Thiotepa-Clofarabine-Busulfan with Allogeneic Stem Cell Transplant for High Risk Malignancies

Protocol No.: **2008-0363**
Principal Investigator: **Laura L. Worth, M.D., Ph.D.**
Phase: **II**

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1.0 Objectives

1.1 The primary objective is to determine the safety and efficacy of a pre-transplant conditioning regimen consisting of thiotepa, busulfan, and clofarabine followed by allogeneic hematopoietic stem cell transplant for high risk malignancies.

1.2 The secondary objectives are to evaluate the engraftment, toxicity, relapse rate, rate and severity of graft-vs-host disease, disease-free and overall survival.

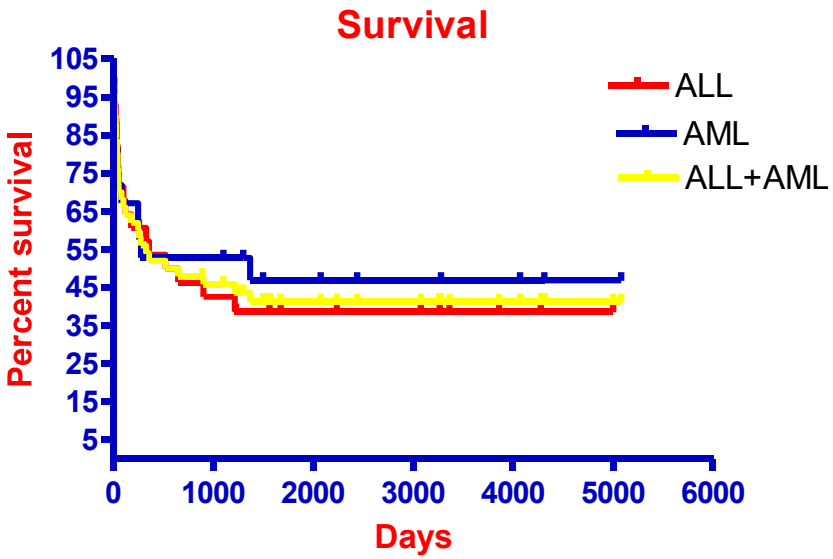
2.0 Background

High dose chemoradiotherapy supported by allogeneic bone marrow, umbilical stem cells, or peripheral blood stem cell transplantation (SCT) is a potentially curative therapy for patients with hematological malignancies. Many preparative regimens for allogeneic stem cell transplant use high-dose chemotherapy with or without total body irradiation (TBI). Comparative studies of TBI and non-TBI based regimens have not shown significant differences in efficacy (1). Because of the known side effects associated with TBI (cataract formation, second malignancies, hormonal deficiencies), chemotherapy only regimens have a potential for major advantages, especially in children.

Thiotepa-Busulfan-Cyclophosphamide

We have extensive experience with the chemotherapy-only regimen consisting of Thiotepa, Busulfan (Bu), and Cyclophosphamide (Cy) with advanced malignancies (2, 3). Bu-Cy is a well established preparative regimen for allogeneic stem cell transplantation for patients with hematological malignancies (4). Thiotepa was added to this regimen because of its ability to cross the blood-brain barrier (5), its in vitro activity against hematological malignancies (6), and its non-overlapping toxicities with BU-CY. More than 50 children were treated with thiotepa (250 mg/m^2 x 3 days), adjusted-dose busulfan x 3 days (AUC 1200-1800/micromol/min/L) and cyclophosphamide (60 mg/kg x 2 days). All stem cell sources were used including related and unrelated peripheral blood stem cells and bone marrow as well as unrelated cord blood (CB) stem cells. Two of the 23 patients receiving CB as a stem cell source failed to engraft and went on to receive a second transplant. Infection was a frequent cause of death in the early years of this study. With the improvement in HLA typing, diagnostic techniques, new antibiotics, and overall supportive care, the morbidity and mortality from all causes declined. Regimen related toxicities (grade 3-4) were predominantly esophagitis, mucositis, emesis, and hepatic dysfunction. Three patients developed veno-occlusive disease (VOD). All three were transplanted prior to the availability of the IV formulation of busulfan. The erratic absorption of BU orally may have contributed to hepatotoxicity since no patients receiving the IV formulation of BU developed VOD. Of the 16 patients treated with active

disease at the time of transplantation, only the patients with AML did well. See survival curve below.



Clofarabine as an antileukemic agent
Clofarabine is a second-generation deoxyadenosine analog designed to improve efficacy and minimize the toxicity of other analogs. It has a cytotoxic activity against a wide variety of leukemias and solid tumors. Although phase I studies are dose finding studies, the pediatric phase I study performed at M D Anderson Cancer Center showed definitive responses (7). Of the 25 patients with multiple relapsed or refractory leukemias, five patients obtained a complete response and an additional 3 patients obtained a partial response for an overall response rate of 32%. The phase II study (8) that enrolled 61 pediatric patients served as the basis of the FDA approval of clofarabine in 2004.

Clofarabine in stem cell transplant
Protocol 2006-0200 (Busulfan-Fludarabine-Clofarabine with allogeneic stem cell transplant for advanced refractory acute myeloid leukemia, myelodysplastic syndrome and advanced, Gleevec refractory chronic myeloid leukemia. A randomized phase II study) is investigating the efficacy of fludarabine and clofarabine in combination with busulfan.

The study is designed to determine which of the following combinations is optimal. Patients are being treated on one of the following 4 treatment arms:
1) Busulfan-fludarabine (30mg/m^2/day) clofarabine (10mg/m^2/day)
2) Busulfan-fludarabine (20mg/m^2/day) clofarabine (20mg/m^2/day)
3) Busulfan-fludarabine (10mg/m^2/day) clofarabine (30mg/m^2/day)

4) Busulfan- clofarabine (40mg/m^2/day)

The busulfan dose is adjusted based on pharmacokinetic data to ensure a constant AUC daily dose of 6,000 microMol-min (± 5%).

To date more than four patients have been enrolled on each treatment arm. All patients treated on this protocol have engrafted. The regimen related toxicities are tolerable (no grade III or IV non-hematological toxicities). Although it is too early to analyze some of secondary endpoints, relapse rate, long-term and overall and disease-free survival, only two patients have had progressive disease.

