, K)

4 – comprehensive metabolic panel consists of albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, creatinine, glucose, lytes (CO2, Cl, Na,, K), total bilirubin, and total protein

5- women of child bearing potential

6- collect 30 ml heparinized bone marrow for research at time of all bone marrow biopsies for clinical care (refer to Section 11.2)

7 -testing only required if symptomatic or prior known impairment

8 –formal follow-up ends 12 months after the NK cell infusion unless unevaluable, withdraws consent, discharged to hospice/not returning to study site or starts new anticancer therapy; however deaths should be recorded upon knowledge per Section 8.8

11.2 Research Related Evaluations

11.2.1 Donor Research Related

	Screen	Day of apheresis
Consent	X	
HLA Typing	X	
Type and Screen (ABO/RH /Indirect Antiglobulin)	X	
BMT Donor Infectious Disease Panel plus NAT testing (HCV, WNV, HIV)	X	X[2]
Donor chimerism (affiliate only to TTL)	X	
Medical History	X	
Physical Exam	X	X
CBC, diff, platelet	X	X
ALT, creatinine	X	
Pregnancy test, if applicable	X	X[2]
Three 9 or 10 ml green top tubes (research related) to TTL		X

1- including CMV Antibody, Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Antibody, HIV 1/2 Antibody, HTLVA 1/2 Antibody, Treponema, and Trypanosoma Cruzi (T. Cruzi) plus HBV, HCV, WNV, HIV by nucleic acid testing (NAT); or per current standard institutional donor screen – must be negative for HIV and active hepatitis B

2- repeat if more than 7 days since previous testing

The research related donor samples are shipped the day of collection (Monday-Thursday) for next day delivery to the Masonic Cancer Center's Translational Therapy Lab (TTL).

Refer to the Laboratory Manual for additional details.

11.2.2 Patient Research Related Evaluations

Day:	Before chemo start	Day 0	Day 0 at least 4 hours after the NK cell infusion		Day +1, Day +2, Day+3, Day+4	Day +5	Day +7	Day +9	Day +10	Days in Reference to the NK Cell infusion					
		Prior to NK cells	ALT-803 injection	Post ALT-803 dose 1						Day +14	Day +21	Day +28	Day +42	Day +60	Month 3
ALT-803			X			X			X						
Toxicity notation [3]	X	X (pre + 1 hr post)	X (pre)	X (2 hour)		X (pre ALT-803 and 30 min post)	X		X (pre ALT-803 and 30 min post)	X					X
Skin Reaction Diary – start day of ALT-803 injection and continue until next injection or for 6 days			X			X	X		X						
Monitoring for Stopping Rule Events			Through Day 42												
Chimerism – PB unseperated	X						X			X[1]	X[1]	X[1]	X[1]	X[1]	
Calculation of NK cell expansion (by study PI)							X			X	X	X	X		
CRP, ferritin[4]	X	X													
Six 9 or 10 ml green top tubes	X	X					X			X	X	X	X	X	X
One 9 or 10 ml of red top tube	X	X				X (pre ALT-803)	X		X (pre ALT-803)	X	X	X	X	X	X
Pharmacokinetics (PKs) 5 ml red top (a minimum of 2 ml blood needed) per time point			X (pre ALT-803)		X	X (pre ALT-803)	X	X							
Immunogenicity 5 ml of serum (1 red top tube)		X								X	X				
30 ml heparinized bone marrow at time of each BM biopsy	X									X		X	X	X	X

1- if donor cells are present at Day 14, chimerism monitoring will recheck Day 21. If present Day 21, check Day 28, If present, then Day 42 and Day 60 or until gone

2 - In the event of a CTCAE grade 4 infusion related toxicity (non-hematologic, excluding fever alone) a sample of banked blood will be tested for HADA

3 – per Section 12.2

4 – CRP and ferritin testing at these time points are research related to better understand the development (or not) of cytokine release syndrome. CPR and ferritin testing at other time points in association with CRS will be SOC as part of clinical care per Section 9.6

Note: if a patient is not abiding by the required clinical care calendar (Section 11.1), the collection schedule of the toxicity data and research related samples may be altered or discontinued on an individual patient basis, as appropriate.

