CIBMTR® CENTER FOR INTERNATIONAL BLOOD & MARROW TRANSPLANT RESEARCH

A Phase II Multicenter Trial of Myeloablative Double Unit Umbilical Cord Blood Transplantation (UCBT) in Adults with Hematologic Malignancy

CIBMTR PROTOCOL 05-DCB
Version 2.0

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PROTOCOL SYNOPSIS – CIBMTR PROTOCOL 05-DCB

A Phase II Multicenter Trial of Myeloablative Double Unit Umbilical Cord Blood Transplantation (UCBT) in Adults with Hematologic Malignancy

Study Chairperson: Juliet Barker, MBBS, (Hons).

Study Design:
This study is a Phase II, open-label, multicenter, prospective study of double unit UCBT in adult patients with hematologic malignancies.

Primary Objective:
The primary aim of this study is to establish the one year overall survival after myeloablative double unit UCBT in a multi-institution setting.

Secondary Objectives:
1) Incidence of donor-derived neutrophil and platelet recovery
2) Contribution of each unit to initial (day +21 BM, +28 PB) and sustained engraftment (day +100 BM; PB at +60, +100, +180, +1 and 2 years)
3) Incidence and severity of acute graft-versus-host disease (GVHD) at 100 days
4) Incidence and severity of chronic GVHD at one year
5) Incidence of day 100 and 180 transplant-related mortality (TRM)
6) Incidence of malignant relapse at one and two years after UCBT
7) Incidence of serious infectious complications in the first year after transplant
8) Incidence of immune reconstitution (serial T, B and NK measurements by flow and Ig levels)
9) Probability of overall survival at one and two years
10) Probabilities of disease-free survival at one and two years after UCBT.

Eligibility Criteria:
1) Age 22 - 50 years.
2) Patients will have one of the following hematological malignancies:
   Acute myelogenous leukemia (AML):
   - Complete first remission (CR1) at high risk for relapse as defined by:
     - Known prior diagnosis of myelodysplasia (MDS); or
     - Therapy related AML; or
     - White cell count at presentation > 100,000; or
     - Presence of extramedullary leukemia at diagnosis; or
     - Unfavorable FAB type (M0, M5-7); or
     - High-risk cytogenetics (those associated with MDS, abnormalities of 5, 7, 8, 11q23 translocations, Philadelphia chromosome, complex karyotype); or
   - Complete second remission (CR2)

   Acute lymphoblastic leukemia (ALL):
   - Complete first remission (CR1) at high risk for relapse as defined by:
     - White cell count at presentation > 50,000; or

NMDP IRB Approved 01/11/2008 through 01/10/2009

- Presence of high-risk cytogenetic abnormality such as t(9;22), t(1;19), t(4;11) or other MLL rearrangements (11q23), t(8;14); or
- Failure to achieve complete morphologic remission after four weeks of induction therapy.

o Complete second remission (CR2)

Acute undifferentiated leukemia (AUL) or biphenotypic leukemia in CR1 or CR2

Myelodysplastic Syndrome (MDS) with one of the following:

o Low and Intermediate-1 International Prognostic Scoring System (IPSS) score with
- Life-threatening neutropenia or thrombocytopenia; or
- Platelet transfusion dependence

o Intermediate-2 or High IPSS score

3) Patients with adequate organ function and performance status criteria measured by:
   o Karnofsky score $\geq$ 70 %
   o Renal: Calculated creatinine clearance $\geq$ 60 mL/min OR if creatinine $\geq$ 1.5 mg/dL or a history of renal dysfunction must have a *measured* creatinine clearance (using 24 hour urine collection) $\geq$ 60 mL/min
   o Hepatic: Total bilirubin < 2.5 mg/dL unless benign congenital hyperbilirubinemia (Gilbert's syndrome) and ALT/AST < 3 x upper limit of normal
   o Albumin $\geq$ 2.5 g/dl
   o Pulmonary: Pulmonary function (spirometry and corrected DLCO) $\geq$ 60% normal
   o Cardiac: Left ventricular ejection fraction $\geq$ 50%

4) Double Unit Umbilical Cord Blood Grafts:
   o Patient must undergo a UCB search at both NMDP banks (Domestic and Co-op) and NYBC at a minimum
   o Each unit must have a cryopreserved dose of at least 1.5 x 10^7 TNC/kg (actual body weight). If the unit contains red cells at time of cryopreservation, the cryopreserved dose must be at least 2.0 x 10^7 TNC/kg. See Appendix B for unit selection details.
   o Each unit must be at least 4/6 HLA-A and B antigen, and DRB1 allele matched with the recipient
   o Each unit must be at least 3/6 HLA-A, B DRB1 antigen matched to each other
   o Above the cell dose threshold of 1.5 x 10^7 TNC/kg (2.0 x 10^7 TNC/kg for red cell containing units), HLA-match will take priority in unit selection. However, within the best available HLA match grade (e.g. 5/6), units with the largest TNC should be chosen.

Exclusion Criteria:

1) Patient with suitable related donor
2) AML, ALL, AUL, biphenotypic leukemia beyond CR2
3) AML evolved from myelofibrosis
4) Any acute leukemia with:
   o Morphologic relapse or persistent disease in the BM (cytogenetic relapse without morphologic evidence of relapse, or cytogenetic persistent disease in the BM is acceptable); or

NMDP IRB Approved 01/11/2008 through 01/10/2009

- Active extra-medullary leukemia including active CNS leukemia; or
- Requiring greater than two cycles of chemotherapy to obtain present remission status

5) Bone marrow aplasia (defined as BM cellularity < 5% at transplant work-up)
6) MDS with 10% or greater bone marrow blasts at pre-transplant workup
7) Prior autologous or allogeneic HSC transplant at any time
8) Prior radiation therapy rendering patient ineligible for TBI
9) Any uncontrolled infection at time of study enrollment
10) Seropositive or NAT positive for HIV or HTLV1
11) Females who are pregnant or breast feeding
12) Patient unable to give informed consent or unable to comply with the treatment protocol including appropriate supportive care, follow-up, and research tests

Treatment Description:
Cyclophosphamide 60 mg/kg/day IV days –7 and –6 (total dose 120 mg/kg)
Fludarabine 25 mg/m^2/day IV days –8 to –6 (total dose 75 mg/m^2)
Hyperfractionated TBI 1320 cGy in 8 165 cGy fractions days –4 to –1

GVHD prophylaxis: cyclosporine A (CSA) day –3 to maintain level 200 - 400 µg/L (ng/mL) until day 100 then taper if no GVHD; mycophenolate mofetil (MMF) 1 gram BID (or 15 mg/kg BID if < 50 kg) day –3 to +45 (must be IV while inpatient and IV at least until day +21).

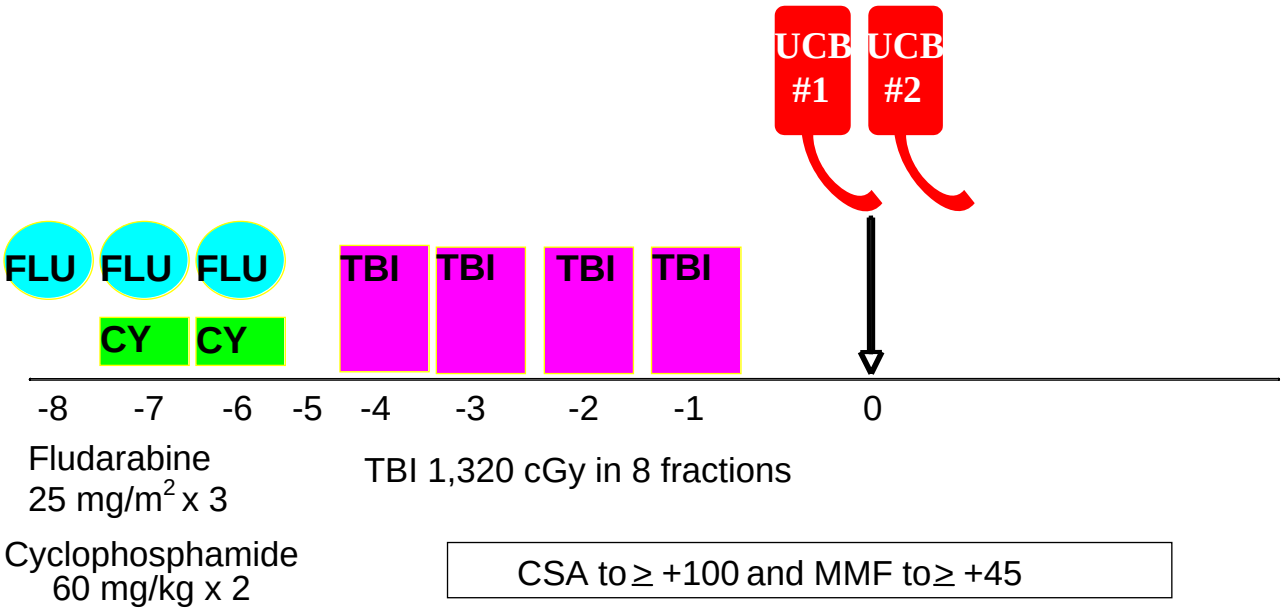
G-CSF 5 mcg/kg/day IV/SQ (maximum 480 mcg; dose rounded to vial size) from day +1 until ANC ≥ 2500/uL x 2 days.

Accrual Objective: The target sample size is 55 patients.

Accrual Period: The estimated accrual period is three years.

Study Duration: Patients will be followed for 24 months post transplant.

TREAMENT SCHEMA



Fludarabine
25 mg/m^2 x 3

Cyclophosphamide
60 mg/kg x 2

TBI 1,320 cGy in 8 fractions

CSA to ≥ +100 and MMF to ≥ +45

TABLE OF CONTENTS

LIST OF APPENDICES

NMDP IRB Approved 01/11/2008 through 01/10/2009

CHAPTER 1

1.0 BACKGROUND AND RATIONALE

Hematopoietic stem cell transplantation (HSCT) is now recognized as an effective form of therapy for an increasing number of malignant and non-malignant disorders.[1] To reconstitute hematopoiesis after intensive myeloablative therapy, the transplantation of pluripotential HSCs is required. Such HSCs are typically recovered from the bone marrow (BM) or peripheral blood (PB) of related or unrelated donors or even from the patients themselves. However, unfortunately transplantation is frequently not possible, either due to the patient's own marrow being damaged from prior chemotherapy or contaminated with tumor cells in the autologous setting, or as suitably HLA-matched allogeneic donors are not available.

UCB is an alternative HSC source that extends the unrelated donor pool.[2-10] However, adult UCBT has been limited by low graft cell dose. This protocol seeks to improve the outcome of adult UCBT by the use of double unit UCBT grafts. Optimizing the success of UCBT in adult patients is important as this will significantly broaden the availability of allogeneic transplant as a treatment modality.