Preliminary results of 2008-0363
We recently presented a poster at ASBMT/IBMTR detailing preliminary results of Protocol 2008-0363. We have 15 patients that are more than 100 days after transplant. All 15 patients have engrafted at a median of 16 days. As expected cord blood recipients engrafted more slowly and peripheral blood stem cell recipients more quickly. Several patients had active disease at the time of transplant. One patient shortly after engraftment had disease progression.

Stem Cell Source	ANC >500/mL Median (range)	Platelets >20,000/mL Median (range)
All Sources (15)	16 (11-29)	28 (12-69)
Double Cord (7)	18 (13-29)	41 (28-61) (n=6)
Single Cord (2)	16 (16)	27 (21-37)
Bone Marrow (3)	14 (11-16)	28 (21-51)
PBSC (n=3)	11 (11)	12 (11-12)

Results
Fifteen patients with advanced malignancies were treated with a preparative regimen containing thiotepa, busulfan and clofarabine prior to allogeneic stem cell transplant.

- All patients engrafted rapidly (median 16 days).
- All evaluable patients had 100% donor chimerism by day +30.
- Regimen related toxicities were as expected or lower for a myeloablative regimen (nausea, vomiting, diarrhea, mucositis).
- Two patients met Jones criteria for VOD (1 – mild, 1 – severe).
    - The mild case resolved with supportive care.
    - The severe case received defibrotide therapy. Despite transient pulmonary and acute renal failure, the patient recovered completely.
- Acute GVHD was seen in 53% of patients.
- Grade III-IV acute GVHD was seen in 20% of patients.

    - 5/15 patients had skin GVHD II that resolved with topical treatment
    - 2/15 patients developed grade III GI GVHD
    - 1/15 patients developed fatal grade IV skin and liver GVHD
- Two non-relapse deaths were secondary to GVHD and aspiration pneumonia.
- Day 30 survival was 100%.
- Day 100 survival was 78%.

3.0 Background Drug Information

3.1. Thiotepa:

CHEMISTRY

Thiotepa, an ethylenimine derivative, is a polyfunctional alkylating agent. The drug is supplied as a sterile lyophilized powder, containing 15 mg of thiotepa. The drug is reconstituted with sterile water for injection resulting in a drug concentration of approximately 10 mg/ml. the reconstituted solution has a pH of approximately 5.5-7.5. The solution should be further diluted with Sodium Chloride Injection before use.

STABILITY

Both thiotepa powder for injection and reconstituted solutions of the drug should be stored at 2-8ºC, protected from light at all times. Reconstituted solutions are stable for 8 hours and solutions further diluted with Sodium Chloride Injection should be used immediately. In order to eliminate haze, solutions should be filtered through a 0.22 micron filter prior to administration.
Reconstituted solutions should be clear. Solutions that remain opaque or precipitate should not be used.

PHARMACOLOGY

Thiotepa, as an alkylating agent, interferes with DNA replication and transcription of RNA, and ultimately results in the disruption of nucleic acid function. Thiotepa also possesses some immunosuppressive activity.

PHARMACOKINETICS

Absorption - Thiotepa is incompletely absorbed from the GI tract. Variable absorption also occurs through serous membranes, such as the pleura and bladder, and from IV injection sites.
Absorption through the bladder mucosa may range from 10% to almost 100% of the instilled dose and is enhanced by extensive tumor infiltration or inflammation. Following IV administration of thiotepa C14, serum concentrations of radioactivity reportedly begin to decline within 10 minutes, but detectable concentrations persist for 72 hours.

KNOWN SIDE EFFECTS AND TOXICITIES
Myelosuppression, nausea, vomiting, dermatitis, and alopecia

3.2 Clofarabine:
CHEMISTRY
Clofarabine purine nucleoside analog. It is formulated as a concentration of 1mg/mL in United States Pharamacopeia (USP) sodium chloride (9mg/mL), and USP water for injection (qs to 1mL). Clofarabine is supplied in 2 vial sizes: a 10mL flint vial and 20mL flint vial. The 10 mL flint vials contain 5mL (5mg) of solution and the 20mL flint vials contain 20mL (20mg). For both vial types, the pH range of the solution is 4.0 to 7.0. The solution is clear with color ranging from colorless to yellow and is free from visible particulate matter.
Expected toxicities: myelosuppression, nausea/vomiting, diarrhea, mucositis, skin rash (particularly hand-foot syndrome), fatigue, mental status changes/coma, allergic reactions (including fever, muscle aches, edema, dyspnea), congestive heart failure, conjunctivitis, anorexia, febrile neutropenia, pruritus, headache, flushing and pyrexia, liver failure.

STABILITY
Clofarabine vials containing undiluted clofarabine for injection should be stored at 25°C or 77°F with temperature excursion permitted to 15 to 30°C or 59 to 86°F. Ongoing self-life stability indicate that clofarabine is stable for 36 months at 25°C (±2°C) and 60% (±5%) relative humidity and for 6 months at 40°C (±2°C) and 75% (±5%) relative humidity.

PHARMACOLOGY
Clofarabine is a purine nucleoside analog. When phosphorylated intra cellularly, it inhibits DNA polymerase a, inhibits ribonucleotide reductase, and activates apoptosis.

KNOWN SIDE EFFECTS AND TOXICITIES
Hepatotoxicity veno-occlusive liver disease, nausea, vomiting, diarrhea, proctalgia, mucositis.
Esophagitis, gastritis. Colitis. Cholestasis. Pancreatitis. Insomnia, coma, and/or changes in mental status (confusion, somnolence). Dysarthria. Skin rashes including hand-foot-syndrome.
Allergic reaction. Hypotension. Renal insufficiency. Congestive heart failure. Myocardial infarction. Atrial fibrillation. Fatigue, flushing, itching, chills, weakness, muscle aches, loss of appetite, peeling hands/feet, pneumonia, constipation, nosebleed, anxiety, pain in arms and legs, shaking, cough, shortness of breath, respiratory failure, palpitations and arrhythmias, arthralgias, dizziness, bone pain. Tingling in hands and feet, back pain, chest pain, changes in taste.
Alopecia. Amnesia. Febrile neutropenia, pyrexia, sepsis. Pneumonia. Bacteremia. Neutropenia. Thrombocytopenia. Myelosuppression. Tumor lysis syndrome. Capillary leak syndrome. Multi-system organ failure. Disseminated intravascular coagulation.

