FRED HUTCHINSON CANCER RESEARCH CENTER
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1. TITLE: Allogeneic hematopoietic cell transplantation for patients with nonmalignant inherited disorders using a Treosulfan based preparative regimen

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Investigator Statement:

I have carefully read Protocol 2256 entitled “Allogeneic hematopoietic cell transplantation for patients with nonmalignant inherited disorders using a Treosulfan based preparative regimen” version date 04/06/2020.

I agree to carry out my responsibilities in accordance with the Protocol, applicable laws and regulations (including 21 CFR Part 312), Good Clinical Practice: Consolidated Guidance (ICH-E6), and applicable policies of Fred Hutch.

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2. INTRODUCTION

Hematopoietic cell transplantation (HCT) has been used to treat life-threatening non-malignant disorders, including certain inherited disorders, by providing normal cells or cellular proteins to replace or substitute for defective components. Until recently conventional myeloablative regimens; primarily busulphan and cyclophosphamide, have been used to establish hematopoietic cell grafts. The significant toxicities associated with conventional regimens often preclude transplantation for those severely affected patients with end organ dysfunction or serious infections. In a broader sense, the risks associated with conventional approaches are deemed unacceptable for many patients with chronic disorders who might otherwise be cured with HCT. In the past 5-10 years, encouraging results have been reported using nonmyeloablative conditioning regimens for patients with nonmalignant disorders.[1-3] Although decreased regimen related toxicities have been seen; graft rejection and/or low donor chimerism with inadequate disease responses have been seen for certain groups of patients. Thus, there is a need to explore other preparative regimens with the goal of decreasing regimen related toxicities and mortalities while preserving engraftment and disease response.

Treosulfan-based regimens are currently being evaluated in Europe as a potential alternative to conventional HCT in patients with malignant and nonmalignant diseases and preliminary results are very encouraging. This busulfan analog has several attractive characteristics including less erratic pharmacokinetics and decreased regimen related toxicities compared to busulfan. The primary objective of this clinical trial is to evaluate, within the limits of a phase II study, the preliminary efficacy as defined by engraftment, of a regimen consisting of treosulfan and fludarabine followed by allogeneic HCT in patients with nonmalignant inherited disorders.

3. BACKGROUND

3A. Conventional Marrow Transplantation for Treatment of Non-Malignant Disorders

1) Primary Immunodeficiency diseases.

HCT is effective in the treatment of a variety of primary immunodeficiency disorders, by restoring normal cells or cellular proteins to the deficient host to correct the underlying genetic deficits. Multiple studies have demonstrated the effectiveness of HCT using myeloablative conditioning for the treatment of patients with primary immunodeficiency disorders (excluding SCID).[4-9] Diagnosis, donor type, age at HCT and pre-HCT co-morbid conditions; in particular infections; were important factors that impacted survival. Death from transplant related causes including infections, GVHD and end organ toxicity were major problems that limited the success of this approach. Therefore, strategies aimed at decreasing toxicity are needed.

2) Metabolic storage diseases.

Marrow transplantation has been used to treat patients with metabolic storage disorders by using transplanted hematopoietic cells to provide a source of deficient enzyme. Conventional transplantation results in cure for a substantial number of patients with certain disorders, although TRM and disease progression continue to be problematic. The largest group of patients studied are those with mucopolysaccharidosis type 1, wherein successful marrow transplantation results in significant reduction of storage material in liver, tonsils, conjunctiva, CSF, and urine, improvement of CNS findings on MRI, normalization of hearing, improved left ventricular function and resolution of arrhythmia.[10,11] Significant amelioration of disease symptoms has been associated with partial as well as complete enzyme replacement.[11]

Peters, et al. reported outcome for 54 patients with Hurler syndrome treated with conventional marrow transplantation from HLA-identical related (n=28) or haploidentical grafts (n=26; 15/26 received T-cell deplete grafts). Patients were conditioned with chemotherapy with or without TBI.[10] Among the 26 evaluable recipients of HLA-identical related grafts, 22 (85%) achieved full donor (n=15) or mixed donor-host (n=7) chimerism, and 4 (15%) failed to engraft. Among the 26 recipients of HLA-haploidentical grafts, 16 (62%) achieved full donor chimerism, 1 (4%) achieved mixed donor-host chimerism, and 9 (35%) failed to engraft. Twenty patients (37%) died of transplant related causes primarily cardiac (n=8) or GVHD +/- infection (n=8). More recently, Souillet et al. reported the outcome of 27 patients with Hurlers syndrome who received related (n=10) or unrelated (n=17) T-replete grafts (n=26) from primarily bone marrow (n=25) following conditioning with busulfan/ cyclophosphamide alone (n=27) or combined with ATG (n=20).[12] Of the 27 patients, 23 engrafted (full donor n=11, mixed n=10) and 4 had primary graft failure. Importantly, disease responses, specifically improvement in enzyme activity levels, cognitive function and clinical improvement in somatic disease were seen in both the fully engrafted and mixed chimera patients. Staba et al. reported on 20 patients with Hurlers syndrome who received busulfan/cyclophosphamide and ATG followed by unrelated donor cord blood grafts.[13] With a median follow up of 905 days, 17 children were alive with an event free survival of 85%. All living recipients had complete donor chimerism, normal enzyme activity levels and demonstrated improvement in neurocognitive performance and decreased somatic features of Hurler's syndrome.

Marrow transplantation has been used to treat other metabolic storage disorders, including Sanfilippo A, Sanfilippo B, Hunter syndrome, metachromatic leukodystrophy, adrenal leukodystrophy, Krabbe's disease, and Neiman-Pick disease.[14-16] Potential for reversing or arresting disease course has depended upon early transplantation, before onset of significant neurologic impairment. Morbidity and mortality from transplant conditioning regimens have contributed to treatment failure.

3) Thalassemia and other hemoglobinopathies.

Several studies have demonstrated efficacy of conventional marrow transplantation using HLA-identical sibling donors[17-25] and unrelated donors[26-28] for cure of thalassemia and other hemoglobinopathies. Stable mixed chimerism has been documented in a number of patients after conventional regimens and has been associated with cure of disease.[20,29] Mortality resulted from infection, pneumonitis, graft rejection, and GVHD. Again, pre-transplant patient characteristics were the most important factors that predicted outcome after HCT. Patients with liver fibrosis or hepatomegaly had a 2-fold increase in risk of mortality. Older age was also associated with worse outcome. Recently, Iannone et al. published results on 7 patients with sickle cell disease (n=6) or thalassemia (n=1) who received bone marrow or peripheral blood stem cell grafts from HLA-identical siblings following conditioning with 200 cGy TBI and fludarabine [30 mg/m^2 x 3 days (n=2) or 5 days (n=5)] alone or combined with ATG (n=2).[30] Although this regimen resulted in minimal toxicity, 6 of the 7 patients lost their grafts following tapering of the immunosuppression. These results suggest that donor engraftment is more difficult to achieve among patients with hemoglobinopathies than what has been found in patients with malignancies. One possible explanation for this is that patients with hemoglobinopathies may have been more sensitized to donor minor histocompatibility antigens by blood transfusions. Therefore, in order to improve the engraftment rate among high-risk patients with thalassemia and sickle cell disease, a more aggressive conditioning regimen may be required in order to overcome these barriers.

4) Osteopetrosis.

Osteopetrosis is an autosomal recessive disease resulting from lack of osteoclast resorption of bone that causes in marrow failure and nerve entrapment, and early death. Normally functioning osteoclasts can be restored by HCT, resulting in amelioration of disease symptoms.[31] Partial as well as full chimerism has been associated with establishment of osteoclast function.[32] Studies have shown 40-80% disease-free survivals for recipients of HLA-matched related grafts, and 30-40% for recipients of unrelated grafts.[31-34] Treatment failure is primarily due to transplant related complications such as infection, GVHD, or toxicity in particular hepatic veno-occlusive disease.[35] Development of a conditioning regimen with decreased toxicity in particular hepatic toxicity would be appealing in this group of patients.

5) Hemophagocytic lymphohistiocytosis (HLH).

Marrow transplantation is the only reported cure for hemophagocytic lymphohistiocytosis (HLH), characterized by progressive organ infiltration of lymphocytes and macrophages associated with hemophagocytosis. Marrow transplantation has been successful in treating both the inherited form, known as familial erythrophagocytic lymphohistiocytosis (FEL), as well as the sporadic form. Henter et al. reported on the outcomes of 65 patients conditioned with primarily busulfan, cyclophosphamide, and etoposide +/- ATG.[36] At a median follow up of 3.1 years, the 3 year probability of overall survival was 62%. Twenty (31%) patients died of transplant related causes prior to day 100. Recently, Baker et al. reported on the outcomes of 91 patients with HLH who were conditioned primarily with BU/CY/Etoposide/ATG followed by unrelated bone marrow (86%) grafts.[37] The 1 and 5 year overall survivals were 52% and 45% respectively after HCT. Day 100 TRM was 33% and was primarily from infections +/-GVHD (n=13) or organ toxicity (n=17). These results support the need for new studies with potentially lower transplant related mortality.

6) Inherited Marrow Failure Syndromes.

Inherited bone marrow failure syndromes are genetic disorders that may present at birth or later in life and are characterized by defective single or multi-lineage hematopoiesis with many patients progressing to develop severe aplastic anemia (SAA) and they are often associated with congenital defects. Patients are at risk for significant infections due to neutropenia; however many patients also have an additional underlying immunodeficiency. In addition, patients with bone marrow failure syndromes are at particularly increased risk for developing malignancies; in particular myelodysplastic syndrome (MDS), acute myeloid leukemia, and solid tumors. For those patients who develop SAA, the primary curative therapy is hematopoietic cell transplantation (HCT). In addition, although not the primary goal for patients with bone marrow failure associated SAA, HCT has been used to try and prevent the development of MDS and myeloid leukemias.

a. Dyskeratosis congenita

Dyskeratosis congenita (DC) is a rare inherited multi-system disorder characterized by the diagnostic triad of reticulated hyperpigmentation of the skin, dystrophic nails, and oral leukoplakia. In addition, several somatic manifestations are seen including skeletal, dental, gastrointestinal, genitourinary, pulmonary, immunological and ocular abnormalities.[38] Autosomal dominant and recessive forms have been described; however over 75% of the cases are an X-linked recessive pattern. The genes identified have been found to encode products involved in telomere maintenance. The primary causes of death are from bone marrow failure culminating in SAA. A recent analysis of data from the dyskeratosis congenita registry found the probability of developing bone marrow failure [one or more

peripheral cytopenia(s)] by age 40 to be around 94%.[38] In addition to death from infections as a result of bone marrow hypoplasia, a large number of patients die from infections resulting from cellular and humoral abnormalities of the immune system. Specifically, studies have found abnormal immunoglobulin production, reduced B and or T lymphocyte numbers and reduced T-cell function in patients with DC.[39] Other causes of death include myeloid and solid tumor malignancies as well as pulmonary failure.

Therapy has consisted of androgens, prednisone, splenectomy and hematopoietic growth factors; however none of these has resulted in a cure of the underlying hematopoietic disorder. The only definitive treatment for those patients who develop bone marrow failure is HCT; however prior studies have demonstrated poor survival due to both early and late complications following HCT.[40] Langston et al published on 8 patients with DC and aplastic anemia who received bone marrow grafts following conditioning with either cyclophosphamide (140-200 mg/kg) +/- ATG (HLA-matched related, n=6) or Cyclophosphamide (120 mg/kg) and 1200 cGy TBI (HLA-matched unrelated, n=2).[41] At the time of publication, 7 of the 8 patients died of overwhelming fungal infections (n=3; day +7, +13, +14), acute and chronic interstitial lung disease (n=3; day 70, 8 years, 20 years), and 1 patient died of acute GVHD. The remaining alive patient has since died of colorectal cancer. Rocha et al published on 5 patients with aplastic anemia associated DC who received HLA-matched related donor grafts following primarily cyclophosphamide (120-200 mg/kg) with irradiation (7 Gy TBI or 6 Gy TAI).[42] All patients engrafted; however 4 of the 5 died of transplant related causes including late veno-occlusive disease (n=2), thrombotic microangiopathic arterial syndrome (n=1), and invasive aspergillosis (n=1). Others have also reported poor outcomes due to significant transplant related complications.[38,40,42]

Unfortunately, the outcomes of allogeneic HCT for patients with SAA associated DC are significantly worse than that seen for patients with SAA alone or even for patients with other marrow failure syndromes such as Fanconi anemia. One possible explanation is the fact that patients with DC often have significant underlying comorbidities as a result of their disease, such as pulmonary disease, which likely accounts for the higher incidences of mortality seen in this patient population. Therefore, due to the high incidences of mortality associated with more ablative preparative regimens, strategies aimed at reducing toxicities are indicated as well as the use of preparative regimens that do not use drugs that are known to be toxic to the lungs such as busulfan.

Several case reports have recently been published using various reduced intensity or nonmyeloablative approaches. Dror et al reported on 2 patients with DC who underwent HCT secondary to severe cytopenias.[43] The preparative regimen consisted of fludarabine (30mg/m^2/day x 5 days), cyclophosphamide (60 mg/kg/day x 2 days), and ATG (40 mg/kg x 4 days) followed by HLA matched unrelated donor bone marrow grafts. Both patients engrafted and are alive with full donor chimerism 15 and 16 months after HCT. Ayas et al. recently published 1 case of a 5 year old boy with DC who received cyclophosphamide (15 mg/kg x 4 days) and ATG (13 mg/kg x 4 days) followed by HLA-matched related graft.[44] He engrafted and had minimal early toxicity. At 6 months post HCT he had 55% and 100% donor chimerism in both the lymphoid and myeloid compartments, respectively. Gungor et al reported on the treatment of a 10 year old female with DC who had neutropenia, thrombocytopenia and combined immunodeficiency with recurrent bacterial pulmonary infections and disseminated refractory molluscum contagiosum skin infections.[45] This patient was conditioned with the nonmyeloablative preparative regimen developed at the hutch [fludarabine (30mg/m^2/day x 3 days) and 2 Gy TBI] followed by G-CSF mobilized peripheral blood stem cell (PBSC) graft. This patient is alive and well 2 years after HCT

with 100% donor chimerism in myeloid and lymphoid lineages and normal T and B cell function. Importantly she has no pulmonary dysfunction.

Due to early and late complications some of which is disease related following more aggressive preparative regimens, the outcome following HCT for DC has been poor. Although the number of patients reported in these case reports is small, the results are encouraging and support further investigation of HCT using reduced intensity preparative regimens for patients with DC.

b. Shwachman-Diamond Syndrome

Shwachman Diamond syndrome (SDS) is a rare autosomal recessive disease characterized by metaphyseal dysostosis, exocrine pancreatic dysfunction, growth retardation and/or short stature and varying degrees of bone marrow dysfunction. The primary causes of death are hemorrhage and infections due to associated hematological abnormalities such as marrow aplasia, neutropenia, and MDS/acute leukemia. Similar to patients with DC, patients with SDS also have abnormalities in their immune system. Specifically, defects in neutrophil mobility, migration and chemotaxis as well as B and T cell abnormalities have been described.[46-48]

Although supportive measures such as transfusions, pancreatic enzymes, antibiotics and G-CSF have been used, the only definitive therapy for marrow aplasia and/or MDS/AML is HCT. However, poor outcomes with HCT have been reported due to excessive cardiac and other organ toxicities which are felt to be due to the preparative regimen.[49,50] Cesaro et al. recently reported on registry data from the European Group for Blood and Bone Marrow Transplantation (EBMT) on 26 patients with SDS and severe bone marrow abnormalities.[51] Various preparative regimens were used however, the majority contained busulphan (54%) followed by primarily T-cell depleted unrelated donor bone marrow grafts. Five (19%) patients failed to engraft. The incidence of grade III-IV and chronic GVHD were 24% and 29%, respectively. With a median follow up of 1.1 years, the 1 year overall survivals were 65%. Day 200 and 1 year transplant-related mortalities were 35% for both and were primarily due to infections +/- GVHD (n=5) or major organ toxicities (n=3). Based on data demonstrating that bone marrow cells of SDS patients have an abnormal susceptibility to Fas-mediated apoptosis,[52] the authors speculated that SDS patients are more susceptible to major organ toxicity and infections and as a result higher transplant related mortalities when more aggressive preparative regimens are used. For this reason, the authors concluded that future studies should evaluate reduced intensity preparative regimens for this group of patients.

3B. Myeloablative HCT for Treatment of Nonmalignant Disorders - Seattle Experience

1) Immunodeficiency disorders.

From 1980 onward, efforts were focused on developing a myeloablative conditioning regimen sufficient for engraftment of marrow from related, and eventually unrelated, donors for treatment of nonmalignant disorders, including immunodeficiency disorders. Busulfan and cyclophosphamide were the backbone of the regimen, which eventually included anti-thymocyte globulin (ATG). Twenty-four patients with primary immunodeficiency disorders were transplanted at the FHCRC between March 1980 and August 2005. Currently, 14 of 24 patients are alive; while 10 patients died from infections +/- GVHD (n=6), veno-occlusive disease (n=2), multi-organ failure (n=1), and graft rejection with subsequent disease

recurrence (n=1). Day 100 and 200 TRM were 29% for both. With a median follow up for the living recipients of 11.3 (range, 2.4-26.1) years, the 12-year overall survival, event free survival and TRM were 58%, 58% and 38% respectively. Further analysis suggested that tolerance of the conditioning regimen depended upon diagnosis. Among the 11 patients with WAS, 10 are surviving a median of 8 years (range 2-26) years after HCT with disease resolution while one patient died of infection. In comparison, 4 of the 13 patients with other immunodeficiency disorders survive long-term. TRM by day 100 for the latter group was 46%, compared to 10% for the WAS patients. These analyses show that, while conventional HCT was an effective therapy for patients with immunodeficiency disorders, early TRM was prohibitive among the subgroup of patients with diagnoses other than WAS. For the most part, the high day 100 TRM can be explained by the presence of infections or co-morbidities at time of HCT.

2) Other inherited disorders.

Fifty-three patients with metabolic storage diseases (n=10), red cell disorders (n=26), Osteopetrosis (n=8), and HLH/Gricellis syndrome (n=9) were transplanted at the FHCRC between October 1981 and October 2007. Day 100 and 200 TRM were 21% and 25%, respectively. The 15-year overall and event free survival and TRM for the entire group was 50%, 45% and 27%, respectively after HCT. An analysis of a subgroup of patients (n=27 metabolic storage diseases, osteopetrosis and HLH) demonstrated that 37% of the patients died by day 200 of transplant related causes. Therefore, efforts at decreasing the toxicity of the conditioning regimen thereby decreasing TRM without compromising engraftment are needed for patients with nonmalignant disorders.

3C. Treosulfan

1) Mechanism of action, activity, pharmacokinetics and toxicity.