1.1. Clinical Results with Unrelated Donor UCBT

Human UCB is one potential source of HSCs that is capable of reconstituting hematopoiesis after intensive myeloablative therapy.[2-10] As a result of the early successes with UCBT from sibling donors, pilot programs for the banking of unrelated donor UCB have been initiated in many countries around the world. Known benefits of banked UCB include: 1) rapid availability, 2) absence of donor risk, 3) absence of donor attrition, and 4) very low risk of transmissible infectious diseases, such as Cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Further, this HSC source may allow us to expand the available donor pool in targeted ethnic and racial minorities. All published studies to date have indicated that the level of HLA match required for a successful unrelated UCBT is less than that required for successful outcome following a volunteer unrelated marrow or peripheral blood HSC donor. [2-10]

The University of Minnesota has analyzed the outcomes of 102 patients (median age 7.4 years) transplanted with an unrelated donor single UCB unit between 1994 and 2001 using myeloablative conditioning for the treatment of malignant (n = 65; 68% high-risk) and non-malignant diseases (n = 37).[7] Median infused cell dose was 3.1 x 10^7 TNC/kg (range 0.7-57.9), and 2.8 x 10^5 CD34+ cells/kg (range 0.4-39.1), and 86% of grafts were 1-3 HLA-A and B antigen and DRB1 allele mismatched. Neutrophil recovery occurred at a median of 23 days (range 9-54) with a cumulative incidence of donor engraftment of 88% (95% CI: 81-95) by day 42. Speed and likelihood of neutrophil recovery were strongly associated with CD34+ cell dose, with a markedly inferior engraftment of 72% at a median of 34 days in patients receiving a CD34+ cell dose < 1.7 x 10^5 cells/kg as compared to that seen in patients receiving higher cell doses ($p < 0.01$). The incidences of grade II-IV and III-IV acute GVHD were 39% (95% CI: 29-49) and 11% (95% CI: 5-17), respectively, at day 100, with 10% (95% CI: 4-14) of patients having chronic GVHD by 1 year. One year TRM was 30% (95% CI: 21-39) and was strongly associated with CD34+ cell dose with a very high TRM in excess of 70% in patients receiving

grafts with a CD34+ cell dose < 1.7 x 10^5 CD34+ cells/kg. One and 2-year survival were 58% (95% CI: 49-70) and 47% (95% CI: 36-57), respectively. Importantly, survival was 70% (95% CI: 49-90) at 1 year in patients with grafts containing > 1.7 x 10^5 CD34+ cells/kg, with very poor survival in patients receiving a graft with < 1.7 x 10^5 CD34+ cells/kg.

Adult single unit UCBT outcomes have recently been compared to those of unrelated volunteer marrow recipients. In this U.S. study, the outcome of 150 UCBT recipients reported to the National Cord Blood Program of the New York Blood Center were compared to those of 6/6 HLA-matched (n = 367) and one antigen mismatched (n = 83) marrow recipients reported to the International Bone Marrow Transplant Registry.[9] UCB recipients had inferior hematopoietic recovery and increased TRM as compared to matched marrow with 95 of 150 (63%) UCBT patients dying of transplant related causes. However, disease-free survival after UCBT was comparable to that after HLA mismatched marrow recipients, introducing UCB as a valid alternative to HLA mismatched volunteer donors.

In summary, the University of Minnesota and other studies have demonstrated that cryopreserved single unit UCB from HLA 0-2 antigen mismatched unrelated donors contains sufficient numbers of transplantable HSC for most pediatric patients.[2, 5-11] In addition to rapid availability and a low rate of viral contamination, UCB has the advantage of low probabilities of severe acute and extensive chronic GVHD despite the use of HLA-mismatched grafts. However, a major disadvantage is that low graft cell dose in adult recipients leads to delayed hematopoietic recovery, an increased risk of graft failure, an increased risk for TRM, and limits the application of UCBT in adults.[2, 5-11] Therefore, for larger adolescents and adult patients, while UCB is a valid alternative to HLA-mismatched BM, efforts must be focused on improving UCBT engraftment and decreasing TRM.

The University of Minnesota has investigated the novel approach of the combined transplantation of two UCB units in a double unit graft after myeloablative conditioning as a strategy to augment graft cell dose.[10] To date, experience in 31 double unit UCBT patients (median age 24 years; range 13-53) with hematologic malignancy has been associated with sustained neutrophil engraftment in all evaluable patients (n = 29) at a median of 23 days (range 14-41). Interestingly, this engraftment was accounted for by one unit with a relatively low cell dose [median infused cell dose of 1.8 x 10^7 TNC/kg (range 0.7-3.6) and 1.7 x 10^5 CD34+ cells/kg (range 0.4-10.4)]. Further, neither nucleated cell dose nor HLA-match predicted unit predominance. The incidences of grade III-IV acute GVHD, chronic GVHD, and TRM were 24% (95% CI: 9-39) at 100 days, 32% (95%CI: 13-51) at 1 year, and 20% (95%CI: 6-34) at 6 months, respectively. With a median follow-up of 1.2 years (range 55 days-3.2 years), the Kaplan-Meier estimate of disease-free survival at 2 years is 60% (95%CI: 41-79) in all patients, and 68% (95%CI: 47-89) in patients (n = 23) transplanted in remission.

This demonstrates that double unit UCBT can be performed safely in adults with improved engraftment and reduced TRM as compared to single unit historical controls. This strategy therefore may extend access to allogeneic transplantation and introduces adult UCBT as a viable alternative to both HLA matched and HLA mismatched unrelated marrow or peripheral blood transplantation. Further, preliminary data from the University of Minnesota suggests that double unit UCBT may be associated with a reduced incidence of relapse[12]. Therefore, double unit

UCBT will be further investigated in this prospective Phase 2 study with the aim to demonstrate that the promising one year survival seen in the single institution series can be replicated in a multi-center setting. Patients with high-risk hematopoietic malignancy will receive myeloablative conditioning with cyclophosphamide (Cy), low dose fludarabine (Flu) and total body irradiation (TBI) with cyclosporine (CSA) and mycophenolate mofetil (MMF) for GVHD prophylaxis. Eligible patients will be aged $\leq$ 50 years with a lower age limit cut-off of 22 years so as not to compete with the randomized CTN study of single vs. double unit UCBT in children. This will be the first multi-institution prospective study of adult UCBT in the United States.

An important aspect of this study is the criteria to be used for unit selection. Firstly, the University of Minnesota data has shown that cell dose does not determine which unit will predominate, but the time to neutrophil recovery is associated with the nucleated cell and CD34+ dose of the winning unit. Therefore, given it is not known which unit will win, the cell dose of each unit is equally important. Therefore, the cell dose threshold for each unit will be the same and will be set at 1.5×10^7 NC/kg. While 1.5 is low by pediatric standards, the cell dose of the graft will be augmented by the use of two units. This will extend access to transplant to larger adults who only have access to units in the range of 1.5-2.0.

Further, there is increasing data that in addition to cell dose, HLA-match is also of critical importance in UCBT. For example, unpublished data from the New York Blood Center (NYBC) have shown that to achieve a one year survival after a myeloablative single unit UCBT of greater than 50%, the cryopreserved cell dose must be at least 2.5×10^7 NC/kg in recipients of 5/6 units. In contrast, recipients of 4/6 units must receive a cell dose of at least 5×10^7 NC/kg to achieve a comparable result. This suggests that improved HLA match can compensate for low cell dose, or conversely, that HLA mismatch must be compensated for by a larger cell dose. This argument is strengthened by the fact that an impact of cell dose on survival cannot be demonstrated after the transplantation of 6/6 units (NYBC, personal communication). These findings are of great significance in unit selection for adult patients who may have smaller but better matched units (e.g. a 5/6 match with a cell dose of 2.0) versus larger 4/6 units (e.g. a 4/6 with a cell dose of 2.6). The data demonstrate that the increase in cell dose that would be required to compensate for a 1 step down in antigen match from 5/6 to 4/6 is sufficiently great (e.g. cell dose must be increased from 2.5 to 5.0) that it cannot be achieved in patients of adult size. Therefore, patients may do better to receive a smaller 5/6 unit over a slightly larger 4/6 unit.

However, in this scenario, efforts must be made to ensure that the smaller but better matched unit will engraft. The double unit transplant experience suggests that this may be achieved with the double unit strategy. Therefore, in this study, above the cell dose threshold of $\geq 1.5 \times 10^7$ NC/kg for each unit, HLA match will be given priority in unit selection. In this way the total graft cell dose will be augmented by the use of double units, but the HLA match of the unit responsible for sustained donor engraftment will be optimized as far as is possible. Given that NYBC data has shown that HLA-match is a critical determinant of post engraftment TRM in engrafting patients[13], it is hoped that this strategy will optimize patient outcome including reducing GVHD and enhancing immune reconstitution.

A further approach that will be investigated in this trial is that of albumin reconstitution for the thaw of UCB units. The traditional method of UCB thaw has been to wash the units as originally

described by Dr Pablo Rubinstein. This approach, developed for the transplantation of children, has been adhered to by most transplant centers for the transplantation of adults as a matter of convention. However, while this technique is appropriate for the transplantation of small children, adult recipients may tolerate a small amount of DMSO without adverse outcome with appropriate supportive care. The albumin reconstitution or dilution methodology has a number of advantages:

- It decreases the potentially significant loss of cells from the wash (5-20% according to unpublished data from the St Louis Cord Blood Bank).
- It is faster than washing and this efficiency translates into less handling of the cells, more rapid infusion into the patient, and cost savings in terms of technologist's time for the Cytotherapy Laboratory.
- In contrast to bedside thaw, it immediately dilutes the DMSO to low levels, negating any toxicity to the HSC, while still allowing for the thaw to be conducted in the controlled environment of the Cytotherapy Laboratory.

For these reasons, this strategy has been adopted as routine practice by a number of transplant centers (Texas Transplant Institute, Loyola, and MSKCC). Therefore, this approach will be mandated by the protocol for all red cell depleted units and the experience with this technique will be reported.

In addition, as part of this protocol, issues associated with red cell containing units will be addressed. Firstly, transplant centers have noticed that the reported nucleated cell dose of red cell containing units does not reflect the ultimate yield in terms of nucleated cells and CD34+ progenitors. Therefore, the nature of the units used (red cell depleted or not), the Cord Blood Bank, and the yield of the units at thaw will be reported. A further issue to be addressed is the technique used to thaw red cell containing units. The Stemcyte bank has recently presented data demonstrating inferior engraftment with Stemcyte units that have been washed as compared to their recommended "no wash" approach. This is consistent with the significant problems encountered in washing red cell containing units by multiple transplant centers (personal communication). However, while some transplant centers have successfully performed bedside thaw and infusion of these units, others do not believe this is a desirable strategy. Therefore, this protocol will allow either bedside thaw or albumin reconstitution methods.

This study will monitor for the traditional outcome measures of donor engraftment, GVHD, TRM, relapse and survival. However, a further contribution to the field will be to monitor for serious infectious complications during the first year after transplant and correlate them with laboratory measures of immune recovery. In addition, the response to post transplant vaccination will be measured for clinically relevant organisms. This data will permit comparison to the experience of transplantation with unrelated volunteer HSC and act as a baseline for future efforts to augment immune reconstitution.

CHAPTER 2

2.0 STUDY DESIGN

2.1. Study Overview

This is a phase II study to establish the one year overall survival after myeloablative double unit UCBT in a multi-institution setting in patients with high-risk hematopoietic malignancy. Fifty-five patients will be recruited over three years and transplant outcomes will be assessed as listed in Section 2.2. Stopping rules are in place for excess toxicity as evidenced by TRM greater than 40% at day 100 or graft failure greater than 10% at day 42. Problems with donor engraftment, severe GVHD, TRM, and other adverse experiences will be monitored throughout the study by the CIBMTR research data managers and reported to the Study Chairperson.

2.2. Hypothesis and Specific Objectives

2.2.1. Hypothesis

Double unit UCBT in adults will be associated with a 1-year survival of at least 40%.

