Clinical Study Protocol
Protocol Number 1401-NK
Sponsor Reference Number MT-2014-02

A Randomized Trial Comparing CD3/CD19 Depleted or CD3 Depleted/CD56 Selected Haploidentical Donor Natural Killer (NK) Cell Based Therapy for Adults With Acute Myelogenous Leukemia Who Have Failed 1 or 2 Induction Attempts

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Confidentiality Statement

Key Roles and Responsibilities/Contact Information

Role	Contact Information
IDE Sponsor/Study Funder	Miltenyi Biotec, Inc. [redacted]
Study PI, Deputy PI and Lead Institution	[redacted] **PI:-** [redacted] **Deputy PI** : - [redacted]
Other Clinical Sites	[redacted]
Clinical and Regulatory Leads at the [redacted]	**Study Coordinator (Clinical):** [redacted] **Affiliate Sites Manager:** [redacted]
ClinicalTrials.gov Registration	**Miltenyi Biotec, Inc.**
Subject Registration	[redacted] via Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database
Subject Randomization	[redacted] Study Coordinator
Data Management (eCRFs, and product lot release)	[redacted] via Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database
Adverse Event Reporting (FDA)	**Miltenyi Biotec, Inc.**
Study Site Monitoring Oversight	**Miltenyi Biotec, Inc.**
Statistical Support	[redacted]
Lab For Research Related Sample Processing And Analysis	[redacted]

Synopsis

Study Design: This is a Simon's optimal two-stage phase II trial of related donor HLA-haploidentical NK-cell based therapy for the treatment of newly diagnosed acute myelogenous leukemia (AML) (except acute promyelocytic leukemia) in persons who failed to achieve a complete remission (CR) after one or two standard induction attempts. Failure is defined as ≥ 30% bone marrow blasts in a bone marrow of at least 20% cellularity at the mid-cycle (~day 14) bone marrow biopsy or residual AML on ~day 28 bone marrow biopsy by morphology, flow, PCR or FISH.

In this study, after a preparative regimen of cyclophosphamide and fludarabine, patients receive a single infusion of CD3-/CD19- NK cells or CD3-/CD56+ NK cells followed by a short course of Interleukin-2 (IL-2) to facilitate NK cell survival and expansion.

The primary endpoint of the study is complete remission (CR and CRi and CRp) by day +42 (±3 days) post NK cell infusion with

- absolute neutrophil count > 1,000/mm^3, platelet count > 100,000/ mm^3, no leukemic blast in the peripheral blood and < 5% blast in the marrow, (CR)
- a morphologic complete remission with incomplete blood count recovery (CRi)
- leukemia clearance (< 5% marrow blast and no circulating peripheral blasts) and neutrophil recovery but with incomplete platelet recovery.

However, a secondary endpoint is the expansion and persistence of NK cells. This endpoint will be used in a randomized sub-study in stage 1 to choose one of two T cell depleted NK cells products (CD3-/CD19- versus CD3-/CD56+) to be used for the remainder of the study.

The first 24 patients enrolled in the study (stage I) will be randomized to either CD3-CD19- or CD3-CD56+ in a parallel comparison of the two products. An additional 17 patients (stage 2) will receive the optimal NK cell product based on success of NK cell expansion from the initial randomized sub-study. Between the 2 stages, 29 patients will receive the chosen product and be evaluable for the primary endpoint of CR, CRi and CRp by day +42.

Patients achieving a remission may proceed to standard of care consolidation or hematopoietic stem cell transplantation at the discretion of the treating physician and independent of this study.

Patients (including those who proceed to transplant or consolidation therapy) will be followed for disease free survival until 1) disease progression, 2) start of a new anti-leukemia therapy, 3) discharge to hospice and then for overall survival for a maximum of 2 years from the NK cell infusion, In addition all patients will be followed through day 100 to rule-out for treatment related morality (early study stopping rule events) and GvHD

Primary Objective: To determine the rate of complete remission defined as CR (leukemia clearance with blood count recovery) CRi (morphologic complete remission with incomplete blood count recovery) and CRp (leukemia clearance with incomplete platelet recovery) by day +42 post NK cell infusion.

