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Title:	Transplantation of Umbilical Cord Blood in Patients with Hematological Malignancies Using a Treosulfan Based Preparative Regimen
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Investigator Statement:

I have carefully read Protocol 2275 entitled "Transplantation of Umbilical Cord Blood in Patients with Hematological Malignancies Using a Treosulfan Based Preparative Regimen" version date 01/24/2019.

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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1. Introduction

Conventional myeloablative allogeneic hematopoietic stem cell transplantation (HCT) is limited by lack of rapidly available HLA matched donors and excess transplant related mortality (TRM). Umbilical cord blood (UCB) is an alternative hematopoietic stem cell source with the advantages of relative tolerance of HLA disparity [1-5] and rapid availability [6]. Emerging data supports the efficacy UCB transplantation (UCBT) to treat hematologic malignancies, including acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and myelodysplastic syndrome (MDS). Optimal conditioning regimens for UCBT, however, particularly in older and infirm patients with high-risk disease, have not yet been defined.

Conditioning regimens play a significant role in the outcome of HCT. While aggressive myeloablative regimens are generally associated with lower relapse risk, they are also associated with increased transplant related mortality (TRM). In contrast, less intensive conditioning regimens that rely more significantly on graft versus disease (GVD) effect to treat disease are generally associated with less toxicity, but are associated with higher relapse risk, particularly among patients with high risk acute leukemias and MDS. Optimizing the balance in conditioning intensity to maximize disease control without increasing TRM is an ongoing challenge in HCT. Treosulfan (TREO) is a busulfan (BU) analog that has several attractive characteristics as compared to BU, including less erratic pharmacokinetics and potential for higher antileukemic activity. TREO is appealing as an agent in a regimen that is of less intensity than conventional myeloablative regimens, but that is capable of generating sufficient anti-leukemic activity to induce sustained remissions in high-risk patients. This protocol will investigate the efficacy of a TREO based conditioning regimen prior to infusion of an unrelated donor cord blood graft.

2. Background and Rationale

Experience with UCBT at the FHCRC is growing rapidly. Since February 2006, 18 patients have been treated on the FHCRC standard myeloablative Protocol 2010 and 13 patients have been treated on the FHCRC reduced-intensity conditioning (RIC) Protocol 2012. Entry criteria for both protocols requires morphologic blast counts less than 5% for AML, ALL, and MDS, but minimal residual disease (MRD) is allowed. Protocol 2010 is open to patients 45 and under, and conditioning is fludarabine (FLU) 75 mg/m^2, cyclophosphamide (CY) 120 mg/kg, and TBI 13.2 Gy. Protocol 2012 is open to patients age 70 and under, and conditioning is FLU 200 mg/m^2, CY 50 mg/kg, and TBI 2 Gy with the addition of ATG 90 mg/kg if there was no exposure to significant chemotherapy in the three months preceding UCBT. With a median follow-up of 390 days among survivors, 17/18 (94%) patients treated on 2010 remain alive and 1/18 (6%) patients has relapsed. Seven of thirteen (54%) patients treated on 2012 remain alive and 5/13 (38%) patients have relapsed.

Outcomes to date in the myeloablative setting have been extremely encouraging. In a high-risk population, we have observed only one relapse among 18 patients and only one death due to TRM. Fifteen of these patients received double cord unit transplantations (three patients received single units). Though the data is preliminary, emerging evidence suggests that double unit UCBT may be associated with reduced risk of relapse for patients with good disease control at the time of transplantation. The University of Minnesota has reported that as compared to single unit UCBT, the introduction of double unit UCBT has markedly reduced the risk of

relapse in AML patients undergoing myeloablative conditioning in morphologic CR [7]. Preliminary analysis of FHCRC data suggests that relapse rate following myeloablative double UCBT may be lower than the relapse rate following conventional donor myeloablative transplant, but longer follow-up and more patients are needed to confirm this observation.

In our RIC approach, used for older patients or patients with significant co-morbidities, relapse has been a larger problem. Five of thirteen patients have relapsed. Four of these patients went to UCBT with MRD or were aplastic following chemotherapy. In the largest series of RIC UCBT reported to date, describing outcomes in 110 RIC UCBTs performed at the University of Minnesota, relapse was the most common cause of death. The incidence of relapse or progression for the 106 patients transplanted for malignant diseases was 31% (95% CI, 21%-41%) and disease progression was the most common cause of death (n=27 versus infection n=12 and GVHD n=6) [8]. Given this apparent increased risk of relapse following RIC, coupled with the observation that double unit UCBT may reduce the risk of relapse among patients with good disease control at the time of UCBT, a preparative regimen with improved anti-leukemic properties but reduced toxicity is particularly appealing.

TREO is a novel agent in RIC regimen that may meet these criteria. TREO (Ovastat®, L-treitol-1,4-bis-methanesulfonate, dihydroxybusulfan, TREO; Medac, Hamburg, Germany) is a prodrug of a bifunctional alkylating agent and a water-soluble intravenous BU analog approved for therapy of advanced ovarian carcinoma in Europe. Under physiologic conditions, TREO is spontaneously activated into epoxide species that cause cross-linking of DNA molecules and cytotoxicity in rapidly proliferating cells, both malignant and non-malignant, such as normal hematopoietic cells [9,10]. Contrary to BU, TREO 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 TREO and BU. BU directly alkylates nucleophylic centers, while TREO induces alkylation of nucleophilic centers by intramolecular epoxide formation. Clinical pharmacokinetic studies have demonstrated that TREO has a similar t½ to BU (1.8 – 2 hours) and a higher, dose-independent, cumulative renal excretion (50% vs. 20%) [11,12]. Pharmacokinetic studies of both single and multiple intravenous infusions of TREO have demonstrated low inter-patient and inter-day variability. There is a high linear correlation between the area under the curve and TREO dose (r2=0.9227), which compares favorably with that of oral and intravenous BU [13,14]. In preclinical studies in mice, rats, dogs and primates, TREO resulted in at least 10 times lower acute and chronic toxicity than BU [unpublished data, Investigator's Brochure]. Preclinical studies have demonstrated the antitumor activity of TREO against breast, lung and renal cell carcinoma, melanoma, myeloma, lymphoma, chronic myeloid leukemia and acute leukemias. TREO induces cell death and apoptosis in human AML cells in vitro [15]. The potent in vivo activity of TREO against human B-cell and T-cell lymphoblasts has been demonstrated in xenograft models in mice [16]. A single dose of 3000 mg/kg TREO or repeated doses of 1000 mg/kg for 3 consecutive days resulted in superior antileukemic activity as compared to maximum tolerated doses of BU or CY in these animal models. The pronounced effect of TREO on primitive and committed hematopoietic stem cells and its immunosuppressive effects have been demonstrated in allogeneic murine transplant models [10]. At doses 80-88% lower than those used for humans in clinical trials, TREO was at least as effective as BU in depleting hematopoietic cell subsets. TREO was capable of inducing full donor engraftment and immune tolerance across MHC barriers. Marrow suppression is the limiting toxicity for conventional chemotherapy with TREO at doses over 10 g/m2 [17]. With autologous stem cell rescue, the TREO dose could be escalated up to 47 g/m2 before dose-

limiting toxicity including mucositis, diarrhea, dermatitis or metabolic acidosis was observed [18]. Severe hepatotoxicity such as veno-occlusive disease (VOD), renal, neurological or pulmonary toxicities known to occur with other high-dose alkylating agents such as BU have not been observed with high-dose TREO.

Several centers outside of the United States have reported encouraging outcomes in small studies with TREO based RIC regimens performed in the traditional donor setting [18-24]. In collaboration with Oregon Health and Science University (OHSU), the FHCRC is participating in the first United States trial of a TREO based regimen in the traditional donor setting. Details of outcomes in TREO studies to date are summarized below.

A total of 83 patients with hematologic malignancies undergoing allogeneic HCT have been treated in Medac-sponsored studies of TREO-containing regimens (Table 1).

Table 1. Preliminary results of studies of treosulfan regimens conducted in Europe.

	Treosulfan/Cyclophosphamide	Treosulfan/Fludarabine
No. Patients	27	56
Age (med, range)	44 (17-64)	50 (18-66)
Diseases	10 AML, 10 ALL, 2 CML, 3 MDS, 1 NHL, 1 Hodgkin	19 AML, 1 ALL, 6 CML, 7 MDS, 8 NHL, 1 Hodgkin, 3 CLL, 11 myeloma
MRD/URD	18/9	28/28
Graft failure	1 (3%)	0
Acute GVHD II-IV	6/27 (22%)	24/56 (43%)
Chronic GVHD	3/15 (20%)	8/42 (19%)
1-year OS	67%	70%
1-year PFS	56%	53%
1-year Relapse	11%	15%
1-year TRM	33%	20%
TRM day 200	20%	15%

Treosulfan and cyclophosphamide

Twenty-seven patients, median age 44 (range 17-64) years, with varied hematologic malignancies received three doses of 12 or 14 g/m^2 Treosulfan and 2 doses of 60 mg/kg Cyclophosphamide followed by HLA-identical sibling (n=18) or matched URD (n=9) marrow or PBSC [19]. GVHD prophylaxis was with cyclosporine and methotrexate. The median time to neutrophil engraftment was 16 (range 11-25) days. One patient did not engraft prior to his death from sepsis on day 24. Acute GVHD of grades II-IV and extensive chronic GVHD were observed in 6/27 (22%) and 3/15 (20%) evaluable patients, respectively. Significant toxicities included NCI grade 3 elevation of liver transaminases (n=12), grade 3 mucositis (n=4), arrhythmia (n=1), grade 4 VOD (n=1) and ARDS (n=2). Three (11%) patients died from relapse. Nine (33%) patients died from non-relapse causes, 6 from infection and 3 from RRT (1 cardiac decompensation/ mucositis, 1 cardiac arrest, 1 multiorgan failure). The 1-year DFS is 56%, with a median follow-up of 12 months.

Treosulfan and fludarabine

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 Fludarabine followed by HLA-identical sibling (n=28) or matched unrelated (n=28) marrow or PBSC [20]. GVHD prophylaxis was with 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/Fludarabine may result in improved RRT and TRM and similar survival compared to Treosulfan /Cyclophosphamide or Busulfan-containing regimens. The relapse rate of 15% is also promising, particularly when considering that 57% of the patients had advanced disease. This rate of relapse is much lower than that reported with Busulfan/Fludarabine studies where 56-57% of the patients had advanced disease. Ongoing European phase I/II studies are evaluating the use of treosulfan-containing regimens for other diseases [*Medac, personal communication, 2007*].

Experience with use of treosulfan in children

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 [23]. 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 adenoleukodystrophy (n=1) were studied. They received three doses of Treosulfan (10 or 12 g/m^2) in combination with other drugs including Fludarabine, Cyclophosphamide, 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 GVHD of grade II was observed in 3 (38%) patients. Extensive chronic GVHD was observed in 1 (13%) patient. Severe or fatal toxicities were not observed. Three patients developed mild 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. Although the number of patients studied is small, this study suggests that the use of Treosulfan in children is feasible and relatively safe, resulting in minimal toxicity or post-transplant complications without an increased risk for relapse. Contrary to the studies on adult patients, this study did not evaluate the potential for using doses of treosulfan higher than 12 g/m^2 in children. Pharmacokinetic studies of Treosulfan in children to date have been limited.

