A Phase I Dose Escalation Trial of T-cell Receptor α/β Depleted Donor Lymphocyte Infusions following CD34+-selected Allogeneic Stem Cell Transplantation from Related and Unrelated Donors for Patients with Lymphoid, Myeloid or Plasma Cell Malignancies

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Principal Investigator
Guenther Koehne, M.D., Ph.D.
Deputy Director of Miami Cancer Institute (MCI)
Miami Cancer Institute
8900 North Kendall Drive
Miami, Florida 33176
Email: GuentherK@baptisthealth.net

Sponsor
Miami Cancer Institute at Baptist Health, Inc.
8900 North Kendall Drive
Miami, FL 33176

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1 CONTENTS

1 PROTOCOL SUMMARY

This is a phase I dose escalating trial designed to identify tolerable, clinically active doses of donor-derived TCR α/β depleted donor lymphocyte infusions (DLI) when administered to patients with lymphoid or myeloid malignancies following a T-cell depleted allogeneic hematopoietic cell transplantation (TCD HSCT). Patients will undergo a preparative regimen with Busulfan, Melphalan, Fludarabine, (Bu/Mel/Flu) or Total Body Irradiation (TBI) plus Thiopeta and Cyclophosphamide (TBI, Thio, Cy) and anti-thymocyte globulin (ATG), followed by a CD34+-selected stem cell transplant from a histo-compatible related or unrelated donor. Hematopoietic stem cell donors for this trial will include individuals who are 10/10 HLA matched or one antigen or allele mismatched at the HLA-A, B, C, DRB1 or DQB1 locus, as defined by high resolution methods. Donors who are 8/10 HLA matched with an antigen or allele mismatched at HLA-DQB1 and at one other locus will also be eligible for the trial. The donor-derived TCR α/β depleted donor lymphocyte infusions post transplantation are administered to induce complete remissions in patients with residual disease, to decrease the rate of relapse and to reduce viral complications/reactivations following allogeneic stem cell transplants. For this study, the TCR α/β depleted donor lymphocyte infusions is obtained from the CD34 negative fractions maintained after the CD34+ selection of the stem cell product.

The primary objective of this study is to assess the safety of TCR α/β depleted DLI derived from the CD34neg fractions from the original transplant donor of the CD34+ selected allogeneic stem cell transplant, when administered to patients with ALL, AML, Myelodysplastic Syndrome, Myelofibrosis or relapsed/refractory multiple myeloma following TCD HSCT. Secondary objectives are: 1) to assess the effects of these adoptively transferred cells depleted of TCR α/β T cells on minimal residual disease and the progression of disease, 2) to quantitate levels of TCR α/β and TCR γ/δ T cells in addition to reconstitution of NK cells and 3) to determine the reconstitution of viral-specific T lymphocytes and associated viral complications following adoptive transfer of the T cells.

Patients eligible for this protocol are patients who are undergoing a CD34+-selected allogeneic transplant for AML, ALL, MDS, MPD or multiple myeloma as indicated on their respective, primary transplant protocols/treatment plans. The patients will be conditioned for transplantation with 1. Busulfan (Busulfex®) (0.8 mg/Kg/dose Q6H x 10-12 doses), Melphalan (70 mg/m^2/day x 2 doses) and Fludarabine (25mg/m^2/day x 5 doses) or with 2. Hyperfractionated total body irradiation to dose of 1375cGy fractions at 4-6 hour intervals three times a day for a total of 11 or 12 doses followed by Thiotepa 5mg/kg/day x 2 (or 10mg/kg/day x 1) and cyclophosphamide 60mg/kg/day x 2 (or Fludarabine 25mg/m2 x 5 if cyclophosphamide is contraindicated). Patients will also receive ATG (Thymoglobulin®) prior to transplant to promote engraftment and to prevent graft-versus host disease post transplantation. The preferred source of stem cells will be peripheral blood stem cells (PBSC) mobilized by treatment of the donor with G-CSF for 5-6 days. PBSC will be isolated, and T-cells depleted by positive selection of CD34+ progenitor cells, using the CliniMACS Cell Selection System. The CD34+-selected, T-cell depleted peripheral blood progenitors will then

be administered to the patients after they have completed cytoreduction. No drug prophylaxis against GvHD will be administered post-transplant. All patients will also receive G-CSF post-transplant to foster engraftment.

The CD34neg cell fraction, usually discarded following the cell processing, containing the CD3+ TCR α/β and TCR γ/δ T cells, NK cells and B cells will then be further processed for TCR α/β depletion. In this process, the CliniMACS® TCR α/β CD3+ Reagent Kit is used to deplete human TCR α/β CD3+ T cells *in vitro* from heterogeneous hematological cell populations for lymphocyte infusions and cell products, depleted of the TCR α/β positive T-cells will be aliquoted for infusions post-transplant. Initially, patients in cohort I are treated with single infusions containing $5x10e^5$ of TCR γ/δ T cells/kg at de-escalating time intervals to determine the earliest tolerated time point to safely administer these infusions to these patients. Thereafter, starting at the earliest tolerated time point of TCR γ/δ T-cell infusions, cohorts of patients are treated with 3 infusions of escalating doses of TCR γ/δ T cells/kg at 3-4 week intervals. The dosing of each infusion is adjusted based on the donors HLA-match or mismatch, respectively, as outlined in detail in this protocol.

The sample size and duration of the study:

This is a single center pilot study.

The duration of this study is estimated to be 18 – 24 months with a total of up to 18 patients for the primary analysis, 9 -12 patients in the HLA-matched group, and 6-9 patients in the HLA-mismatched group. To account for attrition and feasibility of dosing effect on treatment on minimal residual disease and progression of disease, the final sample size will be 24 patients.

2 INTRODUCTION

2.1 BACKGROUND AND RATIONALE

During the last 30 years, allogeneic stem cell transplantation has been developed and improved for the curative treatment of patients with life-threatening hematological and non-hematological malignancies as well as non-malignant diseases. The stem cell transplant is intended to reconstitute a healthy and self-sustaining hematopoietic system after eradication of the underlying disease by chemo- and/or radiotherapy (conditioning). The success of allogeneic stem cell transplantation is limited by the potential development of life-threatening complications associated with the procedure. Infection, relapse, and graft-versus-host disease (GVHD) are still major causes of mortality and morbidity after allogeneic transplantation. Therefore, the speed of reconstitution of T, B, NK and other immune cells is of importance for the reduction of the risk for bacterial, viral and fungal infections as well as for mediating anti-tumor effects, especially in HLA mismatched and haploidentical transplantation. (1-3) Approaches to reduce the risk of GVHD development have been pursued. In particular, GVHD remains a major cause of mortality and morbidity after transplantation with allogeneic peripheral blood or bone marrow for both

matched and mismatched transplants. (4,5) Strategies of graft engineering have been employed with the aim to deplete donor-derived T cells which play a central role in GVHD development following an allogeneic stem cell transplant.

Thus, the early morbidity and mortality associated with acute GvHD is a major factor limiting the success of transplantation, as is the long-term morbidity associated with chronic GvHD. The risk of acute GvHD following allogeneic bone marrow transplantation (BMT) from HLA-matched siblings is 20-60% despite the use of immunosuppressive agents like cyclosporine A (CSA), tacrolimus (FK506), methotrexate (MTX), anti-thymocyte globulin (ATG) and corticosteroids, alone or in combination (6-9). Grades II-IV (moderate to severe) acute GvHD are associated with an increased risk of transplant-related mortality (10,11). Mortality rates among patients who develop GvHD can be as high as 75% when that disease is unresponsive to therapy (11).