It is recognized that with novel therapies as used in this study, the timing of protocol directed research samples may miss important patient specific events. For this reason, up to 3 extra samples for a total of 180 ml of blood may be drawn at additional time points that are not specified above.

Samples, other than for immunogenicity and pharmacokinetics (PK), are shipped the day of collection (Monday-Thursday) for next day delivery to the Masonic Cancer Center's Translational Therapy Lab (TTL). Immunogenicity and PK samples are stored frozen at the study site until the time of batch shipping directly to Altor Bioscience's central laboratory.

Refer to the Laboratory Manual for additional details.

11.2.1 Assessment of Immune Activation (TTL)

Samples to evaluate lymphocyte number and phenotype will be collected as detailed above for the Masonic Cancer Center Translational Therapy Lab (TTL) along with serum (red top tubes) for measure of cytokines that can reflect immune activation. Donor immune cells (green top tubes) and the donor apheresis product will used to understand how donor cells interact with patient cancer and patient immune system.

Flow cytometry analysis of a fraction of the PBMC will detect surface markers that define lymphocyte subsets (NK, NKT, B, and T cells, both CD4 and CD8), as well as intracellular markers that define regulatory T cells (Foxp3) and proliferating cells (Ki67). All remaining PBMC will be cryopreserved in 10% DMSO and stored in liquid nitrogen for future testing, if subject agreed to future storage at the time of initial consent.

11.2.2 Pharmacokinetics (ALTOR)

PK analysis will be performed at Altor BioScience Corporation. Serum samples for PK testing will be collected on all patients. The collection schedule is specified in the Study Calendar. One 5 mL silicone coated (red top) tube per time point will be collected.

The collected blood samples will be processed (centrifuge at 1000-1500 xg for 10 minutes) at the study site and the serum portion will be frozen and shipped to Altor BioScience in batches upon request.

11.2.3 Immunogenicity (ALTOR)

Immunogenicity assays will be performed at Altor BioScience Corporation. Serum samples for immunogenicity testing will be collected on all patients. The collection schedule is specified in the Study Calendar. One 5 mL silicone coated (red top) tube per time point will be collected.

The collected blood samples will be processed (centrifuge at 1000-1500 xg for 10 minutes) at the study site and the serum portion will be frozen and shipped to Altor BioScience in batches upon request for evaluation using validated ELISA methods.

12 Event Monitoring, Documentation, and Reporting

Toxicity and adverse events will be classified and graded according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

An exception to the use of CTCAE will be for the assessment of cytokine release syndrome (CRS). Individual adverse events which are associated with CRS will be graded per CTCAE; however the ultimate assessment will be made using a revised grading system for CRS as presented by Lee et al[23]

CRS Revised Grading System (replaces CTCAE v4 CRS grading)	
Grade	**Toxicity Description**
Grade 1	Symptoms are not life threatening and require symptomatic treatment only, e.g., fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Symptoms require and respond to moderate intervention - Oxygen requirement < 40% or Hypotension responsive to fluids or low dose of one vasopressor or Grade 2 organ toxicity
Grade 3	Grade 3 Symptoms require and respond to aggressive intervention - Oxygen requirement ≥40% or Hypotension requiring high dose or multiple vasopressors or Grade 3 organ toxicity or grade 4 transaminitis
Grade 4	Life-threatening symptoms - Requirement for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
Grade 5	Death

Grades 2-4 refer to CTCAE v4.0 grading.

The following definitions of adverse events (AEs) and serious adverse events (SAEs) will determine whether the event requires expedited reporting via the OnCore SAE Report Form in addition to routine documentation in the OnCore AE case report form (CRF).

The reporting timeframes for SAEs and other reportable events are located in Section 12.6.

Note: throughout this section the generic term “study drug” refers to the study related treatment (the preparative regimen, NK cell infusion, ALT-803 injections).