Phase I/II study of treosulfan and fludarabine in the United States

In collaboration with Oregon Health and Science University (OHSU), the FHCRC has conducted a study to determine the optimum dose of treosulfan, in combination with fludarabine prior to allogeneic HCT with traditional donors in patients with AML, ALL, or MDS. “Optimal” dose was determined by safety and efficacy, as defined by incidence of severe/fatal RRT, graft

failure and TRM. Thirty-six patients (8 children and 28 adults > 18 years), with a median of 41 (5-58) years, and diagnosed with AML in first (n=19), second remission (n=7) or not in remission (n=4), ALL in second remission (n=2) or MDS (n=4) have been treated. Patients were considered to be at high risk for TRM because of aggressive therapy for a prior malignancy, comorbid conditions or previous HCT. Patients received Treosulfan at 12 (n=5) or 14 g/m2/day (n=31) on days -6 to -4, and Fludarabine (30 mg/kg/day) on days -6 to -2. GVHD prophylaxis was with tacrolimus and methotrexate (n=35) or cyclosporine and methotrexate (n=1). Donors were HLA-matched siblings (n=19) or unrelated volunteers (n=17); source of stem cells were bone marrow (n=7) or filgrastim-mobilized peripheral blood cells (n=29). Neutrophil engraftment occurred at a median of 18 (11-30) days. Grade II-IV acute graft-versus-host disease (GVHD) was observed in 9 of 36 patients, and chronic GVHD in 8 of 23 evaluable patients surviving beyond post-transplant day 80. Dose-limiting RRT was observed in 1 of 36 patients (mucositis requiring intubation). With a median follow-up of 118 (20-575) days, estimated one-year overall and disease-free survival, relapse and TRM are 83%, 78%, 22% and 0%, respectively. Day-100 mortality observed to date is 6%. Treosulfan/Fludarabine seems to be a well-tolerated regimen with promising disease control and low rates of RRT and TRM in patients with acute leukemia and MDS.

Treosulfan in cord transplant

While a TREO based UCBT regimen holds appeal, two significant concerns specific to the UCBT setting include probable delayed engraftment and the possibility of graft failure. In Minnesota's initial effort at RIC UCBT, it performed 21 transplants following conditioning with BU 8 mg/kg, FLU 200 mg/m2, and 200 cGy TBI. This regimen was associated with neutrophil engraftment at 26 days (12-30) and this delayed neutrophil engraftment resulted in Minnesota switching to the FLU/CY/TBI +/- ATG regimen that is the basis for FHCRC protocol 2012 [25]. The switch to a FLU/CY/TBI regimen in Minnesota resulted in initial autologous hematopoietic recovery, and a decrease in time to neutrophil engraftment to a median of 12 days (range, 0-32). Similarly, in our experience, median time to engraftment on protocol 2012 is 11 days (6-49) compared to 25 days (14-44) on the myeloablative UCBT FHCRC protocol 2010. Based on experience in the traditional donor setting with TREO based regimens [26], as well as Minnesota's experience with an intermediate dose BU based regimen, we expect that a TREO based UCBT regimen will result in prolonged neutropenia. Given the older age and higher comorbidities of patients generally considered for RIC, this prolonged neutropenia is a significant concern. For patients at high-risk for relapse, however, we believe the potential benefit of the increased anti-leukemic affect outweighs the risks.

Our concern about graft failure in the UCBT setting stems from other centers' experience with BU/FLU based regimens. Minnesota experienced 4 graft failures in their first 21 RIC patients receiving BU/FLU (albeit in patients at risk for graft failure by virtue of less treatment before transplant) [25]. Duke has recently reported very high graft failures (6/10 graft failures) following IV BU 520 mg/m2 (mean AUC 4181 umol-min), FLU 160 mg/m2 [27]. Spain, however, has reported good engraftment using a thiotepa 15mg/kg, IV BU 9.6 mg/kg, FLU 150 mg/m2, ATG 8 mg/kg preparative regimen [28]. Patients undergoing an RIC regimen with cord blood as the donor source are at an increased risk of graft failure. Though TREO is likely more immunosuppressive than BU and may promote enhanced engraftment as compared to BU, we believe that a TREO/FLU based regimen may require additional immunosuppressive therapy to promote engraftment. We therefore propose a TREO/FLU regimen using TREO 42 g/m2 and

FLU 150 mg/m2 similar to that established in the FHCRC/OHSU Phase 1 study in the traditional donor setting with the addition of a single dose of 200 cGy TBI. Dose escalation rules are established to first increase FLU to 200 mg/m2 and then increase TBI to 300, 400, and 450 cGy TBI in the event of excessive graft failures.

Previous experience with RIC UCBT suggests that patients who have had limited prior therapy are at particularly greater risk for graft failure. For the purposes of determining acceptable engraftment rates following TREO/FLU/TBI, patients will be classified as low risk for graft failure (Arm 1) or high risk for graft failure (Arm 2) as follows. For each arm, a true probability of graft failure of 5% (or less) will be accepted without dose escalation.

- Arm 1: Low-risk patients include those who have received ≥ 2 cycles of multiagent chemotherapy and at least one cycle of therapy within the 3 months previous to UCBT.

- Arm 2: High-risk patients include those who have received no multiagent chemotherapy or immune suppressive chemotherapy in the 3 months prior to transplant or have received only a single induction therapy.

We believe that a TREO/FLU based conditioning regimen may improve outcomes for acute leukemia and MDS UCBT patients unable to tolerate high-dose myeloablative therapy but at high risk for relapse. In addition to possibly improving outcomes for those RIC patients at high-risk for relapse, a TREO regimen would provide a higher intensity chemotherapeutic treatment option for patients ineligible for high dose radiation and therefore ineligible for FHCRC protocol 2010. Experience with the regimen in this setting may lead to its extension to a broader group of cord patients.

3. Objectives

A. Primary Objective:

The primary objective of this study is to show that TREO/FLU/TBI as a sufficient conditioning regimen reduces the probability of graft failure as compared to an historical benchmark and that the risk of day -200 NRM is not higher than a relevant historical benchmark. Patients will be divided into two arms based on risk of graft failure as described above.

Primary endpoints are as listed below:

1. Graft failure/rejection and secondary graft failure. Patients will be considered primary graft failure/rejections provided they meet any criteria listed below:
    i. Absence of 3 consecutive days with neutrophils ≥500/ul combined with host CD3 peripheral blood chimerism ≥ 50% at day 42
    ii. Absence of 3 consecutive days with neutrophils ≥500/ul under any circumstances at day 55
    iii. Death after day 28 with neutrophil count <100/ul without any evidence of engraftment (< 5% donor CD3)
    iv. Primary autologous count recovery with < 5% donor CD3 peripheral blood chimerism at count recovery and without relapse

Secondary graft failure is defined as decline of neutrophil count to <500/ul with loss of donor chimerism after day 55

2. Day -200 non-relapse mortality

B. Secondary Endpoints:

1. Platelet engraftment by six months
2. Grade II-IV and III-IV acute GVHD at day 100 and one year
3. Chronic GVHD
4. Clinically significant infections
5. Overall survival
6. Relapse or disease progression
7. **(FHCRC only)** Immune reconstitution
8. **(FHCRC only)** Emergence of a dominant unit

4. Patient Selection

A. Inclusions

1. Disease Criteria
   a. Acute Myeloid Leukemia/Acute Lymphoblastic Leukemia, including Biphenotypic Acute Leukemia or Mixed-Lineage Leukemia: Must have < 5% morphologic marrow blasts in an evaluable marrow sample (>25% of normal cellularity for age collected less than one month prior to start of conditioning. Patients in which adequate marrow/biopsy specimens cannot be obtained to determine remission status by morphologic assessment, but have fulfilled criteria of remission by flow cytometry, recovery of peripheral blood counts with no circulating blasts, and/or normal cytogenetics (if applicable) may still be eligible. Reasonable attempts must be made to obtain an adequate specimen for morphologic assessment, including possible repeat procedures. These patients must be discussed with the Principal Investigator, Filippo Milano (667-5925 or pager, 314-1037) prior to enrollment. Patients persistently aplastic for greater than one month since completing last chemotherapy are also eligible with PI approval.
   b. Myelodysplastic syndrome (MDS): Any 2001 WHO classification subtype (Appendix I). RAEB-2 patients may proceed directly to transplant, but may also be considered for induction chemotherapy before transplant. Patients with ≥20% morphologic marrow blasts require induction therapy to reduce morphologic marrow blasts below 5% before transplant.
   c. Chronic myelogenous leukemia: All types, except refractory blast crisis. Chronic phase patients must have failed or been intolerant to Gleevec or other tyrosine kinase inhibitors.

2. Age, Organ Function and Performance Status Criteria
   a. Subjects must be ≤ 65 years old. Patients ≤ 50 must have performance status score: Karnofsky (for adults) ≥ 70 or ECOG 0-1; Lansky (for children) score ≥ 50 (Appendix II). Patients > 50 must have Karnofsky performance score ≥ 70 or ECOG 0-1 and comorbidity index < 5 (Appendix II) .
   b. Adequate cardiac function defined as absence of decompensated congestive heart failure or uncontrolled arrhythmia AND
      i. left ventricular ejection fraction ≥ 35% OR
      ii. fractional shortening >22%
   c. Adequate pulmonary function defined as absence of O2 requirements ***and*** one of the following:
      i. DLCO corrected ≥70% mm Hg
      ii. DLCO corrected between 60% - 69% mm Hg and pO2 ≥70 mm Hg
      iii. DLCO corrected between 50% - 59% mm Hg and pO2 ≥80 mm Hg

      Pediatric patients unable to perform pulmonary function tests must have O2 saturation ≥ 92% on room air. May not be on supplemental oxygen.
   d. Adequate hepatic function. Patients with clinical or laboratory evidence of liver disease will be evaluated for the cause of liver disease, its clinical severity in terms of liver function, histology, and the degree of portal hypertension. Patients with fulminant liver failure, cirrhosis with evidence of portal hypertension or bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, or correctable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin > 3mg/dL, and symptomatic biliary disease will be excluded.
   e. Adequate renal function defined as creatinine ≤ 2.0 mg/dl (adults) or estimated creatinine clearance > 40 ml/min (pediatrics). All adults with a creatinine > 1.2 or a history of renal dysfunction must have estimated creatinine clearance > 40 ml/min.
   f. If recent mold infection, e.g., *Aspergillus,* must be cleared by infectious disease to proceed.
   g. Prior hematopoietic cell transplant: Must be ≥ 3 months after previous transplant.

B. Exclusions

1. Pregnancy or breastfeeding.
2. Evidence of HIV infection or known HIV positive serology.
3. Uncontrolled viral or bacterial infection at the time of study enrollment.
4. Active or recent (prior 6 month) invasive fungal infection without ID consult and approval.

5. CNS leukemic involvement not clearing with intrathecal chemotherapy and/or cranial radiation prior to initiation of conditioning (day –6).

5. Donor Selection

CB donor selection will be based on institutional guidelines and in general should be selected to optimize both HLA match and cell dose. Additionally, CB grafts shall consist of one or two CB donors based on, but not exclusively determined by, cell dose (TNC/kg and CD34/kg), HLA matching and disease status and indication for transplant. Attending preference will be allowed for single versus double unit as well as the degree of mismatching based on patient specific factors, as long as the following minimum criteria are met:

A. HLA matching:
   i. Minimum requirement: The CB graft(s) must be matched at a minimum at 4/6 HLA-A, B, DRB1 loci with the recipient. Therefore 0-2 mismatches at the A or B or DRB1 loci based on intermediate resolution A, B antigen and DRB1 allele typing for determination of HLA-match is allowed.
   ii. HLA-matching determined by high resolution typing is allowed per institutional guidelines as long as the minimum criteria (#A.i., above) are met.