2.2.2. Study Objectives

The primary aim of this study is to establish the one year overall survival after myeloablative double unit UCBT in a multi-institution setting. Secondary objectives include:

1) Incidence of donor-derived neutrophil and platelet recovery
2) Contribution of each unit to initial (day +21 BM and +28 PB) and sustained engraftment (day +100 BM; PB at +60, +100, +180, +1 and 2 years)
3) Incidence and severity of acute GVHD at 100 days
4) Incidence and severity of chronic GVHD at one year
5) Incidence of day 100 and 180 TRM
6) Incidence of malignant relapse at one and two years after UCBT
7) Incidence of serious infectious complications (Appendix E) in the first year after transplant
8) Incidence of immune reconstitution (serial T, B and NK measurements by flow and Ig levels)
9) Probability of overall survival at one and two years
10) Probabilities of disease-free survival at one and two years after UCBT

2.3. Inclusion Criteria

1) Age 22 - 50 years
2) Patients will have one of the following hematological malignancies:
   Acute myelogenous leukemia (AML):
   - Complete first remission (CR1) at high risk for relapse as defined by:
     - Known prior diagnosis of myelodysplasia (MDS); or
     - Therapy related AML; or
     - White cell count at presentation > 100,000; or

- Presence of extramedullary leukemia at diagnosis; or
- Unfavorable FAB type (M0, M5-7); or
- High-risk cytogenetics (those associated with MDS, abnormalities of 5, 7, 8, 11q23 translocations, Philadelphia chromosome, complex karyotype); or

o Complete second remission (CR2)

Acute lymphoblastic leukemia (ALL):

o Complete first remission (CR1) at high risk for relapse as defined by:
- White cell count at presentation > 50,000; or
- Presence of high-risk cytogenetic abnormality such as t(9;22), t(1;19), t(4;11) or other MLL rearrangements (11q23), t(8;14); or
- Failure to achieve complete morphologic remission after four weeks of induction therapy.

o Complete second remission (CR2)

Acute undifferentiated leukemia (AUL) or biphenotypic leukemia in CR1 or CR2

Myelodysplastic Syndrome (MDS) with one of the following:

o Low and Intermediate-1 International Prognostic Scoring System (IPSS) score with:
- Life-threatening neutropenia or thrombocytopenia; or
- Platelet transfusion dependence

o Intermediate-2 or High IPSS score

3) Patients with adequate organ function and performance status criteria measured by:
   o Karnofsky score $\geq$ 70 % (Appendix A)
   o Renal: Calculated creatinine clearance $\geq$ 60 mL/min OR if creatinine $\geq$ 1.5 mg/dL or a history of renal dysfunction must have a *measured* creatinine clearance (using 24 hour urine collection) $\geq$ 60 mL/min
   o Hepatic: Total bilirubin < 2.5 mg/dL unless benign congenital hyperbilirubinemia (Gilbert's syndrome) and ALT/AST < 3 x upper limit of normal
   o Albumin $\geq$ 2.5 g/dl
   o Pulmonary: Pulmonary function (spirometry and corrected DLCO) $\geq$ 60% normal
   o Cardiac: Left ventricular ejection fraction $\geq$ 50%

2.4. Exclusion Criteria

1) Patient with suitable related donor as defined per institutional guidelines
2) AML, ALL, AUL, biphenotypic leukemia beyond CR2
3) AML evolved from myelofibrosis
4) Any acute leukemia with:
   o Morphologic relapse or persistent disease in the BM (cytogenetic relapse without morphologic evidence of relapse, or cytogenetic persistent disease in the BM is acceptable); or
   o Active extra-medullary leukemia including active CNS leukemia; or
   o Requiring greater than two cycles of chemotherapy to obtain present remission status
5) Bone marrow aplasia (defined as BM cellularity < 5% at transplant work-up)

6) MDS with 10% or greater bone marrow blasts at pre-transplant workup. Patients may receive therapy and if achieve a complete remission be eligible.
7) Prior autologous or allogeneic HSC transplant at any time
8) Prior radiation therapy rendering patient ineligible for TBI
9) Any uncontrolled infection at time of study enrollment
10) Seropositive or NAT positive for HIV or HTLV1
11) Females who are pregnant or breast feeding
12) Patient unable to give informed consent or unable to comply with the treatment protocol including appropriate supportive care, follow-up, and research tests. Basic understanding of the English language is required. Alternatively, centers may have consent forms in the patient's native language available.

2.5. Graft selection

Participating centers must search the NMDP (Domestic and Coop) banks and New York Blood Center at a minimum for suitable units. Netcord and other banks may also be searched for additional units to optimize the cell dose and HLA match of the graft. Prior to unit selection it must be determined if units of interest are red cell depleted or not. All patients must have two suitable UCB units for transplantation that meet the following requirements:

- Each unit must have a cryopreserved dose of at least 1.5 x 10^7 TNC/kg (actual body weight). If the unit contains red cells at time of cryopreservation, the cryopreserved dose of each unit must be at least 2.0 x 10^7 TNC/kg.
- Each unit must be at least 4/6 HLA-A, B antigen and DRB1 allele matched with the recipient
- Double mismatches at any given locus should be avoided
- Each unit must be at least 3/6 HLA-A, B, DRB1 antigen matched to each other
- Above the cell dose threshold of 1.5 x 10^7 TNC/kg (2.0 x 10^7 TNC/kg for red cell containing units), HLA-match will take priority in unit selection. However, within the best available HLA match grade (e.g. 5/6), units with the largest TNC should be chosen.
- The criteria for unit selection are outlined in Appendix B.

2.6. Treatment Plan

All patients will receive the same preparative regimen as shown in Table 2.6.1.

Table 2.6.1. TREATMENT SCHEDULE

Day	Treatment
-8	Fludarabine 25 mg/m^2 IV
-7	Fludarabine 25 mg/m^2 IV Cyclophosphamide 60 mg/kg IV
-6	Fludarabine 25 mg/m^2 IV Cyclophosphamide 60 mg/kg IV
-5	Rest
-4	TBI (165 cGy) x 2
-3	TBI (165 cGy) x 2 Start CSA and MMF
-2	TBI (165 cGy) x 2
-1	TBI (165 cGy) x 2
0	UCBT*
+1	Start G-CSF

* The cord blood units may be infused on Day -1 after TBI administration per institution practice. The day of cord blood infusion is considered Day 0.

2.6.2. Study Drugs

2.6.2.1. Fludarabine

Fludarabine 25 mg/m^2/day IV over approximately thirty minutes x 3 days (days -8, -7, and -6) for a total dose of 75 mg/m^2 followed by Cyclophosphamide on days -7 and -6. Fludarabine administration is dosed based on the patient's actual body weight (ABW). However, for patients weighing more than 125% of their IBW, fludarabine will be dosed based on the adjusted ideal body weight (AIBW) as follows:

Ideal Body Weight (IBW) Formula:
Males IBW = 50 kg + 2.3 kg/inch over 5 feet
Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Adjusted Ideal Body Weight Formula:
AIBW = IBW + [(0.25) x (ABW - IBW)]

2.6.2.2. Cyclophosphamide

- Cyclophosphamide 60 mg/kg/day IV will be administered over approximately two hours x 2 days (days –7 and –6) with Mesna. Cyclophosphamide administration is dosed based on the patient's actual body weight (ABW). However, Cyclophosphamide dose will be adjusted if the patient is > 125% ideal body weight (IBW) and calculated on adjusted ideal body weight (AIBW, see above).

- High volume fluids should commence approximately 6-12 hours prior to drug and continue until 24 hours after second dose. Institution standard practice for prevention of hemorrhagic cystitis is acceptable. A suggested regimen includes at least 200 mL/hour of IV fluids and a Mesna dose of 100% of the total daily cyclophosphamide dose given on days -7 and -6. Use diuretic (e.g. Furosemide) as required for fluid balance and monitor electrolytes and body weight.
- Aprepitant (Emend™) and voriconazole must not be administered during cyclophosphamide due to the potential for hazardous drug interaction. Anti-emetic therapy with serotonin antagonists such as ondansetron is recommended. If dexamethasone is used as an anti-emetic it should be stopped prior to Day 0.

2.6.2.3. Radiotherapy

Patients may be treated either in the AP/PA position and/or in the right and left lateral position. Compensators or blocks may be used to compensate for the thinner parts of the anatomy (neck, head, lower legs, and feet).

Total dose will be 1320 cGy in 8 fractions over 4 days. Dose will be prescribed at the level of the umbilicus at midplane.

To compensate for decreased attenuation through the lungs, partial compensators may be used to prevent the lung dose from exceeding the prescription point dose. No adjustments are made for lower lung density. The estimated lung dose is calculated by measuring the off-axis thickness in the mid-lung area:

- If the patient is treated with AP and PA fields, the lungs may be partially blocked with 50% transmission blocks such that the lung receives an estimated minimum of 675 cGy. With the use of 50% transmission blocks, an anterior and posterior electron chest wall boosts, calculated to D90, where electron energy is selected to place the D90 at the pleural surface, must be employed. 300 cGy per fraction for a total of two fractions will be given to both the anterior and posterior chest wall. Regardless of the partial blocking used, the lung may receive an estimated maximum of the prescription dose (1320 cGy).
- If the patient is treated with right and left lateral fields, separations are taken with the arms placed along the axis of the thoracic cavity, and the tissue deficit calculated (without lung correction). Since the effective thickness at the level of the mid-mediastinum is often greater than the thickness at the umbilicus, this may be all the compensation that is necessary. However, if additional tissue deficit is calculated, lung compensators may also be placed such that the estimated lung dose is between a minimum of 1000 cGy and a maximum of 1320 cGy. (The minimum lung dose allowed with this technique is somewhat higher than the right left lateral technique since, by default, some of the mediastinum and spine will also be under the compensator.)

A total of 8 fractions are given over 4 days (Days -4, -3, -2, and -1). The two fractions are given at a minimum of 6 hours apart from beam on to beam on.

The TBI will be delivered from either a linear accelerator or cobalt source at a dose rate of between 4 and 26 cGy/minute using energies of between 1 and 25 MV.

The skin dose should be at least 90% of the prescribed dose. If a higher energy beam (> 4 MV) is used for the TBI treatments, a beam spoiler should be used to accomplish this or thermoluminescent dosimetry data submitted showing that the skin dose is ≥ 90% of the prescribed dose.

Testicular boosts should be used for all males with ALL (and according to institutional practice for other diseases). The testicular boost may be given in a single 400 cGy fraction, or in multiple fractions per institutional practice, with either electrons prescribed to Dmax or photons prescribed to the midplane of the scrotum. If electrons are used, the energy for the testicular boost depends on the thickness of the testicles and is chosen so that the D90 corresponds to the posterior surface of the scrotum.

2.6.3. Immunosuppressive Therapies

All patients will receive GVHD prophylaxis with Cyclosporine-A (CSA) and Mycophenolate mofetil (MMF).

2.6.3.1. CSA

- CSA must begin on the morning of day -3 with the aim to maintain a level of 200-400 µg/L (ng/mL). Every 12 hour dosing is preferred but may be administered as continuous infusion per institutional standards.
- Dose adjustments will be made on the basis of toxicity or low CSA levels [trough level <200 µg/L (ng/mL)].
- Once the patient can tolerate oral medications and has a normal gastro-intestinal transit time, CSA will be converted to an oral form. CSA dosing will be monitored and altered as clinically appropriate.
- Patients will receive CSA until day +100. In the absence of GVHD, the dose should be tapered by 10% per week and discontinued at Day 180.
- Monitoring for CSA toxicity shall be per institution practice.