12.1 Event Terminology

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Serious Adverse Event: An adverse event is considered “serious” if, in the view of either the investigator or sponsor, 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
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unexpected Event: An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Major Deviation as defined by the Masonic Cancer Center: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject’s willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation as defined by the Masonic Cancer Center: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

12.2 Event Documentation Requirements

Due to the intentional clearing of the marrow with chemotherapy as preparation for the NK cell infusion, it is expected that all patients will experience severe depression of their blood counts and other related toxicities as detailed in appendix IV. Therefore, adverse event documentation for the purposes of this study will focus on:

- targeted expected toxicities felt to be related to the NK cell infusion and/or ALT-803 – refer to worksheet in appendix V
    - Prior to chemotherapy start
    - Prior to NK cell infusion
    - 1 hour (+/- 10 min) post NK cell infusion
    - Prior to ALT-803 dose 1
    - 2 hours (± 30 min) post ALT-803 dose 1
    - 24 hours after the NK cell infusion
    - Prior to ALT-803 dose 2
    - 2 hours (± 30 min) post ALT-803 dose 2
    - Day 7
    - Prior to ALT-803 dose 3
    - 2 hours (± 30 min) post ALT-803 dose 3
    - Day 14
- skin reactions as documented in the patient skin reaction diary (Appendix VI) – This form is to be completed by a member of the research staff while the patient is an inpatient. Each skin reaction diary focuses on a single injection site. That said, in this study on Day +5, a new skin reaction diary form is started for the most recent injection; however, if the Day 0 injection site reaction has not resolved its form continues to be completed until resolution. Refer to instructions in Appendix VI.
- other adverse events not on the targeted event worksheet felt to be related to the NK cell infusion and/or ALT-803
- unexpected adverse events that cannot be attributed to the preparative regimen or other causes (i.e. underlying disease, co-morbidities)

After Day 14, monitoring for adverse events will become less frequent based on the schedule in Section 11 and only events that are unexpected and at least possibly related to either the NK cell infusion and/or ALT-803 will be documented.

Note: if a patient is not abiding by the standard of care study calendar (Section 11.1), collection of the corresponding targeted events (and research related samples) also may be altered or discontinued on an individual patient basis, as appropriate.

12.3 SAE Documentation and Reporting Requirements

Any event meeting the definition of a serious adverse event (SAE) requires documentation using the MCC SAE Report Form in OnCore.

Any event that is both serious and unexpected, and at least possibly related to the study treatment must be reported to Masonic Cancer Center Affiliate Sites Manager and to Altor BioScience within 24 hours of knowledge. All others must be reported within 5 working days of knowledge. Refer to Section 12.6.

12.4 Early Stopping Rule Events Documentation and Reporting Requirements

Excessive toxicity defined as grade 4-5 non-hematologic, non-relapse and non-infectious toxicity (except for fevers alone) by Day 42 is considered an early study stopping rule event and must be reported per Section 12.6 using the Event Form found OnCore under the reports tab.

An event that counts toward an early stopping rule does not necessarily constitute a SAE and should be reported as such only if they meet the criteria for reporting as defined in Section 12.3.

12.5 Other Event Documentation and Reporting Requirements

Deaths, including due to disease, will be recorded as an SAE and reported per Section 12.6. Deaths due to disease should be recorded as a Grade 5 “Neoplasms benign, malignant and unspecified (incl cysts and polyps) – Other (Progressive Disease). In addition, the death date and cause must be reported in the patient follow-up tab in OnCore using the comment field in the survival status section to record the cause.

12.6 Institutional Event Reporting Table

Individual institutional sites will be responsible for reporting any event meeting local reporting requirements to their institutional IRB and/or other research oversight committees.

Event Type	Reporting Timeframe	Form in OnCore to Use	Report to
SAEs Requiring Expedited Reporting (serious, unexpected and at least possibly related to ALT-803)	Within 24 hours of knowledge	SAE Report Form	For Affiliate Sites: Masonic Cancer Center (MCC) Affiliate Sites Manager eorchols@umn.edu Altor BioScience at cancer_trial@altorbioscience.com Local institutional IRB or other entities per institutional policies and guidelines For UMN MCC: Report to the study's regulatory specialist
All other SAEs	Not required	n/a	
Stopping Rule Events	Within 24 hours of knowledge	Event Form	
Major Deviations	Within 5 working days of knowledge	Deviation Report Form	

12.7 MCC Reporting Requirements

As the study sponsor, the Masonic Cancer Center has the following expedited reporting responsibilities for events reported in Section 12.6:

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers
FDA	Unexpected and fatal or unexpected and life threatening suspected adverse reaction	no later than 7 Calendar Days	MCC SAE	Submit to FDA as an amendment to IND with a copy to Altor Bioscience at cancer_trial@altorbioscience.com And each affiliate institution
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	no later than 15 Calendar-Days		
Masonic Cancer Center SAE Coordinator	Events that count toward the early study stopping rule.	At time of reporting	Event Form	mcc-saes@umn.edu

13 Study Data Collection and Monitoring

13.1 Data Management

This study will report clinical data using The Online Enterprise Research Management Environment (OnCore®), a web based Oracle® database utilizing study specific electronic case report forms. Key study personnel are trained on the use of OnCore and will comply with protocol specific

instructions embedded within the OnCore. Patient demographics, patient specific study treatment calendars, targeted expected toxicities and other information required for IND annual reporting will be placed in OnCore and other research databases maintained by MCC IT.

13.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Study Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

13.3 Data and Safety Monitoring Plan (DSMP)

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp.

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore the following requirements will be fulfilled:

- At least quarterly review of the study's progress by the Masonic Cancer Center Data and Safety Monitoring Council (DSMC).
- The PI at each site will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The Masonic Cancer Center PI will oversee the submission of all reportable adverse events per Section 12.7 to the Masonic Cancer Center's SAE Coordinator, the University of Minnesota IRB, and the FDA.
- The PI with the CTO has oversight responsibility for trial monitoring at affiliate sites

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the IND sponsor will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

13.4 Teleconferences – Lead Site and Affiliate

Regular teleconferences to facilitate communication between participating sites regarding the study's progress, patient updates, summary of safety reports, case report form completion, and other issues for discussion. The University of Minnesota Affiliate Manager will be responsible for arranging these teleconferences and preparing the agenda. Meetings will occur at least every 2 weeks; however, these may be scheduled more or less frequently at the discretion of the lead institution. Participation of a minimum of one representative from the affiliate site is required. These teleconferences are in addition to other previously described site interactions including centralized patient registration, institutional and MCC required reporting of safety related issues, case report form completion in the study's central database (OnCore) and affiliate oversight through self-monitoring in compliance with the Masonic Cancer Center's Data and Safety Monitoring plan.

13.5 Affiliate Site Monitoring

The PI (Dr. Brunstein) with the CTO has oversight responsibility for trial monitoring at affiliate sites. Affiliate sites must self-monitor following the University of Minnesota Masonic Cancer Center Data and Safety Monitoring Plan (DSMP) http://z.umn.edu/dmsp

The investigator will permit study-related monitoring, audits, and inspections by the study's Principal Investigator/IND sponsor and/or any designees, the local IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

13.6 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study

correspondence in a secured facility for at least 6 years after the study file is closed with the IRB and FDA.

Please contact the CTO before destroying any study related records.

14 Statistical Considerations

14.1 Study Design, Objectives and Endpoints

The primary objective of this phase II trial is to study the potential efficacy of NK cells and ALT-803 to achieve a complete remission (CR or CRp) by Day 42 while maintaining safety. The primary endpoint, CRp is defined as leukemia clearance (< 5% marrow blast and no circulating peripheral blasts) and neutrophil recovery without platelet recovery. CR is defined as ≤5% blasts in the bone marrow with recovery of neutrophils and platelets.

Secondary endpoints include:

- The incidence of in vivo expansion (≥ 100 donor derived NK cells per uL blood) of NK cells by Day +14
- ALT-803 associated toxicity
- Treatment related mortality (TRM) by 6 months post-therapy

The two stage design will enroll 9 patients in stage 1. If 3 or more patients show clearance out of 9, an additional 15 patients will be enrolled to obtain a more precise estimate of CR/CRp. If 10 or more out of the total 24 patients show clearance, this method will be considered for further investigation.

14.2 Statistical Analysis

The primary endpoint of clearance will be estimated by simple proportions with confidence intervals calculated from asymptotic standard errors. Secondary endpoints of in vivo expansion and various safety/toxicity measures will similarly be estimated with simple proportions. Cumulative incidence will be used to estimate the probability of treatment related mortality treating relapse as a competing event. [24]

The correlation of CR/CRp with in vivo NK cell expansion will be evaluated with a chi-square test. The function of adoptively transferred NK cells will be measured descriptively with non-parametric statistics such as median, range and interquartile range.