B. Selection of two CB units is mandatory when a single cord blood unit does not meet the following criteria in the table below.

	Single Unit Allowed for:
Match Grade	**TNC Dose**
6/6	$\geq 2.5 \times 10^7$/kg
5/6, 4/6	$\geq 4.0 (\pm 0.5) \times 10^7$/kg

If two CB units are used, the total cell dose of the combined units must be at least 3.0×10^7 TNC per kilogram recipient weight based on pre-cryopreservation numbers, with each CB unit containing a MINIMUM of 1.5×10^7 TNC/kg.

C. The minimum *recommended* CD34/kg cell dose should be 2×10^5 CD34/kg, total dose from a single or combined double.

D. The unmanipulated CB unit(s) will be FDA licensed or will be obtained under a separate IND, such as the National Marrow Donor Program (NMDP) Protocol 10-CBA conducted under BB IND-7555 or another IND sponsored by (1) a participating institution or (2) an investigator at FHCRC or one of the participating institutions.

E. **FHCRC only**: Up to 5% of the unmanipulated cord blood product (s), when ready for infusion, may be withheld for research purposes as long as thresholds for infused TNC dose are met. These products will be used to conduct studies involving the kinetics of engraftment and immunobiology of double cord transplantation.

F. UCB Unit Exclusions

1. Any cord blood units with $<1.5 \times 10^7$ total nucleated cells per kilogram recipient weight.

2. Any cord blood units without full maternal testing and negative results for hepatitis B, C, HIV, and HTLV-1 viruses. Any additional available virology results on the unit itself will be reviewed but are not mandated, complete or always available. Cord blood units are presumed to be CMV negative regardless of serologic testing due to passive transmission of maternal CMV antibodies.

6. Evaluation and Counseling of Patient

The patient and donor units will be completely evaluated per standard practice. The protocol will be discussed thoroughly with patient and caregivers, and all known risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible and the risks and hazards of the procedure explained to the patient or, in the case of minors, to the patient's responsible family members. Informed consent from the patient will be obtained using a consent document approved by the Institutional Review Board (IRB) of the Fred Hutchinson Cancer Research Center.

7. Protocol Registration

FHCRC patients: Eligible patients will be identified by the Clinical Coordinators Office. Patients will be registered with the Registration Office (206-667-4728) between 8:30 am and 4:00 p.m. Pacific time, Monday through Friday. After hours, the Registration office can be reached by paging (206) 995-7437.

8. Plan of Treatment

A. Conditioning Regimen

The study has two arms, determined by risk for graft failure as outlined below:

Arm 1: Low-risk patients include patients who have received ≥ 2 cycles of multiagent chemotherapy and at least one cycle of therapy within the 3 months previous to UCBT.

Arm 2: High-risk patients include patients who have received no multiagent chemotherapy or immune suppressive chemotherapy in the 3 months prior to transplant or have received only a single induction therapy.

Conditioning regimen for both arms will be the same initially. As outlined in the Statistical Considerations (Section 14), dose escalations will occur in either arm if sufficient evidence exists to suggest that the true probability of graft failure exceeds 5%. Stepwise dose escalation if graft failures persist will be to first increase fludarabine to 200 mg/m^2 (administered at 40 mg/m2 daily days -6 to -2), then to escalate TBI to 300 cGy, then to 400 cGy, then to 450 cGy.

Day –6	Fludarabine 30 mg/m^2 IV over 1 hour* Treosulfan 14 g/m^2 IV over 2 hours
Day –5	Fludarabine 30 mg/m^2 IV over 1 hour Treosulfan 14 g/m^2 IV over 2 hours
Day -4	Fludarabine 30 mg/m^2 IV over 1 hour Treosulfan 14 g/m^2 IV over 2 hours
Day –3	Fludarabine 30 mg/m^2 IV over 1 hour
Day –2	Fludarabine 30 mg/m^2 IV over 1 hour
Day –1	TBI 200 cGy (or escalated to 300 cGy, 400 cGy, or 450 cGy per guidelines outlined in the statistical considerations Section 14)
Day 0	UCB transplant

* Fludarabine will be increased to 40 mg/m2 daily days -6 to -2 in the event of excessive graft failures

1. Treosulfan administration.

   a. For patients for patients whose body surface area (BSA) > 1.0 m^2, TREO will be administered intravenously at a dose of 14 g/m^2/day over 120 minutes for three consecutive days (day –6 to –4). Doses of 14 g/m^2/day for infants and smaller children with low BSA results in significantly higher treosulfan exposure (AUC); therefore, the following BSA-dependent dosing schema will be used for the treatment of infants and smaller children when their BSA is ≤1.0 m^2. For patients > 120% of ideal weight, BSA will be calculated using adjusted weight.

Body surface area (m^2)	Treosulfan dose (g/m^2)
≤ 0.5	10.0
> 0.5 – 1.0	12.0
> 1.0	14.0

TREO will be supplied to investigators by Medac GmbH, Hamburg Germany under an institution-sponsored IND of the U.S. Food and Drug Administration (IND 72,479, Approved 6/22/2005, Sponsor: Fred Hutch). 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. TREO 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 for injection for a final concentration of 50 mg/ml. The 0.45% sodium chloride must be warmed to 25-30°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 48 hours when stored at room temperature in the original glass bottle.

2. Fludarabine administration.
   a. Fludarabine will be administered at a dose of 30 mg/m^2 IV over one hour once daily on each of 5 consecutive days for a total dose of 150 mg/m2. Patients > 120% of ideal weight, BSA will be calculated using adjusted weight.
   b. If fludarabine has been escalated to 40 mg/m^2, fludarabine dose for adult patients (≥18 years old) with renal impairment (defined as CrCl < 70mL/minute per 24-hour) is 35 mg/m2 daily for five consecutive days given as noted above. Patients > 120% of ideal weight, BSA will be calculated using adjusted weight.
   c. If fludarabine has been escalated to 40 mg/m^2, fludarabine dose may also be reduced to 35 mg/m2 daily if there is prior malignancy involvement of the central nervous system with intrathecal chemotherapy and/or cranio-spinal irradiation. Patients > 120% of ideal weight, BSA will be calculated using adjusted weight.

3. Total Body Irradiation (TBI)
   See Appendix IV for radiation guidelines

B. Immunosuppressive Therapies

All patients will receive prophylaxis for GVHD with 2 drugs as follows:

1. Cyclosporine A
   a. 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.
   b. Dose adjustments will be made on the basis of toxicity and low CSA levels with a 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.
   c. 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.

2. Mycophenolate mofetil (MMF)
   a. All patients will receive MMF at the dose of 15 mg/kg IV (based on adjusted weight) every 8 hours with a maximum of 1 gram/dose starting 4-6 hours after infusion of UCBT on Day 0. If actual body weight is < ideal weight, calculation based on actual weight is allowed. Rounding of the dose to the nearest 250 mg capsule size is also allowed. Once the patient can tolerate oral medications, MMF may be converted to an oral form.
   b. 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. If GVHD has been diagnosed, continued treatment and eligibility for acute GVHD protocols is at the attending physician's discretion. It is recommended in this setting that MMF be tapered after initiation of therapy for acute GVHD has been started and there is documented response. This is at the attending physician's discretion.

c. MMF dosing to be monitored and altered as clinically appropriate.

d. 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.

C. Umbilical Cord Blood Transplant (UCBT)

1. Procedures for requesting, receiving and characterizing the cord blood unit for infusion will be according to institutional protocol. Procedures for transplantation at outside centers will be reviewed and approved by the PI.

2. 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. At the FHCRC up to 5% of cord blood product, when ready for infusion, may be withheld for research purposes as long as thresholds for infused TNC dose are met.

   a. The thawed product (either one or two units) will be delivered to the patient floor/bedside where the product is double-checked by a nurse with the technologist from the Cellular Therapy Laboratory. Visual inspection of the product is also made at this time. The unit(s) is verified according to 1) the infusion order sheets, 2) the patient's identification number on the cell product, 3) the product (cell) identification number and 4) the patient wrist band.

   b. If the cord blood unit(s) fail to pass inspection or if there is insufficient information to verify the cell product for the patient, notify the Director of the Cell Therapy Lab ((206) 667-4004) and the PI, Filippo Milano (206) 667-5925, pager (206) 314-1037) immediately.

   c. The goal infusion time of each cord blood unit is 30 minutes, as clinically possible. 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.

   d. The product is infused via IV drip directly into the central line according to standard practice. Filter through a blood component administration filter set per Standard Practice Guidelines.

   e. Vital signs should be monitored before beginning the infusion and periodically during administration. Notify the attending physician, fellow or PA immediately if the patient exhibits signs or symptoms of a reaction.

   f. 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.

g. If the patient is a double cord blood recipient, the two units may be given consecutively with no wait between infusion of the units**.** However, infusion of the second unit will **not** begin until any acute toxicities from the first unit have been controlled. The start and stop time of each unit should be recorded on the infusion record.

D. CNS prophylaxis and treatment

All patients with ALL, AML or MDS-refractory anemia with excess blasts (RAEB) will have a diagnostic lumbar puncture prior to the preparative regimen. All patients with ALL will receive prophylaxis with intrathecal therapy. Patients with AML or RAEB will receive intrathecal methotrexate if the cerebrospinal fluid is positive for malignant cells or if they have previous history of CNS leukemia. Cranial radiation will be administered for patients with active CNS disease, as per each center's standard practice guidelines. Guidelines for administration of methotrexate (or alternative drug if patient cannot receive methotrexate) are included in Appendix V. Note: If a patient does not clear CNS leukemia by day –6, he/she will not be eligible to enter the study as described in Section 4.B.

E. Testicular radiation

All male patients with ALL will receive external beam testicular irradiation prior to day 0, per each center's standard practice guidelines. Patients with AML will receive testicular radiation at the same dose if they have history of testicular involvement or active disease at the time of pre-transplant evaluation.

F. Supportive Care

Patients will receive transfusions, infection prophylaxis, and therapy according to institutional guidelines (Appendix VI for FHCRC infection guidelines).

G. Growth Factor Support

Patients will be started on G-CSF support at 5mcg/kg (IV/SQ)(round to vial size) daily starting day +1 following UCB 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.

H. Management of Pre-engraftment Immune Reactions

A well recognized clinical entity consisting of skin rash, fever, and, often, loose stools and respiratory distress has been noted to occur prior to engraftment among cord blood patients, generally between Days +7 and +21. This clinical syndrome likely involves cytokine activation, and though clinically similar to acute or hyperacute graft versus host disease, it appears to be a distinct entity, "pre-engraftment syndrome." This syndrome is often controlled with brief steroid bursts, thus avoiding a commitment to extended steroid exposure. Patients should be monitored carefully for this syndrome.

If patients have moderate to severe symptoms as described above and alternative etiologies (i.e., infection) have been excluded or are being appropriately evaluated, recommendations for management are:

1. For patients not on steroid therapy when the syndrome occurs: methylprednisolone should be given at 1 mg/kg IV q day for three days. If symptoms have abated, steroids should be stopped. If symptoms persist, 1 mg/kg

can be continued through six days then stopped if symptoms have abated. If symptoms persist for more than six days, the patient should be considered to have acute/hyperacute GVHD and should be treated with prolonged steroids as deemed appropriate.