Certain methods of T-cell depletion are effective in reducing or preventing acute GvHD in both HLA-matched and HLA-disparate transplant recipients without co-administration of CSA and/or MTX. Depletion of T-cells by positive selection of CD34+ hematopoietic cells from GCSF mobilized peripheral blood leukocytes (PBSC) and transplantation of these CD34+cell-enriched, T-cell depleted cell fractions has been recently studied in the United States, is the CliniMACS system (Miltenyi Biotec) (12-14). In this system, GCSF- mobilized PBSC are introduced into the closed device, which treats the cells with anti CD34 MoAB coated paramagnetic beads. The CD34+ progenitor cells are then separated from other cells by passage through an electromagnetic field, and then washed and eluted. In the large Perugia series, recipients of HLA haplotype disparate CD34+ PBSC isolated on the CliniMACS device had only a 10% probability of developing acute or chronic GVHD (15). In a multicenter trial conducted by the BMT Clinical Trials Network under an FDA IND, this method yielded a progenitor-enriched cell fraction that provided doses of progenitor cells ranging from 2.4-31.3 (med 7.9) x 10^6 CD34+ cells kg and T-cell doses of 1.1-84.9 (median 6.6) x 10^3 CD3+ cells/kg. HLA matched related HSCT fractionated by this approach were administered after cytoreduction with the protocol of hyperfractionated TBI, Thiotepa and Cyclophosphamide. These transplants provided consistent engraftment (no late graft failure in 44 patients transplanted) and were associated with incidences of 20.5% grade II-IV acute GvHD and 7.6% extensive chronic GvHD. Overall survival for patients with AML transplanted in 1° and 2° remission was 74% (16). Overall risk of relapse for patients is 1° or 2° CR was 18%, which compares favorably with results of unmodified transplants for these disease indications.

The highly favorable results of the BMT CTN trial published formed the basis for an application to the FDA for licensure as a device to prevent GvHD in patients transplanted for AML. Approval for use of the CliniMACS CD34 reagent system for processing of hematopoietic progenitor cells was obtained for patients with AML when transplanted in 1st morphologic complete remission and with CD34+-selected cells from an allogeneic, HLA-identical sibling donor. For other indications and for transplants from alternative donors, we performed a clinical trial of T-cell depleted CD34+ PBSC transplants fractionated on the CliniMACS which was recently summarized in a manuscript submitted. In this trial summary, we describe an excellent outcome of pts undergoing CD34+-selected allografts of two chemotherapy-based myeloablative conditioning

regimens that secure consistent engraftment of MRD, MUD and single HLA-A, B, C or DR mismatched donor-derived CD34+TCDHCTs. Compared to our standard TBI-based regimen the incidences of acute, grade 2-4 GVHD and chronic GVHD were significantly lower after Bu/Mel/Flu conditioning. Importantly, none of the patients undergoing CD34+-selected allografts on our trial have received post transplantation immunosuppressive therapies, which therefore provides an ideal platform for the additional administration of post-transplant immunotherapies with donor effector cells, such as T-lymphocytes and/or NK cells.

Unmodified donor lymphocyte infusions (DLI) have been used in T cell depleted and repleted allogeneic transplantation in case of decreasing donor chimerism, signs of minimal residual disease and therapy refractory viral infections. (17,18) GVHD remains the most common and serious adverse event of unmodified DLI after allogeneic transplantation. (19) Different strategies have been used with the aim to dissociate the beneficial effects of DLI's such as graft versus malignancy activity, from the unwanted, harmful effects, such as GVHD. These approaches encompass the use of escalating doses of DLI's, $CD8^+$ T cell depleted DLI's, $CD3^+$ T cell depleted DLI's and NK cell infusions. (21, 22)

In our own studies, we have demonstrated the potential of post-transplant DLI and associated complications such as GvHD (23, 24) An important and significant limitation of post-transplant donor lymphocyte infusions is the time dependence and amount of donor T-cell infused post allo transplant, excluding the safe administration of DLI for prophylaxis within the first 4-6 months post allograft.

To further improve outcomes of CD34+ transplantations and donor lymphocyte infusions, a new method for the depletion of unwanted T cells triggering GVHD was developed, namely the selective depletion of TCR α/β T cells, which constitute a subpopulation of CD3+ cells. Depletion of TCR α/β CD3+ T cells in this setting is very efficient and selective with a 4.5 — 5 log T cell reduction. (25) The efficiency of depletion is comparable to that achieved with CD34+ cell enrichment. This may allow further reduction of pharmacological immunosuppression post-transplantation including its numerous negative short- and long-term side effects. This method of TCR α/β CD3+ cells depletion leads to lymphocyte compositions, which strongly resemble unmanipulated preparations. For example, TCR γ/δ CD3+ T cells and NK cells are preserved. (26)

In-vitro, when TCR γ/δ T cells were cultivated in co-culture with primary leukemia blasts they proliferated and exhibited cytotoxicity against the blasts. (24) In addition, TCR γ/δ+ T cells and NK cells have been shown to synergistically enhance their anti-leukemic and anti-viral effector cell function in vitro. (27) Furthermore, it has been reported that TCR γ/δ+ T cells seem to exert anti-leukemic effects in partially mismatched PBMC in vivo. In a retrospective analysis, patients with a high number of TCR γ/δ+ T cells post-transplantation had an improved 5-year survival compared to those with normal or low counts (70.8% vs. 19.6%). Therefore, a high content of TCR γ/δ +T cells early post T-cell depleted transplants might have a beneficial effect on survival. (27) Since TCR γ/δ+ T cells co-express CD3, but do not present the α/β T cell receptor (TCR α/β), they do not provoke GVHD driven by alloantigen T cell receptor activation. Additionally, TCR γ/δ+ T cells may support engraftment, may provide protection against infections and bridge the time to the immune reconstitution of TCR α/β+ T cells by providing protection against infections and they may exert GVT effects. (27-29) In addition, it has been shown, that similar effects are exerted by NK cells in the early post-transplant time period. (30 - 33)

Taken together, the information suggests that the administration of TCR α/β-depleted donor lymphocyte infusions, including functional TCR γ/δ+ T and NK cells at an early time point following CD34+-selected allografts provides effector cells capable of enhancing graft-versus-malignancy and anti-viral effects, while sparing the induction of GvHD. Most importantly, the CD34 negative cell fraction, remaining following a CD34+-cell selection for transplant, is rich of the effector cells, including TCR γ/δ+ T and NK cells and is usually discarded after CD34+ processing. In addition, in validation trials, we can demonstrate an effective 4-5 log depletion of TCR α/β+ cells from CD34 depleted cell fractions, leading to high purity of TCR γ/δ+ T cell and NK cell fractions.

3 OBJECTIVES AND SCIENTIFIC AIMS

Primary objectives:

1. To assess the safety of TCR α/β depleted DLI derived from the CD34neg fractions from the original transplant donor of the CD34+ selected allogeneic stem cell transplant, when administered to patients with ALL, AML, Myelodysplastic Syndrome, Myelofibrosis or relapsed/refractory multiple myeloma following TCD HSCT.