2.6.3.2. MMF

- MMF must begin on the morning of day –3 and continued through Day +45. Dose is 1 gram q 12 hours IV, or if < 50 kg will be given 15 mg/kg BID to a maximum of 1 gram q 12 hours.
- MMF must be given IV while the patient is hospitalized. It is strongly suggested to give IV MMF until day +21. Subsequently the oral route can be used if the patient is tolerating a normal diet (same dosing as IV). If nausea, vomiting or diarrhea develop, MMF must be restarted IV.
- No dose adjustments for renal or liver disease are needed.
- If the patient has acute GVHD requiring systemic therapy by day +45, MMF should be stopped after day 45 only if control of GVHD has been achieved. Control of GVHD

means resolution of skin rash, vomiting and diarrhea, or hyperbilirubinemia (if attributed to GVHD).

- If the patient is unable to tolerate therapeutic CSA due to renal dysfunction or other reasons do not stop MMF.

2.6.4. UCB Thaw and Administration

- UCB grafts must be received at the institution Cytotherapy/Cell Processing laboratory prior to the start of the preparative regimen.
- On transplant day the units should be thawed in the Cytotherapy Laboratory as per the thaw procedure in Appendix C.
- Total nucleated cells (TNC), CD34+ and CD3+ cell number and viability, and sterility should be measured post-thaw.
- UCB units should be infused intravenously via a central venous catheter. Units should be given consecutively with each unit infused over approximately 30 minutes (red cell containing units of a larger volume may be infused over a longer time period).
- Management and pre-medication per institution practice for cryopreserved product infusion, including vigorous IV hydration for 4 - 6 hours prior and at least 12 hours post UCB graft infusion. IV Hydralazine is the preferred medication for treatment of hypertension associated with UCB infusion.
- Specimens of the product for culture and chimerism studies (bullet sample) must be obtained.

2.6.5. Growth Factors

G-CSF 5 mcg/kg/day IV/SQ (maximum 480 mcg; dose may be rounded to vial size) will be given to all patients from day +1 until ANC ≥ 2500/uL x 2 days. If count recovery is delayed, see "Suggested Guidelines for Management of Slow Engraftment / Graft Failure" (Appendix D).

Use of rHu-KGF for mucositis prophylaxis is allowed. Dosing is per institutional standard.

2.6.6. Prophylaxis Against Infection

- Patients must be given prophylaxis against bacterial, viral and fungal infections per institution practice. Antiviral and antifungal prophylaxis should continue for a minimum of three months.
- Antifungal prophylaxis must include prophylaxis specifically against Aspergillus from Day +1 until the patient's ANC ≥ 0.5 for three days or as clinically indicated. Because of nephrotoxicity, liposomal amphotericin should be avoided as it may compromise the ability to deliver therapeutic doses of CSA. Appropriate drugs include extended spectrum azoles or echinocandins. If voriconazole is used, CSA must be dose adjusted accordingly. Antifungal prophylaxis is also strongly suggested while the patient is receiving systemic therapy for GVHD.
- Antibacterial prophylaxis may be discontinued after patient's ANC ≥ 0.5 for three days. It is suggested that patients requiring corticosteroid therapy for GVHD management be

given antimicrobial prophylaxis, including antibacterial, antiviral and antifungal therapies, for the duration of the corticosteroid use.

- PCP prophylaxis should be given until at least one year post transplant.
- Patients must be monitored for reactivation of CMV at least weekly from days 21-100 (or longer if clinically indicated) using CMV antigenemia or PCR. CMV viremia should be promptly treated with ganciclovir or foscarnet and IVIg / Cytogam per institutional guidelines.
- Reactivation of other viruses, including BK and adenovirus is common post cord blood transplant. EBV viremia and post-transplant lymphoproliferative disorders may also be seen. Monitoring or early consideration of these viruses in the differential diagnosis is recommended.

2.6.7. Transfusions

Following initiation of the pre-transplant conditioning regimen, all blood products for transfusion, with the exception of the stem cell graft, must be irradiated to 3,000 cGy to inactivate lymphocytes capable of initiating transfusion associated GVHD. Blood products are irradiated per institutional guidelines. CMV-negative recipients should receive CMV safe (CMV negative or leukodepleted) products.

2.6.8. Investigational Drugs

Use of investigational drugs is not allowed without Study Chairperson approval.

2.6.9. Risks and Toxicities

2.6.9.1. Total Body Irradiation (TBI)

TBI can cause: nausea and vomiting, diarrhea, parotitis (rapid onset within 24-48 hours, usually self-limited), generalized mild erythema, hyperpigmentation, fever, mucositis, and alopecia.
Late effects include: hypothyroidism, cataracts, probable increased risk of secondary malignant neoplasms, sterility, nephropathy, interstitial pneumonitis and veno-occlusive disease.

2.6.9.2. Cyclophosphamide

Cyclophosphamide side effects include: nausea/vomiting, cardiomyopathy, skin rash, mucositis, stomatitis, sterility, diarrhea, hemorrhagic cystitis, fluid weight gain/edema, alopecia and hemolytic anemia.

2.6.9.3. Fludarabine

Fludarabine may contribute to nausea, vomiting, mouth sores, and diarrhea which are primarily due to the high dose cyclophosphamide and TBI. Pulmonary toxicity, nephropathy, jaundice and elevations of liver enzymes and transient skin rashes have also been described.
Myelosuppression and immune suppression is a major toxicity and is treated by donor stem cell infusion and supportive care.

Effects on the nervous system are not expected at the doses used in this protocol, but if they occur could include confusion, coma, weakness or numbness, loss of balance, difficulty walking, or loss of vision and could be very serious or lethal.

2.6.9.4. Mycophenolate mofetil (MMF)

Side effects include: pancytopenia, nausea, vomiting, diarrhea, hypertension, headache, dizziness, insomnia, hyperglycemia, rash, and leg cramps/bone pain.

2.6.9.4. Cyclosporine-A (CSA)

Cyclosporine may cause: nausea, tremors, nephrotoxicity, seizures, hypertension, hirsutism, thrombotic microangiopathy, electrolyte imbalances, paresthesias/neuropathy, gingival hyperplasia, transient-blindness, and hepatic and renal dysfuction.

2.6.9.5. G-CSF (Neupogen)

G-CSF may cause: bone pain, insomnia, headaches, dyspnea, body aches, rash, fever, splenomegaly, allergic reaction, fatigue, edema and nausea/vomiting.

2.6.9.6. UCB unit infusions

Toxicities potentially associated with the infusion of the UCB graft include DMSO toxicity and side effects from red cells and may include changes in heart rate or rhythm, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, fluid overload, headache, dyspnea, presence of DMSO taste and odor, hemoglobinuria, allergic reaction, and acute renal failure. However due to pre-medication these toxicities are unlikely.

CHAPTER 3

3.0 STUDY ENDPOINTS

3.1. Primary Endpoint

The primary endpoint is one year overall survival after myeloablative double unit UCBT in a multi-institution setting. Disease-free survival at 1 and 2 years will be monitored as a secondary endpoint.

3.2. Secondary Endpoints

3.2.1. Engraftment and Graft Failure (GF)

The day of neutrophil engraftment is defined as the first day of 3 consecutive days of an ANC ≥ 500/μl. The day of platelet recovery is the first of 3 consecutive measurements tested on different days of a platelet count ≥ 20,000 without requiring platelet transfusions in the previous 7 days. Patients will be monitored for donor cell engraftment as evidenced by neutrophil recovery and donor chimerism in the marrow and/or peripheral blood at serial time-points post-transplant. The contribution of each donor to engraftment will be recorded. To account for the possibility of the engraftment of both units the total donor engraftment will be defined as the contribution of each donor unit added together at each time-point.

3.2.2. Primary Graft Failure

Primary graft failure is diagnosed when the patient fails to achieve an ANC ≥ 500/μl for 3 consecutive days within the first 42 days post-transplant regardless of chimerism results. The first of the three measurements may occur on Day 42. Infusion of another source of stem cells prior to Day 42 will also be considered primary graft failure. Patients who die before day 42 without count recovery will be classified as primary graft failure if death occurs after day 28 and as not evaluable if death occurs between days 0-28. In the event that there is disease relapse diagnosed prior to ANC ≥ 500/μL for 3 consecutive days, patients are classified as relapse and not as primary graft failure regardless of chimerism results. Stopping rules are in place to consider cessation of the study if graft failure at day 42 is in excess of 10%.

3.2.3. Secondary Graft Failure

Secondary graft failure is defined as having neutrophil recovery as defined above followed by severe neutropenia (ANC drops to < 500/μl for more than 7 consecutive days unresponsive to hematopoietic growth factors in the absence of relapse), or, there is absence of donor cells from either of the donor units in the marrow, blood or both as demonstrated by chimerism assay in the absence of leukemic relapse.

Patients with suspected graft failure will be evaluated with bone marrow biopsy to assess BM cellularity and to assess for residual or recurrent leukemia. In addition to chimerism assays, aspirates should be sent for bacterial and viral cultures and/or DNA studies for pathogens

potentially causing graft failure including CMV and HHV6. Patients who suffer graft failure will be re-infused with a second hematopoietic graft. A suggested management of graft failure is described in Appendix D.

3.2.4. Graft Versus Host Disease (GVHD)

Standard clinical criteria (Appendix F) and histological grading of skin, liver or gastrointestinal pathology where possible will be used to establish and grade acute GVHD. In the first 100 days after transplant patients will be assessed by a transplant physician for the development of acute GVHD approximately weekly. Patients with moderate to severe acute GVHD (grade II-IV) will be treated with corticosteroids per standard of care. Patients failing to respond to steroids will be considered for treatment with standard or experimental immunosuppressive agents.

Chronic GVHD will be diagnosed and graded according to the criteria in Appendix G and treated with standard or experimental immunosuppressive therapy. Patients will be assessed for chronic GVHD at day 100, and at 6, 9, 12, 18 and 24 months, and more frequently if clinically indicated.

3.2.5. Transplant Related Mortality (TRM)

TRM is defined as death at any time from the commencement of pre-transplant conditioning due to any cause other than malignant relapse (with the exception of accidents such as automobile accidents). Stopping rules are in place to consider cessation of the study if TRM at day 100 is in excess of 40%.

3.2.6. Relapse

Relapse of malignancy is a secondary endpoint of this study and will be defined by an increasing number of blasts/malignant cells of recipient origin in the marrow $\geq$ 5% or with characteristic morphology (e.g. Auer rods), by the presence of circulating peripheral blasts of recipient origin, presence of a persistently abnormal leukemic population by flow cytometry, or by the presence of malignant cells in any extramedullary site. Cytogenetic or PCR evidence of relapse or persistence without any flow cytometric or morphologic evidence of disease will not be defined as relapse.

3.2.7. Infections

The occurrence of serious infections will be collected for the first year after transplant and correlated with immune recovery. Clinically compatible time frames will be used to define one infectious episode from a second episode with the same organism. Serious infections and recurrence intervals are defined in Appendix E.

3.2.8. Immune Reconstitution

Quantitation of peripheral blood T, B, and NK cells by flow cytometry will be performed post transplant at days 60 and 100, and at 6, 12, 18 and 24 months. Immunoglobulin levels will be tested at days 30, 60 and between day 90 and 100 to assist in the guidance for the need of IgG

supplementation. After recovery no longer requiring monthly replacement (i.e. IgG > 400 unsupported) the Ig levels are checked at the same time points as the peripheral blood lymphocyte subset analysis. Patients should be re-immunized at 12 months post-transplant per Appendix H. Measurement of the response to vaccination by pre and post vaccination antibody titers for clinically relevant organisms (pneumococcus, hemophilus, and tetanus at 12 months and seasonal influenza virus) is required (Appendix H).