2. For patients already on steroids for other reasons when the syndrome occurs: methylprednisolone should be given at a dose of 3-5 mg/kg IV (max dose 500 mg) q 12 hours x 48 hours, followed by a rapid taper to 1 mg/kg IV q 12 hours. Patients should be weaned after response as tolerated.

9. Evaluation *(in addition to procedures and evaluations listed below, additional clinical evaluations as directed by the clinical team may be captured for research purposes)*

Refer to FHCRC/SCCA Standard Practice Manual for Pre-Transplant Evaluation Guidelines for Allogeneic Transplant (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) See Appendix XII for schedule of study evaluations.

A. Patient Pre-Study Screening and Evaluation Procedures

1. Medical history, allergies, previous chemotherapy, prior radiotherapy, hormonal or immunotherapy and response to treatment, end-organ toxicity and infections.
2. Karnofsky/ECOG/Lansky performance status (Appendix II).
3. Physical examination.
4. Complete blood and platelet count with leukocyte differential.
5. Basic metabolic and hepatic function panels.
6. Urinalysis.
7. Pregnancy test (blood or urine) in females of childbearing potential.
8. Viral titers (HSV, CMV, HIV, HBsAg, HBcAb, HCV, HTLV1/2).
9. PCR for CMV DNA must be done within 2 weeks prior to the start of conditioning.
10. Bone marrow aspirate (+/- biopsy as clinically indicated) collected less than one month prior to start of conditioning.
11. Electrocardiogram
12. MUGA or echocardiography with measurement of the left ventricular ejection fraction (LVEF).
13. Chest radiograph; CXR not required if chest CT performed
14. Pulmonary function tests.
15. DNA specimen from patient and from UCB unit(s) submitted to Clinical Immunogenetics Laboratory (CIL) (FHCRC patients) for chimerism studies.
16. A chest CT without contrast to exclude occult fungal infection prior to transplant for patients with a history of the following:
    - history of MDS or a history of 2 or more consecutive inductions/re-inductions to treat acute leukemia
    - prolonged neutropenia of at least 2 months immediately preceding transplant
17. Pediatric pretransplant evaluation considerations: for children that are not able to cooperate to have a MUGA and/or pulmonary function tests, an echocardiogram

should be attempted and pulse oximetry with exercise tolerance obtained. If not possible at all, it should clearly be documented in the physician's note.

18. Comorbidity Index score to be completed by research staff (Appendix III).
19. Clinical immune reconstitution studies (see section 9.F).
20. Lymphocytes subset enumeration (T cell subset with B and NK cells) **should be done after a patient signs the consent form.**
21. **FHCRC only**: Blood Samples for Host and Donor Immunologic Interaction Studies (see section 9.E).
22. **FHCRC only:** Immune Reconstitution Research Study (see section 9.F)

B. Patient Evaluations from Day 0 until Engraftment (through Day 30)

See Section 9.G below for study evaluation windows and Appendix XII for schedule of study evaluations.

1. Physical examinations daily or as clinically indicated.
2. Complete blood and platelet count daily, or as clinically indicated, until the absolute neutrophil count (ANC) $\geq$5 X 10^8/L for 3 consecutive measurements. Leukocyte differential is to be performed daily if WBC count > 500.
3. Basic metabolic panel daily or as clinically indicated (at least twice weekly).
4. CMV PCR surveillance as clinically indicated per institutional guidelines (Appendix VI).
5. Urinalysis as clinically indicated.
6. Chest radiographs as clinically indicated.
7. Bone marrow aspirate (+/- as clinically indicated) on Day 28 for assessment of underlying disease and UCB engraftment. Day 28 BM specimen submitted at all centers for whole bone marrow chimerism studies. Please note if patient is a double cord blood recipient. Other tests on BM that are disease appropriate on Day 28.
8. Day 28 peripheral blood for chimerism studies (as possible sorted for CD3, CD14, CD33, CD56 cells).
9. **FHCRC only:**
   a. For patients who received a single cord blood unit – peripheral blood for chimerism studies (sorted for CD3, CD14, CD33, CD56 cells) on Day 28.
   b. For patients who receive two cord blood units, peripheral blood for chimerism studies (sorted for CD3, CD14, CD33, CD56 cells) on Day 14 and 28.
10. GVHD evaluation (Appendix VII) weekly or as clinically indicated.
11. Clinical immune reconstitution studies (see section 9.F).
12. **FHCRC only:** Blood samples for Host and Donor Immunologic Interaction Studies (see section 9.E)
13. **FHCRC only:** Immune Reconstitution Research Studies. (see section 9.F)

C. Patient Evaluations from Engraftment to Day 100

See Section 9.G below for study evaluation windows and Appendix XII for schedule of study evaluations.

1. Physical examinations weekly and/or as clinically indicated.
2. Karnofsky/ECOG/Lansky performance once between Day 80 and 100 (Appendix II).

3. Complete blood and platelet count with leukocyte differential at least weekly and/or as clinically indicated.
4. Basic metabolic panel at least weekly and as clinically indicated.
5. CMV PCR surveillance as clinically indicated per institutional guidelines (Appendix VI).
6. Urinalysis as clinically indicated (at least once prior to d/c with urine creatinine/albumin ratio).
7. Chest radiographs as clinically indicated.
8. Peripheral blood for chimerism studies (sorted for CD3, CD33, CD56) (FHCRC patients) on Days 56 and 80.
9. Bone marrow aspirate and biopsy Day 80 (or prior to departure) for all patients for assessment of disease relapse, chimerism and UCB engraftment. DNA BM specimen submitted for chimerism studies. Please note if patient is a double cord blood recipient.
11. GVHD evaluation (Appendix VII) weekly and as clinically indicated.
12. EBV viral load as clinically indicated through day 100 or discharge from transplant center (see Appendix VIII). If ≥ 1000 copies/mL of whole blood refer to Appendix VIII for management.
13. Clinical immune reconstitution studies (see section 9.F).
14. **FHCRC only:** Blood samples for Host and Donor Immunologic Interaction Studies (see section 9.E).
15. **FHCRC only:** Immune Reconstitution Research Studies (see section 9.F).

D. Patient Evaluations at 6 months, 1 year and 2 years

See Section 9.G below for study evaluation windows and Appendix XII for schedule of study evaluations.

1. Physical examinations at 6 months, 1 year and 2 years.
2. Karnofsky/ECOG/Lansky performance status (Appendix II) at 6 months, 1 year and 2 years.
3. Complete blood count with leukocyte differential and serum chemistry at 6 months, 1 year and 2 years and as clinically indicated.
4. CMV surveillance as clinically indicated per institutional guidelines (Appendix VI).
5. Chest radiographs as clinically indicated.
6. Peripheral blood for chimerism studies (sorted for CD3, CD33, CD56) (FHCRC patients) at 6 months, 1 year and 2 years.
7. Bone marrow aspirate and biopsy at 1 year and, as clinically indicated, at 2 years for assessment of UCB engraftment and evidence of recurrent disease. DNA BM specimen submitted for chimerism studies. Please note if patient is a double cord blood recipient.
8. GVHD evaluation (Appendix VII) at 6 months, 1 year and 2 years.
9. Autopsy report, if available, if death occurs before the 2 year follow-up.
10. Clinical immune reconstitution studies (see section 9.F).
11. **FHCRC only**: Immune Reconstitution Research Studies at 1 year and 2 years (see section 9.F).

E. Research Studies (research blood samples may be used interchangeably, and amount of blood to be drawn may be reduced at the investigator/attending physician's discretion and as dictated by patient safety):

FHCRC only: Host and Donor Immunologic Interaction Studies
For patients larger than 20 kg:

For patients 21-30 kg

1. Prior to the start of conditioning (and after consent obtained): 20 ml of peripheral blood will be collected in green top tubes to generate EBV transformed LCL lines from the patient for research studies evaluating donor/host immunologic reactions.
2. Post transplantation on up to five occasions, 20 ml of peripheral blood may be collected in green top tubes to assay for immune mediated responses occurring between the host and donors. Timing of sample collection will be at investigator discretion and research staff will initiate the orders.
3. Please send samples to D2-335; contact Denise Ziegler: phone (206) 667-5762.
4. Samples should be drawn on MONDAY through FRIDAY ONLY.

For patients larger than 30 kg

1. Prior to the start of conditioning (and after consent obtained): 30 ml of peripheral blood will be collected in green top tubes to generate EBV transformed LCL lines from the patient for research studies evaluating donor/host immunologic reactions.
2. Post transplantation on up to five occasions, 30 ml of peripheral blood may be collected in green top tubes to assay for immune mediated responses occurring between the host and donors. Timing of sample collection will be at investigator discretion and research staff will initiate the orders.
3. Please send samples to D2-335; contact Denise Ziegler: phone (206) 667-5762.
4. Samples should be drawn on MONDAY through FRIDAY ONLY.

F. Immune Reconstitution after UCBT
See Section 9.G below for study evaluation windows and Appendix XII for schedule of study evaluations.

Clinical Studies (to be performed as possible):

1. Quantitative immunoglobulin levels (IgG, IgA, IgM) will be assessed at Day 28, 56, 100, 6 months, 1 year and 2 years.
2. Total T lymphocytes and subset enumeration (Lymphocytes panel) will be assessed pre-transplant and at Day 28, 56, 100, 6 months, 1 year and 2 years.

FHCRC only: Immune Reconstitution Research Studies:

1. Samples will be collected for immunophenotypic evaluation of immune reconstitution will be performed once prior to the start of conditioning, then on Day 28, 56, 100, 6 months, 1 year and 2 years. Collect one 10 ml tube. Send 10 ml (Na Heparin green top) to the Digel Lab. Main contact: Denise Ziegler: phone (206) 667-5762.

G. Study Evaluation Windows

The target dates for post-transplant study evaluations are outlined in the table below:

Evaluation Date Post Transplant	Window
Day +7	± 3 days
Day +14	± 2 days
Day +21	± 2 days
Day +28	± 3 days
Day +56	± 7 days
Day +80	± 7 days
Day +100	± 7 days
Day +180	± 30 days
1 year	*
2 year	*

* Every effort will be made to complete the 1 year and 2 year evaluations as close to these dates as possible, taking into consideration patient's circumstances at these time points.

NOTE: In certain clinical circumstances (e.g., relapse or terminal illness) study tests may be omitted at the physician's or PI's discretion.

10. Drugs, Irradiation and Marrow Administration - Toxicities and Complications.

A. Treatment-Related Toxicities

For the purposes of this protocol, toxicity will be graded using the modified NCI common toxicity scale (Appendix IX)

1. Potential toxicities associated with the Conditioning Regimen

Treosulfan

Very Common (>10%)	Common (1-9%)	Uncommon (0.1-0.9%)	Rare/Unknown

• Infection (bacterial/fungal/viral) • Lowering all blood counts leading to anemia, increased risk for infections and bleeding • Mucositis (inflammation lining of mouth which may be painful) • Diarrhea, nausea, vomiting • Abdominal pain • Fever (often associated with very low white blood count) • Increased bilirubin** • Fatigue/lethargy** • Itching skin*	• Rash • Alopecia (temporary hair loss) • Elevated liver function tests • Dysphagia (difficult swallowing) • Decreased appetite** • Insomnia** • Headache/dizzy** • Heart rhythm abnormalities** • High blood pressure** • Shortness of breath** • Gastritis/constipation** • Generalized pain (muscles and bone)** • Abnormal kidney function**	• High blood glucose** • Confusion** • Peripheral neuropathy** • Low blood pressure** • Pneumonitis** • Severe skin rash with blisters and sloughing of skin** • Dry mouth** • Severe liver function abnormality**	• Acidosis, electrolyte abnormalities • Liver failure/severe liver toxicity • Dry eyes • Kidney failure • Heart failure** • Muscle weakness** • Encephalopathy** • High or Low blood pressure*

* Risks unique to Pediatric Patients
**Risks unique to Adult Patients

There have been isolated reports of seizure in infants (4 months of age or less) after receiving treosulfan combined with fludarabine as in this study or with cyclophosphamide (another chemotherapy drug that is not being used in this study). Very young children will be very closely watched for signs of neurologic side effects.