Secondary objectives:

1) To assess the effects of these adoptively transferred cells depleted of TCR α/β T cells on minimal residual disease and the progression of disease,

2) To quantitate levels of TCR α/β and TCR γ/δ T cells in addition to reconstitution of NK cells

3) To determine the development of transplant-associated viral complications following adoptive transfer of the T cells.

4) To correlate the presence and duration of MRD negative status with the duration of complete remission of these patients.

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is a pilot study designed to identify the safe administration and effects of TCR α/β-depleted donor lymphocyte infusions following CD34+-selected allogeneic stem cell transplant from HLA compatible related or unrelated donors on minimal residual disease status, infectious complication, progression-free and overall survival.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

Subject Inclusion Criteria

Patients with hematologic malignancies that are candidates for CD34+ selected, T-cell depleted allogeneic hematopoietic stem cell transplantation.

The following inclusion criteria are also required:

- Patient's age includes ≥18 to < 75 years old.
- Patients may be of either gender or any ethnic background.
- Patients must have a Karnofsky (adult) Performance Status of at least 70%.
- Patients must have adequate organ function measured by:

  Cardiac: asymptomatic or if symptomatic then LVEF at rest must be 50% and must improve with exercise.

  Hepatic: < 3x ULN AST and: s 1.5 total serum bilirubin, unless there is congenital benign hyperbilirubinemia. Patients with higher bilirubin levels due to causes other than active liver disease is also eligible with Pl approval e.g. patients with PNH, Gilbert's disease or other hemolytic disorders.

  Renal: serum creatinine: s; 1.2 mg/dl or if serum creatinine is outside the normal range, then CrCl > 40 ml/m in (measured or calculated/estimated).

  Pulmonary: asymptomatic or if symptomatic, DLCO 50% of predicted (corrected for hemoglobin).

Each patient must be willing to participate as a research subject and must sign an informed consent form.

5.2 EXCLUSION CRITERIA

Subject Exclusion Criteria

- Patients with active acute GvHD

5.3 STRATEGIES FOR RECRUITMENT

Patients who fulfill the eligibility criteria as listed in Section 5.0 will be recruited for this study by an attending physician of the Hematologic Oncology or BMT Program at Miami Cancer Institute (MCI). This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Initially, patients in cohort I will be treated with infusions of TCR γ/δ T cells at time de-escalating intervals. The first 3 patients who received a CD34+-selected allograft from a HLA-matched related or unrelated donor will obtain an infusion containing 5x10e5/kg TCR γ/δ T cells at 6 -7 weeks post-transplant, the next 3 patients will be infused with 5x10e5/kg TCR γ/δ T cells at 4-5 weeks post-transplant and a third group of 3 patients will be treated with 5x10e5/kg TCR γ/δ T cells 2-3 weeks post-transplant.

The first 3 patients who received a CD34+-selected allograft from a HLA-mismatched donor will be treated with an infusion containing 1x10e5/kg TCR γ/δ T cells at 6 -7 weeks post-transplant, the next 3 patients will be infused with 1x10e5/kg TCR γ/δ T cells at 4-5 weeks post-transplant and a third group of 3 patients will be treated with 1x10e5/kg TCR γ/δ T cells 2-3 weeks post-transplant. No immune suppressive therapies will be administered on this trial for these infusions.

Subsequently, administration of 3 consecutive infusions of TCR α/β depleted DLI per patient cohort will be initiated at the earliest tolerated time point determined through these de-escalating intervals. Thus, cohort II of 3 patients from matched related or unrelated donors will receive the first dose of 5x10e5/kg TCR γ/δ T cells at time point X, followed at 3-4 week intervals by a the second and third infusion 1x10e6/kg TCR γ/δ T cells. Cohort III of 3 patients will receive the first dose of 5x10e5/kg TCR γ/δ T cells at time point X, followed by an infusion of 1x10e6/kg TCR γ/δ T cells at 3-4 weeks later and a third infusion 2x10e6/kg TCR γ/δ T cells 3-4 weeks following the second infusion. Three additional patients will then be treated at the maximal tolerated dose determined. All patients will be re-assessed for acute GvHD prior to each TCR γ/δ T cell infusion, and subsequent infusions will be held if new onset of GvHD is observed.

Cohort II + III of 3 patients from mismatched donors will receive the first dose of 1x10e5/kg TCR γ/δ T cells at time point X, followed at 3-4 week intervals by a the second and third infusion of 5x10e5/kg TCR γ/δ T cells and 1x10e5/kg TCR γ/δ T cells at time point X, followed by an infusion of 5x10e5/kg TCR γ/δ T cells at 3-4 weeks later and a third infusion 1x10e6/kg TCR γ/δ T cells 3-4 weeks following the second infusion, respectively. Three additional patients will then be treated at the maximal tolerated dose determined. All patients will be re-assessed for acute GvHD prior to each TCR γ/δ T cell infusion, and subsequent infusions will be held if new onset of GvHD is observed. A dose variation of ±20% of the indicated cell dose is acceptable for all infusions listed. However, the maximum dose of residual TCR α/β T cells contained within each infusion will be limited to a maximum of 2x10e4 TCR α/β T cells/kg.

Table 1: Dose Levels of TCR γ/δ T cells following TCR α/β depleted DLI

Group	Patients (HLA matched)	TCR γ/δ T cells
I	3 per time point	5 x 10e5/kg at 6-7, 4-5 and 2-3 weeks post-transplant
II	3	5 x 10e5/kg starting time point X, 1 x 10e6/kg 3-4 weeks after first dose, 1 x 10e6/kg 3-4 weeks after second dose.
III	3-6	5 x 10e5/kg starting time point X, 1 x 10e6/kg 3-4 weeks after first dose, 2 x 10e6/kg 3-4 weeks after second dose.

Group	Patients (HLA mismatched)	TCR γ/δ T cells
I	3 per time point	1 x 10e5/kg at 6-7, 4-5 and 2-3 weeks post-transplant
II	3	1 x 10e5/kg starting time point X, 5 x 10e5/kg 3-4 weeks after first dose, 5 x 10e5/kg 3-4 weeks after second dose.
III	3-6	1 x 10e5/kg starting time point X, 5 x 10e5/kg 3-4 weeks after first dose, 1 x 10e6/kg 3-4 weeks after second dose.

On this trial, enrollment of patients will be staggered to provide time intervals sufficiently enough to permit the exclusion of new onset of acute GvHD. We will hold enrollment of the next patient for a minimum of 3-4 weeks after the first infusions with TCR γ/δ T cells of the first two patients of each cohort and will continue to enroll the following patient after such time period expired in the absence of new onset of acute GvHD.

7 THERAPEUTIC/DIAGNOSTIC AGENTS

7.1.1 PROCESSING OF CD34 NEGATIVE FRACTION FOR TCR A/B DEPLETION

Historically, the CD34 negative cell fraction, remaining following a CD34+-cell selection for transplant, has been discarded following the process. However, this cell fraction remains rich of the effector cells, including TCR γ/δ+ T and NK cells. The utilization of the CD34 negative cell fraction also appears logistically appropriate, as its use avoids another request for effector cell infusion from the same stem cell donor. Thus, the infusion of TCR α/β depleted DLI derived from the remaining cell fraction offers several advantages. In our initial validation trial, we demonstrate an effective 4-5 log depletion of TCR α/β+ cells from CD34 depleted cell fractions, leading to high purity of TCR γ/δ+ T cell and NK cell fractions for cryopreservation and planned infusion post-transplant.

