Phase I Study of Post-Transplant Alpha-Beta Depleted T-Cell Infusion Following Haploidentical Hematopoietic Stem Cell Transplantation Using Post-Transplant Cyclophosphamide

Study Protocol and Statistical Analysis Plan

NCT02193880

March 1, 2018

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Bone Marrow Transplantation and Cell Therapy Program
University of Alabama at Birmingham

Protocol Version: 2.0 Dated 10/3/2016
Replaces version: 1.0 dated 06/26/2014

Medical Device: CliniMACS Alpha Beta T-Cell Depletion System
IDE Number: BB IDE 15938

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Protocol Synopsis

TITLE	Phase I Study of Post-transplant Alpha-beta depleted T-cell Infusion Following Haploidentical Hematopoietic Stem Cell Transplantation (Haplo HCT) using Post-Transplant Cyclophosphamide (CY).
SPONSOR	UAB Bone Marrow Transplantation and Cell Therapy Program
NUMBER OF SITES	One
RATIONALE	To investigate the feasibility and safety of the use of alpha-beta depleted T-cell (ABD T-cell) infusion after haploidentical HCT and post-transplant cyclophosphamide without causing severe graft versus host disease (GVHD).
STUDY DESIGN	Single-center single-arm, open-label phase I study.
PRIMARY OBJECTIVES	To determine the safety of using the ABD T- cell infusion following haplo HCT and post-transplant CY.
NUMBER OF SUBJECTS	10 Recipient / Donor Pairs
INVESTIGATIONAL DEVICE	**CliniMACS® TCR α/β Reagent Kit**

1 BACKGROUND

Allogeneic hematopoietic stem cell transplantation (allo HCT) is a potentially curative treatment for many patients with hematological malignancies.[1] The clinically preferred source for stem cells is a human leukocyte antigen (HLA) matched sibling donor or an HLA-matched unrelated donor.[2] Unfortunately many patients, particularly those of ethnic minority groups, do not have an HLA-matched unrelated donor. Registry searches can also be inappropriately time-consuming in some high-risk leukemia patients. Therefore, more alternative sources of HCT grafts have been clinically used. These options include the use of donor cells from a partially HLA matched (haploidentical) family member.[3]

Haploidentical (Haplo) HCT has been shown to achieve long-term survival and cure in patients who require allogeneic HCT with no HLA-matched donor.[3] However, the success of this procedure has been hindered by multiple complications. The HLA disparity between the donor and recipient can induce high risks of graft rejection, graft versus host disease (GVHD), and delayed immune reconstitution with subsequent infectious complications.[4] The use of intense immunosuppression regimens (to prevent GVHD) may, at least in theory, abrogate the graft versus tumor (GVT) effect portending an increased risk of disease relapse. The GVT effect after allo HCT has been shown to correlate with a decreased risk of relapse.[5,6] Thus, the infused donor T-cells can have beneficial effects (engraftment, immune reconstitution, and GVT) and also exert a harmful (and sometimes fatal) effect of GVHD. For these reasons, researchers have looked at ways to engineer cellular therapies that will provide the optimum ratio of T-cell subsets that may provide a sufficient number of cells to maintain engraftment and optimize GVT effect, while minimizing the allo-reactive T-cells that can lead to GVHD.

The majority (~ 80%) of infused donor lymphocytes are T-cells (other infused cells are B-cells and NK cells). T-cells have been shown to be the key player in the post-transplant immune

phenomena (stem cell engraftment, GVHD, GVT, and immune reconstitution) with B-cells and NK cells likely contributing supportive roles.[1,7] The majority (~ 95%) of T-cells carries alpha-beta T-cell receptors (αβ-TCR); referred to as alpha-beta T-cells (AB T-cells). A small proportion of T-cells carry a different T-cell receptor, γδ-TCR, referred to as gamma-delta T-cells (GD T-cells).

1.1 Anti-tumor effect of GD T-cells

The GD T-cells have been shown to have an anti-tumor activity. They are considered to be a part of the innate immune system preventing development of new cancer and also protecting from infections via immune surveillance function.[8] Unlike the common T-cell subtype, AB T-cells, GD T-cells do not require antigen recognition to kill malignant cells.[9] Thus, they have been advocated for use against cancer.[9] Preclinical mice models have shown cytotoxic effect of GD T cells against breast cancer[10] and prostate cancer.[11] In vitro data (cell lines) has also shown cytotoxic activity against human neuroblastoma and ALL cell line.[12,13] A retrospective clinical study showed improved disease-free survival and overall survival in patients undergoing haplo HCT when they have higher GD T cell recovery in the peripheral blood.[14] The NK cells, another type of innate immune cells, have also shown to have an anti-tumor effect and to promote immune reconstitution after HCT without increasing the risk of GVHD.[15,16]

1.2 Other immunological implications of the GD T cells

Preclinical studies showed that donor-derived GD T cells can facilitate the **engraftment** of allogeneic bone marrow in mice models.[17] Earlier preclinical models showed that host-derived (rather than donor-derived) GD T cells are recruited into the donor-derived AB T cell-initiated GVHD lesions and failed to correlate donor GD T cells with pathogenesis of GVHD in their preclinical model.[18] Subsequent preclinical data also suggest that GD T cells are not primary initiators of GVHD and may in fact modulate the GVHD activity of AB T cells.[9] Drobyski et al. have showed that IL-2-expanded GD T cells infused into lethally-irradiated HLA-mismatched mice do not cause GVHD.[19] Ellison et al. found that GD T cells facilitate the cytotoxic effect of NK cells, which is implicated in GVHD reaction in mice model, but found no evidence that GVHD was initiated by GD T cells.[20] Further studies on preclinical model by Drobyski et al. showed that infusion of activated GD T cells and naïve AB T cells exacerbated GVHD compared to naïve AB T cells alone. However, when administration of the naïve AB T cells was delayed by 2 weeks, survival was significantly improved in mice undergoing stem cell transplant plus infusion of GD

T cells suggesting that activated GD T cells can modulate the ability of the AB T cells to cause GVHD.[21]

In vitro data of GD T cell antineoplastic cytotoxicity has shown minimal or no reaction in the mixed lymphocyte culture implying poor alloreactivity of the GD T cells against natural HLA epitopes.[12,13]

Clinical data showing improved survival among haplo HCT patients with higher GD T cell recovery showed no difference in the incidence of GVHD in both low and high GD T cell cohorts.[14]