Fludarabine

Common 21-100 people out of 100	**Less Frequent** 5-20 people out of 100	**Uncommon** <5 people out of 100
Severe suppression of blood counts Diarrhea Anorexia Mucositis Nausea/vomiting Stomatitis Osteoporosis Dysuria	Chills Fever GI bleeding Peripheral edema	Neurotoxicity Agitation and confusion Blurred vision Peripheral neuropathy Hearing loss Headache Cerebellar syndrome Blindness Coma Weakness Depression Insomnia Hemorrhagic cystitis (except in FA) Abnormal renal function test Autoimmune hemolytic anemia Deep venous thrombosis Aneurysms

		Pruritic skin rash Abnormal liver function/Liver failure Constipation Transient ischemic attack Dysphagia Myalgia Arthralgia Renal failure

Total Body Irradiation (TBI)*

Common Occurs in 21-100 people out of 100	**Less Frequent** Occurs in 5-20 people out of every 100
• Nausea (feeling sick to stomach) • Fatigue (feeling tired) • The irradiation dose used may result in sterility	• Mucositis (temporary damage to the lining of the mouth) • Suppression of blood counts • Temporary hair loss • Vomiting (throwing up) • Diarrhea (loose stools) • Painful swelling of the parotid gland (a gland under the chin) for a few days • Cataracts (an opacity or whitening of the lens) may develop in the eye • Secondary cancers

* These side effects may be seen more slightly more commonly at the 400 cGy and 450 cGy dose levels. Regarding recovery of testicular function following radiation exposure, sperm counts normally return to pre-TBI levels. This takes about 2 1/2 years for patients given 300 cGy TBI. It takes about 5 years for patients given 400-450 cGy TBI. Contraceptive techniques must be used for at least one year after transplant to minimize the risk of conceiving genetically damaged offspring.

2. Toxicities potentially associated with the infusion of the UCB graft

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, rhythm or function, changes in blood pressure, changes in oxygenation, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, headache, allergic reaction, presence of DMSO taste and odor, hemoglobinuria, and acute renal failure.

3. Potential toxicities associated with Immunosuppressive Therapies

Cyclosporine A

Nephrotoxicity	Thrombotic thrombocytopenic purpura
Seizures	Electrolyte imbalances
Hypertension	Paresthesias/neuropathy
Hirsutism	Gingival hyperplasia
Increased risk of relapse	Increased risk of opportunistic infection

Mycophenolate Mofetil (MMF)

Pancytopenia	Hypertension
Headache	Dizziness
Insomnia	Hyperglycemia
Electrolyte imbalances	Rash
Leg cramps/bone pain	Nausea/diarrhea
Spontaneous abortion	Birth defects
Progressive multifocal leukoencephalopathy	

Toxicities associated with Growth Factor (G-CSF, Neupogen)

Bone pain	Insomnia
Headaches	Dyspnea
Body aches	Rash
Fatigue	Edema
Nausea/vomiting	

11. Protocol Enrollment and Special Considerations

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	11	13	24
Not Hispanic or Latino	49	57	106
Ethnic Category Total of All Subjects*	60	70	130
Racial Categories			
American Indian / Alaska Native	3	3	6
Asian	13	10	23
Native Hawaiian or Other Pacific Islander	5	6	11
Black or African American	8	8	16
White	31	43	74
Racial Categories: Total of All Subjects*	60	70	130

12. Guidelines for Adverse Event Reporting

A. Monitoring the Progress of Trial and the Safety of Participants

This is a Phase 2 clinical trial that is monitored by the principal investigator (PI), Filippo Milano, M.D. The PI reviews the outcome of the data for each individual patient on an ongoing basis.

The PI of the study will have primary responsibility for ensuring that the protocol is conducted as approved by the Scientific Review Committee and Institutional Review Board. The PI will ensure that the monitoring plan is followed and that all data required for oversight of monitoring are accurately reported to the Data and Safety Monitoring Committee, that all adverse events are reported according to the protocol guidelines, and that any adverse reactions reflecting patient safety concerns are appropriately reported. The PI will personally review with the Research Nurse the clinical course of all the enrolled patients at least twice monthly.

B. Reporting of Adverse Events

1) 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. Hospitalizations commonly associated with transplant including, but not limited, to febrile neutropenia, fevers, infections, and gastrointestinal toxicities will not be recorded as serious adverse events as the majority of patients receiving reduced intensity transplants are hospitalized.
- 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.

2) Monitoring and recording AEs

Grade 3 or 4 adverse events (or highly unusual grade 2 adverse events) and all SAEs, 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 on the Case Report Form (CRF). Clinically significant AEs beyond Day 100 may also be captured at the discretion of the investigator. When a subject experiences relapse or graft failure and goes on to further treatment off protocol, adverse events will no longer be collected with the exception of death. 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 maximum severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure

3) Grading of the severity of an Adverse Event

All AEs will be graded in severity according to the modified (for HSCT) NCI Common Toxicity Criteria for Adverse Events (CTCAE) Version 3.0 Appendix IX (https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf). 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.

4) 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.

5) Adverse Event Reporting Period

AEs will be monitored and recorded in study-specific case report forms (CRFs) from the time of first exposure to an investigational product in this study through day +100 post-transplant. The start of the adverse event reporting for a study subject will coincide with signing of the informed consent. AEs with an onset date prior to the first exposure to an investigational product will not be recorded, except in the case of clinically significant worsening of the AE during the specified monitoring time frame. SAEs that occur after the study-specific informed consent is signed but prior to the first dose of the investigational product will be collected only if they are considered by the investigator to be causally related to the study required procedures. A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

Toxicities meeting the study stopping rule criteria will be reported to the IRB within 10 days of study staff awareness. All other SAEs and deaths not meeting the expedited reporting criteria will be reported to the IRB as part of the annual continuation review report to the IRB.

6) Adverse Event Reporting Requirements

A. Research Site Reporting Requirements

Classification of an event as serious or non-serious (see Section 12.B.1) determines the reporting procedures to be followed by the site for reporting the event to the Sponsor. The investigator must report events to the FHCRC IRB in accordance with the policies of the IRB.

Site to Sponsor reporting requirements for adverse events are summarized in Table below:

Classification		**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's Medical Monitor & ISIOC Administrator	Medical Monitor email: ksbaker@fredhutch.org ISIOC email: ISIOC@fredhutch.org

	All SAEs	Within 2 business days of research team* awareness	Submit completed Institution-Sponsored IND SAE Reporting Form signed by PI or designated sub-Investigator	ISIOC email: ISIOC@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.

The information in the Institution-Sponsored IND SAE Reporting Form 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.
The investigator must report events to the FHCRC IRB in accordance with the policies of the IRB.

B. 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 12.B.1.b), the expectedness of the event (see Section 12.B.1.c), and the relationship to participation in the study (see Section 12.B.4). 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.

Refer to Appendix XI for a list of potential adverse events associated or expected with hematopoietic cell transplantation. All collaborating PIs have fulfilled all NIH requirements for training in human subjects protection.

C. <u>Plans for assuring data accuracy and protocol compliance</u>
Collaborating sites send signed consents and eligibility forms to FHCRC study staff with source documents demonstrating eligibility. CRFs are reviewed for adherence to the protocol, accuracy,

and completeness by the FHCRC study staff. Queries are sent to the collaborating investigators if CRFs are inaccurate or incomplete. 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.

D. Oversight and Review of Safety Monitoring

An annual review of the progress of the study with respect to the monitoring plan will be performed by a Data and Safety Monitoring Committee (DSMC). As part of the annual renewal process, the PI will submit an accounting of patient enrollment and outcomes defining the monitoring plan in sufficient detail as to permit verification of the report through chart audit. The IRB provides a final level of annual review. Approval by the DSMC is a necessary but not sufficient condition for final approval by the IRB. The IRB will review the same continuation application and materials that are reviewed by the DSMC, and make an independent assessment of the progress of the trial and determine whether the perceived risk-benefit ratio continues to be acceptable.

13. Records

Clinical Statistics maintains a patient database at FHCRC to allow storage and retrieval of patient data collected from a wide variety of sources. 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. Patients will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. 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.

14. Statistical Considerations

The goal of this study is to show that the probability of graft failure using TREO/FLU/TBI as a sufficient conditioning regimen is statistically significantly less than the fixed historical benchmark of 10%. In addition, we hope to show that probability of day-200 NRM is consistent with a true probability that does not exceed 25%.

The original rule for escalation of FLU/TBI based on excess graft failure will not be in place in the modified protocol; however the stopping rule for day-200 NRM will be expanded to accommodate the increase in accrual. In particular, a true day-200 NRM of 25% will be considered excessive, and this assessment will be made separately for each treatment arm. The expanded stopping rules will be assessed after every 10^{th} enrolled patient becomes eligible for evaluation. The current distribution of patients enrolled to the two different arms is 34 to Arm 1 and 40 to Arm 2. Given these totals, we expect fewer than a total of 80 patients on either arm by the time a total of 130 has been reached. Therefore, the expanded stopping rules for TRM will be assessed after the 40th and 50th patients have been enrolled to Arms 1 and 2, respectively. In particular, this rule would be triggered in a particular arm if any of the following occurs: 13

NRM events among the first 40 (or fewer) patients; 16 among the first 50 (or fewer) patients; 19 among the first 60 (or fewer) patients; 22 among the first 70 (or fewer) patients; 24 among the first 80 (or fewer) patients. If the true probability of NRM is 0.15, the probability of stopping after 50 or 70 patients is approximately .005 and .005, respectively. If the true probability of day-200 NRM is 0.40, the stopping probabilities are approximately .77 and .85, respectively (estimated from 5,000 simulations).

Secondary endpoints will be evaluated and include overall survival (with 65 patients in an arm, we can be 80% certain that estimated one-year survival is within at least 0.08 of the true one-year survival), non-relapse mortality, platelet engraftment, acute and chronic GVHD, relapse, progression-free survival, and clinically significant infections. Each of these will be treated as a time-to-event endpoint, and Kaplan-Meier and cumulative incidence estimates will be used to summarize each, as appropriate.