3.3 Busulfan (IV Busulfex™):

CHEMISTRY

Busulfan is an antineoplastic alkylating agent. It is a sterile, pyrogen-free solution provided in a mixture of dimethylacetamide (DMA) and polyethyleneglycol 400 (PEG400). It is supplied in 10 ml single use ampoules at a concentration of six (6) mg busulfan per ml. Each ampoule contains 60 mg of busulfan in 3.3 ml of DMA and 6.7 ml of PEG400. When diluted in normal saline or D5W to a concentration of 0.5 mg/ml, the resulting solution must be administered within eight (8) hours of preparation including the three (3) hour infusion of the drug.

STABILITY

Ampoules should be stored refrigerated at 2-8°C (35-46°F). Stable at 4°C for at least twelve (12) months. Additional stability studies are in progress. DO NOT use beyond the expiration date.
DO NOT use if the solution is cloudy or if particulates are present.
Solution Preparation: Prepare the busulfan solution as follows (The patient is to receive a dose of 130 mg/m^2 of busulfan): mix into normal saline to a final concentration of 0.5 mg/mL. In each bag 6.0 mg busulfan (1.0 ml at 6 mg/ml and 11 ml saline) should be added to compensate for drug lost in the tubing with each infusion (approximately 12 ml at 0.5 mg/ml is lost in the tubing when using the controlled rate infusion pump).
CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS.

PHARMACOLOGY

Busulfan interferes with DNA replication and transcription of RNA through DNA alkylation, and ultimately results in the disruption of nucleic acid function.

PHARMACOKINETICS

The pharmacokinetic data suggests that the plasma decay of the formulation fits an open one-compartment model with linear pharmacokinetics in the dose range of 12 mg-130 mg/m^2.
The drug is slowly excreted in the urine, chiefly as methanesulfonic acid. Ten to fifty percent (10-50%) of a dose is excreted as metabolites within twenty-four (24) hours.

KNOWN SIDE EFFECTS AND TOXICITIES

Dose limiting toxicity is expected to be hematological when used without stem cell support. Other toxicities seen frequently following high-dose busulfan in preparative regimens for bone marrow transplantation include: VOD, nausea, vomiting, pulmonary fibrosis, seizures, rash, and an Addison's-like syndrome.

3.4 Thymoglobulin:

Thymoglobulin® (Rabbit antithymocyte globulin, Genzyme Corporation) will be used as an in vivo immunosuppression.

CHEMISTRY
Thymoglobulin is a polyclonal anti-lymphocyte preparation. The drug is supplied as a sterile lyophilized powder, containing 25 mg of antithymocyte globulin. It is reconstituted 50 – 500 mL of saline or dextrose solution.

STABILITY:
The lyophilized powder should be stored in a refrigerator at 2 to 8°C (36 to 46°F). Thymoglobulin should be used within 4 hours after reconstitution if kept at room temperature. For vials containing the unreconstituted lyophilized powder, the product is stable for 36 months at 5-3°C (41 4°F), and 12 months at 37°C (98.6° F). Reconstituted product is stable for 72 hours at room temperature 20 to 25°C (68 to 77°F).
Immediately before intravenous administration, dilute reconstituted Thymoglobulin in isotonic saline or dextrose solution to a total infusion volume of 50 to 500 mL (usually 50 mL of IV admixture solution per 25 mg vial). The recommended route of administration is intravenous infusion through an in-line 0.22 micron filter into a high-flow vein.

PHARMACOLOGY
Mechanism of action: Possible mechanisms by which Thymoglobulin may induce immunosuppression *in vivo* include: T-cell clearance from the circulation and modulation of T-cell activation, homing, and cytotoxic activities. Thymoglobulin is thought to induce T-cell depletion and modulation by a variety of methods, including Fc receptor-mediated complement-dependent lysis, opsonization and phagocytosis by macrophages, and immunomodulation leading to long term depletion via antibody dependent cell-mediated cytotoxicity and activation of apoptosis.

KNOWN SIDE EFFECTS AND TOXICITIES:
The most common adverse reactions are fever, chills, leukopenia, thrombocytopenia, rashes, systemic infections, abnormal renal function tests, and serum sickness-like symptoms. Other reported side effects are arthralgia, chest and/or back pain, diarrhea, dyspnea/apnea, nausea and vomiting.

3.5 Mycophenolate Mofetil (MMF):
THERAPEUTIC CLASSIFICATION
Immunosuppressant for GVHD prophylaxis and treatment.

CHEMISTRY
Oral: 250-mg and 500-mg capsules, and as 200 mg/mL oral suspension. It may also be prepared extemporaneously as an oral suspension (100 mg /mL; in cherry syrup; pH = 6.0 - 6.7; Injectable: 500 mg vial to be reconstituted in 14 mL of D5W to yield about 15 mL containing 33.33 mg/mL of MMF.

PHARMACOLOGY
Oral: stable for at least four months at room temperature. Injectable: Each 500-mg vial should be reconstituted with 14 mL D5W to yield about 15 mL of MMF solution (33.33 mg/mL) as above.

The dose should be further diluted in D5W to a final concentration of about 6 mg/mL. The solution should not be refrigerated and should be administered within 4 hrs of preparation. The dose should be infused over at least two hrs. The manufacturer does not specify the type of container to use for parenteral.

MECHANISM OF ACTION
MMF inhibits T and B cell proliferation by blocking the production of guanosine nucleotides required for DNA synthesis.

SIDE EFFECTS
Constipation, diarrhea, nausea, vomiting, headache, confusion, tremor, gastrointestinal bleeding, hypertension, peripheral edema, cough exacerbation, infection, sepsis and bone marrow suppression including severe neutropenia.

3.6 Filgrastim:
MODE OF ACTION
Filgrastim is a human granulocyte-stimulating factor that acts on hematopoietic cells to stimulate proliferation, differentiation, and some end-cell functional activity.

STORAGE AND STABILITY
Filgrastim should be stored at 2° to 8° C. Prior to injection, Filgrastim may be allowed to reach room temperature, however, any vials left at room temperature for greater than 24 hours should be discarded. Vials should not be shaken. Vials should be inspected for sedimentation or discoloration prior to administration. If sedimentation or discoloration is observed, the vials should not be used.

ROUTE OF ADMINISTRATION
SC Injection-IV Infusion.

INCOMPATIBILITY
No definite incompatibilities are known. However, drugs that may potentiate the release of neutrophils should be used with caution.