14.3 Sample Size

The overall sample size of 24 patients is based on Simon's optimal two-stage design assuming a type-I error of 5% and 80% power.[25] The null hypothesis or the rate considered unworthy of further investigation is 25% clearance. The alternative hypothesis or the rate considered worthy of further investigation is 50%.

If we reach the second stage, the study will go on to enroll an additional 15 patients for a total sample size of 24 patients. Based on prior enrollment in a similar phase I trial, we expect to accrue at least 8 patients per year. Therefore, we anticipate completion of enrollment within 3 years.

14.4 Stopping Rule for Excessive Toxicity

Excess toxicity will be monitored using an early stopping rule. The stopping rule was developed using Pocock stopping boundaries.[26]

Patients with refractory AML are expected to die. Therefore, excess toxicity needs to be carefully evaluated. Based on current experience, grade 4-5 non-hematologic, non-relapse and non-infectious toxicity (except for fevers alone) as defined by the NCI's CTCAE version 4 is expected to be about 10%. The goal is to construct a boundary based on excessive toxicity by Day 42 such that the probability of early stopping is at most 10% if the rate is equal to 10% and our sample size is 24. With these stipulations, the study will be stopped if there is excess toxicity in 2 out 3 patients, 3 out of 8 patients, 4 out of 14 patients, 5 out of 21 patients or 6 patients at any time. If the actual probability of toxicity is 30% or 40%, the probability of reaching the boundary will be 86% and 98% respectively.

15 Ethical and Regulatory Considerations

15.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

15.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study

documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

15.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved Consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

16 References

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Appendix I – Eligibility Checklist – Patient

Haploidentical Donor Natural Killer (NK) Cell Infusion with Subcutaneous ALT-803 in Adults with Refractory or Relapsed Acute Myelogenous Leukemia (AML)

Patient Eligibility Checklist – page 1 of 2

Patient initials ☐☐☐☐ Patient ID ☐☐☐-☐☐☐
(site code – seq #)

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the patient from study entry.

<table>
<tr><th></th><th></th><th>Yes</th><th>No</th></tr>
<tr><td>1.</td><td>≥18 but ≤ 70 years of age</td><td>☐</td><td>☐</td></tr>
<tr><td>2.</td><td>Diagnosis of acute myeloid leukemia (AML) and meets one of the following disease criteria:
▪ Primary induction failure:
○ De novo AML - no CR after 2 or more chemotherapy induction attempts
○ Secondary AML (from MDS or treatment related): no CR after 1 or more chemotherapy induction attempts
▪ Relapse after chemotherapy: not in CR after 1, 2, or 3 re-induction attempts
○ Patients > 60 years of age, the 1 chemotherapy re-induction is not required
▪ Relapse after hematopoietic stem cell transplant:
○ Relapse must have occurred > 18 months after transplant
○ No re-induction required and no more than 1 re-induction attempt is allowed.
Notes:
1) For hypomethylating agents (i.e. decitabine, azacititdine) to count as an induction/re-induction attempt, the patient must have completed a minimum of 3 monthly cycles
2) For targeting agents (i.e. sorafenib) to count as an induction/re-induction attempt, the patient must have completed a minimum of 1 month without attaining CR
3) 7+3 followed by 5+2 counts as TWO induction attempts
4) Use of hydroxyurea is permitted to control blasts until Day -3 per Section 8.7
5) A history of AML related CNS involvement is allowed if CSF analysis is negative on 2 test dates at least 2 weeks apart prior to study treatment. The use of ongoing CNS maintenance therapy is allowed while on study</td><td>☐</td><td>☐</td></tr>
<tr><td>3.</td><td>HLA-haploidentical related donor (aged 12 to 75 years) with donor/recipient match based on a minimum of intermediate resolution DNA based Class I typing of the A and B locus (at least 2/4 class I allele)</td><td>☐</td><td>☐</td></tr>
<tr><td>4.</td><td>Karnofsky Performance Status ≥ 60% PS - ☐☐☐</td><td>☐</td><td>☐</td></tr>
<tr><td>5.</td><td>Adequate organ function within 14 days of study registration (28 days for pulmonary and cardiac) defined as:
<table>
<tr><th>Test</th><th>Required Value</th><th>Patient's Value</th><th>Date of Test Result</th></tr>
<tr><td>serum creatinine</td><td>≤ 2.0 mg/d</td><td>☐.☐ mg/d</td><td>☐☐/☐☐/☐☐☐☐</td></tr>
<tr><td>AST</td><td>< 3 x upper limit of institutional normal value:☐☐☐ u/L</td><td>☐☐☐ u/L</td><td>☐☐/☐☐/☐☐☐☐</td></tr>
<tr><td>ALT</td><td>< 3 x upper limit of institutional normal value:☐☐☐ u/L</td><td>☐☐☐ u/L</td><td>☐☐/☐☐/☐☐☐☐</td></tr>
<tr><td>oxygen saturation</td><td>≥ 90% on room air</td><td>☐☐☐</td><td>☐☐/☐☐/☐☐☐☐</td></tr>
<tr><td>pulmonary function (only if sxs or know impairment)</td><td>>50% corrected DLCO and FEV1</td><td>☐☐.☐%</td><td>☐☐/☐☐/☐☐☐☐</td></tr>
<tr><td>cardiac</td><td>LVEF ≥ 40% by echo, MUGA or MRI, no uncontrolled angina, severe uncontrolled ventricular arrhythmias, or ECG evidence of acute ischemia or active conduction system abnormalities</td><td>☐☐.☐%</td><td>☐☐/☐☐/☐☐☐☐</td></tr>
</table></td><td>☐</td><td>☐</td></tr>
</table>