For the immunophenotyping of TCR α/β- depleted product, we are using BD FacsCanto II and we created our own panel, which include the following CD markers CD45, CD3, CD4, CD8, CD14, CD16, CD19, CD56, TCR α/β and TCR γ/δ plus 7ADD. Identification and enumeration of T lymphocytes with TCR α/β and TCR γ/δ subsets, B lymphocytes and natural killer cells .

Viability testing will be performed by flow cytometry and 7-ADD staining. Viability from the TCR α/β+ depleted product from the negative fraction has been consistently between 90% to 95% and remained 80-85% following cryopreservation and thawing.

The products will be cryopreserved by controlled rate freezing and stored on vapor phase at <130 C with a DMSO concentration of 5 to 7.5% based on total nucleated cells concentration. The cell infusions will be prepared in 5% human albumin and plasmalyte to be injectable in humans.

Supplies and reagents that come into contact with patient product during processing, storage and/or administration must be sterile and of appropriate grade for intended use. Reagents undergo initial qualification for the intended use.

Sterility testing is performed on all products prior to cryopreservation. If products have undergone manipulation, a post processing sterility testing is also performed.

Stability program:

Viability testing will be performed at various test intervals- preprocessing (zero-time point), post processing (inclusive of fresh and cryopreserved products). For cryopreserved products (storage conditions of ≤ -130°C), post thaw viability will be performed using sample aliquots representative of final processed product. This post thaw viability will be performed at time of infusion (as applicable), 1 year post storage, and annually, thereafter for the duration of aliquot availability.

Pre and post processing cell counts to determine product identity, quality and strength (potency).

Pre and post processing sterility testing (gram stain and 14 day culture) to determine purity.

Post processing endotoxin testing to determine purity.

For cryopreserved products, container integrity post thaw.

Potency Program:

Potency will be developed in an incremental approach based on the biological properties of the product. To assess biological function and characterization of product a series of analytical assays will be performed to assess potency at the early preclinical phase, as clinical study progress we would be able to determine the product effectiveness based on the clinical data.

A Certificate of Analysis with release specifications will be generated for each product to demonstrate product activity, quality and consistency throughout product development. This Certificate of Analysis will include the tests used for routine lot release:

Cell counts for Total nucleated cells

Flow cytometry analysis for identification and enumeration of T cells, TCR α/β CD3+ T cells, γ/δTCR CD3+ T cells, natural killer T cells and B cells

Sterility: gram stain and cultures

Endotoxin

Viability (by both manual method and flow cytometry)

7.2 THE CLINIMACS SYSTEM FOR POSITIVE SELECTION OF TCR A/B CD3+ T-CELLS.

The CliniMACS System (Miltenyi Biotec, Auburn, CA) including the CliniMACS^{plus} Instrument, a CliniMACS Tubing Set, the CliniMACS TCR α/β Reagent Kit and the CliniMACS PBS/EDT A Buffer is intended for the selection and enrichment of human TCR α/β CD3+ positive cells from a leukapheresis product.

The CliniMACS TCR α/β Reagent Kit consists of 1 vial of CliniMACS TCR α/β -Biotin and 2 vials of CliniMACS Anti-Biotin Reagent which are used with the CliniMACS System. The CliniMACS TCR α/β Reagent Kit is used to deplete human TCR α/β T cells *in vitro* from heterogeneous hematological cell populations for stem cell transplantation and lymphocyte infusions in cases where this is clinically indicated.
Each CliniMACS TCR α/β Reagent Kit is sufficient to label up to 24 x 10^9 TCR α/β$^+$ T cells out of 60 x 10^9 total white blood cells using an indirect labeling strategy.

The components of the CliniMACS System include:

7.2.1 THE CLINIMACS INSTRUMENT

The CliniMACS instrument is a bench-top instrument consisting of a supporting structure to hold the column/tubing assembly and various bags, a series of valves through which the tubing set is fitted, a magnet between the poles of which the separation column is placed, a peristaltic pump through which a section of tubing is placed, software to control the instrument and user interface and a computer touchpad with a display window. The instrument is operated at ambient temperature and it is intended to be multi-use item.

The software for the CliniMACS Instrument controls the function of the electromechanical components of the instrument and the user interface. Two separate computers, one a micro-controller located on a control board of the CliniMACS Instrument and the second a PC compatible computer which operates the user interface are incorporated with the instrument. Software Version 2.3 1, the current version of software is directly traceable to the version of software utilized in pre-clinical testing and European Safety trials and has been inspected and approved by TOV product services with the CE Mark.

7.2.2 CLINIMACS TUBING SET

The CliniMACS Tubing Set consists of a tubing element combined with a pair of proprietary cell selection columns. These form a closed, sterile system for processing the cells. The separation column is a proprietary component of the CliniMACS System consisting of a plastic column housing with polypropylene fits in each end. The interior of the column housing is filled with a matrix of submillimeter iron beads coated with a heat-cured biocompatible resin. The columns are placed at appropriate locations in the CliniMACS Tubing Set to facilitate the cell selection process. The first column serves as a device to remove components that bind non-specifically to the column. The second column which is placed with in a magnetic field performs the actual cell selection. The columns are incorporated sterile as part of the tubing set and are intended for single use only.

The tubing element consists of a series of tubes, connectors, spikes, Luer locks, and collection bags. The tubing of the tubing element is comprised of materials that have been qualified for use in this application by testing to ISO I 0993. The principal constituents are polyvinyl chloride (PVC) and silicone. The connectors are made of various polymers (e.g., ABS and PVC) suitable for use in a blood contact environment. They are solvent bonded to the PVC tubing. The silicone pump tubing is softened with petroleum ether for manufacturing and mechanically fixed to connectors. The cell wash bags are composed of PVC.

The CliniMACS Tubing Set is packaged in a thermoformed tray and heat-sealed with a Tyvek® lid. The CliniMACS Tubing Set is sterilized by ethylene oxide gas in a validated sterilization cycle and supplied as a single-use component for the CliniMACS Instrument.

7.2.3 CLINIMACS TCR A/B REAGENT

The CliniMACS TCR α/β -Biotin consists of biotinylated TCR α/β monoclonal antibody (mAb). The CliniMACS TCR α/β -Biotin mAb is a mouse IgG_2b monoclonal, which is produced by the hybridoma cell line clone BMA031.H7.

CliniMACS TCR α/β -Biotin is a sterile solution intended for the indirect magnetic labeling of human TCR α/β $CD3^+$ T cells, in combination with the CliniMACS® Anti-Biotin Reagent. It is supplied in single use vials which contain nominally 7.5 mL per vial. The vials used are 10 mL glass injection vials sealed with a chlorobutyl rubber stopper and flip-off aluminum seal.

The TCR α/β $CD3^+$ T positive cells are specifically labeled for selection by incubation with the CliniMACS TCR α/β Reagent. After unbound reagent is washed from the suspension, the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension; the cells are ready for the automated separation process. The CliniMACS System passes the antibody-labeled suspension through a column in which strong magnetic gradients are generated. The Selection Column retains the magnetically labeled TCR α/β positive cells, while unlabeled cells flow through the Selection Column and are collected in the Negative Fraction Bag. The cells in the Negative Fraction Bag containing the TCR γ/δ+ T cells will be

aliquoted and cryopreserved at doses defined in this protocol for post-transplant infusions. The separated TCR α/β positive cells are released from the column by removing the column from the magnetic field and clueing the cells into a separate collection bag and will be discarded.