Treosulfan (Ovastat®, L-treitol-1,4-bis-methanesulfonate, dihydroxybusulfan, TREO; Medac, Hamburg, Germany) is a prodrug of 2 biologically active epoxy-derivatives and a water-soluble intravenous Busulfan analog approved for therapy of advanced ovarian carcinoma in Europe. Under physiologic conditions, Treosulfan is non-enzymatically, in a pH-dependent manner, spontaneously activated into the monoepoxide((2S,3S)-1,2-epoxybutane-3,4-diol-4-methane-sulfonate, S,S-EBDM), which converts to (2S,3S)-1,2:3,4-diepoxybutane (S,S-DEB). The formed epoxy-transformers are responsible for cross-linking of DNA molecules and cytotoxicity in rapidly proliferating cells, both malignant and non-malignant, such as normal hematopoietic cells.[53] Contrary to Busulfan, Treosulfan does not require enzymatic activation, thus bypassing hepatic metabolism. The hydroxyl groups in positions 2 and 3 of the molecule account for differences in pharmacological activity between Treosulfan and Busulfan (Figure 1). Busulfan directly alkylates nucleophilic centers, while Treosulfan induces alkylation of nucleophilic centers by intramolecular epoxide formation. Clinical pharmacokinetic studies have demonstrated that Treosulfan has a similar t½ to Busulfan (1.8 – 2 hours) and a higher, dose-independent, cumulative renal excretion (50% vs. 20%).[54,55] Pharmacokinetic studies of both single and multiple intravenous infusions of Treosulfan have demonstrated low inter-patient and inter-day variability. There is a high linear correlation between the area under the curve and Treosulfan dose (r^2=0.9227), which compares favorably with that of oral and intravenous Busulfan.[56,57] On

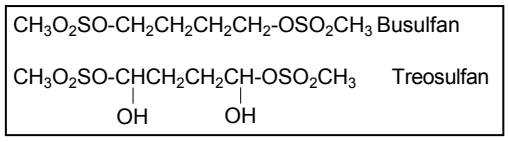

Figure 1. Molecular structures of busulfan and tresoulfan

preclinical studies in mice, rats, dogs and primates, Treosulfan resulted in at least 10 times lower acute and chronic toxicity than Busulfan [unpublished data, Investigator's Brochure].

The pronounced effect of Treosulfan on primitive and committed hematopoietic stem cells and its immunosuppressive effects have been demonstrated in allogeneic murine transplant models.[53] At doses 80-88% lower than those used for humans in clinical trials, Treosulfan was at least as effective as Busulfan in depleting hematopoietic cell subsets (Figure 2). Treosulfan was capable of inducing full donor engraftment and immune tolerance across MHC barriers. Marrow suppression is the limiting toxicity for conventional chemotherapy with Treosulfan at doses over 10 g/m^2. With autologous stem cell rescue, the Treosulfan dose could be escalated up to 47 g/m^2 before dose-limiting toxicity including mucositis, diarrhea, dermatitis or metabolic acidosis was observed.[58]

2) Initial phase I/II studies of Treosulfan -containing regimens for allogeneic HCT – European Experience

2a. <u>Treosulfan and fludarabine</u>

Fifty-six patients, median age 50 (range 18-66) years, with varied hematologic malignancies received three doses of 10, 12 or 14 g/m^2 Treosulfan and 5 doses of 30 mg/kg FLU followed by HLA-identical sibling (n=28) or matched unrelated (n=28) marrow or PBSC.[59] GVHD prophylaxis consisted of cyclosporine with or without thymoglobulin (unrelated recipients only). All patients engrafted, with a median time to neutrophil engraftment of 14 (range 10-23) days. Acute GVHD of grades II-IV and extensive chronic GVHD were observed in 24/56 (43%) and 8/42 (19%) evaluable patients, respectively. Significant grade 3 toxicities included elevation of bilirubin or liver transaminases (n=7), diarrhea (n=3), mucositis (n=2), dyspnea (3) and elevation of creatinine (n=1). Eight (15%) patients died from relapse. Eleven (20%) patients died from non-relapse causes, 8 from infection, 2 from GVHD with or without infection, and 1 from RRT (myocardial infarction). The 1-year DFS is 53%, with a median follow-up of 21 months. These results suggest that the combination of Treosulfan /FLU may result in improved RRT and NRM and similar survival compared to Treosulfan /CY. Recently several other groups have published encouraging results in patients with hematologic malignancies using a Treosulfan/fludarabine based conditioning regimen.[60-62]

<u>2b. Experience with use of Treosulfan in children</u>

The experience with this drug in patients of pediatric age is limited. Wachowiak and colleagues conducted a pilot study of Treosulfan -containing preparative regimens for children. Eight children considered at high risk for toxicity from conventional regimens, of median age 11 (4-13) years, and diagnosis of AML (n=3), ALL (n=1), MDS (n=1), histiocytosis (n=1), Wiskott-Aldrich syndrome (n=1) or adrenoleukodystrophy (n=1) were studied. They received three doses of 10 g/m^2 Treosulfan in combination with other drugs including FLU, CY, melphalan or etoposide, followed by HLA-identical sibling marrow (n=7) or cord blood (n=1). GVHD prophylaxis was with cyclosporine only. All patients engrafted, with a median time to neutrophil engraftment of 19 (range 10-22) days. Acute grade II GVHD was observed in 3 (38%) patients. Extensive chronic GVHD was observed in 1 (13%) patient. Severe or

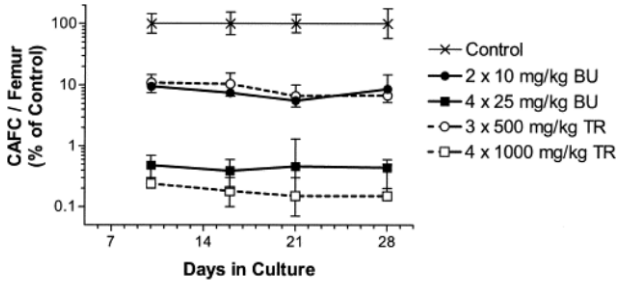

Figure 2. Survival of different CAFC subsets remaining in the femoral bone marrow after treatment with different doses of busulfan and treosulfan.. Error bars=95% CI

fatal toxicities were not observed. Three patients developed mild (Bearman grade I) mucositis or transient elevation of liver transaminases (n=2). Two (25%) patients relapsed. The remaining 6 patients are alive and disease-free, with a median follow-up of 12 months.

More recently, Sauer[63] et al. published on 3 patients with Shwachman-Diamond Syndrome who received treosulfan (12 g/m^2/d x 3), fludarabine (30 mg/m^2/d x 6), and Melphalan (140 mg/m^2/d x 1) in combination with CAMPATH-1H (0.1 mg/kg x 2) in 2 cases and ATG in one case followed by HLA-identical sibling (n=1), HLA-matched unrelated donor (n=1) or 9/10 matched unrelated cord blood graft. All patients engrafted at 24, 31, and 26 days after HCT and had 100% donor chimerism at last follow up. Two of the 3 patients are alive at 9 and 20 months after HCT. The third patient who received a cord blood graft died 3 months after HCT of IPS. Greystoke and colleagues recently published a retrospective analysis of 32 pediatric patients undergoing HCT for nonmalignant diseases including primary immunodeficiency (n=18), metabolic disorders (n=9), osteopetrosis (n=4) and thalassemia (n=1).[64] Patients received a total treosulfan dose of 36 g/m^2 (n=6) or 42 g/m^2 (n=26) given in three divided doses on consecutive days. A variety of other conditioning agents were used depending on the stem cell source and donor type including fludarabine (150 mg/m^2) Cyclophosphamide (200 mg/m^2), plus or minus Campath or ATG. HLA-matched or mismatched related or unrelated bone marrow (n=17), peripheral blood (n=9), and cord blood (n=5) grafts were used as the stem cell source. Post HCT immunosuppression included Cyclosporine alone or combined with Mycophenolate Mofetil, methylprednisolone, or methotrexate. Twenty eight patients (87.5%) established donor cell engraftment; however, 4 patients have required additional transplantation procedures to maintain adequate donor derived hematopoiesis. With a median follow up of 471 days, 27 (84%) of the patients survive. One patient died early (day +4) due to sinusoidal obstructive syndrome and four other deaths were late due to disease progression, GVHD or infection. The incidence of GVHD was very low with 6 (19%) patients developing acute grade II (n=4) or grade III (n=2) acute GVHD and 4 (13%) or patients developing chronic GVHD. Although multiple different donors and conditioning regimens were used, these results are encouraging in pediatric patients with nonmalignant disorders.

Bernardo et al recently published on 20 patients with Thalassemia who were conditioned with Thiotepa (8 mg/kg; day -7), Treosulfan (14 g/m^2; days -6 to -4), and fludarabine (40 mg/m^2; days -6 to -3) followed by HLA matched related (n-3) or unrelated (n=17) bone marrow grafts. GVHD prophylaxis consisted of Cyclosporine and methotrexate. Pretransplantation ATG (10 mg/kg; days -5 to -3) was administered to all the unrelated graft recipients. Two patients experienced secondary graft failure. Cumulative incidence of TRM was 5% and the 2 year probability of survival and thalassemia-free survival was 95% and 85%, respectively.

2c. Phase I/II Study of Treosulfan and fludarabine in the United States- Protocol 1931 -Preliminary Results

Forty-six patients, median age 41 years (5-59 years), with acute myeloid leukemia in first remission (n=22), second remission (n=9), partial remission or relapse (n=5) or ALL in second remission (n=2) or myelodysplastic syndrome (n=8) have been enrolled on study. Eight of the AML patients had secondary (treatment-induced) AML and 5 patients had failed a previous transplant. Most patients had underlying conditions that made them poor candidates for conventional myeloablative regimens. All patients received therapy as per protocol with treosulfan (5 at dose level 1 or 12 g/m^2/day, and 41 at dose level 2 or 14 g/m^2/day) on days -6 to -4 and fludarabine (30 mg/m^2/day) on days -6 to -2. Patients received HLA-matched sibling grafts (n=1 marrow, n=23 PBSC), or unrelated grafts (n=6 marrow, n=16 PBSC) on day 0. Neutrophil engraftment occurred in 100% of 43 evaluable patients at a median of 17 (range, 5-30) days after HCT. Acute graft-versus-host disease

(GVHD) has been observed in 25 of 43 evaluable patients (grade I-II). Chronic GVHD has been observed in 13 of 35 evaluable patients. Dose limiting toxicity has been observed in 1 of 46 patients (**Table 1**). There have been 9 reportable serious adverse events, 2 of which were deemed study drug related. One patient died of systemic aspergillosis on day 10. Ten patients have relapsed at a median of 76 (26-184) days, of which 7 have died of disease recurrence. Thirty-six patients are alive and disease-free, with a median follow-up of 204 (range, 10-711) days after HCT.

Table 1: Outcomes Summary			
Probability (%)	**100-day**	**200-day**	**1-year**
OS	93	81	76
EFS	81	75	72
Relapse	17	23	26
TRM	2	2	2

2d. Treosulfan Pharmacokinetics

Studies have demonstrated highly predictable PK in adult patients; however, limited data are available for pediatric patients. In addition, very little data is available on the relationship between treosulfan exposure and HCT outcomes including early toxicities and engraftment. Van der Stoep recently published on treosulfan pharmacokinetics and the drug's relationship with regimen-related toxicity and early clinical outcome in 77 pediatric patients with hematological malignancies (n=12) or non-malignant diseases (n=65) who underwent HCT. Conditioning consisted of treosulfan, fludarabine, +/- thiotepa (n=52) followed by HLA-matched sibling n=27, matched URD (≥9/10; n=36), or haploidentical (n=14) grafts. Hematopoietic cell sources varied and included bone marrow (n=50), PBSC (n=20), cord blood (n=6), and bone marrow plus cord blood (n=1). Van der Stoep and colleagues reported low intra-patient variability (13.9%) but high inter-patient variability [56%; <1-year old, (n=12) and 33%; ≥1 to 21 years of age (n=65)] among 77 pediatric patients. This is in contrast to that seen in adult patients where there is low intra- and low inter-patient variability. In addition, high treosulfan exposure (>1650 mg*h/L) was associated with an increased risk of developing grade 2 or higher mucositis [OR 4.40; 95% CI 1.19–16.28, p=0.026], increased risk of skin toxicity, including erythematous rash and skin exfoliation, [OR 4.51; 95% CI 1.07-18.93; p=0.040], and increased risk of developing multiple toxicities [OR 4.52; 95%CI 1.32-15.53, p=0.016] compared to the lower exposure (<1350 mg*h/L) group. The authors highlighted that their study included a heterogeneous group of diseases and additional data in more homogeneous and single disease groups with longer follow up are needed in order to better understand the relationship between AUC and long-term outcomes. We plan on collecting PK samples on patients enrolled on this clinical trial in order to gain a better understanding whether the exposure to treosulfan AUC for pediatric patients with nonmalignant diseases impacts engraftment and both acute and long-term toxicities. This information will help guide future studies using treosulfan-based conditioning in patients with non-malignant diseases.

3D. Fludarabine

Fludarabine (Fludara®) is a purine analog that inhibits lymphocyte proliferation, promotes lymphocyte apoptosis, and is effective in the treatment of lymphoid and myeloid malignancies.[65-67] Fludarabine is phosphorylated intracellularly in several steps to its active form 2-fluoroadenosine arabinoside triphosphate, which acts by inhibiting DNA synthesis.[65,66] Fludarabine induces immunosuppression and long-lasting lymphopenia reducing the incidence of GVHD and facilitating engraftment along histocompatibility barriers when used in combination with other chemotherapeutic agents or low-dose TBI.[66,68]

3E. Stored cord blood use

Historically, bone marrow, peripheral blood, and cord blood have all been used as stem cell sources for transplantation. It is becoming more common for parents to store the cord blood of a sibling, particularly if that sibling does not have the same genetic disease and is a match to the patient. If a patient has an HLA-matched sibling donor and the cord blood has been stored, we may combine the bone marrow and cord blood in order to try and improve the transplant outcomes. Soni et al. [69] recently published on 13 patients with hemoglobinopathies who received an HLA-matched sibling cord blood and marrow grafts and demonstrated that this strategy was feasible and safe in this small cohort. Furthermore, this combined graft source may potentially result in faster neutrophil recovery, decreased GVHD, and decreased transplant related mortality compared to either cord blood or bone marrow alone.[69]

3F. Prioritization of Peripheral Blood Stem Cells

Less intense conditioning regimens have led to improved survival and lower transplant related toxicities and mortality in patients with nonmalignant diseases, particularly those with co-morbid conditions. However, less intense regimens have been associated with mixed donor/host chimerism or graft rejection.[70,71] In addition, many patients with nonmalignant disease have increased barriers to engraftment due to characteristics of their underlying disease, which pose further challenges to sustained engraftment, particularly following reduced intensity conditioning. Finally, amelioration of many nonmalignant diseases requires high-level multi-lineage donor chimerism.

Thus, the goal of this study for patients with nonmalignant diseases is to develop a regimen that overcomes the barriers of engraftment without increasing the toxicities and mortality of the transplant procedure. Results of the current trial using a reduced toxicity/intensity regimen of Treosulfan, fludarabine, and rabbit ATG are encouraging, with excellent overall survival and very low transplant-related mortality. However, mixed donor/host chimerism remains a challenge, particularly in patients with diseases that have barriers to engraftment. Several clinical trials have shown that in the setting of reduced intensity or nonmyeloablative conditioning, engraftment is superior when the graft is composed of peripheral blood stem cells (PBSC) compared to bone marrow. [72] Slatter et al. recently published on 160 pediatric patients with primary immunodeficiency diseases who received Treosulfan, fludarabine +/- Campath (n=154) followed by unrelated or related donor grafts. The initial stem cell source was bone marrow; however due to a high proportion of patients with poor engraftment or low-level donor chimerism, the trial transitioned to PBSC grafts. Results show a significant association between high-level donor myeloid chimerism (>95% donor) and use of PBSCs without an increased risk of significant GVHD. [73] Although the number of recipients of PBSC enrolled on protocol 2256 (Regimen A) has been low (n=10), we have not seen an increased risk for GVHD in recipients of PBSC vs bone marrow to date. Therefore, going forward in this clinical trial, PBSCs will be prioritized whenever feasible with the goal of improving donor engraftment, in particular CD33 myeloid engraftment.

4. OBJECTIVES

A. Primary Objectives:

The primary objective of this clinical trial is to evaluate, within the limits of a phase II study, the preliminary efficacy as defined by engraftment, of a regimen consisting of treosulfan and fludarabine followed by allogeneic HCT in patients with nonmalignant inherited disorders.

B. Secondary Objectives:

1. Incidence of non-relapse mortality 200 days and 1 year post-HCT
2. Incidence of grade II-IV acute GVHD
3. Incidence of chronic GVHD – as defined as those patients requiring systemic immunosuppression
4. Donor chimerism on days +28 and +100
5. Assess disease response following HCT
6. Immune reconstitution following HCT
7. Incidence of infections
8. Overall survival

C. Exploratory Objectives:

1. Pharmacokinetic parameters of treosulfan and treosulfan monoepoxide

5. PATIENT SELECTION

5A. Inclusions

1. Age < 50 years with nonmalignant disease treatable by allogeneic HCT.
2. Patients with a known nonmalignant disease that is not clearly defined will need to be discussed with the protocol PI (Dr. Lauri Burroughs) and potentially the Nonmalignant board to determine if they are eligible for HCT on this study.

5B. Exclusions:

1. Patients with Idiopathic Aplastic Anemia and Fanconi Anemia. (Patients with Aplastic Anemia associated with PNH or inherited marrow failure syndromes, except Fanconi Anemia, will be allowed).
2. Patients with the following organ dysfunction:
   a. With impaired cardiac function as evidenced by ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction of <26%) or cardiac insufficiency requiring treatment or symptomatic coronary artery disease. Patients with a shortening fraction < 26% may be enrolled if approved by a cardiologist.
   b. With impaired pulmonary function as evidenced by DLCO < 50% of predicted (or, if unable to perform pulmonary function tests, then O_2 saturation < 92% on room air).
   c. With impaired renal function as evidenced by creatinine-clearance < 50% for age, weight, height or serum creatinine > 2X upper normal limit or dialysis-dependent.
   d. With evidence of synthetic dysfunction or severe cirrhosis requiring deferral of conditioning as recommended by a gastroenterology specialist.

3. Patients with an active infectious disease requiring deferral of conditioning; as recommended by an Infectious Disease specialist.
4. Patients who are positive for human immunodeficiency virus (HIV).
5. Females who are pregnant or breast-feeding.
6. Patients with a known hypersensitivity to treosulfan and/or fludarabine.
7. Receiving another experimental drug within 4 weeks of initiation of conditioning (day -6) unless approved by the PI.

6. DONOR SELECTION

Regimen A: Bone marrow or Peripheral Blood or HLA-matched sibling bone marrow combined with HLA-matched sibling cord blood

A. Inclusions: Donors must be:

1. HLA-identical related donors or
2. Unrelated donors matched for HLA-A, B, C, DRB1, and DQB1 or mismatched for a single allele at HLA-A, B, C, DRB1 or a single DQB1 antigen or allele mismatch by high resolution DNA typing.
3. PBSC is the preferred cell source (when feasible) for fully matched donors. PBSC may also be used for a mismatched donor following discussion with the PI. Bone marrow is allowed when PBSC is not feasible or as determined by the PI.
4. HLA-matched sibling bone marrow in combination with HLA-matched sibling umbilical cord blood if the HLA-matched sibling umbilical cord blood was collected and stored. The HLA-matched sibling bone marrow and cord blood would be matched for HLA-A, B, C, DRB1, and DQB1.