Haploidentical Donor Natural Killer (NK) Cell Infusion with Subcutaneous ALT-803 in Adults with Refractory or Relapsed Acute Myelogenous Leukemia (AML)

Patient Eligibility Checklist – page 2 of 2

Patient initials ☐☐☐☐

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the patient from study entry.

		Yes	No
6.	Able to be off prednisone or other systemic immunosuppressive medications for at least 3 days prior to NK cell infusion (excluding preparative regimen pre-medications)	☐	☐
7.	Sexually active females of child bearing potential and males with partners of child bearing potential must agree to use effective contraception during therapy and for 4 months after completion of therapy	☐	☐
8.	Voluntary written consent prior to the performance of any research related procedures	☐	☐

EXCLUSION CRITERIA

A "YES" response to any of the following disqualifies the patient from study entry.

		Yes	No
9.	Acute leukemias of ambiguous lineage	☐	☐
10.	Pregnant or breastfeeding	☐	☐
11.	Active autoimmune disease requiring systemic immunosuppressive therapy	☐	☐
12.	History of severe asthma and currently on systemic chronic medications (mild asthma requiring inhaled steroids only is eligible)	☐	☐
13.	New or progressive pulmonary infiltrates on screening chest x-ray or chest CT scan unless cleared for study by Pulmonary. Infiltrates attributed to infection must be stable/improving (with associated clinical improvement) after 1 week of appropriate therapy (4 weeks for presumed or documented fungal infections)	☐	☐
14.	Uncontrolled bacterial, fungal or viral infections including HIV-1/2 or active hepatitis C/B - chronic asymptomatic viral hepatitis is allowed.	☐	☐
15.	Received any investigational agent within the 14 days before the start of study treatment (1st dose of fludarabine)	☐	☐
16.	Prior ALT-803	☐	☐

Having obtained consent and reviewed each of the inclusion/exclusion criteria, I verify that this patient is eligible.

_____ _____

Signature of enrolling investigator Date

Appendix II – Eligibility Checklist – Donor

Haploidentical Donor Natural Killer (NK) Cell Infusion with Subcutaneous ALT-803 in Adults with Refractory or Relapsed Acute Myelogenous Leukemia (AML)

Donor Eligibility Checklist – page 1 of 1

Donor initials ☐☐☐☐

INCLUSION CRITERIA

A "NO" response to any of the following disqualifies the donor from study entry.