AVAILABILITY
Commercially available in single-dose, preservative-free vials containing 300 mcg (l mL) and 480 mcg (1.6 mL) of Filgrastim. Used portions should be discarded.

SIDE EFFECTS
Mild to moderate bone pain is possible in patients receiving myelosuppressive therapy. General skin rash, alopecia, fevers, thrombocytopenia, osteoporosis, nausea, vomiting, diarrhea, mucositis, anorexia, inflammation of the blood vessels, and/or cardiac dysrhythmia can occur.
Splenomegaly may result at high doses of Filgrastim. Headaches, wheezing, dyspnea, swelling, and/or increased enzyme levels. May accelerate the growth of tumors or cancers.

3.7 Tacrolimus:
THERAPEUTIC CLASSIFICATION
Cyclic polypeptide immunosuppressive agent

MECHANISM OF ACTION
Macrolide antibiotic produced by Streptomyces tsukubaensis that inhibits T and B cell proliferation.

PHARMACOLOGY
Available in 5 mg/mL vials. Anhydrous is diluted in alcohol dehydrated 80% v/v and polyoxyl 60 hydrogenated castor oil 200 mg/mL. For IV infusion, solution is diluted with 0.9% NaCl or 5% dextrose to a concentration of 4-20 mcg/mL. Also available in tablets of 0.5, 1, and 5 mg.
Disposition is biphasic with a terminal half-life of 10-30 hours. Elimination is mainly by hepatic metabolism and biliary excretion.

STABILITY
The oral from is stable at room temperature protected from light for at least 2 months. The IV preparation is diluted in D5W or NS and is stable for 24 hours.

KNOWN SIDE EFFECTS
Tacrolimus may cause kidney and/or liver damage, high blood pressure, high blood sugar, and/or diabetes. It may cause low levels of potassium, magnesium, and/or phosphate in the blood, which can lead to weakness or other neurological symptoms such as shaking of hands.
The drug may cause nausea, vomiting, constipation, burning feeling in the hands and/or feet, seizures, coma, and/or confusion. An allergic reaction may occur.
The drug may cause headache, difficulty sleeping, diarrhea, high blood pressure, blurred vision, chest pain, higher sensitivity to pain, ringing in the ears, sweating, enlarged heart, and/or weakening of the immune system, which may result in the development of infections. It may cause rapid growth of body hair, loss of appetite, weight loss, wheezing, facial flushing, yellowing and/or darkening of the skin due to bile in the blood, and/or abdominal pain. It may cause an increased risk of cancer of the lymph glands. It may also cause rashes, itching, changes in hearing, fluid in the lungs, and/or back pain.

3.8 Cyclophosphamide (Cytoxan™):

CHEMISTRY
The Distribution (Vd) is 0.48-0.71 L/kg; crosses placenta; crosses into CSF. Protein binding is 10-60%. Hepatically metabolized to active metabolites acrolein, 4-aldophosphamide, 4-hydroperoxycyclophosphamide, and nor-nitrogen mustard. Bioavailability is >75%. Half-life elimination is 3-12 hours. Excretion in urine (<30% as unchanged drug, 85% to 90% as metabolite).

PHARMACOLOGY
Cyclophosphamide is an alkylating agent that prevents cell division by cross-linking DNA strands and decreasing DNA synthesis. It is cell cycle phase non-specific. Cyclophosphamide also possesses potent immunosuppressive properties. It is a pro-drug metabolized by the liver to active metabolites

KNOWN SIDE EFFECTS:
Leukopenia, anemia, alopecia, nausea, vomiting, increased AST, ALT, mucositis, diarrhea, headache, dizziness. Cardiomyopathy, non-specific ST changes on EKG. At doses greater than 200mg/kg, Cy can cause fatal myocardial necrosis with clinical heart failure. Hemorrhagic cystitis, SIADH, fluid retention, hemorrhagic cystitis are possible. Teratogenic, may cause secondary neoplasms, anaphylaxis (rare).

3.9 Mesna (sodium -2-mercapto ethane sulphonate):

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines. At the doses used for uroprotection, mesna is virtually non-toxic. However, adverse effects which may be attributable to mesna include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

4.0 Patient Eligibility

4.1 Inclusion Criteria
4.1.1 Diagnosed with one of the following:
a. Acute myelogenous leukemia (AML) in induction failure, relapse, past first remission, or CR1 considered at risk for relapse.

b. Myelodysplastic syndromes with International Prognostic Scoring System score {IPSS score (9)} >/= 2 or myelodysplasia that has not responded to chemotherapy.
c. Biphenotypic leukemia
d. Acute lymphocytic leukemia with induction failure, first complete remission with high risk cytogenetics (e.g. Philadelphia positive chromosome, t(4:11) Remission requiring more than 2 cycles of chemotherapy to achieve remission, or any stage beyond CR1.
e. Chronic Myelogenous Leukemia (CML): second chronic phase, accelerated phase or blast crises with less than 10% blasts in the bone marrow, or CR1 and resistance to Gleevec or other tyrosine kinase inhibitors.
f. Non-Hodgkin's Lymphoma (NHL) with induction failures, second or third complete remission, or relapse (including relapse post autologous hematopoietic stem cell transplant).
g. Hodgkin's disease - induction failure, second or later complete remission, or relapse (including relapse post autologous hematopoietic stem cell transplant).
h. Chronic Lymphocytic Leukemia (CLL) that has failed induction therapy or Rai Stages 2-4

4.1.2 A related or unrelated donor which is HLA-matched or mismatched in 1 HLA A, B, C, DR, or DQ locus is acceptable (i.e. at least a 9/10 matched related or unrelated donor, matched with molecular high-resolution technique per current standard for the BMT program). Cord blood units must match the patient at 4, 5, or 6/6 HLA class 1 serological and II molecular antigens with a minimum of 2 x $10e^7$ total nucleated cells/kg recipient weight in the pre-thawed fraction. For patient lacking a matched related or unrelated volunteer donor or acceptable cord blood unit(s), a related haploidentical donor (</= 7/8 allele matched at the A, B, C, Dr loci) may be used.
4.1.3 Age </= 60 years.
4.1.4 Lansky performance score >/= 50% for patients </= 16 years of age, or Zubrod performance status score of 0-2 for patients > 16 years of age. (Refer to Appendices H and I)
4.1.5 Cardiac function - left ventricular ejection fraction >/= 40%.
4.1.6 Pulmonary function - diffusion capacity of 50% predicted. Children unable to perform pulmonary function tests (e.g. less than 7 y.o.) pulse oximetry of >/= 92% on room air.
4.1.7 Serum creatinine < 1.6 mg/dL or creatinine clearance >/= 50 ml/min.
4.1.8 SGPT </= 200 IU/mL, serum bilirubin < 1.5 x normal.
4.1.9 Written informed consent and assent as is age appropriate.
4.1.10 No active infection.