Further clinical studies have been done using AB T cell-depleted HCT (wherein graft would have collected dose of GD T cells) with no or abbreviated pharmacologic GVHD prophylaxis showed low risk of GVHD compared to standard of care HCT.[22,23]

1.3 Prior experience with the use of GD T-cells with haploidentical HCT

GD T-cells have been shown to have an anti-leukemic effect in partially mismatched transplant without increasing the risk of GVHD[24,25]. In a retrospective analysis, the 5-year leukemia-free survival (LFS) and overall survival (OS) was higher in patients who recovered with increased GD T-cells as compared to those with normal or decreased numbers; 54 vs 19% ($P < 0.0003$) and 71 vs 20% ($P < 0.0001$) respectively. There were no differences in the incidence of GVHD in both groups ($P = 0.96$).[14] Handgretenger et al used AB T-cell depletion to preserve the GD T-cells with haplo HCT. Their experience showed rapid engraftment with rapid immune reconstitution.[8,26] The rate of engraftment failure with the clinical use of AB T cell/B cell depletion with was comparable to the risk of engraftment failure with T cell depleted haplo HCT.[22,23] In our proposal, we plan to use T cell replete haplo graft with post-transplant cyclophosphamide (as explained below) as a novel method of elimination of T cell alloreactivity (rather than upfront T cell depletion). This method has been associated with favorable outcome of haplo HCT compared to T cell depletion method.

1.4 Medical Device for ex-vivo depletion

Name of Investigational Device –**α/β** T-cell Depletion System

This treatment protocol will require the use of the device CliniMACS with the α/β TCR Reagent Kit and other associated reagents. CliniMACS α/β TCR Reagent is a sterile monoclonal antibody reagent specific for α/β TCR cells (AB T-cells). The depletion of the AB T-cells will be performed according to the manufacturer's instructions and as previously described.[27] In brief, the

leukapheresis product will be incubated with the appropriate antibodies that are conjugated to magnetic particles and then processed using the CliniMACS device (Miltenyi Biotec).
CliniMACS plus Instrument is a software-controlled instrument that processes the blood sample (cell product). The CliniMACS Tubing Set is a single-use, sterile, disposable tubing set with proprietary cell selection columns. The CliniMACS PBS/EDTA Buffer is a sterile, isotonic, phosphate buffered, 1 mM EDTA saline solution used as external wash and transport fluid for the in vitro preparation of blood cells. *PLEASE NOTE*: "The CliniMACS CD34 Reagent system, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved; all other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE)."

1.5 In-vivo T-cell depletion using post-transplant cyclophosphamide (CY) to decrease the risk of GVHD

The administration of CY within a few days after infusion of T-cell replete HCT depletes allo-reactive T-cells of both the donor and host, thus inhibiting both GVHD and graft rejection respectively.[28-32] It is hypothesized that high-dose CY can deplete the proliferating allo-reactive T-cells sparing the non-proliferative (inactive) T-cells.[32] The use of post-transplant cyclophosphamide after haplo HCT has shown promising results by investigators at Johns Hopkins University and Fred Hutchinson Cancer Research Center.[33] The most unfortunate outcome of this approach remained the high relapse rate of 51% at 1 year.[34] The Bone Marrow Transplant Clinical Trials Network (BMT-CTN) conducted a clinical trial (CTN 0603) using the same approach of haplo HCT with a reported relapse rate of 45%.[35] Thus despite the reduction of GVHD using this approach in haplo HCT, the high risk of relapse (45-51%) remained a challenge. The increased risk of relapse may be due to the lack of effective graft versus tumor effect. It is particularly apparent with the use of non-myeloablative regimens, as graft versus leukemia (GVL) effect is the only anti-tumor effect in this setting.

2 THE STUDY HYPOTHESIS

We are proposing the use of a combination of an in-vivo T-cell depletion method (post-transplant cyclophosphamide (CY), detailed above) with an ex-vivo method of cell separation (using the CliniMACS System). The use of post-transplant CY (infusion of CY after infusion of the stem cell

graft) depletes (in-vivo) the allo-reactive T cells that would otherwise increase the risk of GVHD. The ex-vivo AB T-cell depletion will selectively deplete the AB T-cells, thus leaving other graft components including GD T-cells, NK cells, and B-cells.

We hypothesize that boosting the GD T-cells (via infusion of AB T-cell depleted graft) after the post-transplant CY (to reduce allo-reactive T-cells that cause GVHD) is safe and could be beneficial to decrease the risk of relapse of hematological malignancy after haplo HCT.

We propose to collect peripheral blood stem cell (PBSC) grafts from haploidentical donors and divide the cell product into an un-manipulated HCT product that will be given on transplant day (as is standard in HSCT) and an AB depleted (ABD) T-cell product that will be given after engraftment.

3 OBJECTIVES

3.1 Primary Objective

- To determine the safety of using the ABD T-cell infusion following haplo HCT and post-transplant CY.

3.2 Secondary Objectives

- To determine the risk of **viral reactivation** after using the ABD T-cell infusion following haplo HCT and post-transplant CY.
- To determine the **immune reconstitution** pattern after using the ABD T-cell infusion following haplo HCT and post-transplant CY.
- To determine the risk of **severe chronic GVHD** after using the ABD T-cell infusion following haplo HCT and post-transplant CY.

4 ENDPOINTS

4.1 Primary Safety Endpoints

- Grade IV acute GVHD (before day +100 of transplant).
- Severe non-hematological toxicity before day +100 of transplant (as defined under dose-limiting toxicity in section 12.1).

4.2 Secondary Endpoints

- Viral reactivation within 100 days of transplant that requires preemptive therapy.
- Severe chronic GVHD.

5 ELIGIBILITY AND EXCLUSION CRITERIA

5.1 Eligibility Criteria

1. Patients with neoplastic hematological disorders with indication of allogeneic transplant according to the National Comprehensive Cancer Network (NCCN) or other standard guidelines as follows:
    - Acute lymphoblastic leukemia [ALL][36] with high-risk features or relapsed disease.
    - Hodgkin[37] or Non-Hodgkin lymphoma[38] [HL or NHL]: relapsed disease where remission duration is less than 1 year, relapse after previous autologous transplant, or failure to achieve CR with chemotherapy.
    - Myeloid malignancy (acute myeloid leukemia [AML][39] with intermediate/high-risk features (per NCCN criteria) or relapsed disease, OR chronic myeloid leukemia [CML][40] in hematological remission or chronic phase).[39]
    - Myeloid disorder (myelodysplastic syndrome [MDS][41] with intermediate/high risk features or refractory disease or myeloproliferative disorder; primary or secondary if high-risk features or refractory disease)[42].
2. No available suitable HLA-matched donor.
3. Age Criteria: 19 to 65 years in age.
4. Organ Function Criteria: The following organ function testing should be done within 35 days before study registration.
    - Cardiac: LVEF of 50% or above, by MUGA or Echocardiogram.
    - Pulmonary: FVC, FEV1 and DLCO (corrected) should be 50% or above of expected.
    - Renal: serum creatinine level to be < 2 mg/dl or estimated creatinine clearance (CrCl) must be equal or greater than 40 mL/min/1.73 m^2 as calculated by the Cockcroft-Gault Formula.