B. Exclusions: Ineligible donors will be those:

1. Deemed unable to undergo marrow harvesting or PBSC mobilization and leukapheresis.
2. HIV-positive.
3. With active infectious hepatitis.
4. Females with a positive pregnancy test.
5. HLA-matched sibling cord blood exclusions:
   a. Any cord blood units that have not passed donor screening for infectious disease markers as recommended by the National Marrow Donor Project (NMDP) will not be used unless a waiver is signed by the clinical attending allowing use of cord blood unit. Cord blood units are presumed to be CMV negative regardless of serologic testing due to passive transmission of maternal CMV antibodies.

Regimen B: Unrelated Umbilical Cord Blood will be allowed if an HLA-matched related or unrelated donor is not identified or available

A. Unrelated Umbilical Cord Blood Inclusions:

1. Unit selection is based on the cryopreserved total nucleated cell (TNC) dose and matching at HLA-A, B antigen level and DRB1 allele level typing. While HLA-C antigen/allele level typing is not considered in the matching criteria, if available, may be used to optimize unit selection.
2. The patient and the cord blood unit(s) must be matched for at least 4 of 6 loci as defined above.
3. Selection of two UCB units is allowed to provide sufficient cell dose (see 3d below for algorithm to determine single versus double unit transplant). When multiple units are selected, the following rules apply:

a. The UCB unit with the least HLA disparity (with the patient) will be selected first (i.e., selection priority is 6/6 match >5/6 match>4/6 match). Additional UCB units then may be selected to achieve the required cell dose, as outlined below (3e). If a second unit is required, this unit will be the unit that most closely HLA matches the patient and meets minimum size criteria outlined below of at least 1.5 x 10^7 TNC/kg (i.e. a smaller more closely matched unit will be selected over a larger less well matched unit as long as minimum criteria are met).
b. Each UCB unit MUST contain at least 1.5 x 10^7 TNC per kilogram recipient weight.
c. The total cell dose of the combined units must be at least 3.0 x 10^7 TNC per kilogram recipient weight.
d. Algorithm for determining single versus double cord blood transplant: The donor selection algorithm has been designed to favor single cord HCT to decrease the risk of GVHD in the nonmalignant setting while attempting to not compromise engraftment.

	Single Unit Allowed for:	**Multiple Units Required for:**
Match Grade	**TNC Dose**	**TNC Dose**
6/6	≥ 3.0 x 10^7/kg	< 3.0 x 10^7/kg
5/6	≥ 4.0 x 10^7/kg	<4.0 x 10^7/kg
4/6	≥ 6.0 x 10^7/kg	<6.0 x 10^7/kg

4. General Comments:
   a. Units will be selected first based on the TNC dose and HLA matching.
   b. CD34+ cell dose will not be used for unit selection unless 2 units of equal HLA-match grade and similar TNC dose (± 0.5 x 10^7 TNC/kg) are available. In this case, the unit with the larger $CD34^+$ cell dose (if data available) should be selected.
   c. A UCB unit that is 5/6 mismatched but homozygous at the locus of mismatch should be chosen over a 5/6 unit with bidirectional mismatch even if the latter unit is larger (has more cells). This also applies to 4/6 units. This is only applicable to choosing units within a given match grade.
   d. Within an HLA match grade, the unit containing the greatest number of cells will be chosen. If there are two units of equivalent cell dose (± 0.5 x 10^7 TNC/kg) within a match level, choose the unit with match by higher resolution molecular typing, if known.
   e. Other factors to be considered:
      1. Within the same HLA match grade, matching at DR takes preference.
      2. Cord blood banks located in the United States are preferred.
      3. The youngest unit would be preferred.

B. Unrelated UCB Unit Exclusions:

1. Any cord blood units with <1.5 x 10^7 total nucleated cells per kilogram recipient weight.
2. Any cord blood units that have not passed donor screening for infectious disease markers as recommended by NMDP will not be used unless a waiver is signed by the clinical attending allowing use of cord blood unit. Cord blood units are presumed to be CMV negative regardless of serologic testing due to passive transmission of maternal CMV antibodies.

7. INFORMED CONSENT

The attending physician will conduct a conference with the patient and family to discuss this study and alternative treatments available. In a separate conference, the risks of the donation procedure will be outlined to the donor and/or his parent/guardian(s), including the risks of anesthesia, bleeding, and infection. The goals of the study, requirement for data collection, and requirement for release of medical records will be discussed with the patient and/or his/her parent/guardian. All potential risks associated with the use of treosulfan, fludarabine, low dose TBI (cord only), immunosuppressive drugs and allogeneic HCT will be discussed as objectively as possible. Discussion of potential complications should include graft rejection, GVHD, infections, and death.

Informed consent from the patient and donor will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. Informed consent will be obtained by the Principal Investigator, Co-Investigator, or Attending Physician who is familiar with the study but not necessarily an investigator. When patients are less than 18 years of age, consent will be obtained from parents or legal guardian(s). All patients enrolled at collaborating centers will engage in institution-specific informed consent conferences after completion of the pre-transplant evaluation. Informed consent from the donor and patient will be obtained using a form approved by the Institutional Review Board for each treatment center.

8. PROTOCOL REGISTRATION

8A. FHCRC Patients: Eligible patients will be identified by the Clinical Coordinator's Office who will register patients with the Registration office (206-667-4728) between 8:30 am and 4:00 PM, Monday through Friday. After hours, the Registration Office can be reached by paging (206)-995-7437

8B. Collaborating Institutions: Eligible patients will be identified by the principal investigator of the collaborating institution who will register the patient with the FHCRC Registration Office. Registration will include completion of the eligibility checklist and demographic form (**Appendix F of protocol**). This form and a copy of the signed informed consent will be faxed to the Trial Coordinator (206-667-4427). Questions regarding eligibility or protocol information should be directed to the Principal Investigator, Lauri Burroughs (206) 667-2396.

9. PLAN OF TREATMENT/CONDITIONING REGIMEN:

Regimen A: For patients receiving marrow, PBSC or HLA-matched sibling bone marrow combined with HLA-matched sibling cord blood		
Day	**Treatment**	**Dose**
-6	Treosulfan Treosulfan PK sampling[b] Fludarabine	14 grams/m^2/day IV[a] 30 mg/m^2/day IV[a]
-5	Treosulfan Treosulfan PK sampling[c] Fludarabine	14 grams/m^2/day IV[a] 30 mg/m^2/day IV[a]
-4	Treosulfan Fludarabine Rabbit Antithymocyte globulin (ATG; Thymoglobulin®)	14 grams/m^2/day IV[a] 30 mg/m^2/day IV[a] 1.0 mg/kg/day IV[d]
-3	Fludarabine Rabbit Antithymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[a] 2.5 mg/kg/day IV[d]
-2	Fludarabine Rabbit Antithymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[a] 2.5 mg/kg/day IV[d]
-1	Rest[e]	
0	Marrow or PBSC infusion or HLA-matched sibling bone marrow combined with HLA-matched sibling cord blood	

[a] For patients >120% ideal body weight (IBW) BSA will be calculated using adjusted weight
[b] Research Treosulfan PK sampling (7 timepoints): All patients, see **section 14B.7** for details
[c] Research Treosulfan PK sampling (7 timepoints): Only in patients who are < 4 years of age at time of start of conditioning, see **section 14B.7** for details.
[d] Actual body weight
[e] Additional rest days may be added given delays that may occur with stem cell collection, transportation or processing. The actual day of stem cell infusion will remain "Day 0" even if 1-2 days later than originally scheduled

Regimen B: For patients receiving unrelated umbilical cord blood		
Day	**Treatment**	**Dose**
-6	Treosulfan Treosulfan PK sampling[b] Fludarabine	14 grams/m^2/day IV[a] 30 mg/m^2/day IV[a]
-5	Treosulfan Treosulfan PK sampling[c] Fludarabine	14 grams/m^2/day IV[a] 30 mg/m^2/day IV[a]
-4	Treosulfan Fludarabine Rabbit Antithymocyte globulin (ATG; Thymoglobulin®)	14 grams/m^2/day IV[a] 30 mg/m^2/day IV[a] 1.0 mg/kg/day IV[d]
-3	Fludarabine Rabbit Antithymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[a] 2.5 mg/kg/day IV[d]
-2	Fludarabine Rabbit Antithymocyte globulin (ATG; Thymoglobulin®)	30 mg/m^2/day IV[a] 2.5 mg/kg/day IV[d]
-1	**Recipients with underlying T-cell primary Immunodeficiency** or **All other recipients**	TBI 200 cGy TBI 300 cGy
0	Unrelated umbilical cord blood infusion	

[a] For patients >120% ideal body weight (IBW) BSA will be calculated using adjusted weight
[b] Research Treosulfan PK sampling (7 timepoints): All patients, see **section 14B.7** for details
[c] Research Treosulfan PK sampling (7 timepoints): Only in patients who are < 4 years of age at time of start of conditioning, see **section 14B.7** for details.
[d] Actual body weight

Conditioning regimen.

The regimen will consist of:

1. **Treosulfan.**

   Treosulfan will be administered intravenously over 120 minutes for three consecutive days (day –6 to –4). Use actual weight to calculate BSA. For patients >120% of ideal weight, use adjusted weight to calculate BSA. Optional pharmacokinetic evaluation will be performed on days -6 (all patients) and -5 (only in patients who are < 4 years of age at time of start of conditioning; see Section 14B.7).

   Treosulfan will be supplied to investigators by Medac GmbH, Hamburg Germany under an IND held by Fred Hutchinson Cancer Research Center (IND 72,479). The drug will be delivered by courier to each participating centers' pharmacy as a clinical investigational product with a study-specific label on the glass vial, as approved by the FDA. Treosulfan is available as a white crystalline powder in vials of 1000 mg and 5000 mg. The dry product must be stored at room temperature and has a shelf life of 5 years. TREO must be dissolved in 20 ml (1 g vial) or 100 ml (5g vial) of 0.45% sodium chloride solution, for injection for a final concentration of 50 mg/ml. The 0.45% sodium chloride must be warmed to 25-30^{o}C (not higher) using a warmer or water bath. The reconstituted solution should remain at room temperature and be used the day of preparation. The reconstituted solution should not be stored in a refrigerator because crystallization may occur at low temperatures. Acidic media do not influence the stability of treosulfan. In alkaline and neutral solutions treosulfan decomposes to methanesulfonic acid and threitol. The reconstituted solution has a physicochemical stability of at least 1 day when stored at room temperature in the original glass bottle.

   There have been isolated reports of seizure in infants after combined administration of Treosulfan with Fludarabine or cyclophosphamide. Therefore, seizure prophylaxis during Treosulfan administration should be considered for patients ≤ 1 year of age. We recommend the following: for patients ≤ 1 year begin levetiracetam (Keppra) at 10 mg/kg every 12 hours to start the evening prior to Treosulfan administration and continue until 24 hours after the infusion.

2. **Fludarabine.**

   Fludarabine will be administered intravenously at a dose of 30 mg/m^2/day over 60 minutes for five consecutive days (day –6 to –2). On days –6 to –4, fludarabine will be given after the treosulfan dose is given. For patients > 120% of ideal weight, BSA will be calculated using adjusted weight. Fludarabine (Fludara®, Berlex Laboratories, Inc., Richmond, CA) is commercially available in the U.S. Details of the product's description, preparation; storage and stability are found in the drug's package insert.

3. **Rabbit Anti-thymocyte globulin (ATG; Thymoglobulin®)**

   Thymoglobulin will be administered intravenously over three consecutive days (days -4, -3, -2) at a dose of 1.0 mg/kg on day -4, and then 2.5 mg/kg on days -3 and -2 for a total dose of 6.0 mg/kg. Thymoglobulin will be given intravenously over a minimum of 6 hours for the first two doses and 4 hours for the subsequent dose per institutional standard practice. Doses should be based on actual body weight.

Anti-Human Thymocyte Globulin (ATG; Sangstat Medical Corp., Fremont, CA) is an immunosuppressant which alters the function of or eliminates T-cells and NK cells. Rabbit anti-thymocyte globulin (Thymoglobulin®) is a polyclonal immunoglobulin mixture (IgM/IgG) raised in rabbits against human T thymocytes which reduces all circulating lymphocytes or lymphocyte subsets. ATG is used in preparation for hematopoietic cell transplant (HCT), as immunosuppressive therapy in aplastic anemia or MDS, and for GVHD treatment.

Preparation
Thymoglobulin® is provided in 25mg vials by Sangstat. Thymoglobulin® vials should be reconstituted with 5ml sterile water to a concentration of 5mg/ml. It can then be further diluted with D5W or NS to a volume of 50ml per vial. Diluted drug should be used immediately.

Skin Test
Skin tests are not required prior to Thymoglobulin® administration.

Administration
Thymoglobulin® should be administered alone and not run piggyback with any other solution. A 0.22 micron in-line filter should be used during administration. The first and second dose of Thymoglobulin® should be infused over 6 hours, and subsequent doses should be infused over 4 hours.

Pre-Meds:
Acetaminophen, diphenhydramine, and 1 mg/kg of methylprednisolone should be given before every dose of Thymoglobulin®.

Significant Allergic Reaction Precautions
Have recommended medications for significant allergic reactions available at the bedside in case of allergic reaction:

Medication Recommendations For Significant Allergic Reactions

	Pediatric:	**Adults > 40 kg**
Epinephrine (1:1000 dilution) [1 mg/ml]	0.01 mg/kg IM (Every 20 minutes as needed). Maximum dose 0.3 mg	0.3 ml SC (every 20 minutes as needed)
Diphenhydramine	1 mg/kg IV (max 50 mg)	50 mg IV
Hydrocortisone	2 mg/kg IV (max 250 mg)	250 mg IV

4. Total Body Irradiation (TBI)

A.Cord Blood Recipients Only:
Patients with an underlying T-cell immunodeficiency will receive 200 cGy TBI on day -1. All other patients will receive 300cGY TBI on day – 1. See institutional standard practice guidelines for radiation (**Appendix B)**.

10. COLLECTION OF HEMATOPOIETIC STEM CELLS/INFUSION

Related donors will be collected at each participating center and unrelated donors will be collected through the NMDP or other donor collection centers.

A. PBSC: Is the preferred stem cell source for fully matched donors. PBSC may also be used for a mismatched donor following discussion with the PI. PBSC collection and infusion will be according to institutional standard practice guidelines.

B. Bone Marrow: Donors will undergo standard bone marrow harvest following each center's standard practice. The marrow will be infused according to each center's standard practice guidelines.

C. Unrelated Umbilical Cord Blood: will be used if an HLA-matched related or unrelated donor is not identified or available (see matching criteria section 6)

1. Procedures for requesting, receiving and characterizing the cord blood unit for infusion will be according to institutional standard practice.
2. Pre-infusion hydration should be performed per institutional guidelines.
3. For FHCRC patients, the cord blood unit should be thawed and infused per FHCRC standard practice guidelines. Cord blood products should be infused without delay as soon as the product arrives on the unit.
4. The infusion should take no longer than 30 minutes per unit. Pre-medications prior to cord blood infusion will be per institutional standard practice guidelines. Under no circumstances is the cord blood to be irradiated. No medications or fluids should be given piggyback through the catheter lumen that is being used for cord blood infusion.
5. The product is infused via IV drip directly into the central line according to standard practice with gravity filtered tubing.
6. Vital signs should be monitored before beginning the infusion and periodically during administration. Notify the attending physician, fellow, PA, or NP immediately if the patient exhibits signs or symptoms of a reaction.
7. Benadryl, epinephrine, and hydrocortisone should be available at the bedside for emergency use if necessary. Oxygen with nasal prongs for standby use should be present in the room.
8. If the patient is a double cord blood recipient, the two units may be given consecutively with no wait between infusions of the units**.** The start and stop time of each unit should be recorded on the infusion record.

D. Combined bone marrow and cord blood from the same HLA-matched sibling donor: At the discretion of the PI, bone marrow and cord blood cells from the same HLA–matched sibling may be used together for the transplant in cases where such cord blood cells were previously stored and found to be qualified for use as a donor for stem cell transplant.

1. The HLA-matched sibling umbilical cord blood unit will be deemed qualified for use in combination with bone marrow when the matched sibling cord blood unit meets the eligibility criteria outlined in section 6 A.
2. The HLA-matched sibling bone marrow will be infused first followed by HLA-matched sibling cord blood cells. The attending physician will determine the appropriate timing of infusion of the HLA-matched cord blood unit following completion of the HLA-matched sibling bone marrow graft. **However, infusion of the HLA-matched sibling cord blood unit will not begin until any acute toxicities from the bone marrow infusion have been controlled. In addition, the attending physician or Principal investigator may determine that infusion of the HLA-matched sibling cord blood**

is not clinically feasible or safe depending on the clinical circumstances and therefore may decide to NOT infuse the HLA-matched sibling cord blood.
The HLA-matched sibling cord blood unit must undergo rigorous donor screening and testing as well as evaluation for infectious disease markers as is required to release an unrelated donor cord blood unit for clinical use. If the related donor cord blood unit was processed and stored by a FACT accredited cord blood bank, then as standard practice, the documents verifying donor eligibility are available for review prior to shipment of the unit and will accompany the unit. If the cord blood unit was processed and stored by a bank that is NOT FACT accredited, documents regarding verification of HLA typing, cell count (including total nucleated cell count and CD34 if available), pre-cryopreservation viability, and infectious disease testing will be requested and reviewed prior to release of this cord blood unit for clinical use.

3. Procedures for infusion of the HLA-matched sibling bone marrow and HLA-matched sibling cord blood cells will follow institutional standard practice guidelines for each cell product.
4. Procedures for requesting, receiving and characterizing the cord blood unit for infusion will be according to institutional practice.
5. Pre-infusion hydration should be performed per institutional guidelines.
6. Conditioning and GVHD prophylaxis will be per regimen A.
7. Patients receiving HLA-matched sibling bone marrow and HLA-matched sibling cord blood products may have infusions that go over 2 days. Day 0 will be considered the day the 2nd product finishes and administration of methotrexate will be adjusted accordingly.
8. For FHCRC patients, the cord blood unit should be thawed and infused per FHCRC standard practice guidelines. Cord blood products should be infused without delay as soon as the product arrives on the unit. Outside centers should follow institutional standard practice guidelines.
9. The cord blood infusion should take no longer than 30 minutes. Pre-medications (if any) prior to cord blood infusion will be at the discretion of the attending. Under no circumstances is the cord blood to be irradiated. No medications or fluids should be given piggyback through the catheter lumen that is being used for cord blood infusion.
10. The product is infused via IV drip directly into the central line according to standard practice with gravity filtered tubing.
11. Vital signs should be monitored before beginning the bone marrow and cord blood infusion and periodically during administration. Notify the attending physician, fellow, PA or NP immediately if the patient exhibits signs or symptoms of a reaction.
12. Benadryl, epinephrine, and hydrocortisone should be available at the bedside for emergency use if necessary.
13. Oxygen with nasal prongs for standby use should be present in the room.