4.2 Exclusion Criteria

4.2.1 Pregnancy in women of childbearing potential (pregnancy test performed within 2 weeks of study entry)

4.2.2 HIV positive (highly immunosuppressive treatment)
4.2.3 Active CNS leukemia
4.2.4 Chronic or active Hepatitis B or Hepatitis C. If questions about liver health, discuss with PI and strongly consider liver biopsy.

5.0 Pretreatment evaluation

The following standard evaluations will be conducted within the last 4 weeks prior to admission unless otherwise indicated below:

5.1 EKG and Echocardiogram (or other measure of LVEF)
5.2 CT of sinuses
5.3 Dental consult within 3 months of transplant as clinically indicated
5.4 Infectious disease panel
5.5 Serum HCG in all female patients of childbearing potential
5.6 CBC, platelets, differential, SGPT, Calcium, glucose, uric acid, magnesium, serum bilirubin, BUN, creatinine, serum protein, albumin, alkaline phosphatase, electrolytes, PT, PTT
5.7 Chest X-ray
5.8 Pulmonary function test with diffusional lung capacity (pulse oximetry in children too young to perform pulmonary function tests)
5.9 Urinalysis
5.10 Patients with leukemia – bone marrow aspirate and cytogenetics (only if never done before) within 2 weeks of admission
5.11 Patients with lymphoma, CLL, or Hodgkin's disease: CT chest, abdomen, and pelvis; bilateral bone aspirates and biopsies or PET-CT as indicated if active disease at time of admission.

6.0 Treatment Plan

6.1 Preparative Regimen

Thio = Thiotepa
Bu = Busulfan
Clo = clofarabine
*ATG = Rabbit ATG

Treatment Plan for recipients with HLA non-identical or unrelated donors

Day	Treatment
-9	Hydration therapy

-8	Thiotepa 5 mg/kg
-7	IV Bu 0.5 mg/kg test dose for patients 12 and younger (adult test dose 32 mg/m^2 for patients 13 and older)
-6	Clofarabine 40 mg/m^2
-5	IV Bu to AUC 5,000 mcMol-min (or 130 mg/m^2 / alt 3.2 mg/kg BW) +Clofarabine 40 mg/m^2
-4	IV Bu to AUC 5,000 mcMol-min (or 130 mg/m^2 / alt 3.2 mg/kg BW) +Clofarabine 40 mg/m^2 +*rabbit ATG 1.25 mg/Kg
-3	IV Bu to AUC 5,000 mcMol-min (or 130 mg/m^2 / alt 3.2 mg/kg BW) +Clofarabine 40 mg/m^2 +*rabbit ATG 1.75 mg/Kg
-2	Rest
-1	Rest
0	Stem cell

alt = alternatively

*Patients with HLA nonidentical or unrelated donors will receive Thymoglobulin®.

Adjusted dose: the Busulfan dose determined to achieve an average daily AUC of 5,000 µMol-min ± 10% for the entire 3-day treatment period is administered to all patients. If it is not possible to perform pharmacokinetic testing, busulfan 130 mg/m^2 should be administered.

Treatment Plan for haploidentical marrow recipients only

Day	Treatment
-9	Hydration therapy
-8	Thiotepa 5 mg/kg
-7	IV Bu 0.5 mg/kg test dose for patients 12 and younger (adult test dose 32 mg/m^2 for patients 13 and older)
-6	Clofarabine 40 mg/m^2
-5	IV Bu to AUC 5,000 mcMol-min (or 130 mg/m^2 / alt 3.2 mg/kg BW) +Clofarabine 40 mg/m^2
-4	IV Bu to AUC 5,000 mcMol-min (or 130 mg/m^2 / alt 3.2 mg/kg BW) +Clofarabine 40 mg/m^2
-3	IV Bu to AUC 5,000 mcMol-min (or 130 mg/m^2 / alt 3.2 mg/kg BW) +Clofarabine 40 mg/m^2
-2	Rest
-1	Rest
0	Unmanipulated bone marrow infusion
+3	Cyclophosphamide 50 mg/kg
+4	Cyclophosphamide 50 mg/kg

alt = alternatively

Patients with HLA-haploidentical related donors will not receive rabbit ATG (Thymoglobulin®).

6.2.1 Thiotepa administration: Thiotepa will be dosed at 5 mg/kg for patients who are within 20% of their ideal body weight. Patients greater that 20% of their ideal body weight will be dosed using adjusted body weight. Thiotepa will be administered on Day -8 by controlled-rate pump,

6.2.2 Clofarabine administration: Clofarabine will be dosed per actual body weight/actual body surface area. No arbitrary dose adjustment(s) based on a perceived need for such modifications of a chemotherapeutic agent in high-dose chemotherapy is allowed for clofarabine, since insufficient data regarding the impact of such modification(s) on engraftment and toxicity/disease-control are available.

Clofarabine is administered at a dose of 40 mg/m^2 diluted in NS to produce a final concentration of 0.15mg/mL to 0.4mg/mL on each of four (4) consecutive days (Days -6 through -3). The doses of Clofarabine are to be given immediately preceding busulfan. Intravenous fluids should be administered at a rate of approximately 1.5 L/m^2/days (125 cc/hr in adults) starting the evening before the start of this chemotherapy, after the last dose of Busulfan.

6.2.3 Busulfan administration: Pharmacokinetic-guided (PK-guided) treatment: The Busulfan "test dose" of 0.5 mg/kg will be based on actual body weight for pediatric patients 12 years of age and younger, no dose adjustment(s) based on a perceived need for modification is allowed for busulfan. For children 13 years of age and older and for adult patients, the Busulfan "test dose" will be 32 mg/m^2. This busulfan dose will be administered on Day –7, it will be will be given by controlled-rate infusion pump. The pharmacokinetic-guided daily high-dose busulfan dose(s) will be started immediately upon completion of the daily Clofarabine doses (on the day clofarabine is given). The busulfan doses will be diluted in normal saline and administered daily by controlled rate infusion pump.