- Hepatic: serum bilirubin ≤1.5 × upper limit of normal (ULN), Aspartate transaminase (AST)/alanine transaminase (ALT) ≤2.5 × ULN, and alkaline phosphatase ≤ 2.5 × ULN.

5. Performance status: Karnofsky ≥ 80%.
6. Consent: All patients must be informed of the investigational nature of this study and given written informed consent in accordance with institutional and federal guidelines.

5.2 Exclusion Criteria

1. Non-compliant to medications.
2. No appropriate caregivers identified.
3. Uncontrolled medical or psychiatric disorders which may preclude patients to undergo clinical studies (Discretion of the attending physician)
4. Known active central nervous system (CNS) neoplastic involvement.
5. Patients with a known allergy to DMSO.
6. HIV1 (Human Immunodeficiency Virus-1) or HIV2 positive.
7. Pregnant or breastfeeding.

5.3 Donor Eligibility Criteria

1. HLA typing per UAB standard.
2. Suitable Donor – Medically cleared to donate.
3. Eligible Donor – Meets all donor screening and testing requirements related to transmission of infectious disease per current FACT standards.

6 STUDY TREATMENT

6.1 Preparative Chemotherapy Regimen

All patients will receive post-transplant CY (PTCy) of 50 mg/kg on Day +3 and +4. Patients will receive MESNA and hydration for prophylaxis of hemorrhagic cystitis as per institutional guidelines.

All the following defined days of chemotherapy/radiation administration have +/- 2 day window to allow for scheduling conflicts and / or clinical status changes.

All patients will receive the following preparative regimen:

- **Fludarabine/Cyclophosphamide/TBI (FLU/CY/TBI)**: Patients will receive Fludarabine 30 mg/m^2 x 5 doses. Cyclophosphamide 14.5 mg/kg x 2 doses plus total body irradiation 2 Gy.

  Preparative Regimen schema

  - Day -6 Fludarabine 30 mg/m2 IV + CY 14.5 mg/kg IV
  - Day -5 Fludarabine 30 mg/m2 IV + CY 14.5 mg/kg IV
  - Day -4 Fludarabine 30 mg/m2 IV
  - Day -3 Fludarabine 30 mg/m2 IV
  - Day -2 Fludarabine 30 mg/m2 IV
  - Day -1 Total body irradiation (TBI) 2 Gy
  - Day 0 Transplant
  - Day +3 Cyclophosphamide 50mg/kg IV
  - Day +4 Cyclophosphamide 50mg/kg IV

6.2 Chemotherapy dose adjustments

Dosing of chemotherapy will be based on adjusted body weight unless the actual body weight is less than the ideal body weight (IBW), in which case we will use the actual body weight. Body weight will be calculated as follows:

- Ideal Body Weight (IBW):
  - Males: IBW= 50 + ([(Ht. in cm x 0.39) – 60] x 2.3)
  - Females: IBW= 45.5 + ([(Ht. in cm x 0.39) – 60] x 2.3)
- Adjusted weight = IBW + (Actual Weight - IBW) x 0.4

6.3 Donor Selection

Donors will be screened for eligibility and suitability for allogeneic hematopoietic stem cell donation according to requirements mandated by FACT (Foundation for the Accreditation of Cellular Therapy) and the FDA. Donors will be offered the clinical research IRB approved consent form once they have been identified as a potential donor for a patient who has consented for this trial. There are no extra studies or procedures required from the donor to participate in this trial.

All potential donors, irrespective of participation in this clinical trial, also sign the standard donor informed consent form prior to being accepted as a donor.

The standard donor consent form of the UAB BMT-CT program addresses the voluntary nature of stem cell donation, risks associated with the placement of a central venous line, risks and side effects associated with the use of G-CSF, risks associated with leukapheresis including, but not limited to, nausea, vomiting, syncope, hematoma, thrombophlebitis, seizures, blood loss, and infection, and the possibility that the collection may require more than one day. The donor will have a thorough history and physical exam as well as appropriate blood tests in compliance with FACT standards. Donors also will receive post-donation follow-up and care as per standard clinical care of PBSC donors.

6.4 Donor collection

The suitable and eligible haplo donor will undergo peripheral blood apheresis for the collection of stem cells targeting a CD34+ cell dose of 8 $x10^6$ cells/kg of recipient weight (minimum of greater than $4x10^6$ cells/kg). For standard transplant we target a minimum of $4x10^6$ CD34+ cells. If the total stem cell number collected is equal to or less than $4x10^6$ cells/kg, then the product will not be split for additional processing and the patient will be taken off of the study. If a participant is taken off of study before receiving the ABD product, they will receive the post-transplant cytoxan as per their assigned preparative regimen and will be followed up for survival until day +100. Donor will sign both a standard donor consent form and a study participant consent form explaining the collection procedure and the targeted volumes needed for this study.

6.5 Stem cell infusion

A full (un-manipulated) stem cell graft will be infused on day 0. The collected product will be split in order to give a minimum of $4x10^6$ cells/kg on day 0. The rest of the product will undergo ABD process to be given after post-transplant CY and after engraftment.

6.6 Post-transplant CY

Infusion of post-transplant CY (50 mg/kg) will take place on day +3 and day +4. Mesna will be administered as per institutional guidelines to prevent hemorrhagic cystitis.

6.7 ABD product preparation

The ABD product (graft that is depleted of alpha-beta T cells) will be prepared from the mobilized stem cell graft using the CliniMACS device. This device typically depletes 3-4 log of the total AB T-cell content. This ABD product will be quality tested and cryopreserved as per institutional guidelines, and will be infused after neutrophil engraftment post transplant to primarily provide GD T cells (and also additional CD34+ stem cells and NK cells).