7.3 THE CLINIMACS PBS/EDTA BUFFER

The CliniMACS PBS/EDTA Buffer is an isotonic and is hydric buffer solution with a pH-value of 7.2 and osmolality of 290 mosmol/L. Its formulation is shown in the following table.

Table 1 Formulations of the CliniMACS PBS/EDT A Buffer

Ingredient	Compendia	Amount
NaCl	Ph. Eur.	8.0 g/L
KCl	Ph. Eur.	0.19g/L
Na2HP04 anhy.	Ph. Eur.	1.15g/L
KH2P04	Ph. Eur.	0.19 g/L
Na2EDTA	Ph. Eur.	0.37g/L
Water for Injection	Ph. Eur.	ad IL

The CliniMACS PBS/EDTA Buffer is used as external wash and transport fluid for the in vitro preparation of human heterogeneous cell populations intended to be separated with the CliniMACS Cell Selection System.

8 TREATMENT/INTERVENTION PLAN

Patients will be treated as inpatient or outpatient on the Miami Cancer Institute Hematologic Oncology and Blood & Marrow Transplant Service following their allogeneic stem cell transplantation on their respective transplant protocols. The stem cell transplant is considered standard of care. The TCR α/β depleted DLI dosing and timing are the elements under investigation in this trial.

8.1 TCR A/B DEPLETED DONOR LYMPHOCYTE INFUSIONS

8.1.1 DOSING AND TIME INTERVALS OF TCR A/B DEPELETED DLI.

The dosing and time intervals of TCR α/β depleted DLI will be adjusted based on the HLA-match/mismatch of the stem cell donors.

Treatment of the first cohort of patients will be performed to determine the earliest time point post allogeneic stem cell transplant tolerating TCR α/β depleted DLI without complications.

In this cohort the first 3 patients who received a CD34+-selected allograft from a HLA-matched donor will be infused with 5x10e5/kg TCR γ/δ T cells at 6 -7 weeks post-transplant, the next 3 patients will be treated with 5x10e5/kg TCR γ/δ T cells at 4-5 weeks post-transplant and a third group of 3 patients will be treated with 5x10e5/kg TCR γ/δ T cells 2-3 weeks post-transplant.
The first 3 patients who received a stem cell transplant from a HLA-mismatched donor will be treated with an infusion containing 1x10e5/kg TCR γ/δ T cells at 6 -7 weeks post-transplant, the next 3 patients will be infused with 1x10e5/kg TCR γ/δ T cells at 4-5 weeks post-transplant and a third group of 3 patients will be treated with 1x10e5/kg TCR γ/δ T cells 2-3 weeks post-transplant.

Subsequently, stem cell recipients will receive the first infusion of TCR α/β depleted DLI at the earliest tolerated of the patients treated on these de-escalating intervals. Three recipients from matched related or unrelated stem cell donors will receive the first dose of 5x10e5/kg TCR γ/δ T cells at time point X, followed at 3-4 week intervals by a the second and third infusion 1x10e6/kg TCR γ/δ T cells. Recipients who received a stem cell transplant from mismatched donors will receive the first dose of 1x10e5/kg TCR γ/δ T cells at time point X, followed at 3-4 week intervals by a the second and third infusion of 5x10e5/kg TCR γ/δ T cells.

The final cohort of 3 patients of HLA-matched donors will receive the first dose of 5x10e5/kg TCR γ/δ T cells at time point X, followed by an infusion of 1x10e6/kg TCR γ/δ T cells at 3-4 weeks later and a third infusion 2x10e6/kg TCR γ/δ T cells 3-4 weeks following the second infusion. In this group, three additional patients will then be treated at the maximal tolerated dose determined and the final cohort who received a HLA-mismatched transplant will be treated with 1x10e5/kg TCR γ/δ T cells at time point X, followed by an infusion of 5x10e5/kg TCR γ/δ T cells at 3-4 weeks later and a third infusion 1x10e6/kg TCR γ/δ T cells 3-4 weeks following the second infusion. Three additional patients will then be treated at the maximal tolerated dose determined.

8.2 PROPHYLAXIS AGAINST ACUTE GRAFT-VERSUS-HOST DISEASE

No GvHD prophylaxis will be administered other than the CliniMACS TCR α/β depletion performed.

9 EVALUATION POST TCR A/B DEPLETED DONOR LYMPHOCYTE INFUSIONS

9.1 POST-INFUSION EVALUATION

The chart below shows the approximate dates for tests and procedures performed after TCR α/β depleted DLI. This chart also reflects standard of care transplant follow-up procedures. Certain tests may be held or repeated at the discretion of the treating physician if deemed in the best clinical interest of the patient.

Activity	Post TCR α/β Depleted DLI until discharge.	DISCHARGE TO DAY 100	Day 100	6 Months	12 Months	24 Months
Windows	See below for windows	See below for windows	+/- 4 days	+/- 2 weeks	+/- 1 month	+/- 1 month
Blood counts and chemistry (CBC, Comprehensive Metabolic Panel)	CBC: Daily +/- 12 hours CMP: 2 times a week	CBC/CMP: Weekly, 2 times a week	Every 2-4 weeks		x	x
Physical exam for GvHD evaluation	Monitored routinely as per inpatient transplant guidelines	Weekly	Every 2-4 weeks		x	x
Disease evaluation BMA [1,2]	30 - 100 days after transplant, if clinically indicated			x	x	x
T-cell Chimerism[2]	N/A	30 days after transplant	x	x	x	x
Peripheral blood lymphocyte (PBL) phenotyping[3]	Every 2 weeks	Monthly	x	x	x	x
Viral PCR analyses (CMV, EBV, HHV-6)[4]	Twice weekly	Weekly	x	x	x	x
Adverse Event Collection	x On infusion day 1 and discharge		x On day 100	x		

Concomitant Medications	x On infusion day 1		x	x	x	x

1) This should apply to patients with malignant disease in which the BM was previously involved. BM Chimeras should also be assessed whenever possible. BMA may be delayed if patient is clinically unstable.
2) May be done more frequently if clinically indicated. Tests need not be done if patient has a low number of peripheral white cells.
3) PBL phenotyping should be performed until CD4 counts is >200.
4) Will be performed as per standard transplant guidelines.

10 TOXICITIES/SIDE EFFECTS

Patients recruited to this trial are individuals who are either referred by physicians or self-referred for peripheral blood stem cell transplantation as a potentially curative treatment for their malignancy or other life-threatening disorders. All patients will undergo a detailed consultations discussing the risks and potential benefits of an allogeneic stem cell transplantation as well as risks and benefits of the infusions of TCR α/β depleted DLI post transplantation. The risks and potential benefits of the procedure, as well as the participation in any given research, experimental, or therapeutic protocol are also discussed.

10.1 TOXICITIES/SIDE EFFECTS OF THE TRANSPLANT AND THE COMBINED EFFECTS OF THE CONDITIONING REGIMEN AND TRANSPLANT AND TCR A/B DEPELETED DLI.