Busulfan is administered at the dose calculated to achieve a systemic exposure dose of 5,000 µMol-min in normal saline for three (3) consecutive days (Days -5 to -3), starting immediately after the completion of Clofarabine, see below. The Busulfan dose on Day -5 to Day -3 will be based on the pharmacokinetic studies to target an AUC of 5,000 µMol-min ± 10% in this program. This PK-guided, adjusted dose, will be administered in an identical fashion to the previously used 130 mg/m^2 dose.

The PK adjusted dose of busulfan = Target AUC x busulfan mol. wt (0.2463) x busulfan gross clearance normalized to body surface area (L/min)+ the dose uninfused in the IV line.

If it is not feasible to perform pharmacokinetic monitoring, patients ≥ 13 will receive a fixed Busulfan dose of 130 mg/m^2/day for 3 days. PKs are required for children <13 y.o.

Please see special precautions for administration of IV Busulfan. **IT IS MANDATORY, THAT ACETAMINOPHEN NOT BE USED, BETWEEN DAY –9 AND DAY 0, SINCE IT INTERFERES WITH THE METABOLISM OF BUSULFAN AND MAY CONTRIBUTE TO SERIOUS LIVER DAMAGE.**

Other drugs known to interfere with the metabolism of clofarabine and/or busulfan should not to be concomitantly used during the chemotherapy administration up to and including the day of transplantation. In particular, drugs that are known to affect the hepatic cytochrome P450-systems (such as the –azoles) and tyrosine kinase inhibitors should be omitted for at least 5 days prior to the busulfan test dose. It is also recommended that treatment with Mylotarg™ (Gemtuzumab Ozogamicin) is not allowed for at least three weeks prior to admission, since these agents have either a well described interference, or a high propensity for an adverse interaction with busulfan disposition in the human body. As needed these agents can be resumed starting one day following the stem cell transplant SCT day +1.

6.2.4 Anti-thymocyte globulin: Patients with HLA-haploidentical related donors will not receive Thymoglobulin. Patients receiving a graft from a matched unrelated donor or 1-antigen mismatched donor will receive Thymoglobulin; 1.25 mg/kg on Day –4 and 1.75 mg/kg on Day –3. Thymoglobulin will be administered as per regular departmental routines, and will be infused in the afternoon of respective days so not to interfere with the chemotherapy administration.

6.3 PBSC/Marrow Processing and Infusion

NOTE: Section 6.4.1 represents recommended guidelines regarding stem cell products, bone marrow, peripheral blood progenitor cell, or cord blood processing and infusion procedures. The Investigator may follow procedures described in current departmental protocols and guidelines see standard operating procedures for department of Blood and Marrow Transplantation.

Patients may receive bone marrow, peripheral blood progenitor, or CB cells as sources of stem cells for allogeneic transplantation. The products are not T-cell depleted, but may be manipulated as per standard procedures for ABO incompatibility. The cells may be infused fresh or after cryopreservation.

ALLOGENEIC MARROW OR HLA-MATCHED RELATED DONOR STEM CELL TRANSPLANTATION: PBPC or marrow cells may be collected and infused on the day of the transplant or infused after cryopreservation. The

goal is to infuse >3.0 X 10^8 marrow mononuclear cells/kg, 5 X 10^6 CD34+ cells/kg from peripheral blood progenitor cells (PBPC), or a CB with a minimum of 2 x 10^7 total nucleated cells/kg.

For infusion of fresh or cryopreserved hematopoietic stem cells, the patient should be premedicated with IV steroid(s) and IV diphenhydramine per departmental guidelines. A normal saline intravenous drip will be established.

6.4 Supportive Care

NOTE: This section indicates recommended guidelines regarding supportive care. The Investigator may elect to follow other procedures as per departmental routines and guidelines. All concomitant medications (i.e. supportive care) administered to the patient during the study will be documented in the patient's primary medical record.

6.4.1 Antiemetics should be administered per institutional guidelines prior to the first dose of Bu and continued on a fixed schedule through 12-24 hours after the last dose of Bu.

6.4.2 Antiseizure prophylaxis is to be given to all patients according to department guidelines.

6.4.3 All patients should receive supportive care (allopurinol, menstrual suppression, prophylactic antibiotics, empiric antibiotics, IV Ig, transfusions of blood products, hyperalimentation, etc.) as clinically indicated, according to departmental guidelines.

6.4.4 Patients may receive G-CSF, 5 µg/kg/day (may be rounded to nearest vial size) s.c. starting on Day +1 CB recipients and Day +7 for bone marrow and peripheral blood recipients until ANC exceeds 0.5 per microliter for 3 days.

6.4.5 GVHD prophylaxis for peripheral blood and bone marrow recipients will be tacrolimus and methotrexate. Tacrolimus administered at starting dose of 0.015 mg/kg (ideal body weight) as a 24 hour continuous infusion daily, to be changed to oral dosing when tolerated. Tacrolimus is to be tapered as indicated after transplant day 90, if no GVHD is present. Tacrolimus is adjusted trough level of 5-15 ng/mL. Methotrexate 5 mg/m^2 will be administered intravenously on days 1, 3 and 6 and Day +11 post transplant. The Day 11 methotrexate dose may be held as indicated if mucositis is present.

CB recipients will receive tacrolimus as outlined above. Instead of methotrexate, patients with CB receive MMF from Days -3 to +30 (stop at day +30 if GVHD is not present). If the patient has acute GVHD requiring systemic therapy, MMF therapy may be prolonged. MMF is dosed 15 mg/kg

orally twice daily, with a maximum dose of 1 gram twice daily. IV route can be used if PO is not tolerated (same dosing). Patients receiving haploidentical stem cell products will receive tacrolimus at doses described above and MMF will be dosed TID. These agents will start on day +5.

6.4.6 Post transplant cyclophosphamide administration with Mesna:
Premedication: Patients will receive a dose of Mesna 10 mg/kg IVPB just prior to the first dose of cyclophosphamide (Cy), that will be repeated every 4 hours for a total of ten (10) doses. Patients will also receive ondansetron (or a comparable anti-emetic) and dexamethasone prior to each dose of Cy.