15. References

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APPENDIX I: WHO classification and criteria for the myelodysplastic syndromes (MDS)

Disorder	Peripheral Blood	Bone Marrow
Refractory Anemia (RA)	**Anemia** **No or rare blasts**	**Erythroid dysplasia only** **<5 percent blasts** **<15 percent ringed sideroblasts**
Refractory Anemia with Ringed Sideroblasts (RARS)	**Anemia** **No blasts**	**Erythroid dysplasia only** **<5 percent blasts** **≥15 percent ringed sideroblasts**
Refractory Cytopenia with Multilineage Dysplasia (RCMD)	**Bi- or pan-cytopenia** **No or rare blasts** **No Auer rods** **Monocytes** **<1,000/µL**	**Dysplasia in ≥10 percent of cells in two or more myeloid cell lines** **No Auer rods** **<5 percent blasts** **<15 percent ringed sideroblasts**
Refractory Cytopenia with Multilineage Dysplasia and Ringed Sideroblasts (RCMD-RS)	**Bi- or pan-cytopenia** **No or rare blasts** **No Auer rods** **Monocytes** **<1,000/µL**	**Dysplasia in ≥10 percent of cells in two or more myeloid cell lines** **No Auer rods** **<5 percent blasts** **≥15% ringed sideroblasts**
Refractory Anemia with Excess Blasts-1 (RAEB-1)	**Cytopenias** **<5% blastss** **No Auer rods** **Monocytes** **<1,000/µL**	**Unilineage or multilineage dysplasia** **5-9 percent blasts** **No Auer rods**
Refractory Anemia with Excess Blasts-2 (RAEB-2)	**Cytopenias** **5-19% blasts** **Auer rods ±** **Monocytes** **<1,000/µL**	**Unilineage or multilineage dysplasia** **10-19 percent blasts** **Auer rods ±**
MDS-Unclassified (MDS-U)	**Cytopenias** **No or rare blasts** **No Auer rods**	**Unilineage dysplasia in granulocytes or megakaryocytes** **No Auer rods** **<5 percent blasts**
MDS with del(5q) "5q- syndrome"	**Anemia** **<5 percent blasts** **Platelets usually normal or increased**	**Normal to increased megakaryocytes with hypolobulated nuclei** **No Auer rods** **<5 percent blasts Isolated del(5q)**

APPENDIX II: Performance Status Scales

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Lansky Play Performance Status Scale

Lansky Play Performance Scale	
Score	Description
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both, greater restrictions of, and less time spend 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
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 III: The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI)

Instructions: Circle applicable scores and provide actual value or cause of' co-morbidity.

Comorbidities	Definitions	HCT-CI weighted scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventriuclar arrhythmias	1	
Cardiac	Coronary artery disease‡, congestive heart failure, myocardial infarction, or EF≤50%	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis	1	
Diabetes*	Requiring treatment with insulin or oral hypoglycemics, but not diet alone	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular Accident	1	
Psychiatric Disturbance	Depression anxiety requiring psychiatric consult or treatment	1	
Hepatic -mild*	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN	1	
Obesity*	Patients with a body mass index > 35kg/ m^2	1	
Infection*	Requiring continuation of anti-microbial Treatment after day 0	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD Polymyalgia rheumatica	2	
Peptic ulcer*	Requiring treatment	2	
Moderate/severe renal*	serum creatinine>2mg/dl, on dialysis, or prior renal transplantation	2	
Moderate pulmonary*	DLCO and/or FEV, >65%-80% or Dispend on slight activity	2	
Prior solid tumor	*Treated at any time point in the patient's past history, excluding non-melanoma skin cancer*	3	
Heart valve disease*	Except mitral valve prolapse	3	
Severe pulmonary*	DLCO and/or FEV_1 <65% or Dyspnea at rest requiring oxygen	3	
Moderate/severe Hepatic	Liver cirrhosis, Bilirubin>1.5XULN or AST/ALT>2.5XULN	3	
Please provide Karnofsky performance (KPS): Score=_____%		**Total Score =_____**	

Completed by (print):_____ Date completed:_____

Signature:_____

*Comorbidity is currently active or patient requires medical treatment +

‡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 erythematosus; 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 IV: Guidelines for Total Body Irradiation

All patients who have had previous radiation therapy or TBI should be seen by Radiation Oncology prior to entrance on the protocol for approval for additional TBI. TBI may be delivered by local guidelines provided the effective dose is equivalent to what is recommended in the TBI Guidelines.

Patients ineligible for this protocol include those who have had previous irradiation to areas of the body such that the Radiation Oncologist feels that even a relatively small dose of total body irradiation (TBI) cannot safely be given.

For patients previously exposed to total body irradiation (TBI), the Radiation Oncologist will determine whether the patient can safely receive additional TBI based on prior exposure history. In addition, the treating Radiation Oncologist, along with the attending physician, will determine the need for lung and/or kidney shielding from the radiation beam to prevent excessive toxicity and again based on the history of exposure. No other organs may be shielded.

TBI dosing guidelines

Dose level 1:
The treatment dose regimen will be 200 cGy in one fraction. No lung shielding or kidney shielding will be used for this TBI scheme unless patients have experienced previous irradiation (see lung shielding below).

Dose level 2:
The second dose level will be 300 cGy in one fraction for the first TBI dose level. No lung shielding or kidney shielding will be used for this TBI scheme unless patients have experienced previous irradiation (see lung shielding below).

Dose level 3:
The third dose level will be 400 cGy in one fraction. Lung shielding will be used for this TBI scheme as outlined below.

Dose level 4:
The fourth dose level will be 450 cGy in one fraction for patients > 7 years old. Patients ≤ 7 years old will receive 225 cGy BID over one day. Lung shielding will be used for this TBI scheme as described below.

Modality:
High-energy photons with energy ≥ 6MV photons should be utilized. Although there is no upper limit on the energy as long as the skin dose requirements can be met, it is recommended that 18MV or lower be used. The selection of energy is determined by the dose uniformity criterion.

Target Volume:

The total body will be treated including the head and feet in one field (except in certain circumstances). Care should be taken to ensure that the patient is entirely within the 90% isodose decrement line of the beam (i.e., not in the penumbra region of the beam).

Target Dose:
The prescription point is defined as the point along the longitudinal axis of the patient at the midline at the level of the umbilicus (see Prescription **Point 2**). No tissue inhomogeneity correction will be made in the calculation of dose to the prescription point. The absorbed dose along the patient's head to toe axis (line formed by the intersection of the midsagittal plane and the midcoronal plane) shall be within 10% of the prescribed dose. The dose at selected anatomical points shall be calculated and these calculations are to be submitted as part of the quality assurance. Measurements of patient dimensions needed for the calculation of the prescription dose will be made at the time of the simulation for lung blocks.

Measurement and calculations of required monitor units may be necessary for each treatment and will be performed for both the expected upright treatment position (AP-PA fields) and the reclining, lateral decubitus position (AP-PA fields) or lateral field techniques. Pediatric patients may be treated prone and supine under anesthesia by AP-PA fields. In the event the patient proves too ill to receive a fraction in the upright position, dose calculation may be pre-calculated to permit treatment in the lateral decubitus position).

Dose point calculations:
1. **Head (Point 1)**: this reference point is defined along the longitudinal axis of the skull at the greatest mid-separation (immediately superior to the nasal bridge). The depth should be taken as midway between the entrance and exit points of the opposed radiation beams.
2. **Neck (Point 2)**: this reference point is defined along the patient's longitudinal axis at the level of C3/C4 (approximate mid-neck, but chosen for the thinnest mid-separation of the neck). The point is taken to be midway between the entrance and exit point of the beam.
3. **Upper Mediastinum (Point 3)**: this reference point is defined along the patient's longitudinal axis at the level of the angle of Louis. The reference point is midway between the entrance and the exit points of the opposed beams.
4. **Lower Mediastinum (Point 4)**: this reference point is defined along the patient's longitudinal axis at the level of the xiphisternal notch. The reference point is midway between the entrance and exit points of the opposed beams
5. **Umbilicus (Point 5)**: THE PRESCRIPTION POINT is defined along the patient's longitudinal axis at the level of the umbilicus. The prescription point is midway between the entrance and exit points of the opposed beams.
6. **Knee (Point 6):** this reference point is defined along the midline in the midplane of the knee at the level of the patella.
7. **Ankle (Point 7)**: this reference point is defined along the midline at the midplane of the ankle at the level of the lateral malleolus.
8. **Shielded Lung Dose (Point 8)**: this reference point is located on the right chest wall under the lung block. It is centered (both medial/lateral and cephalocaudad) under the lung block as projected on the patient's skin. The depth should be taken as midway between the entrance and exit points of the opposed radiation beams. Dose measurements at this location will be taken during a fraction with lung shielding in place.

9. **Unshielded Lung Dose (Point 9)**: This reference point is the same as point 8. Dose measurements at this location will be taken during a fraction without lung shielding in place. The depth should be taken as midway between the entrance and exit points of the opposed radiation beams.

Prescription Points/Dose Monitoring Points:
1. **Head (Point 1):** this prescription point/dose monitoring point is defined along the longitudinal axis of the skull at the greatest mid-separation (immediately superior to the nasal bridge). The depth should be taken as midway between the entrance and exit points of the opposed radiation beams. Prescription point measured each fraction AP/PA.
2. **Umbilicus (Point 2)**: the prescription point/dose monitoring point is defined along the patient's longitudinal axis at the level of the umbilicus. The prescription point is midway between the entrance and exit points of the opposed beam. Prescription point measured each fraction AP/PA.
3. **Ankle (Point 3)**: this dose monitoring point is defined along the midline at the midplane of the ankle.

Dose Definition:
The absorbed dose is specified as centigray (cGy)-to-muscle.

Dose Rate:
A mid-plane dose rate of between 6 and 15 cGy per minute is required.

Dose Uniformity:
The objective is to keep the dose throughout the body, defined to extend to within 2 mm of the skin surface, to at least 90% of the prescription dose. In addition, the brain dose shall not exceed 107% of the prescription dose. For some AP/PA treatments, partial transmission lung blocks will be used to limit the overall total lung dose. The dose at the midpoint of the thickest part of the body while in the treatment position should be determined and if necessary, modifications made to the treatment to raise the dose in this region to at least 90% of the prescription dose.

In order to satisfy the requirement that the skin dose at a depth of 2 mm is within at least 90% of the prescription dose, beam spoilers or other equally effective devices should be used. The field size shall be such that no part of the patient extends into the portion of the penumbra region where the dose is less than 90% of the central axis dose.

Treatment Technique:
Patients will be treated using AP/PA fields in an upright seated or standing position in a TBI positioning device. Treatment will be delivered with equally weighted parallel opposed portals, with each treatment including both AP and PA fields. If the patient is unable to tolerate the upright position, acceptable alternate arrangements will include equally weighted AP-PA or lateral parallel opposed fields delivered to the patient in a lateral decubitus position on a treatment couch or gurney.

Changes in patient positioning after the patient has started TBI are discouraged. When unavoidable, to ensure compliance to the overall dose and lung shielding parameters, appropriate

changes in lung blocking and dose recalculation will be required. Young patients requiring anesthesia will be treated in an AP/PA configuration at extended distance.

Dose Calculation for the Prescription Point:
The calculation of the treatment time or the monitor units for the prescribed dose can be carried out using standard techniques. However, TBI presents special problems relative to the routine treatment situation in that the field sizes are much larger and the treatment distances much longer. The TBI percent depth dose (PDD) or Tissue Maximum Ratio (TMR) and output factors should be measured under TBI treatment conditions for a range of phantom sizes to establish the database for TBI beam-on time calculations or to validate the calculation methodology.

Typically, a calculation methodology will be adopted which uses PDD or TMR and output factors measured under standard conditions but then modified to account for the larger treatment distance. For example, modified values for inverse square corrected percentage depth dose or tissue-air ratios and tissue phantom ratios are necessary for some treatment units when the patient is positioned at a long distance from the photon source and near the floor or one wall of the room. Also, some deviation from an exact inverse square decrease with distance has been demonstrated for certain geometries.

Measurements of dose at the center of a phantom about the size of the typical patient should be performed and compared to the calculated dose. If differences are found, additional correction factors should be introduced to the calculation method.