The GD T cell dose to be infused will be a minimum of **$1x10^5$** cells/kg and maximum of **$3x10^6$** cells/kg (~1.3 log range). Based on our prior data on the 4 treated patients (version 1.0 of the protocol), the range of GD T cells in the add-back product was $7x10^5$ - $6x10^6$ cells/kg. We thus have selected a dose rage of GD T cells with lower minimum (to allow for expected variable GD T cell yield) with only 1.3 log range.
The maximum AB T cell dose in the add-back product will be $1x10^5$ cells/kg in a clear definable event cluster calculated by flow cytometry which is equivalent to ~3 log reduction. Typical expectation of the procedure is 4-5 log reduction.

The target release criteria for the final product will be

- AB T cell dose $\leq 1x10^5$ cells/kg recipient weight.
- GD T cell dose of a minimum of $1x10^5$ cells/kg and maximum of $3x10^6$ cells/kg recipient weight.

Post-processing studies will include white blood cell (WBC), platelet, and red blood cell (RBC) content in the samples using a Coulter AcTDiff Hematology Analyzer previously validated for this purpose. After an initial gram stain, 1 ml aliquot of unprocessed cell suspension and 1 ml of processed final product will be submitted to the UAB microbiology laboratory for a 14-day sterility assessment using the BAC-T Alert™ system. Testing for endotoxin will be performed using FDA-approved methods. Composition, purity, and viability will be determined by flow cytometric lymphocyte immunophenotyping prior to and at the time the product is released (Table 1).
Table 1.

Tube	mAb1	mAb2	mAb3	mAb4	mAb5	mAb6	mAb7	Function
1	CD3	TCR-γδ	CD16/56	CD19	CD45	TCR-□□	7-AAD	Phenotype

Since we are cryopreserving a portion of the cell product to be given separately, study participants will be exposed to Dimethyl sulfoxide (DMSO) during the infusion of the ABD product. DMSO is a cryoprotectant commonly used to cryopreserve cellular therapy products. DMSO toxicity is a possible complication of cryopreserved product administration. Side effects and symptoms are generally associated with histamine release. Signs and symptoms include coughing, flushing, rash, chest tightness, wheezing, nausea, vomiting, and cardiovascular instability. Standard stem cell infusion precautions are taken to decrease the risk of reaction to the DMSO. These precautions include slowing the rate of infusion, pre-medicating with antihistamines, and continuous monitoring during administration.

6.8 ABD T-cell infusion

The ABD T-cell infusion will take place 3 to 10 days after the first day of neutrophil engraftment post-transplant. Neutrophil engraftment is defined as the first day of 3 consecutive absolute neutrophil counts that are 500 or greater. The product infusion will be performed as per the program's standard order set for the post-transplant infusion of donor cells.

If the patient is in poor condition such as high fever, unstable blood pressure, renal insufficiency post cytoxan, or severe volume overload during the planned infusion window of 3 to 10 days post engraftment the ABD T-cell product may be withheld for 2 additional days at the attending physician's discretion. If the infusion is further delayed, or not to be performed, the PI should be consulted.

6.9 Risk Analysis

Theoretical risks to the patient when using the CliniMACS® TCRα/β Reagent Kit and the CliniMACS® CD19 Reagent are postulated from possible events resulting from system failure, user error, or patient reaction to the depleted product. Furthermore, recipients of allogeneic transplants are subject to risks from the transplant-related procedures and medications utilized. These risks are independent of the TCRα/β+ T cell depletion using the CliniMACS® TCRα/β Reagent Kit.

Graft Failure, EBV-associated post-transplant lymphoproliferative disease, and an increased risk of opportunistic infection related to prolonged neutropenia, defined risks in the investigator's brochure, are risks usually associated with the infusion of a T-cell depleted donor graft, but do not apply in this investigation as the T-Cell depleted product will be infused as a donor lymphocyte product after the infusion of an un-manipulated stem cell graft.

7 GVHD PROPHYLAXIS

- GVHD prophylaxis consists of post-transplant CY (50 mg/kg IV on Day +3 and day +4 post-transplant).
- Other GVHD prophylaxis will include mycophenolate mofetil (MMF, Cellcept) and a calcineurin inhibitor, such as tacrolimus.
    - Cellcept will be given as 15 mg/kg PO 3 times daily (maximum daily dose of 3 gm) starting day +5 to day +35. An intravenous formulation may be used as per physician discretion until reliable PO intake of the patient is established.
    - Tacrolimus will be given as 0.03 mg/kg/day (dosing may be adjusted as is standard for drug interactions with concurrent medications) IV infusion beginning on day +5 and converted to oral tacrolimus when PO intake is tolerated. Tacrolimus will be continued until day +100 and then may be tapered to none by day +180 if there is no evidence of active GVHD.

8 INFECTION PROPHYLAXIS

- The use of prophylactic anti-fungal, anti-bacterial, PCP, and anti-viral therapies will follow institutional guidelines.
- Viral monitoring: CMV, HHV6, adenovirus, EBV surveillance (PCR or antigenemia) will be employed on weekly (±3 days) basis starting on approximately day +20 (±3 days) and continuing until day +100 (expected time of fair T cell recovery). Surveillance can change to every 4 weeks thereafter until patient is off immunosuppression. Risk of viral reactivation after day +100 is uncommon.[43-46] Preemptive therapy will be employed per standard of care guidelines.

9 GROWTH FACTOR USE

As part of our standard of care haplo transplant patients will be started on G-CSF on day +5 after transplant.

10 CHEMOTHERAPY DRUGS

10.1 Cyclophosphamide (Cytoxan, CY)

Chemical Characteristics

CY is a synthetic antineoplastic drug chemically recognized as 2-[bis (2 cholorethyl) amino] tetrahydro-2H-1, 3, 2-oxazaphosphorine 2-oxide monohydrate. The molecular formula of CY is $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$ with a molecular weight of 279.1.

Available Forms

IV CY is available as a sterile white crystalline powder. CY is stored in a single dose vial containing 500mg of the powder.

Storage and Handling

Unopened vials should be stored at or below 25°C (77°F).

Toxicity

Common side effects of CY include: nausea, vomiting, alopecia, leucopenia, interstitial pneumonitis, interstitial pulmonary fibrosis, anaphylactic reactions, amenorrhea, hemorrhagic cystitis, oligospermia or azoospermia and suppression of immune responses. Less common side effects include: secondary malignancies in patients diagnosed with a primary malignancy, fetal harm when administered to pregnant women, acute cardiac toxicity, congestive heart failure, hemorrhagic myocarditis , hemopericardium, and possible cross-sensitivity with other alkylating agents.