Patients will receive Cy on days + 3 and + 4 at a dose of 50 mg/kg per dose. Patients weighing within 20% above their ideal body weight will be dosed according to actual body weight. Patients weighing more than 20% above their ideal body weight will be dosed according to the adjusted body weight. Formula to calculate adjusted body weight: Adjusted BW (Kg) = IBW + 0.5 (Actual body weight-IBW). The first dose of cyclophosphamide must be administered 60 to 72 hours following the start of marrow infusion.

Cy will be mixed in D5W to a maximum concentration of 20mg/ml and given by controlled-rate infusion pump. Patients should be well hydrated. It is recommended to receive IV Fluids at a rate of 150 ml/hour starting the evening (Day +2) before the first dose of Cy (minimum of 8 hours before first dose) and continued for a minimum of 24 hours after the last dose. For pediatric patients, it is recommended to receive IV Fluids at a rate of 125 ml/m^2/hr starting the evening (Day +2) before the first dose of Cy (minimum of 8 hours before first dose) and continued for a minimum of 24 hours after the last dose.

7.0 Evaluation During Study

7.1 STUDY PERIOD: EVALUATION DURING THE INITIAL HOSPITALIZATION (BMT Day -9 THROUGH DISCHARGE)

a) Daily physical examinations.
b) Toxicity grading and evaluation for adverse experiences.
c) Vital signs (VS), intake/output (I/O) per daily routine.
d) Bone marrow biopsy and aspirate (with cytogenetics and/or chimeric studies) at approximately (1) one month (Day 28) (+/-3 days), or as clinically indicated.
e) CBC and platelet counts daily and as clinically indicated.
f) Chemistry profile twice per week

g) Peripheral blood T-cell and myeloid cell chimerism at approximately Day 28 (+/-3 days)

7.2 POST-STUDY SURVEILLANCE: EVALUATION FROM BMT DISCHARGE THROUGH BMT DAY +100.

Note: For this study, post-study surveillance from initial hospital discharge to BMT Day +100 will include data collection of serious adverse experiences and survival. The data as listed below will continue to be collected per institutional transplant guidelines. The following will be performed at least twice monthly:

a) Physical examination.
b) Toxicity grading.
c) Vital signs (VS), and weights
d) CBC and platelet count.
e) Chemistry profile.

Bone marrow aspirate with cytogenetics if indicated and Peripheral blood T-cell and myeloid cell chimerism will be performed between 2 and 3 months post transplant.

7.3 POST-STUDY SURVEILLANCE: EVALUATION AFTER BMT DAY +100 POSTTRANSPLANT

NOTE: For this study, post-study surveillance after BMT Day +100 will include quarterly data collection of patient status and survival. The data as listed below will continue to be collected per departmental transplant guidelines.

a) Physical Examination, screening labs and bone marrow aspirate with cytogenetics and chimerism studies at approximately three (3) months, six (6) months, and twelve (12) months for all patients, or at other time points if clinically indicated.

7.4 Busulfan Pharmacokinetic Studies

Patients will receive a “test dose” of busulfan, at 0.5 mg/kg administered IV on Day –7 for measurement of pharmacokinetic parameters to determine the dose that will give a daily systemic exposure, “AUC,” of 5,000 µMol-min (± 10%) on Days –5 till –3. Blood samples (5ml each) will be drawn on Day –7 and Day -5 at time points per SCTCT Department standards as determined by the Department of Laboratory Medicine. For the full therapeutic (3 hr) busulfan infusion the 15 minute time point will be replaced with a 90 minute time point.

Pharmacokinetic analysis and individualized IV busulfan dose will be calculated and ordered on Day –7. This complete sampling schedule will later be replaced with a limited sampling schedule when a validated limited sampling assay becomes available. All PK samples will be drawn from a peripheral line to minimize the number of venipunctures for each patient,

since blood samples from the CVC line ("alternative port") have been shown to yield a high incidence of erratically high busulfan concentrations, likely due to local turbulence around the tip of the CVC catheter. Plasma concentrations of busulfan will be determined in duplicate for each time point by a sensitive and specific HPLC assay following derivatization, or alternatively by GC-MS when a high-through-put validated busulfan assay becomes available.

8.0 Criteria for Study Evaluation

8.1 Engraftment

Will be recorded as the first day of 3 consecutive days that the ANC exceeds 0.5 X 10^9/L and will be displayed for the group in a life-table format in the manner of Kaplan-Meier and compared with historical controls. treated with our standard conditioning regimen of IV Busulfan-Fludarabine (29).

8.2 Graft Failure

Is defined for the purpose of this trial as the failure to reach an ANC >0.5 X 10^9/L within 30 days after transplantation. Patients who engraft within the first 100 days after transplantation but who develop a sustained ANC <0.5 x 10^9/L (three consecutive days) will be classified as secondary graft failure, unless this is correlated with progression / recurrence of the underlying malignancy.

8.3 Relapse

Will be recorded by the day of detection of histologic diagnosis of recurrent disease. Per standard transplant practices, relapsed patients eligible for and electing to participate in alternative treatment protocols will be withdrawn from participation in this protocol and data beyond that of survival will not be collected.

8.4 Survival

Will be recorded by the day of death and the cause of death will be assessed.

8.5 Response:

8.5.1 Leukemias -

Complete Response will be defined as bone marrow with <5% blasts

8.5.2 Lymphomas -

Complete Response will be defined as disappearance of all evidence of active tumor for a minimum of 8 wks, without symptoms

Partial Response: 50% or greater decrease in the sum of the products of measured lesions persisting for 8 wks or more. No new lesions.

No change: results inferior "partial response," and/or no progression for a minimum of 8 weeks.

Progressive disease: appearance of new lesions or increase in the size of measurable disease.

9.0 Statistical Consideration

Statistical Considerations
We will monitor the data for safety/toxicity following the methods of Thall and Simon (10). The primary outcome of this study will be the survival rate at 100 days post-transplant. We will enroll at most 60 patients.

The toxicities will be monitored and scored on a daily basis. All regimen related toxicities for unexpected non-hematological toxicities will be reviewed with the Chair of the Clinical Research Committee (CRC) after the first three patients and after six patients. If two or more patients out of the first three patients experience a grade IV regimen related toxicity, accrual will be halted for review.

A Bayesian stopping rule will be used to stop the trial if there is a 90% chance that the true toxicity rate exceeds the target toxicity rate of 0.25. We will employ the following monitoring rule for grade 3 and 4 severe adverse events. (See section 10.2.1).