		Yes	No
1.	HLA-haploidentical related donor (aged 12 to 75 years) with donor/recipient match based on a minimum of intermediate resolution DNA based Class I typing of the A and B locus (at least 2/4 class I allele).	☐	☐
2.	Body weight of at least 40 kilograms	☐	☐
3.	In general good health as determined by the medical provider	☐	☐
4.	Adequate organ function defined as: • Hematologic: hemoglobin, WBC, platelet within 10% of upper and lower limit of normal range of test (gender based for hemoglobin) ▪ Hepatic: ALT < 2 x upper limit of normal, ▪ Renal: serum creatinine < 1.8 mg/dl	☐	☐
5.	Performance of a donor infectious disease screen panel including CMV Antibody, Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Antibody, HIV 1/2 Antibody, HTLVA 1/2 Antibody, Treponema and Trypanosoma Cruzi (T. Cruzi) plus HBV, HCV, WNV, HIV by nucleic acid testing (NAT); or per current standard institutional donor screen – must be negative for HIV and active hepatitis B	☐	☐
6.	Not pregnant - females of childbearing potential must have a negative pregnancy test within 7 days of apheresis	☐	☐
7.	Voluntary written consent (and assent if donor < 18 years of age) prior to the performance of any research related procedure	☐	☐

_____

Signature of person completing eligibility checklist

Appendix III - Performance Status Scale

KARNOFSKY PERFORMANCE STATUS SCALE

Percentage	
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

REFERENCE
Karnofsky DA: Meaningful clinical classification of therapeutic responses to anti-cancer drugs. Editorial: Clin Pharmacol Ther 2:709-712, 1961.

Appendix IV – Expected Toxicities of the Preparative Regimen

Cyclophosphamide –

Common	Less Common	Rare, but may be serious
• low white blood cell count with increased risk of infection • hair loss or thinning, including face and body hair (usually grows back after treatment) • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility (inability to have children) in women and men	• low platelet count (mild) with increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if you become pregnant while taking cyclophosphamide	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • kidney damage (renal tubular necrosis) which can lead to kidney failure • heart damage, with trouble getting your breath, swelling of feet, rapid weight gain • scarring of lung tissue, with cough and shortness of breath • second cancer, which can happen years after taking this drug • death from infection, bleeding, heart failure, allergic reaction, or other causes

Fludarabine -

Common	Less Common	Rare, but may be serious
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness • tiredness (fatigue) • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding, severe anemia, skin blistering, or other causes

Appendix V – Targeted Expected Toxicities Worksheet

MT2016-05 CTCAE v4 Refer to Section 12.2 for time points

Patient Initials: _____ **Date of Assessment:** _____ **Assessment Time point:** _____

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Infusion related reaction (NK cells)	None	Mild transient reaction; infusion interruption not indicated; intervention not indicated	Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs	Prolonged (e.g., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptom following initial improvement; hospitalization indicated for clinical sequelae	Life-threatening consequences; urgent intervention indicated
ALT-803 Injection site reaction	None	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Pain; lipodystrophy; edema; phlebitis	Ulceration or necrosis; severe tissue damage; operative intervention indicated	Life-threatening consequences; urgent intervention indicated
Dyspnea	None or no change	Shortness of breath with moderate exertion	Shortness of breath with minimal exertion; limiting instrumental ADL	Shortness of breath at rest; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Hypoxia	None	……	Decreased O_2 saturation with exercise (e.g., pulse oximeter < 88%) intermittent supplemental oxygen	Decreased oxygen saturation at rest (e.g., pulse oximeter < 88% or PaO_2 ≤ 55 mm Hg)	Life-threatening airway compromise; urgent intervention indicated (e.g., tracheotomy or intubation)
Fever	None	38.0 - 39.0◦ C (100.4 - 102.2◦ F)	> 39.0 - 40.0◦C (102.3 - 104.0◦F)	> 40.0 ◦ C (>104.0◦ F) for ≤ 24 hrs	> 40.0 ◦ C (>104.0◦ F) for > 24 hrs
Chills	None	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	……
Hypertension	None	Pre-hypertension (systolic BP 120 - 139 mm Hg or diastolic BP 80 - 89 mm Hg)	Stage 1 hypertension (systolic BP 140 - 159 mm Hg or diastolic BP 90 - 99 mm Hg); medical intervention indicated; recurrent or persistent ≥ 24 hrs); symptomatic increase by >20 mm Hg (diastolic) or to >140/90 mm Hg if previously WNL; monotherapy indicated.	Stage 2 hypertension (systolic BP ≥ 160 mm Hg or diastolic BP ≥ 100 mm Hg); medical intervention indicated; more than one drug or more intensive therapy than previously used indicated.	Life-threatening consequences (e.g., malignant hypertension, transient or permanent neurologic deficit, hypertensive crisis); urgent intervention indicated.
Hypotension	None	Asymptomatic, intervention not indicated	Non-urgent medical intervention indicated	Medical intervention or hospitalization indicated	Life-threatening and urgent intervention indicated
Edema	None	Localized to dependent areas, no disability or functional impairment	Moderate localized edema and intervention indicated; limiting instrumental ADL	Severe localized edema and intervention indicated; limiting self care ADL	……
Pneumonitis	None	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; medical intervention indicated; limiting instrumental ADL	Severe symptoms; limiting self care ADL; oxygen indicated	Life-threatening respiratory compromise; urgent intervention indicated (e.g. intubation or tracheotomy)
Headache	None	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	……
Confusion (Altered Mental Status)	None	Mild disorientation	Moderate disorientation; limiting instrumental ADL	Severe disorientation; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Rash	None	Covering < 10% body surface area (BSA)	Covering 10-30% body surface area (BSA)	>30% body surface area (BSA)	Generalized exfoliative, ulcerative, or bullous dermatitis
Gait Disturbance	None	Mild change in gait (eg, wide-based, limping or hobbling)	Moderate change in gait (eg, wide-based, limping or hobbling); assistance device indicated; limiting instrumental ADL	Disabling; limiting self care ADL	……