10.1.1 INFECTIONS

Transplantation puts the patient at higher risk for bacterial, viral, or fungal infections, which are potentially life threatening. These risks are potentially higher with T-cell depleted (TCD) transplants. Prophylaxis will be initiated and patients will be closely monitored for signs of infections and will receive early and appropriate treatment

10.1.2 GRAFT FAILURE / POOR MARROW FUNCTION

T-cell depletion of donor cells is associated with an increased incidence of graft failure in allogeneic transplant recipients. After allogeneic transplantation, the recipient's marrow function may be poor, and leukopenia, anemia, or thrombocytopenia may result from many causes including graft rejection induced by surviving host immune T-cells, or ongoing suppression of

engrafted donor blood-forming cells by GvHD, infection or marrow suppression or immunosuppressive drugs and other medications. Graft failure may result in death if not reversed. In patients with immune rejection second transplants can be administered with immunosuppressive therapy, including non-myeloablative conditioning regimens. For patients who are engrafted with donor cells but have severe cytopenia affecting one or more blood cell lineages, secondary transplants of CliniMACS fractionated CD34+ T-cell depleted PBSCs may be administered to booster and replenish donor hematopoietic cells without conditioning or after treatment with anti-thymocyte globulin.

10.1.3 GRAFT-VERSUS-HOST DISEASE

Acute or chronic GvHD may develop after allogeneic transplantation that can be disabling and can lead to death. GvHD is thought to be initiated by T-cells contained in the PBSC graft. CD34+ selection and CD3+ depletion reduces the number of T-cells in the PBSC but GvHD can occur after TCD transplants as well as after TCR α/β depleted DLI. Acute and/or chronic GvHD will be treated with immunosuppressive drugs as per the transplant service guidelines.

10.1.4 SINUSOIDAL OBSTRUCTION SYNDROME (SOS) OF THE LIVER

SOS is a manifestation of damage to the liver by the conditioning regimen that usually develops within two weeks after allogeneic transplant and is characterized by at least two of the following:

- Hyperbilirubinemia (total bilirubin > 2 mg/dL)
- Hepatomegaly or right upper quadrant pain, or
- Rapid weight gain (> 5% above baseline)

TCD is not expected to affect the risk of SOS. However, the effect of TCR α/β depleted DLI on the development of SOS is unknown.
Recipients developing SOS will be monitored closely and will receive appropriate supportive care and careful fluid management.

10.1.5 END ORGAN DAMAGE

End organ damage of all or any of the major organs, including the brain, may occur as a result of cumulative toxicity from anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g., infection, GvHD, etc.) and may have a fatal outcome. Toxicities may occur in any individual patient due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function. Data from previous studies do not suggest that the risk of end organ damage is appreciably affected by TCD or by TCR α/β depleted DLI or the preparative regimens to be used in this study. However, the effect of TCR α/β depleted DLI on the development of end organ damage is unknown.

Recipients developing end organ damage will be monitored closely and will receive appropriate supportive care and careful fluid management.

10.1.6 DISEASE RELAPSE

Allogeneic transplantation using T-cell depleted peripheral blood stem cells has, in some cases, been associated with an increased incidence of leukemic relapse in patients with chronic myelogenous leukemia, compared to recipients who receive unmanipulated donor cells. The risk of relapse has not been increased in patients with acute leukemia. Nevertheless, despite cytoreduction, the transplant and TCR α/β depleted DLI, relapse may occur.

10.1.7 DEATH

There is an approximately 5-10% risk of treatment related mortality within the first month of transplant due to the risk of severe regimen related toxicity, hemorrhage, opportunistic infection, or other complications. It is not expected that the regimens to be used in this protocol or TCR α/β depleted DLI will increase this risk.

11 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

11.1 DISEASE RELAPSE AND RECURRENCE

Relapse of leukemia is achieved if one or more of the following criteria are met: greater than 5% circulating leukemic blasts in the marrow or peripheral blood; the presence of blasts in any extra medullary site or; disease determined by clinical assessment. Cytogenetic and/or Molecular analysis of the marrow and/or peripheral blood will also be obtained for the diagnosis of relapse.

11.2 DISEASE-FREE SURVIVAL

DFS is defined as the minimum time interval of times to relapse/recurrence, to death or to the last follow- up, from the time of transplant.

11.3 OVERALL SURVIVAL

Overall survival is defined as time from transplant to death or last follow-up.

12 STATISTICAL CONSIDERATIONS

As the study is confirmatory in nature, the sample size is assessed pragmatically. Because of the small sample size and multiple strata there can be no inferential statistical analysis. As each strata has less than 10 subjects the sample size is too small for any analysis other than descriptive statistics. As such, within each cohort, the percentage of patients who have achieved feasibility will be reported. There are two strata of patients: HLA matched and HLA mismatched (Tables 1 and 2). Within each strata there are three cohorts. For the HLA matched strata (cohort 1), 3 patients will receive infusions containing $5x10e^5$/kg TCR γ/δ T cells at 6-7 weeks post-transplant. The next three patients will be given the same dose at 4-5 weeks post-transplant. A third group of three patients will be treated at the same dose at 2-3 weeks post-transplant. The same timing procedure is applied to those in the HLA mismatched group, the only difference being the infusion dosage which is $1x10e^5$/kg TCR γ/δ T cells.

In both strata after cohort I, the earliest tolerated time point will be selected according to pre-specified criteria. For HLA matched cohort II, 3 patients will receive an infusion using $5x10e^5$/kg TCR γ/δ T cells at the time point post-transplant chosen from cohort I. After this, they will receive 2 $1x10e^6$/kg TCR γ/δ T cells infusion at 3-4 week intervals after the first dose. In the HLA mismatched group, 3 patients will receive an infusion using $1x10e^5$/kg TCR γ/δ T cells at the ideal time point chosen from cohort I. Then they will receive 2 $5x10e^5$/kg TCR γ/δ T cells infusion at 3-4 week intervals after the first dose.

In cohort III, for HLA matched patients, 3 to 6 patients will receive an infusion using $5x10e^5$/kg TCR γ/δ T cells at the ideal time point chosen from cohort I. Then they will receive a $1x10e^6$/kg TCR γ/δ T cells infusion at 3-4 week intervals after the first dose. The third dose will be $2x10e^6$/kg TCR γ/δ T cells infusion 3-4 weeks after the second dose.

In cohort III, HLA mismatched patients, 3 to 6 patients will receive an infusion using $1x10e^5$/kg TCR γ/δ T cells at the ideal time point chosen from cohort I. Then they will receive a $5x10e^5$/kg TCR γ/δ T cells infusion at 3-4 week intervals after the first dose. The third dose will be $1x10e^6$/kg TCR γ/δ T cells infusion 3-4 weeks after the second dose.

Sequential boundaries will be used to monitor dose-limiting toxicity rate. An SAE will be considered as a dose limiting toxicity. The accrual will be halted if excessive numbers of dose-limiting toxicities are seen, that is, if the number of dose-limiting toxicities is equal to or exceeds *b* out of *n* patients with full follow-up (see Table 3).

This is a Pocock-type stopping boundary that yields the probability of crossing the boundary at most 0.05when the rate of dose-limiting toxicity is equal to the acceptable rate [event probability of 0.1]. SAEs, particularly GVHD, will be assessed subjectively to determine if the cause is the infusion of cells or the actual transplant.

The main objective of this study is to assess the safety of TCR α/β depleted DLI derived from the CD34neg fractions from the original transplant donor of the CD34+ selected allogeneic stem cell

transplant, when administered to patients with ALL, AML, Myelodysplastic Syndrome, Myelofibrosis or relapsed/refractory multiple myeloma following TCD HSCT. Due to the study design, the analysis will be descriptive. In each cohort, percentages and number experiencing a dose limiting toxicity will be reported. As the secondary objectives are all qualitative in nature, no statistical analysis will be performed

There is no formal interim analysis planned for this study.