Administration

CY for parenteral use must be prepared by either adding 0.9% sodium chloride solution, if injected directly, or sterile water, if infused. Constituted in water, CY is hypotonic; hence, it should not be

injected directly. Solutions of CY with sodium chloride solution may be injected intravenously, intramuscularly, intraperitoneally, or intrapleurally.

Constituted cylophosphamide is physically and chemically stable for 24 hours at room temperature or six days refrigerated. Prepared solutions do not contain any microbial preservative; hence, sterility of the solutions should be monitored.

10.2 Fludarabine (Fludara)

Chemical Characteristics

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

Available Forms

IV Fludarabine is packaged as a white lyophilized solid cake in a vial. Each vial contains 50mg of fludarabine phosphate and only one vial is enclosed per carton.

Storage and Handling

Unopened vials of fludarabine should be stored at 20° to 25°C (68° TO 77°F); excursions permitted between 15° to 30°C (59° TO 86°F).

Toxicity

Toxicities reported as more than 10% incidence are:

Myelosuppression (neutropenia, thrombocytopenia, and anemia), fever and chills, infections, nausea and vomiting, pain, weakness, cough, pneumonia, dyspnea, diarrhea, anorexia, rash, and edema.

Toxicities with expected incidences between 1% and 10% are:

Malaise, stomatitis, myalgia, paresthesia, visual disturbance, gastrointestinal bleeding, upper respiratory infection, diaphoresis, dysuria, urinary infection, sinusitis, hearing loss, hyperglycemia,

headache, pharyngitis, hemoptysis, esophagitis, mucositis, hematuria, osteoporosis, alopecia, anaphylaxis, hemorrhage, dehydration, sleep disorder, depression, cerebellar syndrome, impaired mentation, allergic pneumonitis, epitaxis, bronchitis, hypoxia, liver failure, abnormal liver function, cholelithiasis, ARDS, respiratory distress, pulmonary hemorrhage, pulmonary fibrosis, respiratory failure, constipation, dysphagia, pruritus, seborrhea, renal failure, abnormal renal function test, protenuria, hesitancy, angina, congestive heart failure, arrhythmia, supraventricular tachycardia, myocardial infarction, deep venous thrombosis, phlebitis, transient ischemic attack, aneurysm, cerebrovascular accident, arthralgia, and tumor lysis syndrome.

Administration

IV fludarabine is prepared by adding sterile water to the white solid cake. Reconstituted in 2mL of sterile water, the solid cake produces a solution with an approximate concentration of 25mg/mL fludarabine phosphate. Follow the institutional guidelines for further preparation and administration procedures of fludarabine.

Reconstituted IV fludarabine contains no antimicrobial preservative; hence, it should be utilized within 8 hours of reconstitution. DO NOT infuse concomitantly with another intravenous solution of unknown compatibility.

10.3 Tacrolimus (Prograf)

Chemical Characteristics

Tacrolimus is a macrolide immunosuppressant produced by Stretocyces Tsukubaensis. Tacrolimus has an empirical formulation of $C_{44}H_{69}NO_{12}$-H_2O and a formula weight of 822.05. Tacrolimus appears as white crystals or crystalline powder. It is practically insoluble in water, freely soluble in ethanol, and very soluble in methanol and chloroform. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism of action is not known. Experimental evidence suggests that tacrolimus binds to an intracellular protein, FKBP-12. A complex of tacrolimus-FKBP-12, calcium, calmodulin, and calcineurin is then formed and the phosphatase activity of calcineurin inhibited. This effect may prevent the dephosphorylation and translocation of nuclear factor of activated T-cells (NF-AT), a nuclear component thought to initiate gene transcription for the

formation of lymphokines (such as interleukin-2, gamma interferon). The net result is the inhibition of T-lymphocyte activation (i.e., immunosuppression).

Available Forms

Tacrolimus is available for oral administration as capsules (tacrolimus capsules) containing the equivalent of 0.5 mg, 1mg, and 5mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg and 1 mg capsule shell contains gelatin and titanium dioxide, and the 5-mg capsule shell contains gelatine, titanium dioxide, and ferric oxide. Tacrolimus is also available as a sterile solution (tacrolimus injection) containing the equivalent of 5mg anhydrous tacrolimus in 1 ml for administration by IV infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v.

Storage and Handling

Tacrolimus capsules should be stored at room temperature between 15° and 30°C (59° and 86°F). Tacrolimus injection should be stored between 5° and 25°C (41° and 77°F).

Toxicity

Possible side effects of tacrolimus include: depressed kidney function, high blood sugar, high blood potassium, skin rash, headache, nausea, and vomiting. Less common side effects are loss of appetite, sleep disturbances, vivid dreams, hallucinations, high blood pressure, seizure, decreased level of consciousness, anemia, agitation, tremors, irritability, slurred speech, tingling in the hands and feet, pain in the palms and soles of the feet, weakness, and abnormal blood cell levels. All of these side effects are reversible by reducing the dose or discontinuing the drug. Rare fatal cases of severe allergic reactions have been reported in patients receiving cyclosporine and it is possible that similar reactions could also occur in patients receiving tacrolimus.

Administration

Tacrolimus (Prograf injection) must be diluted with NS or D5W before use. Tacrolimus is administered as a continuous infusion. Oral preparation will be administered on empty stomach every 12 hours.

10.4 Mycophenolate Mofetil (MMF, Cellcept)

Chemical Characteristics

CellCept (mycophenolate mofetil) is the 2-morpholinoethyl ester of mycophenolic acid (MPA), an immunosuppressive agent, inosine monophosphate dehydrogenase (IMPDH) inhibitor. The chemical name for mycophenolate mofetil (MMF) is 2-morpholinoethyl (E)-6-(l,3-dihydro-4-hydroxy-6-methoxy-7- methyl-3-oxo-5-isobenzofuranyl)-4-methyl-4-hexenoate. It has an empirical formula of $C_{23}H_{31}NO_7$ and a molecular weight of 433.50. Mycophenolate mofetil is a white to off-white crystalline powder. It is slightly soluble in water (43 µg/mL at pH 7.4); the solubility increases in an acidic medium (4.27 mg/mL at pH 3.6). It is freely soluble in acetone, soluble in methanol, and sparingly soluble in ethanol. The apparent partition coefficient in 1-octanol/water (pH 7.4) buffer solution is 238. The pKa values for mycophenolate mofetil are 5.6 for the morpholino group and 8.5 for the phenolic group. Mycophenolate mofetil hydrochloride has a solubility of 65.8 mg/mL in D5W. The pH of the reconstituted solution is 2.4 to 4.1.