CHAPTER 4

4.0 PATIENT REGISTRATION, ENROLLMENT AND EVALUATION

4.1. Screening for Patient Eligibility

The following tests must be performed within 30 days prior to starting pre-transplant conditioning regimen:

1. Complete history, review of systems, physical exam (including performance status)
2. CBC with differential, comprehensive metabolic panel including albumin, LDH, serum uric acid, PT/PTT
3. If creatinine ≥ 1.5 mg/dL or a history of renal dysfunction must have a *measured* creatinine clearance (using 24 hour urine collection).
4. Bone marrow aspirate, and trephine core if clinically indicated for morphology (must be done if possibility of myelofibrosis), surface markers (if warranted), cytogenetics, FISH and molecular studies (if warranted) for documentation of disease status
5. Spinal or intra-Ommaya tap for evaluation of evidence of CNS leukemia in patients with acute leukemia at risk for CNS disease
6. Urinalysis
7. Red blood cell type and screen (ABO blood type)
8. Evaluation for dental disease; full dental exam if evidence of disease present
9. EKG
10. Echocardiogram, MUGA scan, or cardiac MRI with measurement of left ventricular ejection fraction
11. Chest X-Ray
12. Radiographic studies as clinically indicated for diagnosis
13. Chest CT scan without contrast for all patients to exclude occult fungal infection prior to transplant
14. Pulmonary Function Test (spirometry and corrected DLCO)
15. Infectious disease markers (according to institutional guideline) to include at a minimum: CMV titer, Hepatitis panel (HepB sAb, HepB sAg, HepB cAb, HepC Ab), HIV 1/2 (serology **and** HIV-1 NAT or p24 antigen), and HTLV-1/2, HSV, toxoplasmosis, and RPR serology testing.
16. Pregnancy test for females of childbearing age (serum or urine HCG).
17. Peripheral blood from the patient submitted to the Diagnostic Molecular Pathology laboratory for future chimerism studies.

4.2. Post-Transplant Evaluations

Post-transplant evaluations are summarized in Table 4.2.1. Scheduled evaluations for day 21 and 28 should be performed +/-2 days; for day 60 and 100 should be performed +/-7 days; for 6 and 9 months should be performed +/-14 days; and for 12, 18, 20 and 24 months should be performed +/-30 days of the targeted date. Evaluations may be withheld if the treating physician feels that there is a strong contra-indication to perform the study (e.g. patient has relapsed and is terminally ill). Additional tests will be performed as clinically indicated.

Table 4.2.1. REQUIRED POST-TRANSPLANT EVALUATIONS

Activity	Day +1 to 100	Long term Follow-up
History and Physical	Inpatient: Daily Outpatient: Twice weekly until Day +60; Weekly Day +60 - 100	Month +6, 9, 12, 18, 24
Karnofsky score	Day +100	Month +6, 9, 12, 18, 24
Chemistry	Inpatient: Daily* Outpatient: Weekly (CMP)	Month +6, 9, 12, 18, 24 (CMP)
WBC/differential	Inpatient: Daily (with differential when WBC $\geq$ 500) Outpatient: Weekly	Month +6, 9, 12, 18, 24
BM biopsy	Days +21 (aspirate and core) Day +100 (aspirate; and core if clinically indicated)	As clinically indicated
Chimerism: blood	Days +28, 60 and 100	Month +6, 12, 24
GVHD evaluation	Inpatient: Daily Outpatient: Weekly	Month +6, 9, 12, 18, 24
Ig levels	Day +30, 60, 90-100	Monthly until IgG > 400 then Month +6, 12, 18, 24
Lymphoid phenotyping	Day +60, 100	Month +6, 12, 18, 24
Vaccination Response	N/A	Pre-vaccination titers at Month +12; response titers at Month +20**

*Daily chemistry will be standard electrolyte panel with comprehensive metabolic panel (CMP) including liver function tests biweekly (or more frequently as indicated).

**Response titers may be drawn at the Month 18 follow-up visit if patient is not scheduled for a Month 20 visit.

During the first 100 days post-transplant patients who are hospitalized must be closely monitored with daily history and physical examinations, blood counts and chemistries and at least biweekly liver function tests. After discharge, clinic visits are required a minimum of twice weekly until day 60 and a minimum of weekly until day 100. Blood counts and chemistries are required weekly post-discharge until day 100. Long term follow-up includes physical exams, blood counts and chemistries at 6, 9, 12, 18 and 24 months.

Bone marrow evaluation (aspiration and biopsy) with analysis for chimerism (and cytogenetics if indicated) must be performed on day 21 to assess donor hematopoietic engraftment and marrow

cellularity. This should be repeated at day 28 only if there are concerns about engraftment. Bone marrow aspirate should be done at day 100 on all patients to monitor donor cell engraftment.

Analysis for chimerism in peripheral blood and disease status assessment should be performed at days 28, 60 and 100, and at 6, 12 and 24 months, and as clinically indicated.

Acute GVHD will be assessed and graded according to the criteria in Appendix F. To determine acute GVHD grading, clinical data will be collected at least weekly until Day 100. Chronic GVHD will be diagnosed and graded according to the criteria in Appendix G. Assessments will be obtained at approximately day 100, and at 6, 9, 12, 18 and 24 months, and at additional time points as clinically indicated.

Ig levels will be monitored at days 30, 60, 90-100 to guide need for Ig replacement. After recovery no longer requiring monthly replacement (i.e. IgG > 400 unsupported) the Ig levels must be done at a minimum of 6, 12, 18 and 24 months.

Lymphoid phenotyping will be evaluated at days 60 and 100, and at 6, 12, 18 and 24 months. Patients will be vaccinated at 12 months after transplant and the response to vaccination for organisms of immediate clinical relevance must be documented (Appendix H).

4.3. Serious Adverse Event (SAE) Reporting

All patients will be followed for safety and toxicity related to the study. Toxicities will be graded using CTC guidelines (v3.0). This will occur continuously during the first 100 days after transplant, and then at a minimum of 6 and 9 months and 1 year after transplant, and annually thereafter. Serious adverse experiences will be graded regarding the severity, relation to the study, action required (if any) and outcome. Potentially serious toxicities are an expected part of transplant therapy. However, for the purposes of this study, serious adverse events (SAEs) which will be reportable will be defined as:

1) Primary graft failure (within the first 42 days after UCB infusion) or secondary graft failure (at any time after primary donor engraftment);

2) Grade IV acute GVHD within the first 100 days;

3) Death of the patient due to transplant-related causes (TRM) or death due to relapse of their original disease at any time up to one year post-transplant

CHAPTER 5

5.0 STATISTICAL CONSIDERATIONS

5.1. Study Design

The study is a Phase II, single arm, multicenter trial. It is designed to assess whether the anticipated endpoints for a myeloablative double unit UCBT are likely to be achieved in a multicenter study conducted by the CIBMTR. The study population is adult patients with high-risk hematologic malignancies. The sample size is 55 patients for this trial.

5.2. Accrual

It is estimated that three years of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, gender, and age.

5.3 Study Duration

Patients will be followed for a minimum of two years post-transplant.

5.4. Randomization

There is no randomization aspect to this trial.

5.5. Primary Objective

The primary objective is to estimate the overall survival (OS) probability at one year post-transplant.

5.6. Sample Size and Power Considerations

The sample size is 55 patients for this trial. The OS probability estimate will be based on the Kaplan-Meier product limit estimator using Greenwood's formula as the variance estimate. In the absence of censoring, the Kaplan-Meier estimate reduces to the simple binomial proportion. Ninety-five percent confidence intervals were calculated for varying observed probabilities based on the sample size of 55 patients, using the exact binomial distribution. Table 5.6.1 provides confidence intervals for a variety of observed proportions. Of particular interest is where the OS probability is 60%, which is the anticipated 1 year OS probability. For this setting, the confidence interval length is approximately 27%. The percentages above and below 60% are meant to represent other plausible OS percentages.

TABLE 5.6.1: CONFIDENCE INTERVAL LENGTHS AND POSSIBLE CONFIDENCE INTERVALS FOR VARIOUS UNDERLYING OVERALL SURVIVAL PROBABILITIES

N	Number alive at one year (%)	Length of 95% Confidence Interval	Possible Confidence Intervals	
55	39 (71%)	25.3	57.1	82.4
55	36 (65%)	26.4	51.4	77.8
55	33 (60%)	27.1	45.9	73.0
55	30 (55%)	27.5	40.5	68.0
55	27 (49%)	27.6	35.3	62.9
55	22 (40%)	27.1	27.0	54.1

The lower bound of the confidence interval can be viewed as a lower bound on the overall survival rate, so that we can rule out (with 95% confidence) OS percentages below that value. We propose declaring the treatment efficacious if we can demonstrate that the overall survival at 1 year is greater than 40%. If we observe at least 30/55 patients alive at one year, we have 95% confidence that the true 1 year overall survival is > 40%. The probability to rule out OS percentages of a certain size is known as "power". Table 5.6.2 provides the probability (or power) that the lower bound of a 95% two-sided confidence interval for the OS probability will be greater than thresholds ranging from 70% to 40%, as a function of the true OS probability. When the true OS percentage is 60%, there is 83% power to rule out an OS percentage of <= 40% (bolded entry).

This can also be viewed in a hypothesis testing framework, where we test the null hypothesis: H_o: $p \leq 0.40$ versus the alternativeH_1: $p > 0.40$. Based on the table below, there is 83% power at $\alpha = .025$ (one-sided) to reject the null if the true percentage is 60%.

TABLE 5.6.2: PROBABILITY OF RULING OUT A THRESHOLD OF SIZE T OR LARGER FOR VARIOUS TRUE UNDERLYING OVERALL SURVIVAL PERCENTAGES

	Probability of ruling out OS Percentages of size T or smaller						
True OS	0.70	0.65	0.60	0.55	0.50	0.45	0.40
0.70		0.07	0.28	0.62	0.81	0.96	0.99
0.65	0.12		0.09	0.31	0.53	0.82	0.96
0.60	0.34	0.11		0.11	0.25	0.56	**0.83**
0.55	0.63	0.32	0.10		0.08	0.27	0.58
0.50	0.86	0.61	0.30	0.09		0.09	0.30
0.45	0.97	0.85	0.58	0.27	0.08		0.10
0.40	1.00	0.96	0.83	0.56	0.25	0.11	

5.7. Interim Analysis and Stopping Guidelines

Monitoring of two key safety endpoints (Treatment-related mortality and graft failure) will be conducted monthly. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal "stopping rules" that would mandate automatic closure of study enrollment.

Treatment-related mortality

Treatment-related mortality in this trial is anticipated to be <=25% at 100 days. The stopping rule for treatment-related mortality will be triggered if there is significant evidence that the 100 day treatment-related mortality rate is more than 40% based on the exact binomial test. The stopping rule is summarized in the following table.

TABLE 5.7.1: STOPPING RULE FOR TREATMENT-RELATED MORTALITY BY DAY 100

Number of patients in the trial	Stop if TRM occurs in
5-7	5
8-10	6
11-13	7
14-16	8
17-19	9
20-22	10
23-25	11
26-28	12
29-31	13
32-34	14
35-38	15
39-41	16
42-44	17
45-47	18
48-50	19
51-53	20
54-55	21

The actual operating characteristics of this stopping rule, shown in the table below, were determined in a simulation study that assumed uniform accrual of 55 individuals over a three-year time period.