Person Completing Form: _____ ADL = activities of daily living

Appendix VI – ALT-803 Injection Site Reactions Diary

The Diary is to be completed by the patient as a self-assessment in association with each dose of ALT-803.

A new diary must be started for each ALT-803 injection. If the previous injection reaction has not resolved, continue to collect information on its diary based on the days from that injection. In this study, the Day 5 post-injection for the Day 0 injection will be the same date as date of injection for the Day +5 injection and both forms will be completed assessing the form's targeted injection site. If at Day 6 post-injection, an injection site reaction is still present continue to collect data for items 1-4 daily until the reaction resolves..

For inpatients, the diary is completed by study personnel.

Patient Number*		**Date of Study Drug Injection***	_____/_____/_____	*To be completed by the site.

Please answer all questions below <u>daily</u> for 7 days, beginning with day of treatment. Be sure to bring back this completed diary to your next clinic visit.

	Instructions	**Day of Study Drug Injection** ___/___/_____	**Day 1 Post Injection** ___/___/_____	**Day 2 Post Injection** ___/___/_____	**Day 3 Post Injection** ___/___/_____	**Day 4 Post Injection** ___/___/_____	**Day 5 Post Injection** ___/___/_____	**Day 6 Post Injection** ___/___/_____
1. Is there redness at the injection site?	Check: ☐Yes or ☐No If yes, measure longest diameter in cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm	☐Yes ☐No _____cm
2. Is there firmness or swelling at the injection site?	Check: ☐Yes or ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No	☐Yes ☐No
3. Have you experienced any pain or itching at the injection site?	Check: ☐Yes or ☐No If yes, tell us if the pain and/or itching is mild, moderate or severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe	☐ Pain ☐ Itch Mild Mild Mod Mod Severe Severe
4. Have you taken or applied any medication for injection site pain or itching?	Check: ☐Yes or ☐No Provide name of medication(s)	☐Yes ☐No Name(s):	☐Yes ☐No Name(s):	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:	☐Yes ☐No Name:
5. Have you experienced any chills?	Check: ☐Yes or ☐No If yes, tell us if the chills are mild, moderate or severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe	☐Yes ☐No Mild Moderate Severe
6. Record your daily temperature upon waking (do not drink anything 5 minutes before taking your temperature)	Check: ☐Yes or ☐No If your temperature is 101°F for more than 24 hours, call your doctor.	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM	☐Yes ☐No _____°F Time: ___:___ AM / PM

Grading Injection Site Pain or Itching
Mild – Noticeable, does not interfere with activity
Moderate – Interferes with activity, limiting activities of daily living
Severe – Severely limiting self-care activities of daily living, incapacitating

Grading Chills
Mild – Mild sensitive of cold, shivering, chattering of teeth
Moderate – Moderate tremor of entire body, medication taken
Severe – Prolonged or severe, does not respond to medication