Available Forms

CellCept is available for oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/mL mycophenolate mofetil

Toxicity

Toxicities reported as more than 20% incidence are:

Hypertension, hypotension, peripheral edema, chest pain, tachycardia, pain, headache, insomnia, fever, dizziness, anxiety, rash, hyperglycemia, hypercholesterolemia, hypomagnesemia, hypokalemia, hypocalcemia, hyperkalemia, abdominal pain, nausea, diarrhea, constipation, vomiting, anorexia, dyspepsia, urinary tract infection, leukopenia, anemia, leukocytosis, thrombocytopenia, liver function tests abnormality, ascites , back pain, weakness, tremor, paresthesia, abnormal kidney function, dyspnea, respiratory tract infection, pleural effusion, cough, lung disorder, sinusitis, infection, sepsis, and lactate dehydrogenase increase.

Toxicities with expected incidences ***between*** 3% ***and*** 20% are:

Angina, arrhythmia, arterial thrombosis, atrial fibrillation, atrial flutter, bradycardia, cardiac arrest, cardiac failure, CHF, extrasystole, facial edema, hyper-/hypovolemia, pallor, palpitation, pericardial effusion, peripheral vascular disorder, postural hypotension, supraventricular extrasystoles, supraventricular tachycardia, syncope, thrombosis, vasodilation, vasospasm, venous pressure increased, ventricular extrasystole, ventricular tachycardia, chills with fever, confusion, delirium, depression, emotional lability, hallucinations, hypoesthesia, malaise, nervousness, psychosis, seizure, somnolence, thinking abnormal, vertigo , acne, alopecia, bruising, cellulitis, fungal dermatitis, hirsutism, petechia, pruritus, skin carcinoma, skin hypertrophy, skin ulcer, vesiculobullous rash, acidosis, alkalosis, Cushing's syndrome, dehydration, diabetes mellitus, gout, hypercalcemia, hyper-hypophosphatemia, hyperlipemia, hyperuricemia, hypochloremia, hypoglycemia, hyponatremia, hypoproteinemia, hypothyroidism, parathyroid disorder, abdomen enlarged, dysphagia, esophagitis, flatulence, gastritis, gastroenteritis, gastrointestinal hemorrhage, gastrointestinal moniliasis, gingivitis, gum hyperplasia, ileus, melena, mouth ulceration, oral moniliasis, stomach disorder, stomach ulcer, stomatitis, xerostomia, weight gain/loss, impotence, nocturia, pelvic pain, prostatic disorder, scrotal edema, urinary frequency, urinary incontinence, urinary retention, urinary tract disorder, coagulation disorder, hemorrhage, neutropenia, pancytopenia, polycythemia, prothrombin time increased, thromboplastin time increase, alkaline phosphatase increased, bilirubinemia, cholangitis, cholestatic jaundice, GGT increased, hepatitis, jaundice, liver damage, transaminases increase, abscess, arthralgia, hypertonia, joint disorder, leg cramps, myalgia, myasthenia, neck pain, neuropathy, osteoporosis, amblyopia, cataract, conjunctivitis, eye hemorrhage, lacrimation disorder, vision abnormality, deafness, ear disorder, ear pain, tinnitus, albuminuria, creatinine increased, dysuria, hematuria, hydronephrosis, oliguria, pyelonephritis, renal failure, renal tubular necrosis, apnea, asthma, atelectasis, bronchitis, epistaxis, hemoptysis, hiccup, hyperventilation, hypoxia, respiratory acidosis, pharyngitis, pneumonia, pneumothorax, pulmonary edema, pulmonary hypertension, respiratory moniliasis, rhinitis, sputum increased, voice alteration, and thirst.

Administration

Oral dosage formulations (tablet, capsule, suspension) should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter); oral suspension should not be

mixed with other medications. Delayed release tablets should not be crushed, cut, or chewed. Intravenous solutions should be administered over at least 2 hours (either peripheral or central vein); do not administer intravenous solution by rapid or bolus injection.

10.5 Filgrastim (or biosimilar)

Chemical Characteristics

Neupogen is the trademark name for filgrastim, representing recombinant methionyl human granulocyte colony-stimulating factor (r-methHuG-CSF). Neupogen is a 175 amino acid protein produced by recombinant DNA technology utilizing *Escherichia coli (E. coli)*. Neupogen has a molecular weight of 18,800 daltons and an amino acid sequence similar to that of natural human DNA except for the additional methonine at the N-terminal, necessary for expression in *E. coli*.

Available Forms

Neupogen is marketed as a sterile, clear, colorless preservative-free liquid. It is packaged in vials and prefilled syringes. The vials are single-dose, preservative-free and contain 300 mcg/mL of filgrastim. The prefilled syringes are single-dose, preservative-free, and contain 600 mcg/mL of filgrastim.

Storage and Handling

Neupogen must be stored refrigerated at 2° to 8°C (36° TO 46°F). Avoid shaking.

Preceding injection, neupogen must reach room temperature for at most 24 hours. Discard any vials or prefilled syringes left at room temperature for more than 24 hours. Avoid use if particulate matter or discoloration is observed prior to administration

Toxicity

Common side effects include: nausea, vomiting, skeletal pain, spontaneous reversible elevations in uric acid, lactate dehydrogenase and alkaline phosphatase, alopecia, palpable splenomegaly and petechiae. Other side effects include: diarrhea, neutropenic fever, mucositis, fever, fatigue, thrombocytopenia, anorexia, dyspnea, headache, epistaxis, transfusion reaction, cough, skin rash,

chest pain, generalized weakness, sore throat, stomatitis, pain, myocardial infarctions, constipation, arrhythmias, hypotension, hemorrhagic events, renal insufficiency, capillary leak syndrome, hepatomegaly, arthralgia, osteoporosis, cutaneous vaculitis, hematuria/proteinuria, exacerbation of some pre-existing skin disporders, splenic rapture, sickle cell crisis, sweet's syndrome, and decreased bone density.

Administration

Neupogen may be administered as an IV or a subcutaneous infusion. It is recommended that neupogen be administered at least 24 hours after bone marrow infusion, with dosage modifications determined by neutrophil response.

If necessary, neupogen maybe diluted in 5% dextrose with the addition of Albumin(human) to prevent absorption to plastic materials. Dilution to final concentration less than 5mcg/mL is not recommended at any time. Do not dilute with saline as the product may precipitate.

When using either vials or prefilled syringes, do not save unused drugs for later administration. Dispose of all unused portions.