11. IMMUNOSUPPRESSION:

Regimen A: Bone Marrow or PBSC or recipients of HLA-matched sibling bone marrow combined with HLA-matched sibling cord blood

All patients who receive bone marrow or peripheral blood stem cells will receive 2 agents for GVHD prophylaxis as follows:

A. Tacrolimus

1. Tacrolimus should be initiated per Standard Practice Guidelines as an IV continuous infusion on day –1 at a dose of 0.03 mg/kg/day. Dosing should be based on adjusted body weight. If actual weight is less than adjusted body weight, dosing should be based on actual weight. The same lumen of the central catheter should be used for all tacrolimus infusions. Conversion to the oral formulation (IV:PO ratio 1:4) should be made when oral feeding is established. Oral tacrolimus is available in 0.5, 1 and 5 mg capsules and as a compounded suspension of 1 mg/mL. Oral tacrolimus should be given in two divided daily doses every 12 hours. If the patient vomits within one hour of taking the medication, repeat the dose. If vomiting persists, the medication should be given intravenously at the appropriate dose (PO:IV ratio 4:1). Beverages containing the enzyme bergamottin (grapefruit juice, Sunny Delight, Fresca and Squirt) should be avoided. If possible, oral tacrolimus should be taken on an empty stomach at a consistent time each day.

2. In the absence of GVHD, we recommend that the tacrolimus be tapered starting on day +50. In the absence of GVHD, the dose should be tapered by 5% each week for liquid and 20% each month for capsules. In the presence of GVHD, or if the patient is receiving corticosteroids or other therapy for GVHD, tacrolimus should not be tapered at day +50, and dosing should be maintained to target therapeutic levels. Long-term administration of tacrolimus will be per the recommendations of the Long-term Follow-up Department (LTFU).

3. *Tacrolimus levels*: Tacrolimus levels should be maintained in the range of 5-15 ng/ml. In patients who are also taking sirolimus, it is generally recommended that tacrolimus trough levels not exceed 10ng/ml. Tacrolimus levels will be performed weekly starting on day +2 through day +50 per Standard Practice Guidelines, see Calcineurin Inhibitor Therapy (Cyclosporine, Tacrolimus) section. To avoid contamination, all levels should be drawn from the central catheter port opposite the lumen used to infuse tacrolimus. If a tacrolimus taper is initiated at day +50, and the patient has adequate oral fluid intake and stable volume status, checking of blood levels may generally be discontinued once tacrolimus has been reduced by 25%. If a tacrolimus taper is not initiated, levels should be performed weekly through day +100. Tacrolimus levels should be performed more frequently ***1)*** when the medication is converted from oral to IV or IV to oral; ***2)*** when dose adjustments are made due to levels outside the therapeutic range; or ***3)*** if toxicity is suspected. Steady state levels will not be achieved for at least 72 hours after any change in dosing, so levels obtained earlier than this may not reflect an accurate steady state concentration.

4. Tacrolimus Toxicities:

Common, No Specific Therapy	Common, Intervention Usual	Uncommon, May Require Intervention	Uncommon or Serious, Intervention Usual
• Hypertension (controlled) • Tremor (mild) • Hyperlipidemia	• Hypertension (poorly controlled) • Tremor (severe) • Hyperkalemia • Hypomagnesemia • Elevated Creatinine • Elevated BUN	• Anorexia, nausea, vomiting • Diarrhea • Anemia • Back pain • Rash, pruritis • Atelectasis • Insomnia • Asthenia • Hyperglycemia • Hyperbilirubinemia • Elevated Transaminases	• Pleural effusion • Coma, delirium • Hemolytic Uremic Syndrome • Dysarthria • Seizures • Hallucinations

B. Methotrexate

1. Administration:

   a. In adults and children > 10 kg, methotrexate should be administered as an IV push at a dose of 15 mg/m^2 on day +1 after transplant and at 10 mg/m^2 on days +3, +6 and +11**.** Calculation of m^2 will be per Standard Practice Guidelines.

   b. Infants who weigh $\leq$ 10 kg should have methotrexate dosed on a per kilogram (actual weight) basis rather than per meter squared. Therefore, the methotrexate dose is 0.5 mg/kg on Day + 1 and 0.33 mg/kg on Days +3, +6 and +11.

   c. The first dose of methotrexate should be given approximately 24 hours after completion of the initial stem cell infusion, but no sooner than 24 hours later.

2. Toxicities: In this setting, mucositis is the primary toxicity related to methotrexate. In the setting of renal or hepatic dysfunction, where there is impaired clearance of the medication, methotrexate may contribute to delayed neutrophil engraftment. Methotrexate may cause an elevation in serum transaminase.

3. Dose adjustments - Every attempt should be made to administer methotrexate as prescribed, as dose reduction or omission may increase the risk of acute GVHD. Patients at greatest risk for methotrexate -related toxicities are those with either decreased renal function or significant fluid collection (i.e. ascites, pleural effusions, etc.), where methotrexate can accumulate and, thus, delay clearance. Renal dysfunction or third space fluid accumulation are not strict contraindications to the administration of methotrexate, although dose reduction (75–50%) and rescue with leucovorin (citrovorum factor) should be considered. Similarly, while one may want to discontinue methotrexate in patients who require intubation because of severe mucositis, generally mucositis should not be a contraindication for the use of methotrexate.

4. Methotrexate levels should be monitored in patients at risk for the development of toxicity. Acceptable methotrexate levels at 24 hours or later after the administration of

methotrexate are <0.04 x 10^{-6} molar (see **Figure 3**). If toxic levels are present, leucovorin should be given per our **Standard Practice Guidelines**. The standard dose of leucovorin is 10 mg/m^2 IV every 6 hours.

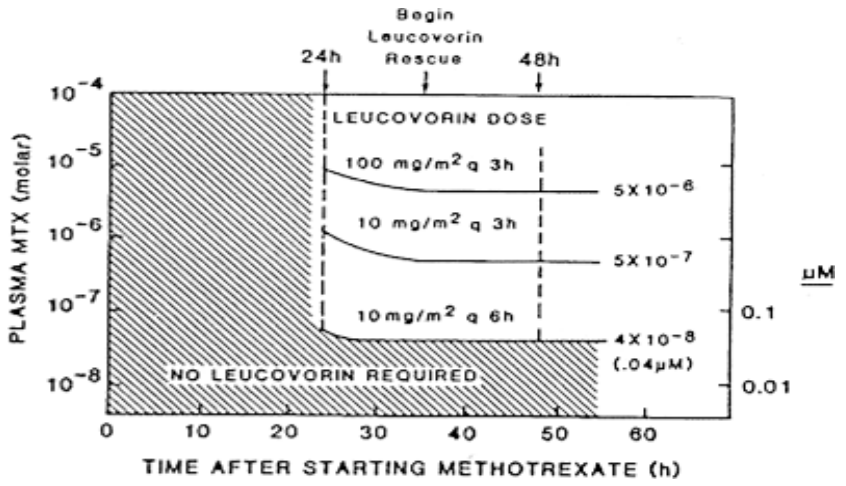

Figure 3. Methotrexate (MTX) plasma levels and leucovorin (citrovorum) "rescue".

Regimen B: Unrelated Umbilical Cord Blood:

All recipients of unrelated umbilical cord blood will receive prophylaxis for GVHD with 2 drugs as follows:

A. Cyclosporine A

1. Patients will receive cyclosporine A (CSA) therapy beginning on Day -3 maintaining a trough level between 200 and 400 ng/mL by HPLC analysis (250 and 500 ng/ml by immunoassay). For adults the initial dose will be 2.5 mg/kg IV over 1 hour every 12 hours. For children < 40 kg the initial dose will be 2.5 mg/kg IV over 1 hour every 8 hours. The initial dose will be based on adjusted body weight.
2. Dose adjustments will be made on the basis of toxicity and low CSA levels with trough level of <200 mg/L (HPLC). Once the patient can tolerate oral medications, CSA will be converted to an oral form. CSA dosing will be monitored and altered as clinically appropriate.
3. Patients will receive CSA until Day +100. If there is no evidence of GVHD, the dose will then be tapered 10% per week starting on Day +101 and discontinued no sooner than 6 months post-transplant.
4. Drugs that may affect CSP levels are:

Decrease CSP levels	Increase CSP levels	
Phenytoin	Erythromycin	Caspofungin
Phenobarbital	Ketoconazole	Azithromycin
Carbamazepine	Acetazolamide	Diltiazem
Primidone	Fluconazole*	Clarithromycin
Rifampicin	Colchicine	Verapamil
Nafcillin	Itraconazole*	Doxycycline
Octreotide	Fluoroquinolones	Nicardipine
Sulfonamides	Voriconazole	Nifedipine
Trimethoprim	Imipenem	Alcohol
Metoclopramide		
*Discontinuation of fluconazole or itraconazole may lower CSP levels, and if used for antifungal prophylaxis, then changes in these drugs should be avoided during the first 2 months post-transplant.		

B. Mycophenolate mofetil (MMF)

1. MMF will be administered at 15 mg/kg every 8 hours with a maximum of 1 gram/dose beginning the evening of day 0 (i.e. first dose to follow 4-6 hours after UCBT). The dose will be based on adjusted body weight.
2. MMF will be given every 8 hrs daily until day 40 post-transplant and then in the absence of GVHD, tapered by 12%/week with MMF discontinued after day + 96.
3. Markedly low (<40%) donor T cell chimerism after UCBT may indicate impending graft rejection. MMF should be continued at full dose or, if MMF taper has been initiated, reinstitution of full dose MMF should occur. If MMF has been discontinued, MMF should be reinitiated at full dose.

12. SUPPORTIVE CARE

All supportive care will be provided per each center's standard practice guidelines and will be dictated by the physician and transplant team caring for the patient. There are no restrictions or limitations to supportive care measures. Some Fred Hutch guidelines are included in **Appendix B**.

13. POST–HCT GROWTH FACTORS

Regimen A: Bone Marrow or PBSC -
Patients who receive bone marrow or PBSC should in general not receive post-transplant growth factors during the first 3 weeks after HCT. Growth factors should not be given unless neutropenia develops or persists past day 21 post-transplant (ANC <500/μL).

Regimen B: Unrelated Umbilical Cord Blood –
Patients who receive unrelated umbilical cord grafts will be started on G-CSF support at 5mcg/kg (IV/SQ; round to vial size) daily starting day +1 following unrelated umbilical cord blood infusion until ANC > 2500/μL for 2 consecutive days. Once a patient has met these criteria, the ANC will be monitored and G-CSF restarted if ANC falls to < 1000/μL.

Note: We will not give G-CSF to those patients who receive both bone marrow and cord blood from an HLA-matched sibling donor.

14. PATIENT AND DONOR CLINICAL AND LABORATORY EVALUATIONS

14A. Donor Evaluations: Please refer to the instructions below and Standard Practice Manual guidelines or institutional guidelines:

1. HLA-matched related bone marrow or peripheral blood donor:
Related donors will undergo standard evaluation for allogeneic stem cell donation, which should include but not limited to:

a. Complete history and physical examination to include the following:
- i) Medical problems, including pulmonary and upper airway disease, cardiovascular disease, diabetes, arthritis, or abnormalities of the spine.
- ii) Last menstrual period
- iii) Previous exposure to anesthetics, and family history of anesthesia-associated complications
- iv) Blood transfusions
- v) Medications
- vi) Vaccinations
- vii) Allergies
- viii) Height and weight

b. Lab tests:
- i) CBC with differential including platelet counts.
- ii) Serum sodium, potassium, Chloride, CO2, Glucose, Bun, Creatinine, Calcium, Magnesium, Phosphorus, Uric acid, LDH, alkaline phosphatase, Total bilirubin, AST, ALT and albumin
- iii) Hepatitis screen, CMV, syphilis, HIV and HTLV I serologies (tests must be completed within roughly 30 days of transplantation)
- iv) ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic cross match between patient and donor (HLA Laboratory) will be performed.
- v) Female donors of menstruation age: serum HCG
- vi) HLA typing - See Standard Practice Manual
  - A) Serotyping (HLA-A, B, C) and DNA typing (HLA-A, B, C, DRB1, DQB1 of patient and donor.
  - B) Leukocyte and/or florescence activated cell sorter cross match between the patient and donor.
- vii) Sickle cell analysis for any patients of African-American ethnicity per standard practice

c. The donor will be reevaluated with a directed history and physical examination the day after the bone marrow harvest is completed.

d. Attainment of 10 cc heparinized blood sample for subsequent determination of donor chimerism to the cytogenetics lab or the Clinical Immunogenetics Lab as outlined in section 14B3k.

2. HLA-matched unrelated bone marrow or PBSC donor:
Unrelated donors will undergo evaluation for allogeneic hematopoietic cell donation at the collection center by NMDP standard. The attending physician of the collection center will review the results of the donor evaluation. Evaluations typically include:
a. Complete history and physical examination.
b. Lab tests:
i) CBC with differential and platelet counts
ii) Serum sodium, potassium, Chloride, CO2, Glucose, Bun, Creatinine, Calcium, Magnesium, Phosphorus, Uric acid, LDH, alkaline phosphatase, Total bilirubin,

AST, ALT and albumin
iii) Hepatitis screen, CMV, syphilis, HIV and HTLV I serologies
iv) ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic cross match between patient and donor (HLA Laboratory) will be performed.
c. The donor will be reevaluated the day after the marrow aspiration. CBC with platelet count should be checked.

14B. Recommended Patient Evaluation:

Refer to FHCRC/SCCA Standard Practice Manual for Pre-Transplant Evaluation Guidelines for Allogeneic Transplant as clinically indicated (results of tests and/or procedures conducted as per standard of care for pre-transplant workups may be used for eligibility determination if conducted within an appropriate window prior to screening)

Recommended Pre-transplant Baseline Evaluation

1. History: A complete history (approximately 1 month prior to conditioning) with full details of the patient's prior treatment and response including the following:
a) Hematologic findings at diagnosis (including biochemical markers, cytogenetic and molecular markers, disease process, and immunologic criteria)
b) Transfusion history (including type of donor, i.e. random or family donor)
c) Current medical problems
d) Current medications
e) Female patients who have gone through puberty – pregnancy history, menstrual history, and date of last sexual intercourse
f) Karnofsky/ Lansky scoring (Appendix A)
g) Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) scoring (Appendix H)

2. Physical Exam: Careful physical examination

3. Laboratory evaluation (Approximately 1 month prior to conditioning)**:**
a) CBC with differential
b) Reticulocyte count if clinically indicated
c) BUN/Creatinine
d) Total bilirubin, fractionated bilirubin, alkaline phosphatase, AST, ALT, GGT
e) ABO/Rh typing, direct coombs
f) Quantitative serum immunoglobulins
g) Ferritin – for patients with a history of transfusions, bone marrow failure, or hemoglobinopathies
h) Pre-transplant viral testing:

Pre Transplant Viral Testing	All 2256 Patients	Patients with Primary Immune Deficiency Disorders	Patients with Antibody Deficiencies or on IVIG		If Clinically Indicated
Hepatitis Screen	X				
CMV Serology	X				
VZV Serology	X				
HSV Serology	X				
Toxoplasma Serology	X				
Anti HIV Serology	X				
Hepatitis B PCR			X		
Hepatitis C PCR			X		
HIV PCR or p24 antigen testing			X		
CMV PCR	X*				
EBV PCR		X*			X*
Adenovirus PCR		X*			X*
VZV PCR					X
HSV PCR					X
Toxoplasma PCR					X

*Approximately 1 month prior to conditioning

i) Urine analysis
j) As a pre-transplant reference for subsequent determination of donor chimerism 10 cc of heparinized peripheral blood from the patient (and the donor) will be drawn. For FHCRC patients: Send to Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Outside institutions may use VNTR analysis (sex-matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).

4. See Recommended Disease Staging Tables for further evaluations

5. Radiological evaluation: The following studies should be obtained prior to the start of conditioning.

a) CXR-PA and lateral view (Other x-rays as clinically indicated) within approximately 1 month of HCT or as clinically indicated. If a CT scan of the chest has been done a CXR is not required.
b) CT scan of the chest, abdomen and pelvis within approximately 1 month of HCT if clinically indicated
c) EKG within approximately 3 months of HCT. We recommend that all patients with Shwachman Diamond Syndrome have an EKG within approximately 1 month of HCT.
d) Echo or MUGA for calculation of cardiac ejection fraction within approximately 3 months of HCT. If unable to obtain ejection fraction, then please obtain shortening fraction calculation. We recommend that all patients with Shwachman Diamond Syndrome have an ECHO or MUGA within approximately 1 month of HCT.
e) Pulmonary function tests if clinically indicated within approximately 1 month of HCT if older than age 6 and able to have pulmonary function testing done: This is particularly relevant for patients with Dyskeratosis Congenita and Shwachman Diamond Syndrome.
f) Renal Ultrasound: We recommend that all patients with Dyskeratosis Congenita and Shwachman Diamond Syndrome have had a renal ultrasound pre HCT if this has not already been performed.

6. Bone marrow evaluation:
A bone marrow aspirate and biopsy should be obtained within approximately 4 weeks of start of transplant conditioning for morphology, flow cytometry, and cytogenetics unless clinical concerns. Patients with bone marrow failure disorders should also have the MDS FISH panel sent on their bone marrow in addition to morphology, flow cytometry, and cytogenetics. If a patient's transplant is delayed, the attending should discuss with the PI as to whether a bone marrow aspirate needs to be repeated.

7. Pharmacokinetic (PK) evaluation:
PK sampling and analysis will be done on days -6 (all patients) and -5 (patients aged <4 years at start of conditioning) in patients who agree to participate in the PK research studies. Same sample will be used for both treosulfan and treosulfan monoepoxide quantitation (samples will be split in PK Lab during analysis process).

a. Day -6, n=7 samples (all patients), with window as noted below
   1. Pre-infusion (within 10 minutes before starting treosulfan infusion)
   2. End-of-infusion (window: end of infusion +5 minutes)
   3. End-of-infusion + 20 minutes (window: +/- 5 minutes)
   4. End-of-infusion + 40 minutes (window: +/- 5 minutes)
   5. 3.5 hours after start of infusion (window: +/- 10 minutes)
   6. 6 hours after start of infusion (window: +/- 10 minutes)
   7. 9 hours after start of infusion (window: +/- 10 minutes)

b. Day -5, n=7 samples (only for patients < 4 years of age at the time of start of conditioning), with window as noted below
   8. Pre-infusion (within 10 minutes before starting treosulfan infusion)
   9. End-of-infusion (window: end of infusion +5 minutes)
   10. End-of-infusion + 20 minutes (window: +/- 5 minutes)
   11. End-of-infusion + 40 minutes (window: +/- 5 minutes)
   12. 3.5 hours after start of infusion (window: +/- 10 minutes)
   13. 6 hours after start of infusion (window: +/- 10 minutes)
   14. 9 hours after start of infusion (window: +/- 10 minutes)

Recommended Post HCT Evaluation:

Once patients are discharged, they should be evaluated at least twice weekly in the outpatient department until day 28 and then weekly. Patients with GVHD or graft rejection should have more frequent follow up. Please refer to instructions below and in the Standard Practice Manual for guidelines regarding evaluation of patients following HCT.

Note: We recommend the following studies be done following HCT and would optimally like all Day + 28, 84, etc. evaluations to be performed. This is a recommended evaluation schedule. The clinical team can decide to not do these studies if clinically indicated.