12.1 STUDY POPULATION DEFINITION

- Safety Population: All subjects who received at least one dose of TCR α/β depleted DLI.
- Efficacy Evaluable Population: All subjects who meet all eligibility criteria and received at least one cycles of intervention.

12.2 SUBJECTS DISPOSITION

Subject disposition (analysis population allocation, entered, discontinued, along with primary reason for discontinuation) will be summarized using frequency and percent for both treatment and follow-up phases. A summary of subjects enrolled by site will be provided. Protocol deviations will be summarized using frequency tabulations.

12.3 EFFICACY ANALYSIS

If study compliance becomes a problem efficacy analysis will not be performed. Data for those who completed the study according to protocol will be used. Sample size permitting, all analyses previously specified will be applied to this subgroup.

12.4 SAFETY ANALYSIS

The safety population will be included in the safety analyses.

Safety and tolerability will be monitored through continuous reporting of adverse events and serious adverse events, laboratory abnormalities, and incidence of subjects experiencing dose modifications, dose interruptions, and/or premature discontinuation. Data from all subjects who receive one or more doses will be included in the safety analyses. Adverse events, physical examinations (including vital sign measurements), clinical laboratory information, and concomitant medications/procedures will be tabulated and summarized. Descriptive statistics will be presented.

For both AEs and SAEs, the PI must assess the severity / intensity of the event. The severity / intensity of AEs will be graded based upon the subject's symptoms according to the current active minor version of the CTCAE, Version 4.0;
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40
AEs that are not defined in the CTCAE should be evaluated for severity / intensity according to the following scale:

- Grade 1 = Mild – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required;
- Grade 2 = Moderate – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required;
- Grade 3 = Severe – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible;
- Grade 4 = Life threatening – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable;
- Grade 5 = Death - the event results in death.

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is not the same as "serious" which is based on subject/event outcome or action criteria associated with events that pose a threat to a subject's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory obligations.

Table 2. Infusion Regimen for HLA matched donors

Cohort	**Number of patients**	**Infusion:** TCR γ/δ T cells
I		
	3	5x10e^5/kg at 6-7 weeks post-transplant
	3	5*10e^5/kg at 4-5 weeks post-transplant
	3	5*10e^5/kg at 2-3 weeks post-transplant
II	3	5*10e^5/kg at X* weeks post-transplant, 1*10e^6/kg at 3-4 weeks post-first dose, 1*10e^6/kg at 3-4 weeks post-second dose
III	3-6	5*10e^5/kg at X* weeks post-transplant, 1*10e^6/kg at 3-4 weeks post-first dose,

		2*10e^6/kg at 3-4 weeks post-second dose
	*X represents the time which is chosen from cohort one based on pre-specified criteria.	

Table 2. Infusion Regimen for HLA mismatched donors

Cohort	**Number of patients**	**Infusion:** TCR γ/δ T cells
I		
	3	1*10e^5/kg at 6-7 weeks post-transplant
	3	1*10e^5/kg at 4-5 weeks post-transplant
	3	1*10e^5/kg at 2-3 weeks post-transplant
II	3	1*10e^5/kg at X* weeks post-transplant, 5*10e^5/kg at 3-4 weeks post-first dose, 5*10e^5/kg at 3-4 weeks post-second dose
III	3-6	1*10e^5/kg at X* weeks post-transplant, 5*10e^5/kg at 3-4 weeks post-first dose, 1*10e^6/kg at 3-4 weeks post-second dose
	*X represents the time which is chosen from cohort one based on pre-specified criteria.	

Table 3. Table of stopping boundaries

Number of patients (n)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Boundary (Any SAE) (b)	-	2	3	3	3	3	4	4	4	4	4	4	5	5	5	5	5	6

13 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

13.1 RESEARCH PARTICIPANT REGISTRATION

Confirm eligibility as defined in the section entitled Study Population.

Obtain informed consent by following procedures defined in section entitled Informed Consent Process.

During the registration process, registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Clinical Research Office (CRO) at MCI. The signed consent form and other relevant documents will be uploaded into OnCore, MCI's Clinical Trial Management System (CTMS) using the electronic database (eDC) and electronic case report form (eCRF).

13.2 RANDOMIZATION

This research study does not require a randomization.

13.3 ENROLLMENT INTO COHORTS

Enrollment into each cohort will follow the regimen outlined in Table 2 above according to whether the patient is receiving either a matched or mismatched donor cells. The cohorts will be filled sequentially.

14 DATA MANAGEMENT ISSUES

A Clinical Research Coordinator (CRC) will be assigned to the study and will be responsible for adult accruals. The responsibilities of the CRC and principal investigator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

14.1 QUALITY ASSURANCE

Real-time quality control activities will be conducted to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action. All necessary precautions will be taken to avoid missing data due to small sample size. In addition, imputation of missing data will be done after assessing for feasibility.

Random-sample data quality and protocol compliance audits will be conducted by the study team.

14.2 DATA AND SAFETY MONITORING

The MCI Data Safety and Monitoring Committee (DSMC) will monitor this clinical trial according to the MCI Data and Safety Monitoring Plan (DSMP). In its oversight capacity, the MCI DSMC bears responsibility for suspending or recommending this study.

DSMC oversight of study conduct includes ongoing review of adverse event data, and periodic review of viral complications associated with transplant. The guidelines appearing in Section 16.3 are offered for DSMC consideration in assessing adverse events. In addition, the DSMC will review reports from all audits, site visits, or study reviews pertaining to this clinical trial and take appropriate action.

Risk Based Monitoring

Routine monitoring or audit activities for this study will be conducted by the MCI OCR Office of Research Integrity authorized personnel in accordance with current FDA Regulations, ICH GCP guidelines, site's Standard Operating Procedures (SOPs), IRB and the respective local and national government regulations. The general scope of such visits would be to inspect study data including but not limited to, regulatory requirements, source documentation, original medical records/files and eCRF completion, as applicable, following a risk-based monitoring approach.

The study will be monitored based on procedures established in the Risk-Based Monitoring Plan at the time of protocol activation. A series of monitoring forms, tools and templates including

MCI Clinical Research Monitoring/Audit Deficiencies Guidance and Summary Monitoring Letters are utilized to ensure standard and consistent monitoring practices. The monitoring frequency may also be adjusted as per the monitor's discretion based on accrual rate, trial complexity, major deficiency findings, visit rating and safety reporting.

To ensure compliance with current federal regulations and the ICH GCP guidelines, data generated by this study must be available for inspection upon request by representatives of the FDA, national and local health authorities, and duly authorized representatives of any entity providing support for this trial.

15 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

15.1 INFORMED CONSENT PROCESS

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their Inclusion in the study. Participants will also be informed that they are free to withdraw

from the study at any time. All participants must sign an IRB -approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant will receive a copy of the signed informed consent form.

15.2 CRITERIA FOR REMOVAL FROM STUDY

Research participants may be removed from the study if requested by the research participant. Management will depend on where they are in their treatment course. Such research participants will receive appropriate supportive care. Patients may also be removed from the study at any point deemed appropriate by the principal investigator.