Critical Organ Dose Points:
The required dose calculations should be performed for at least 3 points referenced above, including the dose monitoring points. The midline dose at these locations should be recorded on the TBI Summary Form. The dose can be calculated based on the thickness at each location and factors appropriate to the TBI treatment conditions.

It is recommended that entrance and exit TLDs or diodes be placed on the patient at each required dose assessment location. The midline dose can be calculated from these measurements making the appropriate corrections to the readings and then averaging the corrected values.
In younger patients it is also recommended that TLDs or diodes be placed underneath the lung blocks to document the transmission dose and scatter dose.

Lung Shielding:
Lung shielding shall be used in all patients who receive a dose $\geq$ 400 cGy total TBI. One half value layer transmission (HVL) blocks will be used for **Dose Levels 3 and 4**. In addition, for patients receiving 200 or 300 cGy of TBI who have received previous irradiation, the treating Radiation Oncologist will determine whether the patient can safely receive additional TBI based on prior exposure history. In addition, the treating Radiation Oncologist, along with the attending physician, will determine the need for lung and/or kidney shielding from the radiation beam to prevent excessive toxicity and again based on the history of exposure. If the lungs should be shielded, one half value layer lung blocks will be used to decrease the photon contribution to the lung dose to approximately half that of the prescription point.

In the event that the Radiation Oncologist feels it is necessary, one half value layer POSTERIOR kidney blocks may be used to decrease the photon contribution to the kidneys to approximately 75% that of the prescription point.

Lung-Block Design:
Lung blocks will confirm to the following guidelines: The lateral edges will be 1.0 – 1.5 cm from the inner border of the ribs; the inferior edges will be 1.0 – 1.5 cm from the dome of the apex of the diaphragm; the superior borders will be 1.0 – 1.5 cm below the clavicles; the medial border 2.0 – 2.5 cm from the lateral edges of the thoracic vertebral bodies. No contouring of the lung shields will be done around the hilum unless there is a residual abnormal hilar adenopathy, in which case the margins around the hilar mass will be 1.0 – 1.5 cm.

Lung-Block Timing:
Lung blocks will be employed for the treatment(s) listed below. Should patient infirmity preclude upright positioning during a fraction when lung shielding is prescribed, that patient may be treated in the lateral decubitus position without lung shielding, and lung shielding can be deferred until the next treatment fraction. For children receiving TBI under anesthesia, treatments will be performed in a modified supine and prone position, with the appropriate lung shielding as specified in the protocol.

Lung blocks will be used for the first fraction of the first incremental dose escalation over 300 cGy, i.e., 400 cGy for one fraction, or 450 cGy for one fraction, or both fractions for pediatric patients treated with 225 cGy x 2. This schema is calculated to deliver a nominal dose of approximately 200 cGy for **Dose Level 3** (400 x 50% = 200); and 225 cGy for **Dose Level 4** (450 x 50% = 225 cGy). No compensatory electron boost of that portion of the chest wall shielded by the lung blocks is required.

APPENDIX V: Intrathecal Diagnostics and Therapeutics

Disease:	Intervention:
ALL – remission or relapse • Positive cytology on LP • Previous history of positive CNS • Negative history and cytology	Lumbar puncture with therapy 2 pre-transplant IT and 6 post-transplant + cranial XRT 2 pre-transplant IT and 6 post-transplant 2 pre-transplant IT and 4 post-transplant
AML – remission or relapse • Positive cytology on LP • Previous history of positive CNS • Negative history and cytology	Diagnostic lumbar puncture 2 pre-transplant IT and 6 post-transplant 2 pre-transplant IT and 6 post-transplant No therapy
MDS • RAEB • RA or RARS	 Treat as AML No LP or IT therapy indicated

Methotrexate

Standard intrathecal methotrexate dosing (serum creatinine < 1.5 baseline)

Age	Lumbar Puncture	Ommaya Reservoir (1/2 of Lumbar Puncture Dose)
Age < 2 yrs	4 mg	2 mg
Age 2-3 yrs	6 mg	3 mg
Age ≥ 4 yrs	12 mg	6 mg

Monitoring of serum methotrexate levels
1. Renal insufficiency: if serum creatinine is 1.5 to 2 times the pre-transplant baseline, administer Intrathecal
methotrexate and follow serum levels.
2. Meningeal Inflammation: patients with active meningeal inflammation (due to malignant disease and/or prior therapy) may have significant leakage of methotrexate into the systemic circulation; such patients may require monitoring of serum methotrexate levels, particularly during the posttransplant period, to avoid marrow toxicity.
3. Suspected methotrexate toxicity: monitoring of serum methotrexate level following intrathecal therapy is appropriate in patients who have had suspected systemic methotrexate toxicity (hematologic or hepatic) following previous intrathecal doses.
4. Significant accumulations of third space fluid (e.g. ascites, pleural or pericardial effusions) may be associated with impaired clearance of methotrexate. The decision to administer Intrathecal methotrexate in these clinical settings should be made by the attending physician. Monitoring of serum methotrexate levels is appropriate when methotrexate is administered in the setting of clinically evident third space fluid accumulation, since the effective half-life of the drug may be prolonged.
5. Leucovorin should be given if the methotrexate level exceeds 0.04 micromolar at 24 hours. Dosing is 10 mg/m2 every 6 hours IV or PO (tablets available as 5 mg, 10 mg, 25 mg), and should be continued until a methotrexate level < 0.04 micromolar is documented. IV and oral doses are bioequivalent.
6. Liver dysfunction: If administering Intrathecal methotrexate in the setting of liver dysfunction with bilirubin > 20 mg/dl, monitor serum methotrexate levels.
7. Methotrexate levels and/or automatic leucovorin rescue are not routinely indicated in patients with normal renal function receiving prophylactic therapy.

Alternative Intrathecal therapy – cytarabine (Ara-C)

Alternative intrathecal therapy with cytarabine (Ara-C) should be considered in the following circumstances:
1. Significant renal insufficiency with serum creatinine > 2x baseline.
2. Toxicity with previous doses of Intrathecal methotrexate has been demonstrated.
3. Persistent third space fluid collections (ascites or pleural effusion).
4. Meningeal inflammation felt to be secondary to methotrexate therapy.
5. Significant liver dysfunction.

Standard Intrathecal Cytarabine (Ara-C) Dosing

Age	Lumbar Puncture	Ommaya Reservoir
Age < 2 yrs	16 mg	16 mg
Age 2-3 yrs	20 mg	20 mg
Age ≥ 4 yrs	24 mg	24 mg

Alternative Intrathecal therapy – Triple Therapy with Methotrexate, Hydrocortisone, and cytarabine (Ara-C)
Triple intrathecal therapy with methotrexate, hydrocortisone (hydrocortisone), and cytarabine (Ara-C) is sometimes given to patients who have CNS disease that is refractory or poorly responsive to monotherapy with either methotrexate or cytarabine (Ara-C). Such patients should be treated twice weekly until their spinal
fluid (CSF) becomes negative for malignant cells.

Standard Triple Intrathecal Dosing

Age	Lumbar Puncture			Ommaya Reservoir		
	Methotrexate	Hydrocortisone	Ara-C	methotrexate	Hydrocortisone	Ara-C
Age < 2 yrs	4 mg	8 mg	16 mg	2 mg	8 mg	16 mg
Age 2-3 yrs	6 mg	10 mg	20 mg	3 mg	10 mg	20 mg
Age ≥ 4 yrs	12 mg	12 mg	24 mg	6 mg	12 mg	24 mg

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APPENDIX VI: Infection Treatment and Prophylaxis Guidelines

CMV screening and pre-emptive treatment

CMV.pdf

Anti-fungal treatment

Antifungal_Therapy.pdf

PCP prophylaxis

PCP.pdf

HSV and VZV prophylaxis and treatment

HSV and VZV.pdf

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APPENDIX VII: GVHD Staging and Grading

ACUTE GVHD ASSESSMENT

Staging by Individual Organ Involvement

SKIN: measured by rash first appearing generally between 10 and 70 days after transplant. (excludes rashes of known viral or other origin)

Stage	Description
1	Maculopapular rash <25% BSA
2	Maculopapular rash 25 – 50% BSA
3	Generalized erythroderma
4	Generalized erythroderma with bullous formation and desquamation

LIVER*: measured by total serum bilirubin

Stage	Description
1	2.0 – 2.9 mg/dL
2	3.0 – 5.9 mg/dL
3	6.0 – 14.9 mg/dL
4	≥ 15.0 mg/dL

GUT:** includes only diarrhea occurring after Day +21

Score	Adult	Pediatric***
1	upper GI (anorexia, nausea, vomiting) with diarrhea of <1000 mL/day	upper GI (anorexia, nausea, vomiting) with diarrhea of <555 mL/m^2/day
2	1000 – 1499 mL/day diarrhea	556-833 mL/m^2/day diarrhea
3	≥ 1500 mL/day diarrhea	>833 mL/m^2/day diarrhea
4	severe abdominal cramping, bleeding or ileus caused by GVHD	

* In cases where another cause of hyperbilirubinemia antedated the onset of rash, the liver score should be decreased by one stage.

** In cases where peak GI symptoms are exacerbated by a cause other than GVHD, the gut score should be decreased by one stage.

*** Pediatric patients <17 years of age

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ACUTE GVHD ASSESSMENT

Overall Grade

The determination of an overall GVHD grade should be based on the organ stage, response to treatment and whether GVHD was a major cause of death.

Overall Grade	Organ Stage	Qualifying Conditions	Additional Qualifying Conditions
I	Stage 1 -2 skin	No liver or gut	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD.
II	Stage 3 skin or Stage 1 liver or Stage 1 gut	N/A	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD, but glucocorticoid treatment after the onset of GVHD was generally sufficient to control the disease.
III	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**without** GVHD as a major contributing cause of death	Indicates that the prophylactic immunosuppressive regimen was not sufficient to prevent all manifestations of aGVHD and that additional treatment after the onset of GVHD did not readily control the disease.
IV	Stage 4 skin or Stage 2-4 liver or Stage 2-4 gut	**with** GVHD as a major contributing cause of death	GVHD was resistant to both the prophylactic immunosuppressive regimen and any additional treatment after the onset of the disease.

APPENDIX VII: GVHD Staging and Grading (continued)

CHRONIC GVHD

In the past, any manifestation of GVHD that was present (or continued) at 100 days after HCT or thereafter was arbitrarily defined as chronic GVHD even if the clinical manifestation was indistinguishable from that of acute GVHD. Advances in HCT practice in the past 2 decades have profoundly altered the presentation and natural history of both acute and chronic GVHD and bring previous definitions into question. For instance, acute GVHD may present beyond 3 months in patients who have received reduced-intensity conditioning whereas manifestations of acute and chronic GVHD can be present simultaneously. Therefore, the current consensus is that clinical manifestations, and not the time to symptomatic onset after transplantation, determine whether the clinical syndrome of GVHD is considered acute or chronic.[1]

Chronic GVHD will therefore be defined according to the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. Diagnosis of chronic GVHD will require the presence of at least 1 diagnostic clinical sign of chronic GVHD or presence of at least 1 distinctive manifestation confirmed by pertinent biopsy or other relevant tests after the exclusion of other possible diagnoses. Chronic GVHD will be described as mild, moderate, or severe as graded according to the attached Organ Scoring Sheet. Symptoms developing after day 100 but consistent with acute GVHD only will be considered persistent, recurrent, or late-onset acute GVHD. Symptoms consistent with both chronic and acute GVHD occurring after day 100 will be considered overlap chronic GVHD syndrome.

a. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host-disease: 1. Diagnosis and Staging Working Group Report. Bio Blood and Marrow Transplant 2005;11:945-955.