Secondary Objectives:

- Expansion and persistence of NK cells - this endpoint will be used in a randomized sub-study in stage 1 to identify the preferred cell product processing method
- Incidence and severity of adverse events
- Incidence of infusional toxicities
- Incidence of treatment related mortality
- Incidence of "proceeding" to hematopoietic cell transplantation
- Incidence of acute graft versus host disease (GvHD) by day +42
- Overall survival at 12 and 24 months
- Disease-free survival at 12 and 24 months

Correlative Objectives:

- Incidence of successful *in vivo* expansion of adoptively transferred haploidentical NK cells as measured by the percentage of patients achieving 40% donor DNA and 40% of lymphocytes are NK cells at day 7 OR 20% donor DNA and 20% of lymphocytes are NK cells at day 14
- Feasibility of using the appropriate CliniMACS® Reagent System to deplete CD3+ cell and deplete CD19+ cells (CD3-/CD19-), or to deplete CD3+ cells and enrich CD56+ cells(CD3-/CD56+) to consistently obtain the product specific targeted NK cell dose
- Incidence of minimal residual disease

Disease Related Criteria:

Newly diagnosed acute myelogenous leukemia (except acute promyelocytic leukemia) and has failed to achieve remission after 1 or 2 standard induction attempts with failure defined as

- ≥ 30% bone marrow blasts in a bone marrow of at least 20% cellularity on the mid-course (~day 14) bone marrow biopsy or
- residual AML at ~day 28 bone marrow biopsy by morphology, flow, PCR or FISH

AML that has progressed from MDS is eligible provided no prior MDS directed treatment.

Patients enrolling after only 1 failed induction attempt must meet at least one of the following additional eligibility criteria of high risk: 1) ≥ 60 years of age 2) adverse cytogenetics or molecular characteristics as defined in section 4.1

Other Key Inclusion Criteria:

- Available related HLA haploidentical NK cell donor aged 12-70 years
- ≥ 18, but < 75 years of age, Karnofsky performance status ≥ 60%
- Adequate organ function within 14 days of study registration (30 days for pulmonary and cardiac) as defined in section 4.5
- Ability to be off prednisone and other immunosuppressive drugs for at least 3 days prior to the NK cell infusion (excluding preparative regimen pre-meds)
- No prior hematopoietic transplant
- Not pregnant or lactating
- Sexually active females of childbearing potential and males with partners of child bearing potential must agree to use birth control

Statistical Considerations:

This trial uses a Simon's two-stage design to estimate the complete remission (CR, includes CRi, CRp) rate at day +42 post NK cell infusion. In addition, the trial includes an initial randomized sub-study of 24 patients during stage 1 to choose which of the enriched NK cell products (CD3$^-$/CD19$^-$ versus CD3$^-$/CD56$^+$) should be used to complete the trial. Twelve patients will be randomized to each product. As resources are limited, the selection of the best product will partly be a clinical decision, based in part on which meets the correlative endpoint of successful *in vivo* NK cell expansion. This is defined as 40% donor DNA and 40% of lymphocytes are NK cells at day 7 post infusion OR 20% donor DNA and 20% of lymphocytes are NK cells at day 14 post infusion. As the CD56$^+$ is a more pure product, CD3$^-$/CD19$^-$ processing only will be used if there is dramatically better NK cell expansion and if the safety profile is acceptable. If neither product achieves success at the end of stage 1, the study will stop and the platform redesigned. If the trial continues after stage 1, an additional 17 patients will be enrolled using the optimal NK cell product to complete the trial.

The null hypothesis is that the CR (including CRi, CRp) rate is ≤ 38% and the alternative hypothesis is that CR rate is ≥ 63% at day +42. If 16 or more patients (of 29 using the optimal product) have achieved CR, the regimen will be deemed worthy of further study.

Sample Size:

A maximum of 29 evaluable (defined as alive at day +14 and receiving all study therapy with at least 3 doses of IL-2) patients receiving treatment with the optimal product will be sufficient to maintain an overall type I error of 6.5% while providing 80% statistical power. Including Part I where the two products are compared side by side, the maximum number of patients will be 41 and the minimum number of patients will be 24.

Enrollment Plan:

Based on enrolling 8 to 10 patients per year, accrual should be completed within 3 to 4 years