TABLE 5.7.2: OPERATING CHARACTERISTICS OF STOPPING RULE FOR TREATMENT-RELATED MORTALITY FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

True 100-Day Rate	25%	30%	35%	40%
Probability Reject Null	0.097	0.286	0.558	0.798
Mean Month Stopped	34.0	30.5	25.1	19.4
Mean # Endpoints in 100 Days	13.2	14.2	13.6	11.9
Mean # Patients Enrolled	51.9	46.6	38.4	29.6

The testing procedure for treatment-related mortality rejects the null hypothesis in favor of the alternative 10% of the time when the true 100 day treatment-related mortality rate is 25%, and 80% of the time when the rate is 40%. This corresponds to a type I error rate of $\alpha = 0.10$ and a type II error rate of $\beta = 0.2$. When the true 100 day treatment-related mortality rate is 40%, on average, the stopping rule will be triggered 19 months after opening, when 11.9 events have been observed in 30 patients.

Graft Failure

Graft failure in this trial is anticipated to be <=10% at 42 days. The stopping rule for graft failure will be triggered if there is significant evidence that the day 42 graft failure rate is more than 10% based on the exact binomial test. The stopping rule is summarized in the following table.

TABLE 5.7.3: STOPPING RULE FOR GRAFT FAILURE (GF) BY DAY 42

Number of patients in the trial	Stop if GF occurs in
3-7	3
8-13	4
14-19	5
20-25	6
26-31	7
32-37	8
38-43	9
44-49	10
50-55	11

The actual operating characteristics of this stopping rule, shown in the table below, were determined in a simulation study that assumed uniform accrual of 55 individuals over a three-year time period.

TABLE 5.7.4: OPERATING CHARACTERISTICS OF STOPPING RULE FOR GRAFT FAILURE FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

True 100-Day Rate	10%	15%	20%	25%
Probability Reject Null	0.092	0.350	0.694	0.907
Mean Month Stopped	33.8	28.5	20.8	14.2
Mean # Endpoints in 100 Days	5.3	6.6	6.4	5.5
Mean # Patients Enrolled	51.7	43.6	31.8	21.7

The testing procedure for treatment-related mortality rejects the null hypothesis in favor of the alternative <10% of the time when the true graft failure rate is 10%, and 90% of the time when the rate is 25%. This corresponds to a type I error rate of $\alpha = 0.10$ and a type II error rate of $\beta = 0.1$. When the true graft failure rate is 25%, on average, the stopping rule will be triggered 14 months after opening, when 5.5 events have been observed in 22 patients.

5.8. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, disease stage, HLA match, cell dose.

5.9. Analysis of Primary Endpoint

The primary analysis will consist of estimating the 1 year OS probability based on the Kaplan-Meier product limit estimator. The 1 year OS probability and confidence interval will be calculated. All transplanted patients will be considered for this analysis.

5.10. Analysis of Secondary Endpoints

- **Time to Neutrophil Engraftment:** To assess the incidence of neutrophil engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to neutrophil engraftment will be considered as a competing risk.

- **Time to Platelet Engraftment:** To assess the incidence of platelet engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to platelet engraftment will be considered as a competing risk.

- **Time to Acute GVHD:** To assess the incidence of grades II-IV and grades III-IV acute GVHD from day of transplant. The first day of acute GVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that acute GVHD grade. An overall cumulative incidence curve will be computed along with a 95% confidence interval at 100 days post-transplant with death considered as a competing risk.

- **Time to First Clinical Onset of Chronic GVHD:** To assess the incidence and severity of chronic GVHD from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at two years post-transplant. Death prior to occurrence of chronic GVHD will be considered as a competing risk.

- **Transplant Related Mortality (TRM):** TRM is death occurring in patients in continuous complete remission. The TRM distribution will be estimated by the cumulative incidence curve, with relapse considered as a competing risk.

- **Time to Leukemia Relapse:** To assess the incidence of leukemia relapse from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to relapse will be considered as a competing risk.

- **Disease-free Survival:** The disease-free survival distribution will be estimated by the Kaplan-Meier curve. All patients will be followed for a minimum of two years post-transplant for relapse and mortality.

- **Serious infection:** All serious infections as defined in Section 3.2 will be described in terms of organism, site, and time of onset. In addition, to assess the incidence of one or more serious infections from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at one year post-transplant. Death prior

to occurrence of serious infection will be considered as a competing risk. In addition, the number and type of serious infections at given post-transplant intervals will be described.

Appendix A KARNOFSKY PEFORMANCE STATUS SCALE

The score is defined by the phrase which best describes the activity status of the recipient.

Able to carry on normal activity; no special care is needed.

100 Normal; no complaints; no evidence of disease.
90 Able to carry on normal activity.
80 Normal activity with effort.

Unable to work; able to live at home, care for most personal needs; a varying amount of assistance is needed.

70 Cares for self; unable to carry on normal activity or to do active work.
60 Requires occasional assistance but is able to care for most needs.
50 Requires considerable assistance and frequent medical care.

Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.

40 Disabled; requires special care and assistance.
30 Severely disabled; hospitalization indicated, although death not imminent.
20 Very sick; hospitalization necessary.
10 Moribund; fatal process progressing rapidly.

Appendix B DOUBLE UNIT SELECTION

1.0 Unit Selection

1.1. Transplant centers should search the NMDP banks (domestic and Coop) and the NYBC for suitable units at a minimum. Netcord can also be searched to broaden the search. Unit selection should be based on cryopreserved nucleated cell (NC) dose & HLA-A, B antigen DRB1 allele match.

1.2. While unit selection will be primarily based on A, B antigen and DRB1 allele match, the match at A and B alleles may be considered in unit selection. Therefore, all units should be typed at A, B, and DRB1 alleles.

1.3 Units should be typed twice (i.e. initial typing and confirmatory typing). Confirmatory typing should be in a lab that is ASHI/EFI accredited and must come from an *attached* segment. If CT is not done on an attached segment by the TC, NMDP reference laboratory or the UCB bank, it is strongly suggested an alternate cord blood unit be selected.

1.4 The transplant center should determine if potentially suitable units are red blood cell (RBC) depleted or not. Units that are not RBC depleted are those from StemCyte and some units from Europe (e.g. some units from France, Belgium). The final volume of the cryopreserved product is a helpful guide as to whether the units are RBC depleted or not. RBC depleted units are typically less than 50 mLs. A large volume unit such as 100-200 mLs will not be RBC depleted.

1.5 The minimum required cell dose for each unit is dependent on how the units are processed prior to cryopreservation (i.e. contain RBC or not).

- All red blood cell **depleted** units must be $\geq 1.5 \times 10^7$ NC/kg (actual body weight).
- All red blood cell **containing** units must be $\geq 2.0 \times 10^7$ NC/kg (actual body weight).
- When comparing RBC depleted and RBC containing units above the minimum cell dose threshold, the reported cell dose for RBC containing units should be corrected downward by 30%. The formula for this correction is:
  Corrected dose = reported RBC containing unit dose x 0.7. This corrected dose should then be used to compare the cell dose of that unit to another when selecting units.
- Transplantation using two RBC containing units is allowed but not preferred.

1.6. All units must be at least 4/6 HLA-A and B *antigen* and DRB1 *allele* matched with the patient. Double mismatches at the same locus should be avoided. Units must also be at least 3/6 HLA-antigen matched to each other.

NMDP IRB Approved 01/11/2008 through 01/10/2009

1.7. Over the cell dose threshold of 1.5 x 10^7 NC/kg (2.0 x 10^7 NC/kg for RBC containing units), HLA match should take priority in unit selection (e.g. select the first unit over the second in the example below).

	A	B	DRB1	Match	NC Dose
Patient	0101 0101	5701 7301	0402 0405	-	-
Potential Unit 1	0101 **0201**	5701 7301	0402 0405	5/6	2.9
Potential Unit 2	0101 0101	5701 **5201**	0402 **1502**	4/6	3.7

1.8. If a potentially suitable UCB unit is a 5/6 match because it is homozygous at a particular locus (i.e. a "no rejection" mismatch" to the patient), this unit should be chosen over a 5/6 unit with a bidirectional mismatch even if the latter unit has a higher cell dose. For example, in the scenario below unit 1 should be selected rather than unit 2:

	A	B	DRB1	Match	NC Dose
Patient	0201 8001	0702 0705	1101 1501	-	-
Potential Unit 1	0201 **0201**	0702 0702	1101 1501	5/6	2.9
Potential Unit 2	0201 **3201**	0702 0702	1101 1501	5/6	3.5

This "no rejection mismatch" priority also applies to 4/6 units. Therefore, above the cell dose thresholds, if there are no 5/6 units, of potential 4/6s units, choose a unit with two no rejection mismatches over two bidirectional mismatches regardless of dose (e.g. select unit 2 in example below).

	A	B	DRB1	Match	NC Dose
Patient	0201 2402	1517 5101	0803 1602	-	-
Potential Unit 1	0203 2402	5101 **4601**	0803 **0901**	4/6	3.0
Potential Unit 2	0201 **0201**	**5101** 5101	0803 1602	4/6	2.1

1.9 If there are no units with a "no rejection mismatch" available, and there are multiple units within a given degree of HLA-match, the unit with the largest TNC should be selected. If many units with similar HLA-A,B antigen and DRB1 allele match are available and are within 0.3 NC of each other, units that are allele matched at A and/or B should be selected.

1.10. If unit selection occurs > 60 days before patient clearance for transplant, the UCB search should be re-run using updated patient weight (if changed) to identify any additional UCB units that may be superior to the ones already selected.

1.11. In summary, UCB units should be selected according to the following algorithm:

Step 1: Identify all units at least 1.5 x 10^7 NC/kg (or 2.0 x 10^7 NC/kg for RBC containing units) and rank in order of best to worst HLA-A, B antigen, DRB1 allele match to the patient. Take care to correct the cell dose of red cell containing units using the x 0.7 formula, and to identify homozygous units with no rejection mismatches which take priority over bidirectional mismatches.

Step 2: To select unit #1:

- Select largest unit at least 1.5 x 10^7 NC/kg (or 2.0 x 10^7 NC/kg for RBC replete units) that is 6/6 HLA-matched to the patient.
- If no 6/6 units, proceed to selecting largest 5/6 unit.
- If no 5/6 units, select largest 4/6 unit.
- Avoid double mismatches at the same locus. Also, select a unit with a no rejection mismatch (see above) over a bidirectional mismatch.
- Otherwise, within a given match grade, choose the largest TNC.
- If many units with similar HLA-A,B antigen and DRB1 allele match are available and are within 0.3 NC of each other, the unit with the best allele match at A and B should be selected.

Step 3: Repeat the above procedure to select unit #2. In addition to above rules, unit 2 must be at least 3/6 HLA-antigen match to unit #1.

Step 4: Select back-up (see section below).

2.0 Back-up Grafts

2.1. Back-up UCB unit(s) should be strongly considered if the patient's double unit graft includes 1 or 2 units in which confirmatory typing was done on a specimen other than an attached segment in case the repeat typing on day of thaw does not confirm unit identity.

2.2. In addition, a plan for non-engraftment must be in place prior to the start of the patient's preparative regimen. This could be UCB, haplo-identical HSC or autologous cells previously cryopreserved.

2.3. If the back-up graft is to be UCB, ideally the unit(s) should be confirmatory typed pre-transplant, and remain reserved until the patient has engrafted.

2.4. At least one back-up unit should have typing done on an attached segment, if possible.

3.0 Infectious Disease Markers, Sterility and Hemoglobinopathy Screens

3.1. Maternal blood must be tested for Hepatitis B and C, HIV, HTLV, CMV, and syphilis.

3.2. All maternal samples must be screening test negative, with the exception of CMV IgG, anti-HBc and STS. Units from mothers that are anti-HBc or HBsAb positive will be accepted if mothers are HBsAg negative, or mother or unit is HBV NAT negative (when available). Mothers that are STS screen positive, confirmed negative will be accepted. If maternal CMV total is positive then CMV IgM or culture or PCR should be negative.