1. History and physical exam:
a) Once outpatient: clinic evaluation twice weekly until day 28, then weekly. More frequent evaluations are indicated for patients with signs of GVHD or graft failure.
b) Weekly weights

2. Laboratory Evaluation:
a) **CBC daily from day 0 until ANC > 500/uL for 3 days after nadir reached.** Thereafter, a CBC should be checked three times a week until day +28. After day +28, a CBC should be checked twice weekly until 2 months post-transplant and later if clinically indicated. Daily platelet counts if the platelet count is <20,000/μl.

b) **Electrolyte panel and serum BUN/creatinine** should be checked 3 times a week until day 28 and then twice weekly while on full dose Cyclosporine/Tacrolimus or per attending until Cyclosporine/Tacrolimus is discontinued.
c) **Calcium, magnesium, phosphate** should be checked twice weekly until day 28 and then weekly.
d) **Serum albumin** should be checked weekly.
e) **Hepatic function** including AST, ALT, alkaline phosphatase, and total and direct bilirubin should be checked two times a week until day +28 and then every week.
f) **Patients on steroids** should have surveillance blood cultures checked two times a week until steroids have been tapered to less than 0.5 mg/kg/day
g) **Iron studies:** Ferritin level, binding capacity including serum iron, total iron binding capacity, direct and % transferring saturation checked around Day + 84, and 365 and yearly x 5 years as clinically indicated.
h) **Quantitative Immunoglobulins** per standard practice.
i) Infection monitoring per institutional standard practice, although the following is recommended.

i. CMV surveillance:

	CMV status pre HCT	**Post HCT CMV PCR monitoring (All patients due to ATG administration and per standard practice)**
Bone Marrow Recipients **+** ***Peripheral Blood Recipients***	Recipient and donor CMV Negative (-) pre HCT	CMV PCR weekly up to 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter
	Recipient and/or donor CMV Positive (+) pre HCT	CMV PCR weekly until off ISP. However, if off ISP prior to 6 months post-transplant then continue CMV PCR weekly monitoring until 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter
	Any patient treated for CMV before day 100	CMV PCR weekly until off ISP. However, if off ISP prior to 6 months post-transplant then continue CMV PCR weekly monitoring until 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter
Cord Blood Recipients	Recipient CMV Negative (-)	CMV PCR weekly up to 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter
	Recipient CMV Positive (+)	CMV PCR Day 0-100 twice weekly, Day 101 - 1 year once weekly

For those patients receiving both bone marrow and cord blood, we recommend CMV monitoring according to institutional standard practice for bone marrow recipients or for bone marrow recipients as detailed above.

ii. Adenovirus surveillance:

Post HCT adenovirus PCR monitoring (all patients due to ATG administration)
Adenovirus PCR weekly up to 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter

iii. **EBV lymphoproliferative syndrome surveillance:** See section 16. EBV Monitoring and Treatment for details on treatment of EBV reactivation.

	Post HCT EBV PCR monitoring (all patients due to ATG administration)
If stable copies by PCR	EBV PCR weekly up to 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter
If rising copies by PCR	EBV PCR twice weekly up to 6 months post last ATG dose, or Absolute lymphocyte count >300 cells/microliter

j) Patients should be assessed for the need of IVIG monitoring and replacement therapy per Institutional Guidelines

For FHCRC patients: blood to clinical flow lab for sorting, then to the chimerism lab for quantifying of peripheral blood. Send to Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor:

Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).

3. Bone marrow evaluation:
All patients should have a bone marrow aspiration and biopsy for morphology, flow cytometry, and cytogenetics (and FISH if clinically indicated) on approximately day +84 and 1 year post-transplant.

4. See Recommended Disease Staging Tables for further evaluation

5. Radiology evaluation: Two view chest x-ray (PA and lateral) as clinically indicated

6. Additional evaluation at day 80-100:
Please refer to instructions below and Standard Practice Manual Guidelines or institutional guidelines.
a. A patient with an uncomplicated HCT would be discharged after the day +80-100 workup and screening for chronic GVHD are completed and analyzed. Since the patient may be discharged prior to starting the Tacrolimus and MMF taper, instructions should be provided for preventing and detecting GVHD as per standard practice of collaborating institution.

GVHD evaluation guidelines are as follows (also see **Appendix C & Appendix D)**:

a) History and physical exam
b) CBC, serum IgG, total bilirubin, alkaline phosphatase, ALT and AST
c) GVHD evaluation as directed by the attending physician
d) Pulmonary function test (if old enough to perform this test)
e) Oral medicine evaluation
f) Dietitian assessment
g) Gynecological assessment (adult female)
h) Staging workup, as indicated by staging table and text

7. Additional evaluation guidelines following 100 Days post HCT

a) First month – weekly evaluations by referring physician, including physical examination and weights (children <17 years – record height and weight every 3 months
b) Weekly - CBC, BUN, creatinine, ALT, AST, Alkaline phosphatase, Total bilirubin and direct bilirubin. If stable, monthly evaluations as above for 2 years, then annually
c) Following discharge from the transplant center, we would like to track the patient's medical condition for the rest of their life in conjunction with their primary care physician and the guidance of the Long-Term Follow-Up department.

RECOMMENDED DISEASE STAGING TABLES

Timing of studies: Post-transplant time points represent guidelines for recommended evaluations. Due to numerous factors influencing scheduling (patient and provider availability, testing services limitation, as well as other factors) variation in evaluation performance dates is anticipated and acceptable to the protocol. The following is the recommendation; however, as stated above, we recognize that patients may follow up outside these windows (e.g., within +/- 7 days of time points < Day 100; +/- 30 days for time points > Day 100 to 1 year and +/- 3 months for time points > 1 year).

ALL DISEASES

	Pre	Day -6	Day -5	Day +28	Day +56	Day +84	Day +180	Day +365	18 months	24 months	Years 3-5
History and physical exam[1]	X[2]										
Laboratory Evaluation (See baseline and post-HCT Laboratory sections)	X										
Reference for subsequent determination of donor chimerism[3]	X										
BMA w/ pathology, flow cytometry, cytogenetics, and FISH (see baseline and post-HCT bone marrow sections)	X[4]					X[5]		X[5]			
PB chimerism[6]	X[7]			X[8]		X[8, 9]	X[8, 9]	X[8, 9]	X[8, 9]	X[8, 9]	X[10]
Radiological evaluation (see baseline and post-HCT radiological evaluation sections)	X										
Iron Studies (Ferritin, Iron, Total Iron Binding Capacity, Transferrin, Transferrin Saturation)	X[11]					X		X		X	X
Optional Research: Treosulfan PK Evaluations		X[12]	X[13]								

[1] Once outpatient, twice weekly until day +28, then weekly. More frequent evaluations are indicated for patients with signs of GVHD or graft failure. Weekly weights.

[2] Including hematologic findings at diagnosis (including biochemical markers, cytogenetic and molecular markers, disease process, and immunologic criteria), transfusion history (including type of donor, i.e. random or family donor), current medical problems, current medications, and for female patients who have gone through puberty – pregnancy history, menstrual history, and date of last sexual intercourse

[3] 10 cc of heparinized peripheral blood from the patient (and the donor) will be drawn. For FHCRC patients: Send to Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).

[4] A bone marrow aspirate should be obtained within approximately 4 weeks of start of transplant conditioning for morphology, flow cytometry, cytogenetics unless clinical concerns. Patients with bone marrow failure disorders should have the MDS FISH panel sent on their bone marrow in addition to morphology, flow cytometry, and cytogenetics. If a patient's transplant is delayed, the attending should discuss with the PI as to whether a bone marrow aspirate needs to be repeated

[5] All patients should have a bone marrow aspiration and biopsy for morphology, flow cytometry, and cytogenetics (and FISH if clinically indicated) on approximately day +84 and 1 year post-transplant.

[6] For FHCRC patients: blood to clinical flow lab for sorting, then to chimerism lab for quantifying of peripheral blood. Send to Clinical Immunogenetics Lab (206-667-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor:

Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).

[7] For DNA storage only

[8] CD3, CD33, CD56

[9] CD19 recommended for PID patients or as clinically indicated

[10] CD3 and CD33 only. If mixed chimerism present at previous timepoint then CD56 recommended. For PID patients CD19 recommended if mixed chimerism present at previous timepoint or as clinically indicated.

[11] We recommend that patients with a history of transfusions, bone marrow failure disorders, or hemoglobinopathies have a ferritin checked pre-transplant.

[12] 1 cc of blood will be drawn at 7 timepoints for treosulfan PK evaluations. PK sampling kit will be provided by SCCA Pharmacokinetics Lab, 206-606-7389 (after hours 206-994-5942): All patients

[13] 1 cc of blood will be drawn at 7 timepoints for treosulfan PK evaluations. PK sampling kit will be provided by SCCA Pharmacokinetics Lab, 206-606-7389 (after hours 206-994-5942): only in patients who are < 4 years of age at time of start of conditioning.

Primary Immunodeficiency Diseases

The following tests are recommended for all primary immunodeficiency diseases						
	Pre	**Day +28**	**Day +56**	**Day +84**	**Day +365**	**Yearly x5**
Consultation with Immunologist (recommend)	X[1]			X	X	X[11]
Consultation with Infectious Disease (recommend)[12]	X[1]					
Lymphocyte (phenotype) subsets[3, 13]	X[1]			X	X	X[2, 5]
Lymphocyte function analysis[4, 13]	X[1]			X	X	X[2, 5]
Quantitative Immunoglobulins (Serum troughs)	X	X	X	X	X	X[2]
Disease Specific Tests: The following tests are only recommended if the patient has the specific primary immunodeficiency disease listed below:						
Chronic Granulomatous Disease						
• Neutrophil Oxidative Burst[6]	X[1]			X[2]	X[2]	[5]
• Genetic analysis of cytosolic components of NADPH oxidase system[7]	X[1]					
Leukocyte Adhesion defect						
• Analysis of CD 18 expression[8]	X[1]			X[2]	X[2]	[5]
Wiskott-Aldrich Syndrome						
• Gene sequencing[1, 9]	X[1]					
• Determination of WASP expression by flow cytometry[10]	X[1]			X	X	[5]
IPEX						
• Determination of FOXP3 expression by flow cytometry[10]	X[1]			X[2]	X[2]	[5]
CD40LD						
• Determination of CD40 ligand expression by flow cytometry[10]	X[1]			X[2]	X[2]	[5]

[1] If not already completed pre-conditioning
[2] As clinically indicated
[3] 2-3 ml ETDA Seattle Children's - Cell Marker Lab (206) 987-2560 for flow cytometry to determine number of CD3, CD4, CD8, CD19/20, and CD16/56 cells. For patients <20 kg where blood volume is concern, please call Cell Marker lab for recommendations. Of note, patients with chronic granulomatous disease and leukocyte adhesion deficiency should not have lymphocyte phenotype testing.
[4] 10 ml in Na Heparin; to Seattle Children's Cell Marker Lab (206) 987-2560 for proliferation to mitogens (PHA & CD3 only) testing. For patients <20 kg where blood volume is concern, please call Cell Marker lab for recommendations. For outside centers send to appropriate lab. Of note, patients with chronic granulomatous disease, leukocyte adhesion deficiency, and HLH should not have lymphocyte function

testing.
[5] If most recent result abnormal
[6] 1-2 ml in heparin to Seattle Children's Cell Marker Lab (206-987-2560) or appropriate outside lab
[7] 5 ml blood in EDTA (purple top) to Genedx (www.genedx.com)
[8] 5ml in heparin to Seattle Children's Cell Marker Lab
[9] Send 10mL blood in EDTA tube to Immunology Diagnostic Laboratory Seattle Children's Research Institute 1900 9th Avenue Seattle 98101
[10] Send 5-10 ml (green top) to Immunology Diagnostics Laboratory, Seattle Children's Research Institute 1900 9th Avenue Seattle 98101
[11] As directed by immunology
[12] If the infectious disease team determines that a formal consult is not needed, an infectious disease consult does not need to occur.
[13] If blood volume is an issue, the priority for tests is as follows (we recommend that these blood draws be spaced out during the pre-transplant evaluation period. If not all tests are able to be performed due to issues regarding blood volume contact Dr. Burroughs at (206) 667-2396)
1. Lymphocyte phenotype
2. Lymphocyte function

Marrow Failure Syndromes

The following tests are recommended for all marrow failure syndromes						
	Pre	**Day +28**	**Day +56**	**Day +84**	**Day +365**	**Yearly x5**
• Quantitative Immunoglobulins	X[2]	X	X	X	X	X[1]
• Consultation with Marrow Failure Specialist/Hematology (recommend)	X[2]			X	X	
• Appropriate genetic testing	X[2]					
Disease Specific Tests: The following tests are only recommended if the patient has the specific marrow failure syndrome listed below:						
Dyskeratosis Congenita						
• Telomere length analysis	X[2]					

[1] If clinically indicated
[2] If not already completed pre-conditioning

Other Diseases

	Pre	Day +28	Day +56	Day +84	Day +365	Yearly X5
Mucopolysaccharidoses						
• Disease specific enzyme level	X[2]	X	X	X	X	X
• Consultation with Pediatric Specialist in Metabolic Disorders	X[2]			X	X	X
• Marrow, skin, liver, and/or gut biopsy as indicated	X[2]			X	X	
• MRI of Head if indicated	X[2]			X	X	
• LP for storage determination if indicated	X[2]			X	X	
Sphingolipidoses						
• Consultation with Pediatric Specialist in Metabolic Disorders	X[2]			X	X	X
• Nerve Conduction studies	X[2]				X	
• Consultation with Pediatric Neurologist	X[2]			X	X	X
• MRI of Head	X[2]	X	X	X	X	X
Osteopetrosis						
• Bone biopsy for pathology[1]	X[2]			X	X	X
• Head MRI	X[2]			X	X	X
Hemophagocytosis Lymphohistiocytosis (HLH)						
• Lymphocyte phenotype (subsets)[5]	X[2]			X	X	X[3]
• Soluble Interleukin-2 Receptor/ Interleukin 2	X[2]			X	X	
Sickle Cell Disease						
• % Hemoglobin S	X[6]			X	X	
Other Diseases						
• Disease specific markers[4]	X[2]					

[1] For FHCRC patients send to CHMC pathology.
[2] If not already completed pre conditioning
[3] If abnormal at the previous interval or if clinically indicated.
[4] Send after consultation with a specialist in the disorder
[5] For FHCRC patients, send 2-3 ml in EDTA to Seattle Children's Cell Marker Lab (206) 987-2560 for flow cytometry to determine number of CD3, CD4, CD8, CD19/20, and CD16/56 cells. For patients <20 kg where blood volume is a concern, please call Seattle Children's Cell Marker Lab for recommendations regarding the total blood volume needed to complete this study.
[6] On arrival and then within 1 week prior to start of conditioning.

15. DRUGS AND GRAFT ADMINISTRATION - TOXICITIES AND COMPLICATIONS.

15A. Treosulfan

Treosulfan Toxicities in Adults			
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon (0.1% - 0.9%)**	**Rare/unknown frequency**
Infections (bacterial, viral, fungal)	Sepsis, septic shock	Hyperglycemia	Treatment-related second malignancy
Pancytopenia	Hypersensitivity	Confusional state	Acidosis
Febrile neutropenia	Decreased appetite		Glucose tolerance impaired
Leukopenia	Insomnia	Peripheral sensory neuropathy	Electrolyte imbalance
Neutropenia	Headache	Hematoma	Agitation
Thrombocytopenia	Dizziness	Hypotension	Encephalopathy
Anemia	Cardiac arrhythmias (atrial fibrillation, sinus tachycardia)	Pneumonitis	Intracranial hemorrhage
Stomatitis/mucositis	Hypertension	Pleural effusion	Extrapyramidal disorder
Diarrhea	Flushing	Pharyngeal, lung, or laryngeal inflammation	Syncope
Nausea	Dyspnea	Cough	Paresthesia
Vomiting	Epistaxis	Laryngeal pain	Dry eye
Abdominal pain	Oral pain	Hiccups	Cardiac arrest
Asthenic conditions (fatigue, asthenia, lethargy)	Gastritis	Mouth hemorrhage	Cardiac failure
Bilirubin increased	Dyspepsia	Abdominal distension	Myocardial infarction
	Constipation	Esophageal or gastrointestinal pain	Pericardial effusion
	Dysphagia	Dry mouth	Embolism
	Maculo-papular rash	Veno-occlusive liver disease	Hemorrhage
	Purpura	Hepatotoxicity	Oropharyngeal pain
	Erythema	Erythema multiforme	Hypoxia
	Palmar-plantar erythrodysesthesia syndrome	Dermatitis acneiform	Dysphonia
	Pruritus	Rash	Gastrointestinal hemorrhage
	Alopecia	Hyperhidrosis	Neutropenic colitis
	Pain in extremities	Non-cardiac chest pain	Esophagitis
	Back pain	Pain	Anal inflammation
	Bone pain	Pulmonary hemorrhage	Mouth ulceration
	Arthralgia		Hepatic failure
	Myalgia		Hepatomegaly
	Acute kidney injury		Hepatic pain
	Hematuria		Generalized erythema

Treosulfan Toxicities in Adults			
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon (0.1% - 0.9%)**	**Rare/unknown frequency**
	Edema		Dermatitis
	Pyrexia		Skin necrosis or ulcer
	Chills		Skin hyperpigmentation
	ALT increased		Dry skin
	AST increased		Muscular weakness
	GGT increased		Renal failure
	Blood alkaline phosphatase increased		Cystitis
	CRP increased		Dysuria
	Weight decreased		Injection site reaction
	Weight increased		Feeling cold
			Blood creatinine increased
			Blood LDH increased

Treosulfan Toxicities in Children		
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon/rare/unknown frequency**
Infections (bacterial, viral, fungal)	Oropharyngeal pain	Treatment-related second malignancy
Pancytopenia	Epistaxis	Febrile neutropenia
Leukopenia	Dysphagia	Alkalosis
Neutropenia	Oral pain	Electrolyte imbalance
Thrombocytopenia	Dermatitis exfoliative	Hypomagnesemia
Anemia	Maculopapular rash	Headache
Stomatitis/mucositis	Rash	Paresthesia
Diarrhea	Erythema	Seizure
Nausea	Pain of skin	Conjunctival hemorrhage
Vomiting	Skin hyperpigmentation	Dry eye
Abdominal pain	Alopecia	Capillary leak syndrome
Pruritus	Transaminases (ALT/AST) increased	Hypertension
Pyrexia	Bilirubin increased	Hypotension
	Abnormal creatinine	Hypoxia
	Edema	Neutropenic colitis
	Abnormal heartbeat, heart failure	Anal inflammation
		Dyspepsia
		Proctitis
		Gastrointestinal pain
		Constipation
		Veno-occlusive liver disease
		Hepatomegaly
		Hepatotoxicity
		Skin ulcer
		Erythema multiforme
		Urticaria
		Dermatitis bullous
		Dermatitis acneiform
		Palmar-plantar erythrodysesthesia syndrome

Treosulfan Toxicities in Children		
Very Common (10% or Greater)	**Common (1% - 9%)**	**Uncommon/rare/unknown frequency**
		Dermatitis diaper
		Pain in extremities
		Acute kidney injury
		Renal failure
		Noninfective cystitis
		Scrotal erythema
		Chills
		Fatigue
		Pain
		GGT increased
		Pulmonary hemorrhage
		Pericardial effusion
		Pleural effusion
		Cerebral hemorrhage
		Lung failure or pneumonia

Treosulfan may affect fertility in men and women.