We will stop the trial if P(toxicity > 25% | data) > 0.90. That is, given the outcomes from the patients who have already been evaluated, if we determine that there is more than a 90% chance that the toxicity rate is more than 25% we will stop the trial. This decision rule gives the following stopping rule. We assume a beta (0.5, 1.5) prior distribution for the toxicity rate. This prior distribution has a mean of 0.25 and a standard deviation of 0.25. Stop the trial if

[# of pts with toxicity / # of pts evaluated]

>/= 5/10, 6/12, 7/15, 8/18, 9/21, 10/24, 11/28, 12/31, 13/34, 14/38, 15/41, 16/44, 17/48, 18/51, 19/55, 20/58.

The operating characteristics of this study design are shown in Table 1.

Table 1. Operating Characteristics of Safety Monitoring Rule

Rate of Toxicity	Probability of Stopping	Sample Size		
		P25	P50	P75
0.15	0.024	40	40	40
0.20	0.102	40	40	40
0.25	0.285	39	40	40
0.30	0.553	14	40	40
0.35	0.806	11	20	40
0.40	0.938	10	13	26

Formal continuous interim monitoring for safety will be conducted using the above stopping rule.

Once we have completed the study we will estimate the toxicity rate with a 90% credible interval. For example, if we complete the study with 10 of 60 patients with toxicity, then the 90% credible interval for the toxicity rate will be 0.09 to 0.25. We will also report the posterior probability that the toxicity rate is greater than 25%. We will use the Kaplan-Meier (11) product limit method to estimate overall survival and relapse-free survival rates with 95% confidence intervals at the conclusion of the study.

10.0 Adverse Events

10.1 Definitions

An **Adverse Event (AE)** is defined as any untoward medical event, including worsening of increased frequency of an event present at baseline, in a subject registered in this study and receiving either of the preparative regimens in combination with stem cell transplantation.

Transplant related adverse events:

For the purpose of this study, common transplant related AE are those known to be related to the preparative regimen and stem cell infusion occurring up to 30 days post transplant. The most common are listed below.

Neutropenic Fever without infection, Non-Neutropenic Fever, Infections associated with grade 3 or 4 neutropenia, Nausea and vomiting, readmission during the active treatment period, transfusions of platelets and RBCs, Low blood pressure due to dehydration requiring fluid replacement, Mucositis, cytokine storm. These events will be monitored and captured in the database during the first 30 days post transplant.

The following common transplant related events will be captured in the database any time when observed: cytopenias post-transplant, including secondary graft

failure whether or not leading to death, hemorrhagic cystitis, liver function test abnormalities associated with VOD, TTP, significant infections and graft vs. host disease.

Serious Adverse Event: any adverse event occurring that results in any of the following outcomes:

- Death
- Life-threatening
- Persistent or significant disability/incapacity
- In patient hospitalization or prolongation of existing hospitalization
- Congenital anomaly/birth defect
- Based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Attribution

The Investigator will determine if an adverse event is in some way related to the use of the study drugs or procedures and/or participation in this study. This relationship will be described as follows:

Definite: The event is clearly related.
Probable: The event is likely related.
Possible: The event may be related.
Unlikely: The event is doubtfully related.
Unrelated: The event is clearly NOT related.

10.2 Assessment of Adverse Event

Adverse events will be assessed and graded according to NCI's Common Terminology Criteria for Adverse Events (CTCAE) v3.0.

10.2.1 Adverse Events Not Considered Serious (regardless of the grade, these events are expected to be reversible):

1. Related to myelosuppression: thrombocytopenia, bleeding, platelets and RBCs transfusions.
2. Fever: Non Neutropenic or Neutropenic without infection
3. Infections in the presence or absence of neutropenia
4. Readmissions (lasting <10 days)
5. Cytopenias post transplant including secondary graft failure
6. Low blood pressure due to dehydration requiring fluid replacement
7. Fluid overload leading to cardiac dysfunction
8. Mucositis
9. GI related: nausea, vomiting, diarrhea
10. Organ dysfunction: cardiac, pulmonary, hepatic, CNS and/ or renal.
11. Fatigue
12. Neurologic: seizures, neuropathies

13. Stem Cell Transplant Syndromes: Cytokine Storm, VOD, TTP, hemorrhagic cystitis, interstitial pneumonitis (including pulmonary hemorrhage), GVHD (acute and chronic).

10.2.2 Adverse Events Considered Serious:

1. Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).
2. Readmissions from any cause resulting in a prolonged hospitalization (>10 days).
3. Graft Failure/ rejection.
4. Any expected or unexpected event resulting in an irreversible condition and/or leading to death.

10.2.3 Abnormal Lab Findings Due to Underlying Disease

In this study, patients are expected to experience changes in laboratory parameters such as electrolyte imbalances, uric acid changes, liver function abnormalities, including elevations of GPT, GOT, LDH and alkaline phosphatase. These changes are due to the underlying disease and the nature of the treatment including transplantation and chemotherapy. These expected changes will not be considered Adverse Events and will not be recorded in the eCRF unless in the view of the investigator they are judged clinically significant

10.2.4 Study specific AE relationship: Based on the objectives of this study incidence of GVHD and graft failure will be considered as adverse events definitive related to the study.

10.3 AE Recording

The onset date, resolution date, and maximum grade of adverse events will be collected. Intermittent events should be labeled as such and followed until resolution. If a subject is taken off study while an event is unresolved, this will be followed until resolution unless another therapy is initiated. Pre-existing medical conditions will be recorded only if the condition worsens during active treatment.

The Investigator or Designee will complete and maintain adequate source documentation which may include progress notes, laboratory reports, discharge summaries, and other original records. The information will be stored in a secured location.

10.4 AE Reporting

Upon the Investigator's awareness of adverse events occurring during subject participation, these events will be reported to the Institutional Review Board (IRB) according to institutional guidelines and the BMT AE Reporting Policy (refer to Appendix E).

This study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety, and well-being of the patient requires immediate intervention, based on the judgment of the Investigator or designee.

11.0 Criteria for Removal from the Study

Subjects may be withdrawn from the study for the following reasons:

- Parent, guardian, or patient withdraws consent
- Subject's noncompliance
- Disease progression
- Death
- Discretion of the Principal Investigator
- Termination of study.

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