10.6 Total Body Irradiation (TBI)

Toxicity

TBI will be administered per standard of care procedure as implemented by radiation oncologists. TBI alone for post-pubescent patients with dose/fractionation not exceeding 2 Gy x 6 is well within the tolerance of most normal organs for < 5% risk of severe late toxicity (organ failure or major dysfunction) by 5 years. Notable exceptions are risks of cataract development, bone marrow suppression, and ovarian and testicular dysfunction. Also, there is a small risk of second malignancy. The most common acute effects include nausea, vomiting, diarrhea, and painful swelling of the parotid glands.

When TBI is given in conjunction with other therapies in the transplant setting, there is additional risk of side effects including loss of appetite, dry mouth, difficult or painful swallowing , headache, stomatitis (sore throat/mouth), altered skin integrity, hair loss, swelling, increased risk for infection

and/or bleeding, possible lung failure, dry cough, fatigue, anxiety, fever, possible liver failure, lung scarring, loss of vision, shortness of breath, sterility, heartburn, cystitis, sleep disturbances, altered gastrointestinal and genitourinary function, neuropathy, fistulas, altered endocrine function, pericarditis, and increased risk of a second cancer. Overall, the incidence of most major toxicity when radiation is given in conjunction with other therapy as outlined above is still low, rare, serious side effects are possible.

11 POST-TRANSPLANT THERAPY

Post-transplant therapy will be implemented only if this is part of the standard of care. Patients who receive non-standard maintenance treatment will be taken off the study and their data will be censored at this point.

- Examples of standard post-transplant therapy are:
    a. Tyrosine kinase inhibitors (TKI) for Philadelphia-positive ALL for 2 years after transplant.
    b. Intrathecal (IT) therapy for ALL (for a minimum of 3 treatments) after transplant.
    c. TKI in CML if there is evidence of molecular or cytogenetic relapse or progression after transplant.
- Post-transplant consolidation radiation treatment is allowed if this is planned ahead of transplant or deemed appropriate consolidation treatment after transplant.

12 REQUIRED OBSERVATIONS

The following observations will be monitored (see appendix A study calendar).

Pre-Transplant required observations: (within 35 days prior to study registration / day of transplant final evaluation)

1. History and physical exam (includes Karnofsky Performance Status score).
2. CBC, BUN, Creatinine, AST, ALT, Total bilirubin.
3. Echocardiogram or MUGA
4. Pulmonary function testing: FVC, FEV1, DLCO (corrected for hemoglobin).
5. Unilateral bone marrow aspirate and biopsy (for acute leukemia patients), morphology, and cytogenetics.
6. CT scans or Whole Body CT/PET scans if appropriate for disease status assessment.

7. Lumbar puncture will be done in patients with ALL. Pre-transplant intrathecal treatment(s) may be administered at the discretion of the attending physician.
8. Pregnancy test (if childbearing potential)
9. HIV, HBV and HCV testing

Post-transplant required observations and follow-up plans:

1. Clinical monitoring for GVHD and organ toxicity. This will be done by history-taking and physical examination with lab assessments daily until engraftment and then weekly (± 3 days) until Day +30, and then at least every 2 weeks (± 5 days) until day +100. GVHD will then be evaluated at Day +180 (± 14 days) and +365 (± 45 days) post transplant.
    - Acute GVHD assessment will be done using the modified Keystone (Glucksberg) consensus criteria.[47]
    - Chronic GVHD assessment will be done using the conventional criteria.[48]
2. Pulmonary function test on day +100.
3. Viral monitoring: as outlined under "Infection prophylaxis / viral monitoring section".
4. Immune Reconstitution studies will be performed as per institutional guidelines. The recommended timing is at Day +30 (±7), Day +60 (±7), Day +100 (±14), Day +180 (±21), and at 1 year (±45 days) post-transplant. This panel will include measurement of the percentage and absolute count of CD3+, CD4+, CD8+, CD56+, CD19+, Treg (CD4+/CD25+) effector/memory, and GD T-cells.

The patient will be followed up until day +100 after transplant to monitor for GVHD and organ toxicities and then followed until day +365 for chronic GVHD.

13 STATISTICAL CONSIDERATIONS

This is a single-center, single-arm, open-label phase I study whose primary aim is to evaluate the safety of the infusion of ABD T-cells after HCT and post-transplant CY.

13.1 Study Design and Sample Size

This phase I study will be performed on an initial cohort of 3 patients with an allowed extension to another 3-patient cohort if dose-limiting toxicity (DLT) (as defined below) occurs in 33% in the first cohort. If DLT in the first cohort is < 33% (= none was seen), then the phase I will be

completed with no further cohort enrollment. If DLT in the first cohort is seen in only 1 patient (= 33%), then another cohort of 3 patients will be enrolled. If DLT in the first cohort is seen in 2 patients, the study will be closed and data analyzed.
The first 2 patients can be enrolled simultaneously. A third patient can only be enrolled after both patients pass day +100. If an extension to total of 6 patients is required, then each patient in the second cohort (i.e. subsequent 3-patient cohort) will be followed up until day +100 before enrolling subsequent patient.

This phase I study aims at the demonstration of the safety of the haplo HSCT followed by ABD T-cell infusion. This infusion will be deemed safe if DLT occurred in not more than one third (33%) of patients. DLT of this treatment will be defined as the occurrence of grade IV acute GVHD or other grade IV non-hematological toxicity (using NCI CTCAE version 4.03), that is definitely or probably related to T-cell infusion within 100 days post-transplant. Grade IV hematological toxicity is expected in all patients undergoing allogeneic stem cell transplantation. It is to be noted that the expected risk of grade III-IV aGVHD using haplo PBSC HCT and PTCy (as standard of care) is up to 23%.[49]

Evaluation of safety will be done on evaluable patients (patients who achieve engraftment with the haplo transplant). Patients who do not engraft and undergo salvage HCT will not be evaluable. But data of engraftment failure (when ABD product given before engraftment) will be monitored.

This trial has treated 4 patients with inconclusive results because of occurrence of engraftment failure in 2 patients (not uncommon after standard of care haploidentical transplant). One of these 2 patients (with engraftment failure) was associated with reactivation of HHV-6 virus. We thus opted to modify the protocol to infuse the "add-back" product after engraftment.

We plan to enroll a total of 10 patients (4 enrolled, planning for 3 more and possibly additional 3 patients – using 3+3 design). We expect to enroll the up to 6 remaining patients within 12 months.

13.2 Statistical Analysis Methods

The Data of the phase I study will be descriptive and summarized with the type and grade of toxicity and the proportion of patients who developed toxicities. The proportion of the events (GVHD and toxicity) will be calculated with the corresponding exact 95% confidence interval.