15B. Fludarabine

1. Constitutional: Fever/chills, malaise, headache, myalgia, myelosuppression, immunosuppression, reduced performance status, allergic reactions (rare).
2. Cardiovascular: Hypotension, cardiac arrhythmia, cardiomyopathy, cardiac failure (all rare).
3. Dermatologic: Alopecia, rash.
4. Gastro-intestinal: Mucositis, anorexia, nausea, vomiting, diarrhea, GI bleeding (rare).
5. Hematologic/Immunologic: Myelosuppression, immunosuppression.
6. Hepatic: Elevation of liver transaminases and/or bilirubin; rarely ascites.
7. Neurologic: Paresthesia, weakness or CNS demyelination (all rare).
8. Ophthalmologic: Blurred vision, diplopia, photophobia (all rare).
9. Pulmonary: Dyspnea or pneumonitis (all rare).

15C. Rabbit Anti-Thymocyte Globulin (Thymoglobulin®)

1. Chills and Fever
   Chills and fever commonly occur in patients receiving Thymoglobulin®. Minor toxicities can usually be managed by symptomatic treatment and temporary slowing of the infusion. If during infusion the patient develops fever or chills or both, the patient should be medicated with diphenhydramine 25-50 mg I.V. (pediatric 1 mg/kg) and an antipyretic, e.g. acetaminophen, pediatric 10-15 mg/kg/dose, max dose 650mg orally every 4 hours as needed. For severe fever and chills, meperidine 25-50 mg (pediatric 1 mg/kg) I.V. every 4-6 hours as need may also be required.

2. Pruritus
   Itching and erythema occasionally develop. Symptoms are generally controlled with diphenhydramine.

3. Respiratory Distress
   Respiratory distress may be a sign of significant allergic reaction or anaphylaxis. Infusion should be discontinued. If reaction persists, diphenhydramine, epinephrine or hydrocortisone or all three should be administered.

4. Hypotension
   Hypotension occurs rarely and may be a sign of significant allergic reaction or anaphylaxis. Discontinue infusion and treat accordingly.

5. Serum Sickness
   More common with ATGAM®, it is a syndrome of fever, arthralgias, and rash. Usually occurs with some delay after initial ATG administration. Treat with steroids.

6. Leukopenia, Neutropenia, or Thrombocytopenia
   Neutropenia and thrombocytopenia may initially occur. Leukopenia is the expected consequence of ATG, ATGAM® therapy.

7. Other
   Pain in chest, flank or back may be a sign of significant allergic reaction or anaphylaxis or hemolysis. Infusion should be discontinued. Further work up and therapy as clinically indicated.

15D. TBI – Regimen B only Unrelated Cord blood grafts

TBI given at high doses in conventional transplants may cause nausea, vomiting, diarrhea, temporary hair loss, and painful swelling of the salivary glands for a few days. TBI may destroy normal bone marrow cells in addition to the cancer cells. *The doses of TBI (200 cGy and 300 cGy) used in this protocol is about one-sixth and one-fourth, respectively, of that used in conventional transplant protocols, and severe acute side effects have so far not been observed. TBI has been associated with causing sterility and there is a risk of major genetic damage to any children conceived after transplantation. There is a risk that a small percentage of patients may develop a secondary cancer resulting from this treatment.

15E. Toxicities associated with marrow/PBSC infusion

The following are potential side effects associated with the transplant in general:

1. Constitutional: Fever/chills, malaise, fatigue, headache, pain, weakness.
2. Cardiovascular: Edema, cardiac dysrhythmia, hypertension, thrombosis, cardiac failure.
3. Dermatologic: Alopecia, rash, pruritus, skin depigmentation or hypopigmentation, photosensitivity.
4. Gastro-intestinal: Anorexia, nausea, vomiting, diarrhea, mucositis, dry mouth.
5. Hematologic/Immunologic: Myelosuppression, immunosuppression, infections/sepsis, hemorrhage, acute and chronic GVHD, graft failure.
6. Hepatic: Elevation of liver transaminases and/or bilirubin, VOD, hepatic failure.
7. Metabolic: Electrolyte abnormalities, hyperglycemia.
8. Neurologic: Dizziness, confusion, anxiety, depression; rarely seizures, encephalopathy, demyelination.
9. Pulmonary: Cough, dyspnea, interstitial pneumonitis.
10. Renal/Bladder: Elevation of BUN and/or creatinine, hemorrhagic cystitis, renal failure.

15F. <u>Toxicities associated with umbilical cord blood infusion (both related and unrelated)</u>

Potential toxicities associated with the infusion include DMSO toxicity and side effects from red cells. DMSO toxicity and side effect of red cells may include changes in heart rate or rhythm, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, headache, allergic reaction, presence of DMSO taste and odor, hemoglobinuria, and acute renal failure.

15G. <u>Toxicities associated with immunosuppression</u>

Regimen A: bone marrow or peripheral blood recipients or recipients of HLA-matched sibling bone marrow combined with HLA-matched sibling cord blood:

1. Tacrolimus

Side effects are generally reversible and may include:

- **a)** Renal – **Rise in serum creatinine, hemolytic uremic syndrome.**
- **b)** Neurological – **Peripheral: paresthesia, tremor. Central: seizures, headache, insomnia, dizziness, depression, confusion, hallucinations, psychosis, myoclonus, neuropathy, agitation.**
- **c)** Gastrointestinal – **Nausea, vomiting, anorexia, constipation, diarrhea.**
- **d)** Cardiovascular – **Hypertension, myocardial hypertrophy.**
- **e)** Endocrine – **Hyperglycemia, hyper/hypokalemia, hypophosphatemia, hypomagnesemia.**
- **f)** Integument – **Itching, rash.**
- **g)** Hematologic – **Leukocytosis, thrombocytopenia, leukopenia, anemia, PTLD, thrombotic microangiopathy.**
- **h)** Liver – **Abnormal liver function tests.**
- **i)** Ocular – **Blurred vision, photophobia.**
- **j)** **Respiratory –** Pleural effusion, atelectasis, cough, dyspnea.
- **k)** **Musculoskeletal –** Arthralgias**.**

2. Methotrexate

Toxicities: In this setting, mucositis is the primary toxicity related to methotrexate. In the setting of renal or hepatic dysfunction, where there is impaired clearance of the medication, methotrexate may contribute to delayed neutrophil engraftment. Methotrexate may cause an elevation in serum transaminase.

Regimen B: Unrelated umbilical cord blood recipients

3. Cyclosporine

See section 11 for information about administration and dosage adjustments. Side effects are generally reversible, and may include renal insufficiency, hypomagnesemia, paresthesias, tremor, seizures, visual disturbances, paresis, disorientation, depression, confusion, somnolence, coma, nausea, hypertension, hemolytic-uremic syndrome, hyperglycemia, gynecomastia, and hypertrichosis. (See Standard Practice Manual)

4. Mycophenolate Mofetil (MMF) – See **section 11** for information about administration and dosage adjustments.

a) Description

MMF is the morpholinylethylester of mycophenolic acid (MPA) and reversibly inhibits inosine monophosphate dehydrogenase, particularly the type II isoform that

is more prominent in activated lymphocytes. As a result of the inhibition of de novo purine synthesis, proliferation of B and T lymphocytes is blocked and antibody production is inhibited.

b) Storage and Administration

MMF is available in an oral and an intravenous formulation. The oral formulation is supplied in 250mg hard gelatin capsules and can be stored at room temperature. MMF for IV administration is supplied as a lyophilized powder in a glass vial containing the equivalent of 500mg.

c) Side Effects and Toxicity

Precautions: MMF has been studied extensively among patients after nonmyeloablative HCT. Previous clinical studies in patients after allografting suggest that the principal adverse reactions associated with the administration of MMF include nausea, vomiting, neutropenia, diarrhea, and on one occasion bloody diarrhea. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in gastrointestinal and hematologic parameters. MMF has an increased incidence of digestive system adverse events, including GI tract ulceration, and hemorrhage (3% of patients receiving MMF). GI tract perforations have rarely been observed. Most patients in these studies were also on other drugs known to be associated with these complications. Up to 2% of patients receiving MMF for prevention of rejection developed severe neutropenia (ANC <500). The development of neutropenia may be related to MMF itself, concomitant medications, viral infections or some combination of these causes. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the principal investigator and documented in the medical records and the clinical reporting form (CRF).

16. EBV Monitoring and Treatment

Patients will have EBV by PCR plasma samples collected once a week until day 100 following transplantation. If there is documented evidence of rising copy number of EBV by PCR, monitoring will increase to twice per week through day 100 following HCT. EBV monitoring by PCR will be done every other week from Day 101 until Month 6. If there is evidence of a rising copy number, the frequency of the monitoring will be increased to once or twice a week as clinically indicated according to institutional standard.

A) Patients who develop a viral load of > 1000 copies per mL plasma will receive further individualized monitoring, evaluation by Transplant Infectious Disease service according to institutional practice and/or therapy as per institutional standards and Infectious Disease recommendations.

B) If the patient develops increasing viral load, it is suggested that he or she be treated with either preemptive Rituximab 375 mg/m2 IV weekly x 4 weeks or an EBV reactivation protocol according to local institution practice and ID recommendation. It is suggested that subjects who develop EBV reactivation and/or asymptomatic increasing viral load by PCR undergo flow cytometry of the peripheral blood and CT or MRI imaging of chest, abdomen, and pelvis to assess the development of lymphadenopathy, organomegaly, or other evidence of the development of PTLD. Whenever possible, the clinical diagnosis of PTLD should be confirmed by tissue biopsy.

17. DATA AND SAFETY MONITORING PLAN AND ADVERSE EVENT REPORTING

17A. Monitoring the Progress of Trials and the Safety of Participants

The principal investigators (PI) monitor multi-institutional clinical trials, with oversight by the Medical Monitor, a Data Safety and Monitoring Board (DSMB), the Data and Safety Monitoring Committee (DSMC) and the Institutional Review Board (IRB). The PI reviews outcome data for each individual patient on an ongoing basis.

This multi-center protocol has a DSMB responsible for monitoring patient safety by reviewing protocol data and safety endpoints. The DSMB meets twice a year and all outcome data is reviewed including all adverse events reported to the coordinating center (FHCRC) along with those officially reported to the FHCRC IRO. The DSMB confirms that the trial has not met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCRC IRB, the IND sponsor, the DSMC Chairman, Protocol Office, and the trial coordinators/local PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual. Furthermore, the FHCRC also has a DSMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval is also required.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the FHCRC Trial Coordinator. At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. Thus, multiple health care providers provide independent observations and participate in monitoring this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the primary health care provider (fellow or physician assistant), floor or outpatient nurse and the PI or other attending clinician involved with the patient averting possible conflict of interest having the PI as the attending clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene on an ad hoc basis as necessary to review the primary data and render a decision.

17B. Adverse Event Definitions

a. Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

b. Serious Adverse Event

A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:

- Death.
- Life-threatening situation (ie, with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
- In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.

- Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect.
- An important medical event that requires intervention to prevent one of the above outcomes.

c. Unexpected Adverse Event

An unexpected adverse event is defined as an event that has a nature or severity, or frequency that is not consistent with the applicable investigator brochure. “Unexpected,” as used in this definition, refers to an adverse drug experience that has not been previously observed and reported rather than an experience that has not been anticipated based on the pharmacological properties of the study drug.

17C. Monitoring and recording AEs

Adverse events will be collected and graded using the **modified (for HCT)** NCI Common Toxicity Criteria (**Appendix G of the protocol**). All grade 3 and 4 adverse events (or highly unusual grade 2 adverse events), which occur from the start of study treatment (pre-transplant conditioning) through day 100 post-transplant, will be assessed by the investigator or qualified designee and recorded in the CRF, whether or not attributed to the study intervention. Adverse events that occur between the start of conditioning and day 30 will be assessed separately to identify early regimen related toxicities. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution)
- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure
- The action taken due to the adverse event
- The outcome of the adverse event

The adverse event reporting in this multi-institution clinical trial will follow both the sponsor and IRB guidelines for serious adverse event (SAE) reporting. The classification of an adverse event determines what reporting procedure to follow for reporting the adverse event. (See **Table 2 and 3)**.

17D: Grading of the severity of an Adverse Event

Adverse events will be graded according to CTCAE criteria. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event.

Attribution of Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

- Definite: The event follows a reasonable temporal sequence from exposure to the investigational agent, has been previously described in association with the investigational agent, and cannot reasonably be attributed to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications; AND the event disappears or improves with withdrawal of the investigational agent and/or re-appears on re-exposure (e.g., in the event of an infusion reaction).
- Probable: The event follows a reasonable temporal sequence from exposure to the investigational agent and has been previously been described in association with the investigational agent OR cannot reasonably be attributed to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Possible: The event follows a reasonable temporal sequence from exposure to the investigational agent, but could be attributable to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Unlikely: Toxicity is doubtfully related to the investigational agent(s). The event may be attributable to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.
- Unrelated: The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant medications.

For general AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated. For determination of IND safety reporting, AE attribution will be assessed according to the suspected adverse reaction definition described in 21 CFR 312.32 as an AE for which there is a reasonable possibility that the drug caused the adverse event where "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reactions that are both serious and unexpected will be reported to the FDA as an IND safety report, in accordance with regulations under 21 CFR 312.32.

17E: Adverse Event Reporting Period

All grade 3 and 4 adverse events (or highly unusual grade 2 adverse events), which occur from the start of study treatment (pre-transplant conditioning) through day 100 post-transplant, will be assessed by the investigator or qualified designee and recorded in the CRFs, whether or not attributed to the study intervention. AEs with an onset date prior to the start of study treatment will not be recorded, except in the case of clinically significant worsening of the AE during the specified monitoring time frame. The end of the adverse event reporting period occurs through day 100 post-transplant, or when any ongoing drug-related adverse events and/or serious adverse events have resolved or become stable. A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

All adverse events reportable to the IRB will be collected according to current reporting FHCRC IRB Policies for Reportable Events. Definitions, instructions and forms associated with reportable events can be found on the FHCRC's Institutional Review Office (IRO) extranet website: http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html. All investigators must report adverse events to their respective IRB as locally mandated.

All events which in the opinion of the PI are (1) unexpected, and (2) related or possibly related to the research and (3) serious or suggest that the research places research participants or others at a greater risk of physical or psychological harm than was previously known or recognized will be submitted to the IRB within 10 calendar days (see Tables 2, 3, and 4 below). In addition, all serious adverse events are reported to the IRB at the time of annual renewal. The PI for a study is responsible for this reporting and the IRO assures adverse event reporting on an annual basis. The PI in the annual application for grant continuation will summarize reports of toxicities. Furthermore, stopping rules and interim analysis provides an additional safeguard for adverse event analysis and reporting in this protocol. All collaborating PIs have fulfilled all NIH requirements for training in human subject's protection.

The FHCRC PI and research staff will meet regularly to review all reported events. If the event meets FHCRC IRB current reporting obligations, it will be sent to the FHCRC IRB. After the FHCRC IRB has reviewed the event report it will be disseminated to all participating sites/investigators, the DSMB, and the study sponsor.

When a patient experiences disease recurrence or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death.

Patients enrolled in this study are receiving treatments that are generally associated with high rates of expected adverse events (outlined in **Appendix I**).

17.F. Reporting to Coordinating Center and Fred Hutch IRB

For patients being cared for at the FHCRC, heath care providers communicate with the PI or research staff as events occur triggering subsequent reporting. For patients not being cared for at the FHCRC the outside facilities communicate with the PI or research study staff for these reporting purposes.

As FHCRC is the coordinating center, all reportable events will be collected by the FHCRC PI and/or study staff for submission to FHCRC IRB. All reportable events should be submitted to the coordinating center on the relevant FHCRC IRB forms. (See **Tables 2 and 3** for FHCRC IRB Reporting Policies and Forms for Reporting)

Table 2 FHCRC IRB Policies for Reportable Events (Relevant FHCRC policies include, but are not limited to the following documents. Please also refer to the FHCRC IRO website.)

IRB Policy 2.6	Unanticipated Problems Involving Risks to Subjects or Others (Adverse Events)	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.11	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and Investigational Device Exemptions (IDE)	http://extranet. fhcrc.org/EN/sections/iro/irb/policy/index.html

Table 3 Fred Hutch IRB Forms for Reporting

Adverse Event Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html
Expedited Reporting Form for Unanticipated Problems or Noncompliance	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html

17.G Research Site Reporting Requirements to Sponsor

Classification of an event as serious or non-serious determines the reporting procedures to be followed by the site for reporting the event to the Sponsor.

The information in the Institution-Sponsored IND SAE Reporting Form (**see Appendix E**) must match or be reconciled with the information recorded in the adverse events section of the CRF and study database. For example, the same adverse event term should be used on both forms.

All SAEs must be reported to the Sponsor from the time of study drug administration until SAE duration as outlined in the protocol. SAEs that occur after the study-specific informed consent is signed but prior to the first dose of the investigational agent will be collected only if they are considered by the investigator to be causally related to the study required procedures. SAEs will be reported by the PI or Research Nurse to the Sponsor according to the timelines described in **Table 4**.

Table 4: Reporting Requirements to Sponsor for Adverse Events

Classification	Reporting time	Reporting Time	Reporting Action	Contact Information
Serious Adverse Event (SAE)	Fatal or life-threatening	Within 24 hours of research team* awareness	Email notification to sponsor and to both the Sponsor's Medical Monitor and the PI	IND Sponsor's Medical Monitor email: cdelaney@fredhutch.org IND Sponsor's email: ISIOC@fredhutch.org PI's Email: lburroug@fredhutch.org
	All SAEs	Within 2 business days of research team* awareness	Submit completed IND SAE Reporting Form signed by PI or designated sub-Investigator to both the IND Sponsor and the PI	IND Sponsor's email: ISIOC@fredhutch.org PI's Email: lburroug@fredhutch.org
Non-serious Adverse Event		Per CRF completion guidelines	Record information on appropriate CRFs	N/A

*Research team is defined as the individuals listed on the delegation of authority log. Physicians listed on the study's delegation of authority log as transplant service attending physicians delegated authority to administer informed consent will not be considered part of the research team unless additional responsibilities related to the conduct of the study have been delegated to them by the Principal Investigator.

17H: FHCRC Sponsor Reporting Requirements

The sponsor assumes responsibility for IND safety reporting to the FDA and participating investigators, in accordance with regulations under 21 CFR 312.32.
Each serious adverse event report received from the investigator will be evaluated by the Medical Monitor who will assess the seriousness of the event (see Section 17B.b), the expectedness of the event (see Section 17.B.c), and the relationship to participation in the study (see Section 17.D). For regulatory reporting purposes, the Sponsor will determine expectedness relating to treosulfan using safety information specified in the treosulfan investigator brochure. An event will be classified as related if either the investigator or the Sponsor determines that the event may be related to the study drug.