Chronic GVHD Organ Scoring Sheet

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	SCORE 0	SCORE 1	SCORE 2	SCORE 3
PERFORMANCE SCORE: ☐ **KPS ECOG LPS**	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* ☐ Maculopapular rash ☐ Lichen planus-like features ☐ Papulosquamous lesions or ichthyosis ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Keratosis pilaris ☐ Erythema ☐ Erythroderma ☐ Poikiloderma ☐ Sclerotic features ☐ Pruritus ☐ Hair involvement ☐ Nail involvement **% BSA Involved** ☐ ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No Symptoms	☐ <18% BSA with disease signs but **NO** sclerotic features	☐ 19-50% BSA **OR** involvement with superficial sclerotic features "not hidebound" (able to pinch)	☐ >50% BSA **OR** deep sclerotic features "hidebound" (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH *Diagnostic/distinctive features* ☐ Present ☐ Absent ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No symptoms	☐ Mild symptoms with disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
EYES Mean tear test (mm): ☐ >10 ☐ 6-10 ☐ ≤5 ☐ Not done ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	☐ Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision caused by keratoconjunctivitis sicca
GI TRACT ☐ *Abnormality present but NOT thought to represent GVHD*	☐ No symptoms	☐ Symptoms such as nausea, vomiting,anorexia,dysphagia, abdominal pain or diarrhea without significant weight loss (<5%)	☐ Symptoms associated with mild to moderate weight loss (5-15%)	☐ Symptoms associated with significant weight loss >15%, require nutritional supplement for most calorie needs **OR** esophagea dilation

	SCORE 0	SCORE 1	SCORE 2	SCORE 3
LIVER	☐ Normal LFT	☐ Elevated Bilirubin, AP*, AST or ALT <2 x ULN	☐ Bilirubin >3 mg/dl or Bilirubin, enzymes 2-5 x ULN	☐ Bilirubin or enzymes > 5 x ULN
☐ *Abnormality present but NOT thought to represent GVHD*				
LUNGS ‡ ☐ **PFTs not done** **FEV1** ☐ **DLCO** ☐	☐ No symptoms ☐ FEV1 > 80% **OR** LFS=2	☐ Mild symptoms (shortness of breath after climbing one flight of steps) ☐ FEV1 60-79% **OR** LFS 3-5	☐ Moderate symptoms (shortness of breath after walking on flat ground) ☐ FEV1 40-59% **OR** LFS 6-9	☐ Severe symptoms (shortness of breath at rest; requiring 0_2) ☐ FEV1 ≤39% **OR** LFS 10-12
☐ *Abnormality present but NOT thought to represent GVHD*				
JOINTS AND FASCIA	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM ***AND*** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
☐ *Abnormality present but NOT thought to represent GVHD*				
GENITAL TRACT *Diagnostic/ Distinctive features*: ☐ Present ☐ Absent ☐ Not examined	☐ No symptoms	☐ Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	☐ Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	☐ Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum
☐ *Abnormality present but NOT thought to represent GVHD*				

Other indicators, clinical manifestations or complications related to chronic GVHD (check all that apply):

☐ Weight loss ☐ Bronchiolitis obliterans ☐ Bronchiolitis obliterans with organizing pneumonia

☐ Esophageal stricture or web ☐ Pericardial Effusion ☐ Pleural Effusion(s) ☐ Ascites (serositis)

☐ Nephrotic syndrome ☐ Peripheral Neuropathy ☐ Myasthenia Gravis ☐ Polymyositis

☐ Malabsorption ☐ Cardiac conduction defects ☐ Coronary artery involvement ☐ Cardiomyopathy

☐ Eosinophilia >500/microliter ☐ Other: _____ ☐ **None**

Biopsy obtained: ☐**Yes** ☐**No** **Organ system(s) biopsied:**_____ **GVHD confirmed by histology:** ☐**Yes** ☐**No**

OVERALL severity of GVHD: ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Change from previous evaluation: ☐ **No GVHD** ☐ **Improved** ☐ **Stable** ☐ **Worse** ☐ **N/A (baseline)**

Completed by (print):_____ **Date completed:**_____

Signature:_____

‡ Pulmonary scoring should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO (carbon monoxide diffusion capacity corrected for hemoglobin) is not available, grading using FEV1 (forced expiratory volume) should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established.[28] The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: > 80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; < 40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

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APPENDIX VIII: EBV Viral Load Monitoring and Management

1. Monitoring of EBV viral load: monitor EBV viral load every 2 weeks between Day 30 and Day 100 or discharge from transplant center

    - If viral load positive (≥ 1000 copies/mL of whole blood)

        i. Complete physical exam looking for peripheral adenopathy and splenomegaly

        ii. CT chest, abdomen, and pelvis, if possible with contrast

        iii. If no active GVHD start tapering immune suppression. If EBV viral load stable or increasing try to taper all immune suppression over 3-5 weeks.

        iv. Repeat viral load weekly X 2 (7 and 14 days)

        v. Start Acyclovir 10mg/kg IV TID or 800mg PO 5 x daily (if not already on it)

        vi. Biopsy any new suspicious lesions or adenopathy whenever feasible. If enough material available, the biopsy sample should also be sent for flow cytometry.

        vii. Bone marrow biopsy should be obtained if clinically indicated (e.g. falling counts) and, in addition to regular evaluation, be sent for flow cytometry.

2. Intervention: preemptive and active rituximab treatment

    - Negative physical and/or CT's: rituximab 375 mg/m2 single dose

        i. If viral load falls: back to monitoring

    - Negative physical and/or CT's: rituximab 375 mg/m2 single dose

        i. If viral load stable or climbing treat as item "c" below

    - Positive physical and/or CT's: rituximab 375 mg/m2 4 doses

3. Treatment for patients with presentation as aggressive lymphoma and/or not responding to rituximab alone

    - This patients should be considered for multiagent chemotherapy with rituximab

APPENDIX IX: Adapted from COMMON TOXICITY CRITERIA (CTC)

ALLERGY/IMMUNOLOGY		
Adverse Event	**Grade 3**	**Grade 4**
Allergic reaction/ hypersensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy related edema/angioedema	Anaphylaxis
Vasculitis Requiring steroids	Requiring steroids	Ischemic changes or requiring amputation
Allergy/Immunology – Other (specify): _____	Severe	Life-threatening or disabling
BLOOD/BONE MARROW		
Adverse Event	**Grade 3**	**Grade 4**
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		
Adverse Event	**Grade 3**	**Grade 4**
Cardiovascular/Arrhythmia - Other (specify): _____	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
CARDIOVASCULAR – GENERAL		
Adverse Event	**Grade 3**	**Grade 4**
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
Cardiac troponin T (cTnT)	≥ 0.1 - <0.2ng/mL	≥ 0.2ng/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 vain thrombosis, requiring	Embolic event including

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	anticoagulant therapy	pulmonaryembolism
Vein/artery operative injury is graded as Operative injury of vein/artery in the Cardiovascular (general) category. Other (specify):_____		
Cardiovascular/General –	Severe	Life-threatening or disabling
COAGULATION		
Adverse Event	**Grade 3**	**Grade 4**
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		
Adverse Event	**Grade 3**	**Grade 4**
Weight gain associated with Veno-Occlusive Disease (VOD) for BMT studies, if specified in the protocol. Also consider Ascites Edema, Pleural effusion (non-malignant).	>10% or as ascites	>10% or fluid retention resulting in pulmonary failure
DERMATOLOGY/SKIN		
Adverse Event	**Grade 3**	**Grade 4**
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)
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,	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation

	or desquamation covering ≥50% of body surface area.	
GASTROINTESTINAL		
Adverse Event	**Grade 3**	**Grade 4**
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
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 surgeryv
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.	Abdominal pain with pancreatic enzyme elevation	Complicated by shock (acute circulatory failure)

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Note: Amylase is graded in theMETABOLIC/LABORATORY category.		
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)
HEMORRHAGE		
Notes: *Transfusion in this section refers to pRBC infusion.* *For any bleeding with grade 3 or 4 platelets (<50,000), always 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.*		
Adverse Event	**Grade 3**	**Grade 4**
Hemorrhage/bleeding with grade 3 or 4 theombocytopenia 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
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

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Hemorrhage – Other (specify site): _____	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
HEPATIC		
Adverse Event	**Grade 3**	**Grade 4**
Bilirubin Bilirubin associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	>3.0 – 10.0 x ULN >6 - <15mg/100mL	>10.0 x ULN >15mg/100mL
INFECTION/FEBRILE NEUTROPENIA		
Adverse Event	**Grade 3**	**Grade 4**
Febrile neutropenia (fever or 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.		
Adverse Event	**Grade 3**	**Grade 4**
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		
Adverse Event	**Grade 3**	**Grade 4**
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
RENAL/GENITOURINARY		

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Adverse Event	Grade 3	Grade 4
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		
Adverse Event	**Grade 3**	**Grade 4**
Secondary Malignancy – Other (specify type): _____ *Excludes metastasis from initial primary.*	-	Present

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APPENDIX X: FHCRC IRB Policies

http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

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Appendix XI: 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. Cyclosporine may cause elevation of ALT/AST.

7. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from cyclosporine 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 marrow. 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 after TBI. 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 to be 5-9 weeks in duration.

APPENDIX XII: Schedule of Study Evaluations

	Screen	Day 1 to engraftment					Days 31-100			Long-Term Follow-up		
		daily	weekly	Day 14*** (±2 days)	Day 28 (± 3 days)	weekly	Day 56 (± 7 days)	Day 80 (± 7 days)	Day 100 (± 7 days)	6 months (± 30 days)	1 year	2 years
Informed consent	X											
Medical history	X											
Physical exam	X	X				X				X	X	X
Performance status	X							X Day 80-100		X	X	X
Height/Weight	X							X				
GVHD evaluation			X			X				X	X	X
Adverse events		X				X						
CBC with diff	X	X				X				X	X	X
Basic Metabolic Panel	X	X				X				X	X	X
Hepatic Function Panel	X	As clinically indicated or per standard practice										
Viral screening including CMV PCR	Per standard practice											
CMV Surveillance by PCR	Per standard practice											
Pregnancy test	X											
EBV monitoring							See Appendix VIII					
Urinalysis	X	As clinically indicated or per standard practice										
EKG	X	As clinically indicated or per standard practice										
MUGA or echocardiogram	X	As clinically indicated or per standard practice										
Chest x-ray or CT	X	As clinically indicated or per standard practice										
PFT	X	As clinically indicated or per standard practice										
Bone marrow bx/asp **	X				X			X			X	
Chimerism – BM					X			X			X	
Chimerism – PB				X FHCRC	X		X	X		X	X	X
IgG, IgA, IgM (as possible)					X		X		X	X	X	X
Host/donor studies (FHCRC)	Investigator discretion (See Section 9.E)											
Lymphocyte panel (as possible)	X				X		X		X	X	X	X
Immunophenotypic évaluation (FHCRC)	X				X		X		X	X	X	X

Check Section 9: Evaluation for details. Every effort will be made to complete the 1- and 2-year evaluations as close to these dates as possible, taking into consideration patient's circumstances at these time points.
**as clinically indicated ***For patients who received double cord blood units
NOTE: In certain circumstances (e.g., relapse or terminal illness), study evaluations may be omitted at the physician's or PI's discretion