14 ADVERSE EVENT REPORTING

Adverse events occurring after study registration, but prior to the beginning of the conditioning regimen will not be reported. Adverse events will be reported until day +100 post-transplant.

14.1 Definitions of Adverse Events

This trial utilizes the use of manipulated T-cells. An investigational Device Exemption (IDE) approval has been granted from the Food and Drug Administration (FDA) (BB IDE 15938). This research is sponsored by the UAB BMT-CT program, therefore the PI functions as the investigator and the sponsor for reporting responsibilities. The investigational device exemption (IDE) regulations define an unanticipated adverse device effect (UADE) as "any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects" (21 CFR 812.3(s)). UADEs will be reported by the clinical investigator to the sponsor and/or the reviewing IRB, as described below:

- For device studies, investigators are required to submit a report of a UADE to the sponsor and the reviewing IRB as soon as possible, but in no event later than 10 working days after the investigator first learns of the event (§ 812.150(a)(1)).
- Sponsor of the study (PI) must immediately conduct an evaluation of a UADE and must report the results of the evaluation to the FDA, all reviewing IRBs, and participating investigators within 10 working days after the sponsor first receives notice of the effect (§§ 812.46(b), 812.150(b)(1)).

The IDE regulations, therefore, require investigators / sponsors to submit reports to the IRB in a manner consistent with the recommendations for the reporting of unanticipated problems (adverse events) under the IND regulations.

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject whether or not it may have a causal relationship with this treatment. An AE includes significant exacerbation of any baseline medical condition including, but not limited to, the disease under study. Reporting requirements may include the following considerations:

1) The characteristics of the adverse event including the grade (severity).
2) The relationship to the study therapy (attribution).
3) The prior experience (expectedness) of the adverse event.

Except where otherwise specified, Common Terminology Criteria for Adverse Events (CTCAE) v.4.03 will be used to grade adverse events in this study.

14.2 Required Adverse Events Reporting

Therapy for hematological malignancies, with or without stem cell transplantation, is associated with significant toxicity. These toxicities are generally viewed as an anticipated consequence of therapy rather than an adverse event. To summarize, adverse events with severity grades 1, 2, 3, and all expected grade 4 toxicities will not be reported to the IRB, as they are expected in patients undergoing stem cell transplantation for hematological malignancies. Only unexpected grade 4 non-hematologic toxicity events with a possible, probable or definite relation to the study and all grade 5 events will be reported to IRB. Any adverse event occurring that, in the opinion of the principal investigator, is both unexpected and related or possibly related to the research will be reported to the IRB as soon as possible, but within 10 working days.

14.3 Serious Adverse Event Reporting Procedures

All serious adverse events (SAE) which require reporting must be reported immediately (i.e. within 24 hours of awareness) to the Principal Investigator or designee, followed by written documentation to the IRB from the PI (including the PI's or designee's medical summary of the SAE) within 7 days of the PI's knowledge of occurrence. A serious adverse event (SAE) is defined as: Any adverse event (experience) occurring that results in **ANY** of the following outcomes:

1. Death.
2. A life-threatening adverse drug experience.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon 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 (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

The research staff of BMT-CT Program will coordinate the reporting process between the investigators and the IRB as well as other applicable reporting agencies (FDA, CTEP, and NIH). Copies of all correspondence and reporting documents will be maintained in a regulatory file held by the research staff of BMT-CT Program.

15 DATA SAFETY AND MONITORING

15.1 Data and Safety Monitoring Plan

The Data and Safety Monitoring Board (DSMB) of The University of Alabama at Birmingham Comprehensive Cancer Center (CCC) is the DSMB for this study. The Clinical Trials Monitoring Committee (CTMC) is responsible for direct oversight and monitoring of the trial in adherence with the Data Safety Monitoring Plan (DSMP) of the CCC. This committee is responsible for the monthly review and monitoring of the study's scientific progress, accrual rate, and any serious adverse events. The CTMC provides reports to the Associate Director for Clinical Research and the Quality Assurance (QA) Program. The QA program operates independently from the BMTCT program and the CCC and is responsible for monitoring patient safety, AE reporting requirements, protocol compliance, and DSMP compliance.

In addition to the Cancer Center CTMC, the BMT-CT Program will form a Data and Safety Monitoring Committee (DSMC) for the study. This committee will be composed of the PI, co-

investigator(s), data manager or study coordinator, and other members of the study staff involved in the conduct of the trial. During the committee's quarterly meeting, the PI will discuss matters related to the following:

- Enrollment rate relative to expectations, characteristics of participants
- Safety of study participants including:
    - Serious Adverse Event & Adverse Event reporting
    - Engraftment dates
    - GVHD Occurrence and Severity
    - Results of product viability testing
    - Relapse Occurrence
    - Occurrence of any positive culture results of product
- Adherence to protocol (protocol deviations)
- Completeness, validity, and integrity of study data
- Retention of study participants

These meetings are to be documented by the data manager or study coordinator using the Protocol Specific Data and Safety Monitoring Report (DSMR), and signed by the PI or co-investigator. The completed DSMR is part of the monthly CTMC report and is reported to the IRB via the investigator's progress report.

Similarly, protocol deviations are to be documented using the Protocol Deviation Log and requires the signatures of either the data manager or study coordinator and the PI or co-investigator. These reports are to be sent to the IRB within 7 calendar days of awareness of the event and on a quarterly basis to the DSMC with the Protocol Specific DSMR.

15.2 Quality Assurance and Audits

The Quality Assurance Review Committee (QARC) of The University of Alabama at Birmingham Comprehensive Cancer Center performs quality assurance audits of investigator-initiated clinical trials. Audits provide assurance that trials are conducted in compliance with the protocol. Further, they ensure that study data are collected, documented, and reported in compliance with Good Clinical Practices (GCP) Guidelines and regulatory requirements.

A QARC audit of each clinical trial is conducted annually. Audits occur within the month of the study's initial IRB approval (provided the trial is open, and study accrual is greater than five subjects).

All audit findings are reported by the QARC to the DSMB. These findings are followed-up by the DSMB until they have been resolved.

The DSMB can also request QARC for a 'for cause' audit of the trial if the board identifies a need for a more rigorous evaluation of study-related issues.

A regulatory authority (e.g. FDA) may also wish to conduct an inspection of the study, during its conduct or even after its completion. If an inspection has been requested by a regulatory authority, the PI must immediately inform the IRB/DSMB that such a request has been made.

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