The Sponsor or its designee will provide all investigators with a safety letter notifying them of an event that meets FDA IND Safety Reporting criteria. Investigators will be requested to provide written notification of safety report to the FHCRC IRB as soon as is practical, consistent with IRB requirements.

17I. Plans for Assuring that any Action Resulting in a Temporary or Permanent Suspension of an NCI-funded Clinical Trial is Reported to the NCI Grant Program Director Responsible for the grant
This clinical research trial is associated with an Investigational New Drug (IND). Any temporary or permanent suspension, as determined by the PI, IRB, IND Sponsor, or DSMC, of this clinical research trial will be reported to the NCI grant program director by the PI.

17J. Plans for Assuring Data Accuracy and Protocol Compliance
The study has dedicated research staff who follow patients to confirm eligibility; reporting of adverse events; reporting of events, which are part of the safety-monitoring plan, and protocol adherence. The PI, data coordinators, and research staff are responsible for review and maintenance of all patient records to ensure data integrity and protocol adherence.

At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations in the medical record. This documentation is extracted by study research staff via chart review and entered into an electronic Case Report Forms (CRF's). The study staff also continue to follow patients after day 100, review source documentation, and complete CRFs at 6 months, 1 year, 1.5 years, and then annually per protocol. The CRFs are printed directly from the database, and the PI reviews the CRFs and the primary source documents for data accuracy. After the CRFs are verified, they are signed by the PI. Thus, multiple health care providers provide independent observations and participate in assessments on this trial.

Outside center clinical trial participant's information is transmitted to the FHCRC Trial Coordinator. Clinical outcome data are summarized and transmitted from collaborating centers as CRFs. CRFs from external sites are verified and signed by the local investigators at that site. The CRFs are generated from the collaborating centers at protocol defined time points. When possible, primary source documents regarding patient outcomes are collected with patients' names removed and replaced by Unique Patient Numbers (UPNs). The data are incorporated into a central database by the data manager. Collaborating sites send signed consents, eligibility forms, and CRFs with source documents demonstrating eligibility, treatment, and serious adverse events (if applicable) to the study staff. These are reviewed for eligibility, adherence to the protocol, accuracy, and completeness by the study staff.

The study is monitored under the FHCRC Monitoring Plan. The FHCRC Data and Safety Monitoring Plan details the full scope and extent of monitoring and provides for immediate action in the event of the discovery of major deviations.

18. RECORDS
Clinical records will be maintained as confidentially as possible by all collaborating institutions. Data will be collected from the collaborating institutions and will be maintained by Clinical Statistics. The investigator will ensure that data collected conform to all established guidelines for coding, collection, key entry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room. They are maintained by the FHCRC data collection staff, which is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research.

19. STATISTICAL CONSIDERATIONS AND TERMINATION OF STUDY
The primary objective of this clinical trial is to evaluate, within the limits of a phase II study, the preliminary efficacy as defined by engraftment, of a regimen consisting of treosulfan and fludarabine followed by allogeneic HCT in patients with nonmalignant inherited disorders. We plan to enroll at least 30 patients on this study. A minimum of 30 patients will be enrolled onto Arm A. The protocol will continue to accrue until this goal is met. There will be no set limit for Arm B: the cord blood arm. Analyses will be carried out separately between Arm A and B. We will be encouraged to continue study of this treatment if at least 27 of 30 patients engraft. Table 5 shows the probability of observing 27 or more successes among 30 patients for several possible true rates of engraftment.

The protocol was modified in January 2011 and September 2011. Due to a higher than anticipated incidence of acute grade III-IV GVHD for both arms in the first 13 patients (5/13 = 38%; 3/8 bone marrow recipients, 2/5 cord blood recipients) the protocol was modified to add rabbit ATG (thymoglobulin) to both arms initially for unrelated recipients (January 2011) and subsequently for the related recipients (September 2011). In addition, due to graft rejection (n=1) and low donor chimerism/inadequate disease response (n=1) in 2 of the 5 patients enrolled onto the cord blood arm, the cord blood arm was modified to increase the TBI from 200 cGy to 300 cGy TBI for non T-cell deficient patients. The revised protocol will reset the target accrual to at least 30 patients as of January 2011, with a minimum enrollment of 30 onto Regimen A (as stated above). The efficacy analysis will be performed separately for the initial 13 patients (Regimen A: n=8, Regimen B: n=5) and those accrued under the modified regimen. Only patients enrolled after this protocol modification will be counted towards the stopping rules.

Table 5. Probability of observing 27 or more successes in 30 patients

True rate of engraftment	Pr(≥ 27 successes)
70%	0.01
80%	0.12
90%	0.65
95%	0.94

The incidence of day 200 transplant-related mortality (TRM) will be monitored in all patients. If there is sufficient evidence suggesting that the true incidence of day 200 TRM exceeds 25% on either arm, enrollment on that arm will be suspended and the DSMB will be convened to make recommendations regarding protocol modification. Sufficient evidence will be an observed rate of day 200 TRM that corresponds to a one-sided 80% confidence interval with a lower limit greater than 25%. These limits will be examined after every 5th enrolled patient becomes eligible for evaluation. In practice, the study arm will be suspended if we observe the following number of TRM in study patients: 3/5, 5/10, 6/15, 8/20, 9/25, 10/30, 12/35, 13/40, 15/45 or 16/50 per disease subgroup per study arm (Regimen A versus Regimen B). Table 6 summarizes the operating characteristics of this stopping rule, for enrollment of up to 50 patients per disease subgroup per study arm (Regimen A versus Regimen B) as estimated from 5,000 Monte Carlo simulations. We will apply stopping rules to regimens A and B separately.

The protocol related to regimen B was modified in April 2012. Stopping rules for excess day 200 TRM were met in October 2011 due to 3 deaths occurring in the 5 patients enrolled since the January 2011 protocol modifications (1 due to multi-system organ failure; 1 due to ARDS/diffuse alveolar damage; and 1 due to gram positive sepsis (strep viridans) with multi-system organ failure, questioned possible sinusoidal obstructive syndrome/veno-occlusive disease). All deaths occurred before day 100, with two very early on post-transplant day 1 and day 7. However, the causes of death did not appear to be related to any single part of the regimen. The Data Safety Monitoring Board met and reviewed the data and recommended the following: The revised protocol will allow up to 5 more patients to be enrolled on regimen B before reconvening the DSMB, with each patient enrolled no sooner than 28 days following the date of transplantation (defined as the date of stem cell infusion) of the previous patient. The stopping rules for this arm (Regimen B) were not reset and will continue as stated in the protocol. The next stopping rule would be triggered at 5/10. Therefore, if 2 of the next 5 patients enrolled dies on Regimen B (cord blood), the stopping rule would be triggered and the DSMB will reconvene.

Table 6. Probability of stopping the trial for excess day 200 TRM

Number of patients	True rate of event	Probability of stopping
10	15%	0.03
15	15%	0.04
20	15%	0.04
25	15%	0.05
10	35%	0.32
15	35%	0.49
20	35%	0.54
25	35%	0.63
10	45%	0.57
15	45%	0.78
20	45%	0.84
20	45%	0.91

The incidence of graft rejection will also be monitored in all patients. Regimen A and B will be monitored/analyzed separately. If there is sufficient evidence to suggest that the true incidence of graft rejection exceeds within the first 365 days 10% on either arm, enrollment will be suspended on that arm and the DSMB will be convened to make recommendations regarding protocol modification and/or dose escalation. Sufficient evidence will be an observed rate of graft rejection that corresponds to a one-sided 80% confidence interval with a lower limit greater than 10%. These limits will be examined after every 5th enrolled patient becomes eligible for evaluation. In practice, the study arm will be suspended if we observe the following number of graft rejections in study patients: 2/5, 3/10, 3/15, 4/20, 5/25, 5/30, 6/35, 7/40, 7/45 or 8/50 per disease subgroup per study arm (Regimen A versus Regimen B). Table 7 summarizes the operating characteristics of this stopping rule for enrollment of up to 50 patients per disease subgroup, as estimated from 5,000 Monte Carlo simulations.

Table 7. Probability of stopping the trial for excess graft rejection

Number of patients	True rate of event	Probability of stopping
10	5%	0.03
15	5%	0.05
20	5%	0.05
25	5%	0.05
10	20%	0.38
15	20%	0.62
20	20%	0.68
25	20%	0.72
10	30%	0.67
15	30%	0.88
20	30%	0.93
25	30%	0.95

Therefore, **stopping rules** will be imposed for:

Day 200 TRM > 25% (3/5, 5/10, 6/15, 8/20, 9/25, 10/30, 12/35, 13/40, 15/45, 16/50) per disease subgroup per study arm (Regimen A versus Regimen B).

Day 365 Graft rejection (defined as CD3 T cell chimerism < 5%) > 10% (2/5, 3/10, 3/15, 4/20, 5/25, 5/30, 6/35, 7/40, 7/45, 8/50) per disease subgroup per study arm (Regimen A versus Regimen B)

Day 100 Grade III/IV GVHD >15% for Arm A +rATG PBSC grafts 2/5, 3/10, 4/15, 5/20, 6/25, 7/30, 8/35, 9/40, 10/45, or 11/50

The dosing of treosulfan on protocol 2256 was modified in June 2013, specifically the dose of Treosulfan was decreased for smaller children. However, three of the first five patients treated with the reduced dose have experienced poor engraftment. Therefore the original dose of treosulfan was reinstituted in April 2014. The protocol accrual will continue to a total of 60 patients in Arm A with Treosulfan/Fludarabine/rATG, so as to ensure adequate numbers of smaller children will be treated at the original dose, since among the original 30 patients only a small number were <0.5 m2 body surface area. As stated above, the initial 8 patients transplanted on Regimen A with Treosulfan and fludarabine only do not count towards the accrual goal of 60. Therefore the total number of patients accrued on Regimen A will be 68. The same stopping rules for TRM and rejection applied to the first 30 patients in Arm A will be applied separately to the additional 30 patients in Arm A; however, accrual to Arm B will continue as before under the same stopping rules.

In October/November 2015 Regimen A of the protocol was modified to increase accrual to a minimum of 20 patients for each subgroup of patients (for example, primary immunodeficiency diseases, hemophagocytic disorders, hemoglobinopathies, and marrow failure disorders) who receive Treosulfan, fludarabine, and rabbit ATG. The protocol will continue to accrue until a minimum of 20 patients are in each subgroup, in order to better evaluate the safety and effectiveness of the regimen in each subgroup. There is no limit to the overall accrual to Regimen A as long as the minimum of 20 patients per subgroup has not been reached and each subgroup will continue to accrue until all subgroups reach a minimum of 20 patients. We estimate the overall accrual will be 100-120 patients. Stopping rules for TRM and graft rejection will be applied separately in each disease subgroup of patients who receive Treosulfan, fludarabine, and rabbit ATG conditioning. There are additional patients with nonmalignant diseases that do not fit into one group that have enrolled onto the protocol and will continue to enroll onto the protocol such as metabolic disorders, autoimmune disorders, or other rare nonmalignant diseases. We will continue to enroll these patients but they will not count towards the stopping rules or enrollment since we do not anticipate very many of these patients. However, they will be reviewed by the data safety monitoring board. Accrual to Arm B will continue as before under the same stopping rules.

In March 2017, the protocol was modified to allow the use of both bone marrow and cord blood from the same HLA-matched sibling donor. Specifically, at the discretion of the PI, bone marrow and cord blood cells from the same HLA-matched sibling donor may be used together for transplantation in cases where such cord blood cells were previously stored. The patients enrolled onto the protocol who receive both bone marrow and cord blood from the same HLA-matched sibling donor will be enrolled onto Regimen A of the protocol and analyzed with this group.

In March 2018, the protocol was modified to prioritize PBSC (Regimen A) as the preferred stem cell source when feasible. This change was made with the goal of improving donor chimerism. PBSC has a higher risk of graft versus host disease; therefore, a stopping rule for grade III-IV GVHD will be added to the protocol – Regimen A only. Since bone marrow will still be allowed on Regimen A, we will apply this stopping rule to PBSC transplants. If there is sufficient evidence to suggest that the true incidence of grade III-IV GVHD at day 100 exceeds 15% (Regimen A), enrollment will be suspended on that arm and the DSMB will be convened to make recommendations regarding protocol modification. The stopping rule would be triggered if 2 of 5, 3 of 10, 4 of 15, 5 of 20, 6 of 25, 7 of 30, 8 of 35, 9 of 40, 10 of 45, or 11 of 50 experience grade III-IV GVHD. This stopping rule will not be by disease subgroup but for all PBSC transplants. The current PBSC transplants on the protocol will be included in this stopping rule.

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as of 11-05-2019

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73. Slatter MA, Rao K, Abd Hamid IJ, et al. Treosulfan and fludarabine conditioning for hematopoietic stem cell transplantation in children with primary immunodeficiency: UK experience. *Biol Blood Marrow Transplant*. 2017;24(3):529-536.

APPENDICES – TABLE OF CONTENTS

APPENDIX A
Karnofsky and Lansky Performance Status Scales

Karnofsky Performance Status Scale

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

REFERENCE

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

Lansky Play Performance Status Scale

Percentage	
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of, and less time spent in, play activities
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play; able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	Unresponsive
0	Dead

APPENDIX B
STANDARD PRACTICE GUIDANCE DOCUMENTS

The protocol references institutional standard practice for trial conduct. The standard practice documents utilized at Fred Hutch include the following:

- Herpes Simplex and Varicella Zoster Virus Prevention and Treatment, 6/6/18
- CMV Prevention: Surveillance and Preemptive Therapy, 08/16/17
- CMV Disease: Diagnosis and Treatment, 10/19/16
- Antifungal Therapy Guidelines, 03/16/16
- Pneumonia/Pneumocystis Carnii Prophylaxis, 10/19/16
- Antibiotic Prophylaxis for Encapsulated Bacteria in Allogeneic Patients with Chronic GVHD requiring Immunosuppressive Therapy, 10/19/16
- Vaccinations, 6/21/18
- Foscarnet, 08/16/17
- TBI Adult Non Myeloablative
- TBI Pediatric NON Myeloablative

Most recent versions of these documents can be obtained upon request to the coordinating center.

APPENDIX C
ACUTE GRAFT-VERSUS-HOST-DISEASE STAGING AND GRADING TABLES

Clinical Stage of Acute GVHD According to Organ System

Stage	Skin	Liver	Intestine [(1)]
1	Maculopapular rash <25% of body surface	Bilirubin 2-3 mg/dl	>500-1000 mL diarrhea per day or (nausea, anorexia or vomiting with biopsy (EGD) confirmation of upper GI GVHD
2	Maculopapular rash 25-50% of body surface	Bilirubin 3.1-6 mg/dl	>1000 -1500 mL diarrhea per day
3	Maculopapular rash >50% body surface area or Generalized erythroderma	Bilirubin 6.1-15 mg/dl	>1500 mL diarrhea per day
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/dl	Severe abdominal pain with or without ileus

Overall Clinical Grading of Severity of Acute GVHD

Grade	Skin	Liver	GI
I	1-2	0	0
II[A]	3 and/or	1 and/or	1
III[A,B]	4 and/or	2-4 and/or	2-4
IV[A,C]	4 and/or	2-4 and/or	2-4

A. Grade II-IV GVHD with only single organ involvement should be biopsy confirmed.
B. Non-fatal GVHD
C. Fatal GVHD

[(1)] For pediatric patients the following diarrhea volumes will apply:
Stage 1 - > 10 ml/kg/day
Stage 2 - > 20 ml/kg/day
Stage 3 - > 30 ml/kg/day

APPENDIX D
EVALUATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE[a]

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores, 60%, > 15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below.

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nail	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase $\geq$ 3x upper limit of normal, AST or ALT $\geq$4x upper limit of normal or total serum bilirubin $\geq$ 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary comprise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

Appendix D (continued)

Laboratory testing and diagnostic indicators of chronic GVHD[a]

Eye	Schirmer's test with a mean value ≤ 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as FEV_1 < 80% of predicted with either an FEF_{25-75} <65% of predicted or RV >120% of predicted, or a decrease of FEV_1/FVC by > 12% within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans o the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/μl), eosinophilia, hypo-gammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

[a] From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

Appendix D (continued)

GRADING OF CHRONIC GRAFT-VERSUS-HOST DISEASE

A Clinical limited chronic GVHD

1. Oral abnormalities consistent with chronic GVHD, a positive skin or lip biopsy, and no other manifestations of chronic GVHD
2. Mild liver test abnormalities (alkaline phosphatase ≤ 2x upper limit of normal, AST or ALT ≤ 3x upper limit of normal and total bilirubin ≤ 1.6) with positive skin or lip biopsy, and no other manifestation of chronic GVHD
3. Less than six papulosquamous plaques or limited skin rash or dyspigmentation (< 20% of the body surface), positive skin biopsy, and no other manifestations of chronic GVHD
4. Ocular sicca (Schirmer's test ≤ 5mm), positive skin or lip biopsy, and no other manifestations of chronic GVHD
5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GVHD

B. Clinical extensive chronic GVHD

1. Involvement of two or more organs with symptoms or signs of chronic GVHD, with biopsy documentation of chronic GVHD in any organ
2. > 15% base line body weight loss not due to other causes, with biopsy documentation of chronic GVHD in any organ
3. Skin involvement more extensive than defined for limited chronic GVHD, confirmed by biopsy
4. Scleroderma or morphea
5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
6. Decreased range of motion in wrist of ankle extension due to fasciitis caused by chronic GVHD
7. Contractures thought to represent chronic GVHD
8. Bronchiolitis obliterans
9. Positive liver biopsy; abnormal liver function tests not due to other causes with alkaline phosphatase > 2x upper limit of normal, AST or ALT > 3x upper limit of normal, or total bilirubin > 1.6, and documentation of chronic GVHD in any organ
10. Pericarditis or pleuritis not due to other causes
11. Positive upper or lower GI biopsy

[a] From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by
Long Term Follow-Up at the FHCRC

APPENDIX E
Institution-Sponsored IND SAE Reporting Form

ISIOC SAE
form_v4_23May2018

APPENDIX F

Protocol 2256 Patient Demographics and Eligibility Form

Please Fax this completed form to (206)-667-4427 for patient registration.
Questions regarding eligibility should go to Lauri Burroughs, M.D., 206-667-2396.

UPN:_____

Patient Name: _____ _____ ____
(Last) *(First)* *(MI)*

Date of Birth: _____ /_____/_____ **Age**:_____
(Mo) (Day) (Year)

Gender *(choose one):*
☐ Male ☐ Female ☐ Unknown

Diagnosis:_____

Planned Day 0: _____/_____/_____
(Mo) (Day) (Year)

Ethnicity *(choose one): Instruct the patient to select one of the following.*

- ☐ **Hispanic** *(A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. Term "Spanish Origin" can also be used in addition to "Hispanic" or "Latino".)*
- ☐ **Not Hispanic or Latino**
- ☐ **Declined to Report**
- ☐ **Research Subject does not know ethnicity**

Race *(check all that apply): Instruct the patient to select one or more of the following.*

- ☐ **American Indian/Alaska Native** *(A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment).*
- ☐ **Asian** *(A person having origins in any of the original peoples of the Far East, Southeast, Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam).*
- ☐ **Black or African American** *(A person having origins in any of the black racial groups of Africa.*
- ☐ **Native Hawaiian/Pacific Islander** *(A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands).*
- ☐ **White** *(A person having origins in any of the original peoples of Europe, the Middle East or North Africa).*
- ☐ **Research subject does not know**
- ☐ **race Declined to report**

Protocol 2256 Eligibility (continued)

Inclusion Criteria:

1) Yes ☐ No ☐ Patient signed IRB approved consent form. Date: _____

IRB File Number: _____

For FH patients: ☐ Consent A (Regimen A: PB/BM donor)
☐ Consent D (Regimen B: Unrelated Cord blood donor)
☐ Consent C (Regimen A: HLA matched sibling bone marrow combined with HLA-matched sibling cord blood)

2) Yes ☐ No ☐ The consent was approved by your institution's IRB on Date: _____

The following criteria questions must be marked "Yes" or "N/A" for the patient to enter on 2256.