Patients may be removed from study, and not followed further, if one or more of the following events occur:

- Significant noncompliance on the part of the patient
- Refusal of the patient to continue treatment or observations
- Progressive disease or relapse
- Decision by the investigator that termination is in the patient's best medical interest
- Unrelated medical illness or complication
- Lost to follow-up

A participant will be considered lost to follow-up if he or she fails to return for no more than two scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 3 business days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

15.3 CRITERIA FOR SUSPENSION OR PREMATURE TERMINATION OF THE STUDY

Miami Cancer Institute (MCI) at Baptist Health, Inc., Principal Investigator (PI) or regulatory authority may suspend or prematurely terminate, at the investigation sites for which they are responsible, the clinical investigation for significant and documented reasons. If suspicion of an unacceptable risk to subjects arises during the clinical investigation, or when so instructed by the regulatory authorities, MCI shall suspend the clinical investigation while the risk is assessed. MCI shall terminate the clinical investigation if an unacceptable risk is confirmed.

MCI shall consider terminating or suspending the participation of a particular investigation site or investigator in the clinical investigation if monitoring or auditing identifies serious or repeated deviation. If suspension or premature termination occurs, the terminating party shall justify its decision in writing and promptly inform the other parties with whom they are in direct communication. The Principal Investigator and MCI shall keep each other informed of any communication received from the regulatory authority.

If, for any reason, MCI suspends or prematurely terminates the investigation at an individual investigation site, MCI shall inform the responsible regulatory authority as appropriate. If the suspension or premature termination was in the interest of safety, MCI shall inform all other Principal Investigators.

If suspension or premature termination occurs,

- MCI shall remain responsible for providing resources to fulfill the obligations from the protocol and existing agreements for following up the subjects enrolled in the clinical investigation, and

- The Principal Investigator or authorized designee shall promptly inform the enrolled subjects at his/her investigation site, if appropriate.

When MCI concludes an analysis of the reason for the suspension, implements the necessary corrective actions, decides to lift the temporary suspension, MCI shall inform the Principal Investigator, and, where appropriate, the regulatory authority of the rationale, and provide them with the relevant data supporting this decision. The regulatory authorities shall also provide confirmation of information before the clinical investigation resumes. If subjects have been informed of the suspension, the Principal Investigator or authorized designee shall inform them of the reasons for resumption.

16 PROTECTION OF HUMAN SUBJECTS

The risks associated with a T-cell depleted transplant followed by TCR α/β depleted DLI on this study are those associated with the toxicities, as detailed in Section 10, as well as the risks of an allogeneic transplant, particularly graft failure, or graft vs. host disease, as also detailed in Section 10.

To protect against the toxicities, the patient will be transplanted in a single room, HEPA filtered environment. Organ toxicities such as mucositis, enteritis and hepatic dysfunction as well as infectious complications will be treated by standard procedures developed for transplantation to support our patients. Blood and platelet counts will be supported by transfusion. Graft failure might necessitate a second transplant, after additional conditioning. Approaches to the diagnosis and treatment of graft failure that secure consistent engraftment have been developed by the Transplantation Services. Similarly, advanced treatments will be instituted in the event the patient develops graft vs. host disease.

Despite a transplant and TCR α/β depleted DLI, the patient's disease may recur. In this case, standard and/or experimental therapies, such as phase I /II drugs, antibodies or cell therapies, will be available to the patient for consideration as treatment options.

16.1 BENEFITS

A transplant is administered with curative intent. The integration of post-transplant TCR α/β depleted DLI is intended to improve the outcome. The approaches being evaluated may achieve this goal and may also be effective in preventing infections, acute and chronic graft vs. host disease and disease relapse.

The results of this study will also define risks and benefits of T-cell depleted grafts and TCR α/β depleted DLI and will greatly accelerate further development of graft manipulations employing these approaches.

Consent Process: Participation in this study is voluntary. All patients will be required to sign a statement of informed consent, which must conform to MCI IRB guidelines.

Alternatives: Enrollment in this study is voluntary. Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may include getting a transplant from a volunteer unrelated donor, if one is available; getting treatment for the cancer with either chemotherapy or a transplant without being on a study; taking part in another study; or getting no treatment.

Costs: The patient's health plan/insurance company will need to pay for all of the costs of treatment in this study. The patient will be responsible for the costs of standard medical care, all hospitalizations and any transplant complications. Pre-authorization for the transplant will be cleared with the health plan/insurance company prior to admission. Patients will not be paid for taking part in this study.

Research tests will be done at no cost to the patient.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential.

16.2 PRIVACY

MCI's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB (IRB).

16.3 SERIOUS ADVERSE EVENT (SAE) REPORTING

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in 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 (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant starts investigational treatment/intervention. SAE reporting is required for the participant's duration of the study in accordance with the study calendar (Section 9).

Please note: Any SAE that occurs prior to the start of investigational treatment/intervention and is related to a screening test or procedure (i.e., a screening biopsy) must be reported.
All SAEs must be submitted to the research team at MCI.

The report should contain the following information:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment(s)
- If the AE was expected
- Detailed text that includes the following
  - An explanation of how the AE was handled
  - A description of the participant's condition
  - Indication if the participant remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

All SAEs will be reviewed by the PI and entered in OnCore within 48 business hours of awareness of the event. A follow up SAE report must be entered in OnCore within 5 business days for all inpatient hospitalizations graded as 4 and 5.

For all inpatient hospitalizations graded as 1, 2, and 3, a follow up SAE report must entered in OnCore within 10 business days.

All SAEs will be reported to the IRB of record in accordance with its policies and procedures.

The IRB requires a SAE report be submitted electronically to the IRB Office. The report should contain the following information:

- Subject ID
- Disease/histology (if applicable)
- Protocol number and title
- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)

- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - An explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

16.5 DEFINITION OF AN SAE

An SAE is an undesirable experience that meets one of the follow in criteria:

- ls fatal or life-threatening
- Is disabling
- Results in a congenital anomaly or occurrence of malignancy
- Important medical event that jeopardizes the participant AND requires medical or surgical intervention to prevent one of the outcomes above

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

Attribution:

- Unrelated : The AE is clearly NOT related to the TCR α/β depleted DLI
- Unlikely: The AE is doubtfully related to the TCR α/β depleted DLI
- Possible: The AE may be related to the TCR α/β depleted DLI
- Probable: The AE is likely related to the TCR α/β depleted DLI
- Definite: The AE is clearly related to the TCR α/β depleted DLI

Expected and Unexpected Event:

- Expected: Any experience previously reported (in nature, severity, or incidence) in the in the safety information of the study drugs general investigational plan.
- Unexpected: Any experience not previously reported (in nature, severity, or incidence) in the safety information of the study drugs general investigational plan.

REPORTABLE EVENTS:

- Grades 1-2: Adverse Event Reporting NOT required.
- Grades 3-4: Possible, Probable or Definitely attributed to the study drug (as detailed as 1 1.2.1), will be reported *.
- Grades 5: Regardless of Attribution will be reported*.

*Reportable events are those, which occur within 30 days of the last dose of treatment on protocol. Events beyond 30 days will be reported at the discretion of the PI.

For IND protocols:
The AE report should be completed as above, and the FDA assigned IND number written at the top of the report. If appropriate, the report will be forwarded to the FDA by the SAE staff through the Office of Clinical Research (OCR).

17.3.1 IND REPORTING

This protocol has an IND; therefore, the SAE will also be reported to the FDA through the OCR and the report will include the FDA assigned IND number and name.

All FDA correspondence will occur in accordance with the requirements as set forth in 21CFR Part 312.

18 PUBLICATION POLICY

The results of the clinical investigation will be made publicly available through clinicaltrials.gov or via other publishing bodies, in case of positive or negative results upon study completion.

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