3) Yes ☐ No ☐ Age < 50 years with nonmalignant disease treatable by allogeneic HCT.

4) Yes ☐ No ☐ NA ☐ Patient has a known nonmalignant disease that is not clearly defined and has been discussed and approved for enrollment with the protocol PI (Dr. Lauri Burroughs), and potentially by the Non-Malignant Board

Graft Criteria: One of the following graft sources must be marked "YES"

5a) Yes ☐ No ☐ Patients with HLA-matched related donor

OR

5b) Yes ☐ No ☐ Patient with unrelated donors matched for HLA-A, B, C, DRB1, and DQB1 or mismatched for a single allele at HLA-A, B, C, DRB1 or a single DQB1 antigen or allele mismatch by high resolution DNA typing.

Yes ☐ No ☐ Have a negative anti-donor cytotoxic crossmatch.

N/A☐ Cytotoxic crossmatch not done as patient and donor are phenotypically identical by molecular methods.

Fill in HLA typing for patient and HLA-matched related or unrelated donor
Patient A: _____ A: _____ C:_____ C:_____ B: _____ B: _____ DRB1:_____ DRB1:_____ DQB1: _____ DQB1: _____
Donor A: _____ A: _____ C:_____ C:_____ B: _____ B: _____ DRB1:_____ DRB1:_____ DQB1: _____ DQB1: _____

OR

Protocol 2256 Eligibility (continued)

5c) Yes ☐ No ☐ Patient with unrelated umbilical cord blood graft: Unrelated UCB units will be selected according to current institutional standard Practice. One or 2 UCB units may be used to achieve the required cell dose.

Yes ☐ No ☐ The unrelated UCB graft is matched at 4-6 HLA-A, B, DRB1 antigens with the recipient. This may include 0-2 antigen mismatches at the A or B or DRB1 loci. Unit selection based on cryopreserved nucleated cell dose and HLA-A, B, DRB1 using intermediate resolution A, B antigen and DRB1 allele typing.

Fill in HLA typing for patient and unrelated umbilical cord unit(s)					
Patient					
A: _____	A: _____	C: _____	C: _____	B: _____	B: _____
DRB1: _____	DRB1: _____				
Unit #1					
A: _____	A: _____	C: _____	C: _____	B: _____	B:_____
DRB1: _____	DRB1: _____				
Unit #2 N/A ☐					
A: _____	A: _____	C: _____	C: _____	B: _____	B:_____
DRB1: _____	DRB1: _____				

Exclusion criteria:
Each of the following questions must be marked "No" Or "N/A" for the patient to enroll on 2256.

6) Yes ☐ No ☐ Patients with Idiopathic Aplastic Anemia and Fanconi Anemia. (Patients with Aplastic Anemia associated with PNH or inherited marrow failure syndromes, except Fanconi Anemia, will be allowed).

7) Organ dysfunction. Please check yes if patient meets any of the following:

a. Yes ☐ No ☐ Impaired cardiac function as evidenced by ejection fraction < 35% (or, if unable to obtain ejection fraction, shortening fraction <26%) or cardiac insufficiency requiring treatment or symptomatic coronary artery disease. Patients with a shortening fraction < 26% must be seen by cardiology for approval.

Date:___/____/___

Ejection Fraction Value:_____% Shortening Fraction Value:_____%

b. Yes ☐ No ☐ Impaired pulmonary function as evidenced by DLCO < 50% of predicted (or, if unable to perform pulmonary function tests, then O_2 saturation < 92% on room air).

Date:___/____/___ DLCO Value:_____% O_2 Saturation Value: ____%

Protocol 2256 Eligibility (continued)

c. Yes ☐ No ☐ Impaired renal function as evidenced by creatinine-clearance < 50% for age, weight, height or serum creatinine > 2X upper normal limit or dialysis-dependent.

Date:_____/____/___ Value:_____mg/dL or CrCl:_____ml/min

d. Yes ☐ No ☐ Evidence of synthetic dysfunction or severe cirrhosis requiring deferral of conditioning as recommended by a gastroenterology specialist.
Date:____/____/__
ALT:_____U/L AST:_____U/L
Total Bilirubin:_____mg/dL Alk.phos:_____ U/L

8) Yes ☐ No ☐ Patients with an active infectious disease requiring deferral of conditioning; as recommended by an Infectious Disease specialist.

9) Yes ☐ No ☐ Patients who are positive for human immunodeficiency virus (HIV).

10) Yes ☐ No ☐ Females who are pregnant or breast-feeding.

11) Yes ☐ No ☐ Patients with a known hypersensitivity to treosulfan and/or fludarabine.

12) Yes ☐ No ☐ Receiving another experimental drug within 4 weeks of initiation of conditioning (day -6) unless approved by the PI..

TBI Dose Assignment

For Regimen B patients only, one of the following must be designated.

☐ 200 cGy TBI (for unrelated cord blood recipients with an underlying T-cell primary immunodeficiency)

OR

☐ 300 cGy TBI (for all other unrelated cord blood recipients)

Signature of **Local** Principal Investigator**:**_____ Date:_____
(or Designee)

Transplant Center _____

Signature of **FHCRC** Principal Investigator_____ Date:_____
(or Designee)

Signature of person completing form:_____ Date: _____

☐ **FHCRC Patients:**
Signature of Principal Investigator:_____ Date:_____
(or Designee)

OR

☐ **Outside Center Patients:**

Signature of **Local** Principal Investigator:_____ Date:_____
(or Designee)
Signature of **FHCRC** Principal Investigator:_____ Date:_____
(or Designee)

APPENDIX G
Adapted from
COMMON TOXICITY CRITERIA (CTC) for HCT

	Grade	
Adverse Event	**3**	**4**
ALLERGY/IMMUNOLOGY		
Allergic reaction/hypersensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis
Vasculitis	Requiring steroids	Ischemic changes or requiring amputation
Allergy/Immunology – Other - Specify,_____)	Severe	Life-threatening or disabling
BLOOD/BONE MARROW		
Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis, other)	Requiring transfusion and/or medical intervention (e.g., steroids)	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)
For BMT studies, if specified in the protocol. For pediatric BMT studies, if specified in the protocol.	>4 u pRBC in 24 hours >30mL/kg in 24 hours	Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin
CARDIOVASCULAR (ARRHYTHMIA)		
Cardiovascular/Arrhythmia -Other (Specify,_____)	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
CARDIOVASCULAR (GENERAL)		
Acute vascular leak syndrome	Respiratory compromise or requiring fluids	Life-threatening; requiring pressor support and/or ventilatory/support
Cardiac-ischemia/infarction	Angina without evidence of infarction	Acute myocardial infarction

APPENDIX G continued

Adverse Event	Grade 3	Grade 4
CARDIOVASCULAR (GENERAL) continued		
Cardiac left ventricular function	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Cardiac troponin I (cTnI)	Levels consistent with unstable angina as defined by the manufacturer	Levels consistent with myocardial infarction as defined by the manufacturer
Cardiac troponin T (cTnT)	≥ 0.1 - <0.2 ng/mL	≥ 0.2 ng/mL
Hypotension	Requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	Shock (associated with acidemia and impairing vital organ function due to tissue hypoperfusion)
Myocarditis	CHF responsive to treatment	Severe or refractory CHF
Pericardial effusion/ pericarditis	With physiologic consequences	Tamponade (drainage or pericardial window required)
Syncope (fainting) is graded in the NEUROLOGY category.	-	-
Thrombosis/embolism	Deep vein thrombosis, requiring anticoagulant therapy	Embolic event including pulmonary embolism
Vein/artery operative injury is graded as Operative injury of vein/artery in the CARDIOVASCULAR (GENEARL) category.		
Cardiovascular/General – Other (Specify,_____)	Severe	Life-threatening or disabling

APPENDIX G continued

Adverse Event	Grade 3	Grade 4
COAGULATION		
DIC (disseminated intravascular coagulation) Also consider Platelets. Note: Must have increased fibrin split products or D-dimer in order to grade as DIC.	Laboratory findings present with no bleeding	Laboratory findings and bleeding
Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura/TTA or hemolytic uremic syndrome/HUS) Also consider Hemoglobin, Platelets, Creatinine. Note: Must have microangiopathic changes on blood smear (e.g., schistocytes, helmet cells, red cell fragments).	Laboratory findings present without clinical consequences Evidence of RBC destruction with creatinine (>3 x ULN) not requiring dialysis	Laboratory findings and clinical consequences, (e.g., CNS hemorrhage/bleeding or thrombosis/embolism or renal failure) requiring therapeutic intervention Evidence of RBC destruction with renal failure requiring dialysis and/or encephalopathy
Coagulation - Other (Specify,_____)	Severe	Life-threatening or disabling
CONSTITUTIONAL SYMPTOMS		
Weight gain associated with Veno-Occlusive Disease (VOD) for BMT studies, if specified in the protocol. Also consider Ascites Edema, Pleural effusion (non-malignant).	0% or as ascites	>10% or fluid retention resulting in pulmonary failure
DERMATOLOGY/SKIN		
Erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)	Severe or requiring IV fluids (e.g., generalized rash or painful stomatitis)	Life-threatening (e.g., exfoliative or ulcerating dermatitis or requiring enteral or parenteral nutritional support)

APPENDIX G continued

Grade		
Adverse Event	**3**	**4**
DERMATOLOGY/SKIN continued		
Rash/desquamation associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	Symptomatic generalized erythroderma or symptomatic macular, papular or vesicular eruption, with bullous formation, or desquamation covering ≥50% of body surface area	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation
GASTROINTESTINAL		
Ascites(none-malignant)	Symptomatic, requiring therapeutic paracentesis	Life-threatening physiologic consequences
Colitis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Melena/GI bleeding, Rectal bleeding/hematochezia, Hypotension.	Abdominal pain, fever, change in bowel habits with ileus or peritoneal signs, and radiographic or biopsy documentation	Perforation or requiring surgery or toxic megacolon
Diarrhea associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol. *For pediatric BMT studies, if specified in the protocol.* Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Pain, Dehydration, Hypotension.	>1500mL of diarrhea/day *>15mL/kg of diarrhea/day*	Severe abdominal pain with or without ileus

APPENDIX G continued

Grade		
Adverse Event	**3**	**4**
GASTROINTESTINAL continued		
Duodenal ulcer (requires radiographic or endoscopic documentation)	Uncontrolled by outpatient medical management; requiring hospitalization	Perforation or bleeding, requiring emergency surgery
Gastric ulcer (requires radiographic or endoscopic documentation) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Bleeding without perforation, uncontrolled by outpatient medical management; requiring hospitalization or surgery	Perforation or bleeding, requiring emergency surgery
Gastritis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Uncontrolled by out-patient medical management; requiring hospitalization or surgery	Life-threatening bleeding, requiring emergency surgery
Pancreatitis Also consider Hypotension. Note: Amylase is graded in the METABOLIC/LABORATORY category.	Abdominal pain with pancreatic enzyme elevation	Complicated by shock (acute circulatory failure)

APPENDIX G continued

Grade		
Adverse Event	**3**	**4**
GASTROINTESTINAL continued		
Mucositis Note: Radiation-related mucositis is graded as Mucositis due to radiation.	Painless erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia
Typhlitis (inflammation of the cecum) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Hypotension, Febrile neutropenia.	Abdominal pain, diarrhea, fever, and radiographic or biopsy documentation	Perforation, bleeding or necrosis or other life-threatening complication requiring surgical intervention (e.g., colostomy)

Appendix G continued

Grade		
Adverse Event	**3**	**4**
HEMORRHAGE		
Notes: Transfusion in this section refers to pRBC infusion. For <u>any</u> bleeding with grade 3 or 4 platelets (<50,000), <u>always</u> grade Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia. Also consider Platelets, Transfusion: pRBCs, and Transfusion: platelets in addition to grading severity by grading the site or type of bleeding. If the site or type of Hemorrhage/bleeding is listed, also use the grading that incorporates the site of bleeding: NS Hemorrhage/bleeding, Hematuria, Hematemesis, Hemoptysis, Hemorrhage/bleeding with surgery, Melena/lower GI bleeding, Petechiae/purpura (Hemorrhage/bleeding into skin), Rectal bleeding/hematochezia, Vaginal bleeding.		
Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia Also consider Platelets, Hemoglobin, Transfusion: platelets, Transfusion: pRBCs, site or type of bleeding. If the site is not listed, grade as Hemorrhage – Other (Specify site,_____). Note: This adverse event must be graded for any bleeding with grade 3 or 4 thrombocytopenia.	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia Also consider Platelets, Hemoglobin, Transfusion: platelets, Transfusion: pRBCs, Hemorrhage – Other (Specify site,_____). Note: Bleeding in the absence of grade 3 or 4 thrombocytopenia is graded here only if the specific site or type of bleeding is not listed elsewhere in the HEMORRHAGE category. Also grade as Other in the HEMORRHAGE category.	Requiring transfusion	Catastrophic bleeding requiring major non-elective intervention

Appendix G continued

Grade		
Adverse Event	**3**	**4**
HEMORRHAGE cont'd		
CNS hemorrhage/bleeding	Bleeding noted on CT or other scan with no clinical consequences	Hemorrhagic stroke or hemorrhagic vascular event (CVA) with neurologic signs and symptoms
Hemoptysis	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Melena/GI bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Rectal bleeding/hematochezia	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Vaginal bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage – Other (Specify site,_____)	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
HEPATIC		
Bilirubin Bilirubin associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	>3.0 – 10.0 x ULN >6 - <15 mg/100mL	>10.0 x ULN >15 mg/100mL

Appendix G continued

Grade		
Adverse Event	**3**	**4**
INFECTION/FEBRILE NEUTROPENIA		
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection)	Present	Life-threatening sepsis (e.g., septic shock)
Infection/Febrile Neutropenia – Other (Specify,_____)	Severe	Life-threatening or disabling
NEUROLOGY		
Aphasia, receptive and/or expressive, is graded under Speech impairment in the NEUROLOGY category.		
CNS cerebrovascular ischemia	Transient ischemic event or attack (TIA)	Permanent event (e.g., cerebral vascular accident)
Leukoencephalopathy associated radiological findings	Severe increase in SAS; severe ventriculomegaly; near total white matter T2 hyperintensities or diffuse low attenuation (CT); focal white matter necrosis (cystic)	Severe increase in SAS; severe ventriculomegaly; diffuse low attenuation with calcification (CT); diffuse white matter necrosis (MRI)
Seizure(s)	Seizure(s) in which consciousness is altered	Seizures of any type which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)
PULMONARY		
Adult Respiratory Distress Syndrome (ARDS)	-	Present
Apnea	Present	Requiring intubation
Carbon monoxide diffusion capacity (DLCO)	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
FEV1	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
Hypoxia	Decreased O2 saturation at rest, requiring supplemental oxygen	Decreased O2 saturation, requiring pressure support (CPAP) or assisted ventilation

Appendix G continued

Grade		
Adverse Event	**3**	**4**
RENAL/GENITOURINARY		
Creatinine *Note: Adjust to age-appropriate levels for pediatric patients.*	>3.0- 6.0 x ULN	>6.0 x ULN
Renal failure	Requiring dialysis, but reversible	Requiring dialysis and irreversible
SECONDARY MALIGNANCY		
Secondary Malignancy – Other (Specify type,_____) excludes metastasis from initial primary	-	present

Appendix H

The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) 9/7/10

Assign scores appropriately if the patient has any of these comorbidities

*Patient*_____*(name), UPN*_____*Date*_____

Comorbidities	Definitions	HCT-CI scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmias requiring treatment *in the patient's past history*	1	
Cardiac	Coronary artery disease†, congestive heart failure, myocardial infarction *in patient's past history* or EF of ≤50% *at time of HCT*	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis requiring treatment *in the patient's past history*	1	
Diabetes	Requiring treatment with insulin or oral hypoglycemic, but not diet alone, *at time of HCT*	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular accident *in patient's past history*	1	
Psychiatric disturbance	Depression/anxiety requiring psychiatric consult or treatment *at time of HCT*	1	
Hepatic – mild	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN *at time of HCT*	1	
Obesity	Patients with a BMI of >35 for adults or with BMI-for-age percentile of ≥ 95th percentile for children *at time of HCT*	1	
Infection	Documented infection or fever of unknown etiology requiring anti-microbial treatment *before, during and after* the start of conditioning regimen	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica *in patient's past history*	2	
Peptic ulcer	Requiring treatment *in patient's past history*	2	
Renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation *at time of HCT*	2	
Moderate pulmonary	DLco and/or FEV_1 >65%-80% or Dyspnea on slight activity *at time of HCT*	2	
Prior solid tumor	Treated at any time point *in the patient's past history*, excluding non-melanoma skin cancer	3	
Heart valve disease	*At time of HCT* excluding mitral valve prolapse	3	
Severe pulmonary	DLco and/or FEV_1 ≤65% or Dyspnea at rest or requiring oxygen *at time of HCT*	3	
Moderate/severe hepatic	Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN *at time of HCT*	3	
Please provide (KPS):	**Karnofsky Performance Score =_____%**	***Total Score*** =____	**Signature of Provider:**

†One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft. EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV_1, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Appendix I
Adverse Events Associated or Expected with Hematopoietic Cell Transplantation

1. Graft versus host disease: GVHD is a major toxicity associated with the infusion of allogeneic donor stem cells. GVHD may be acute or chronic and may affect multiple organ systems, including the skin, liver, and GI tract.

2. Opportunistic infections, including viral and fungal infections, can result in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

3. Gastrointestinal toxicity. Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

4. Cardiac toxicity. Cardiotoxicity (congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with the chemotherapy agents and TBI used in the regimen and these sequelae may prove lethal.

5. Pulmonary toxicity. Diffuse interstitial pneumonitis of unknown etiology and diffuse alveolar hemorrhage occur with some regularity after BMT and interstitial fibrosis occurs much more rarely. Both are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

6. Hepatic toxicity. Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Tacrolimus/Cyclosporine may cause elevation of ALT/AST.

7. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from cyclosporine/tacrolimus and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive multi-agent conditioning plus TBI.

8. Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

9. Central nervous system toxicity. Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

10. Marrow aplasia. Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days following infusion of the graft. Transfusion of platelets and red blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

11. Miscellaneous. Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible following conditioning. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone and increased susceptibility to infection. MMF can cause spontaneous abortions and birth defects. Hospitalization during conditioning and recovery period is expected.