3.3. Screening for hemoglobinopathies (sickle cell disease and thalassemia) should be performed on the UCB or the newborn using a method that distinguishes hemoglobin A, A2, S, C and alpha and beta thalassemia disease and/or trait (solubility assay is not acceptable). Units homozygous for either sickle cell disease or thalassemia or heterozygous for both sickle cell and thalassemia trait will be deferred. Units heterozygous for either sickle cell trait or thalassemia will be accepted if no other options are available.

Appendix C THAWING PROCEDURE FOR UCB

General

- The UCB search coordinator should supply the Cytotherapy Laboratory with the expected date and time of arrival of the cord blood units. Units must be shipped prior to the start of conditioning. Any problems occurring during shipping must be resolved before the start of cytoreduction.
- Coordinate the thaw with patient readiness for infusion to ensure prompt infusion post-thaw. Aim should be to infuse units within 2 hours of thaw (except for units that require urgent HLA type on day of thaw that should be infused within approximately 5 hours).
- During the thaw handle each unit separately to avoid mix up. If a single technologist thaws two units, they should be in separate biosafety cabinets, or done one at a time.

Thaw of *Red Cell Depleted* Units

Red cell depleted units must be thawed using a reconstitutive method (i.e. albumin dilution). This decreases the manipulation of the cells, decreases the loss of cells from centrifugation, is faster and technically easier than a wash, and deceases time from thaw to infusion, while still allowing samples to be taken in the controlled environment of the Cytotherapy Laboratory before products are infused. Initially add reconstitutive solution equal to the volume of the cord blood unit (i.e. 1:1 dilution). Allow 1-2 minutes for equilibration. Then continue with the dilution procedure to a minimum of 5:1 fold dilution.

Suggested Procedure for Albumin Dilution of Red Cell Depleted Units

- Reagents and equipment:

25% Human Serum Albumin (25% HSA);
10% Dextran 40 (Molecular Weight 40,000);
Transfer packs and transfer sets;
Zip-Loc Bags;
Disposable syringes, 1, 3, 5, 30, & 60 mL and needles;
37°C water bath;
Microscope;
12 x 75 mm tubes;
Pipets and tips;
Trypan blue stain (0.2%);
Tubing heat sealer;
Needleless adapters;
MedSep Cell Wash/Infusion set

- Prepare reconstitution stock solution at room temperature in a 5:1 ratio of 10% Dextran 40 and 25% albumin. For example, combine 250 mL 10% Dextran 40 and 50 mL 25% albumin.
- Draw up a volume of reconstitution solution that is a minimum of 5 times the volume of the unit. A greater volume can be used if desired. For example, if the unit is 25 mLs then you could draw up 175 mLs of reconstitution solution to make a final volume of diluted product of 200 mLs in a 300 mL transfer pack. Note if the cryopreserved volume of the

unit is larger then a larger volume transfer pack will be required (e.g. for a 50 mL unit would dilute with at least 250 mLs = final volume of at least 300 mLs).

- After confirmation of product identity, place unit inside a Ziploc bag, zip bag closed and submerge in 37°C water bath. Gently agitate bag and knead contents until the product is slushy. After ensuring unit cryobag is intact, remove from ziploc and transfer to hood.
- *If the unit is in a single compartment*, slowly add reconstitution solution to unit cryobag and drain contents into appropriately sized transfer pack so that the original unit cryobag is repeatedly rinsed of its contents into the transfer pack.
- *If the unit is a MedSep bag,* attach appropriate MedSep tubing (e.g. as provided by bank or, for example, Cell Wash/ Infusion Bag Set by Pall Medical, Code 791, Lot #05533) so that reconstitution solution will drain and wash both compartments of the MedSep bag, and drain thawed contents into the transfer pack.
- Thawed unit should be tested for cell count, viability, CD34, CD3, chimerism and sterility.

Thaw of *Red Cell Containing* Units

General

- Units containing red cells can either be thawed using the albumin dilution technique or can be thawed at the bedside per the bank's instructions (TC will need to report which method used). Red cell containing units should not be washed provided patient weighs at least 40 kg.

- If the albumin dilution technique is used, the unit must be diluted to a minimum of 3:1.Initially add reconstitutive solution equal to the volume of the cord blood unit (i.e. 1:1 dilution). Allow 1-2 minutes for equilibration. Then continue with the dilution procedure to a minimum of 3:1 fold dilution. For example, if the unit is cryopreserved in two 100 mL bags then you should aim to dilute each bag with a minimum of 300 mLs to a volume of at least 400 mLs/ bag (final volume of the unit at least 800 mLs).

- Careful observation of the patient for volume overload is required if such a volume is to be delivered. Also, vigorous hydration should be given for at least 12 hours post unit infusion if the unit has a major ABO incompatibility.

Suggested Procedure for Albumin Dilution of Red Cell Containing Units

Reagents and equipment:

- Reagents and equipment as for red cell depleted units.

- 300 mL or 600 mL transfer packs depending on the volume of the cryopreserved unit.

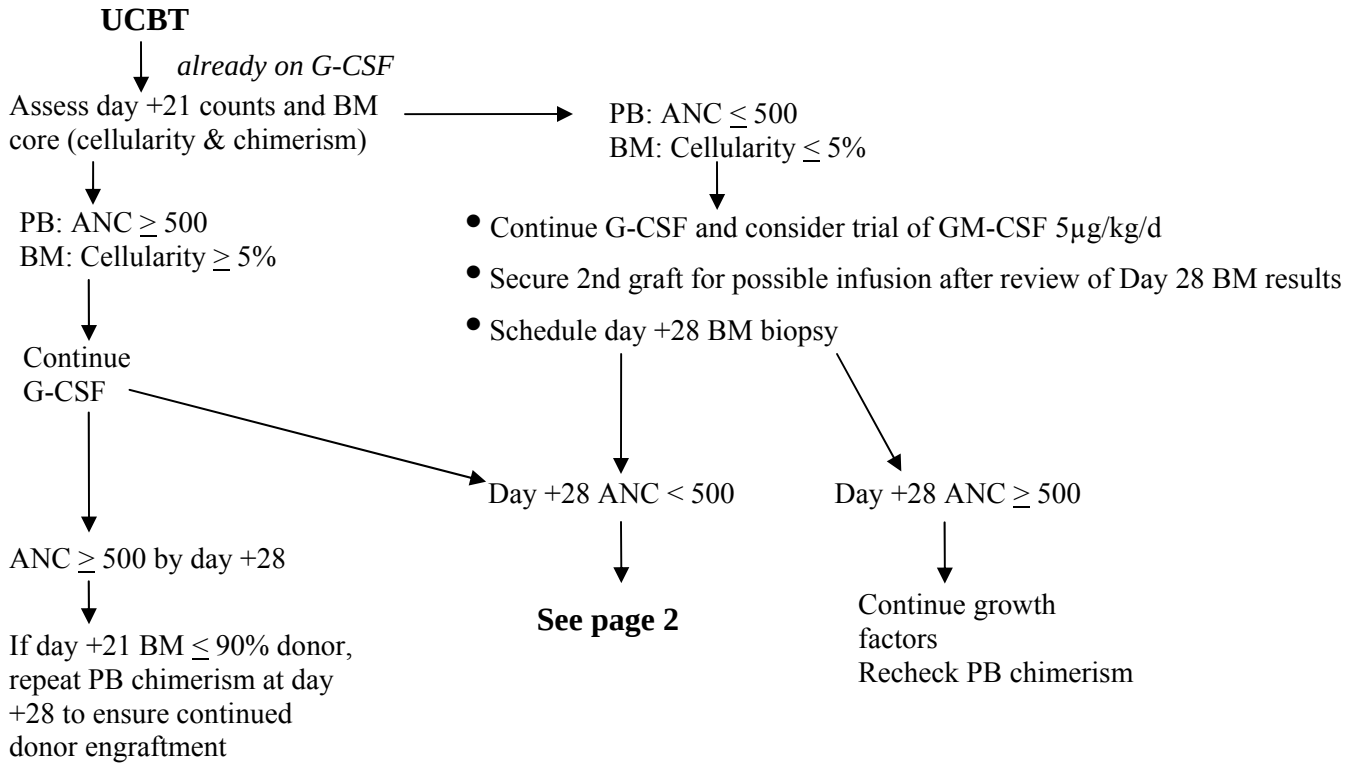
- 1 liter infusion bags depending on the final volume of the unit.

- 2 sets of tubing with double spike (Double Spike Plasma Transfer Kit, Baxter: Lot A05F08112).

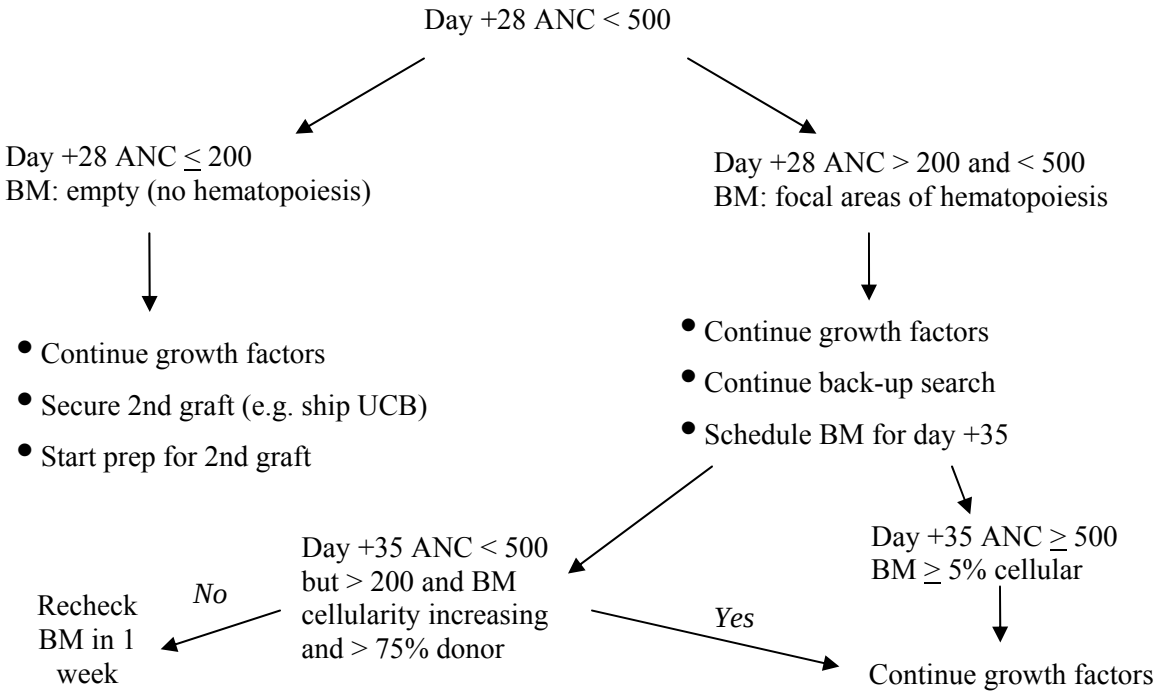
Procedure:

- Calculate how much reconstitution stock solution will be needed. Prepare at 5:1 ratio of 10% Dextran 40 / 25% albumin at room temperature (e.g. 500 mLs 10% Dextran 40 and 100 mL 25% albumin).
- If the unit is cryopreserved in two freezing bags, thaw one bag at a time or two technologists do one each.
- Draw up an amount of reconstitution solution equal to at least three times the total volume of the bag to be thawed and place in an appropriately sized transfer pack. For example, if the bag to be thawed is 45 mLs then draw up at least 135 mLs (could round to 155 mLs to make final volume of 200 mLs), or if bag to be thawed is 100 mLs, then draw up 300 mLs of stock solution into the transfer pack.
- Place bag to be thawed inside a Ziploc bag, zip closed and submerge in 37ºC water bath. Gently agitate and knead contents until the product is slushy.
- Ensuring unit cryobag is intact, remove from ziploc and place in hood.
- Connect the unit and the transfer pack with <u>two</u> double spike plasma transfer kits and open both.
- Hold the unit in one hand and the transfer pack in the other, and raise the transfer pack allowing the albumin dilution solution to drain from the transfer bag into the unit cryobag by gravity via both plasma transfer kit tubes.
- Once somewhat diluted, raise the cryobag so that the cell suspension drains back into the transfer pack.
- Repeat back and forth as needed until the cryobag has been completely rinsed and the entire volume of the cryobag is in the transfer pack.
- During this process watch both sets of tubing to ensure that both continue to drain. If one clogs, ensure that the unit is draining via the other. If there are visible clots "floating" in the cryobag try to avoid their passing through the tubing by nipping them back with your fingers. If the transfer tubing gets clogged, gently squeeze the base of the connection of the spike to see if this will loosen any clots that may be obstructing the flow.
- If 2 hands are needed to manipulate the cryobag, the transfer bag can be placed on a rotator during the thaw procedure. If not, a second person may be needed to hold one of the bags while the primary technician manipulates the other bag.
- After the freezing bag has drained, inspect the diluted product in the transfer bag for clots. If there are large clots visible, consider transferring diluted unit into another transfer bag, by a similar procedure, so that you can keep the clots from getting into the final cell suspension.
- If the unit was frozen in 2 bags store the first half of the thawed unit at 4-10°C until the second half is thawed. Repeat the above procedure for thaw of the second half of the unit. Then combine the 2 halves into one large bag before any samples are removed for cell counts etc. Note: this is important as the 2 "halves" may not contain the same number of cells. Therefore, counts from one "half" may not reflect what is in the other.
- Remove aliquots from the final products as per red cell depleted units.

Appendix D SUGGESTED GUIDELINES FOR SLOW ENGRAFTMENT / GRAFT FAILURE



Suggested Guidelines for Slow Engraftment / Graft Failure (continued)



Appendix E DEFINITION OF INFECTION SEVERITY AND RECURRENCE INTERVALS

The occurrence of serious infections (severe, life-threatening or fatal) will be collected from the time of admission to one year after transplant. For the purpose of data collection, infections will be reported in the following time periods: admit to day 30, 31-60, 61-100, 101-180, 181-1 year). Data collected will include the date of infection diagnosis, the infection type and pathogen (if available), the site of infection, if the infection is associated with a specific syndrome (e.g. septic shock), and the infection severity. The criteria that guide designation of infection severity, and that define one infection versus two with the same organism (recurrence intervals) are provided below.

Fatal Infections:
Any infection clearly linked to death within two weeks.

Life-threatening Infections:
1. Septic shock, need for pressors or intubation
2. Any of the following:
    - Any proven/probable pulmonary or disseminated mold infection (e.g. aspergillus)
    - CMV pneumonitis (CXR infiltrate + recovery of virus in BAL specimen or lung biopsy evidence)
    - Disseminated CMV
    - Respiratory virus pneumonitis
    - Influenza/RSV/Parainfluenza virus of lung (CXR infiltrate + recovery)
    - HHV-6 in central nervous system (CNS)
    - Toxoplasma in brain or CNS
    - PCP in lung

Severe Infections:
1. Deep tissue (invasive) infection requiring IV or oral antibiotics used to treat infection
2. Any infection requiring hospitalization, if outpatient at onset
3. Any infection requiring oxygen or fluids to support BP
4. Any of the following:
    - Any proven or probable sinus (limited) mold infection
    - Pulmonary nodules that decrease in size after a minimum 4 week course of antifungal medications active against Aspergillus
    - Any Bacteremia, catheter-related bloodstream infection (excluding Coagulase negative staphylococcus and Diptheroids which are MODERATE infections)
    - Any infection that requires adjunctive surgical intervention
    - Any Pneumonia not requiring ventilatory support (see life-threatening category for specific viral pneumonias)
    - Upper airway (limited) respiratory viruses (firm diagnosis; e.g. metapneumovirus) if hospitalization required. Outpatient URIs are not included.
    - CMV antigenemia or PCR positivity treated with a course of antiviral therapy

- Hemorrhagic cystitis due to BK virus requiring therapy (either hospitalization with supportive care and/or cidofovir)
- Adenovirus infection (blood, bladder, gut) requiring hospitalization for supportive care and/or specific viral therapy
- HHV6 viremia requiring hospitalization and therapy (incidentally found HHV6 that did not require therapy is not included)
- Pseudomembranous colitis due to C. difficile
- Typhlitis
- Osteomyelitis
- Meningitis
- Disseminated or complicated zoster (i.e., ophthalmic)
- EBV viremia requiring therapy

Recurrence Intervals to Determine Whether an Infection is the Same or New:

1. CMV, HSV: 2 months (≤ 60 days)
2. VZV, HZV: 2 weeks (≤ 14 days)
3. Bacterial, non-C. difficile: 1 week (≤ 7 days)
4. Bacterial, C. difficile: 1 month (≤ 30 days)
5. Yeast: 2 weeks (≤ 14 days)
6. Molds: 3 months (≤ 90 days)
7. Helicobacter: 1 year (≤ 365 days)
8. Adenovirus, Enterovirus, Influenza, RSV, Parainfluenza, Rhinovirus: 2 weeks (≤ 14 days)
9. Polyomavirus: 2 months (≤ 60 days)

For infections documented as "disseminated", any infection with the same organism but different site within the recurrence interval for that organism will be counted as part of the disseminated infection.

Appendix F CLINICAL STAGING AND GRADING OF ACUTE GRAFT VERSUS HOST DISEASE

GVHD STAGING

STAGE	Skin	GI	Liver
1	< 25% rash	Diarrhea > 500 mL/d or persistent nausea	Bilirubin 2 - 3 mg/dl
2	25-50 %	> 1000 mL/d	Bilirubin 3 - 6 mg/dl
3	> 50 %	> 1500 mL/d	Bilirubin 6 - 15 mg/dl
4	Generalized erythroderma with bullae	Large volume diarrhea and severe abdominal pain ± ileus	Bilirubin > 15 mg/dl

GVHD GRADING

GRADE	Skin	Liver	Gut
0	None	None	None
I	Stage 1-2	None	None
II	Stage 3 and/or	Stage 1 and/or	Stage 1
III	None or Stage 3	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	NA

STAGING
For skin GVHD: Use "Rule of Nines" or burn chart to determine extent of rash.
For liver GVHD: Range of bilirubin given as total bilirubin. Downgrade one stage if an additional cause of hyperbilirubinemia is documented.
For gut GVHD: Downgrade one stage if an additional cause of diarrhea is documented. Stage 1 is persistent nausea, vomiting and anorexia in the absence of other known cause unless histology is negative.
GRADING Criteria for grading given as minimum degree of organ involvement required to confer that grade.

APPENDIX G CHRONIC GVHD SCORING TABLE

Check all that apply	**Score 0** - None	**Score 1** - Mild	**Score 2** - Moderate	**Score 3** - Severe
Skin: *Clinical features:* ❑Maculopapular rash ❑Lichen planus-like features ❑Papulosquamous lesions or ichthyosis ❑Hyperpigmentation ❑Hypopigmentation ❑Keratosis pilaris ❑Erythema ❑Erythroderma ❑Sclerotic features ❑Poikiloderma ❑Pruritus ❑Hair Involvement ❑Nail Involvement **%** BSA involved **__%**	❑ No symptoms	❑ < 18% BSA with disease signs but NO sclerotic features	❑ 19-50% BSA, ❑ Involvement with superficial sclerotic features "not hidebound" (able to pinch)	❑ > 50% BSA ❑ Deep sclerotic features "hidebound" (unable to pinch) ❑ Impaired mobility, ulceration or severe pruritis
Mouth:	❑ 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 WITH **major** limitation of oral intake
Eyes: Mean tear test (mm): ❑ > 10 ❑ 6-10 ❑ ≤ 5 ❑ Not done	❑	❑ Mild dry eyes symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) ❑ Asymptomatic signs of keratoconjunctivitis sicca	❑ Moderate dry eyes symptoms partially affecting ADL (requiring eyedrops > 3 x per day or punctual plugs) WITHOUT vision impairment	❑ Severe dry eyes symptoms significantly affecting ADL (special eyewear to relieve pain) ❑ Unable to work because of ocular symptoms ❑ Loss of vision caused by keratoconjuctivitis sicca
Pulmonary **FEV1** ❑ Not done **Pulmonary Fibrosis** **Bronchiolitis Obliterans** **Supplemental O2 required?** ❑ Yes ❑ No	❑ No symptoms ❑ FEV1> 80% ❑ None ❑ Not assessed ❑ None ❑ Yes, clinical ❑ Yes, histologic ❑ Not assessed	❑ Mild symptoms (shortness of breath after climbing one flight of steps) ❑ FEV1 60-78% ❑ Minimal radiographic findings	❑ Moderate symptoms (shortness of breath after walking on flat ground) ❑ FEV1 40-51% ❑ Patchy or bi-basilar radiographic findings	❑ Severe symptoms (shortness of breath at rest; requiring O_2) ❑ FEV1 ≤ 39% ❑ Extensive radiographic findings
GI Tract:	❑ No symptoms	❑ Symptoms such as dysphagia, anorexia, nausea, vomiting, 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% ❑ Requires nutritional supplement for most caloric needs ❑ Esophageal dilation
Liver:	❑ Normal LFT	❑ Elevated Bilirubin, AP, AST or ALT < 2 x ULN	❑ Bilirubin 3 – 10 mg/dl; liver enzymes 2-5 x ULN	❑ Bilirubin > 10 mg/dL; liver enzymes > 5 x ULN
Genital Tract:	❑ No symptoms	❑ Symptomatic with mild signs on exam AND no effect on coitus and minimal discomfort with gynecological exam	❑ Symptomatic with moderate signs on exam AND with mild dyspareunia or discomfort with gynecological exam	❑ Symptomatic WITH advanced signs (stricture, labial agglutination or severe ulceration) AND severe pain with coitus or inability to insert vaginal speculum

Check all that apply	**Score 0** - None	**Score 1** - Mild	**Score 2** - Moderate	**Score 3** - Severe
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 ❑ Joint contractures, erythema thought due to fascitis, moderate decreased ROM AND mild to moderate limitation of ADL	❑ Contractures WITH significant decrease of ROM AND significant limitation of ADLs (unable to tie shoes, button shirts, dress self etc.)

Other indicators, clinical manifestations or complications related to Chronic GvHD (check all that apply).
Assign a score to it's severity based on functional impact, where applicable (0= none, 1=mild, 2 = moderate, 3= severe)

❑ Ascites (serositis) ___	❑ Esophageal stricture or web___	❑ Nephrotic syndrome ___	❑ Pleural effusions ___
❑ Cardiac conduction defects ___	❑ Eosinophilia > 500 µl ___	❑ Pericardial effusion ___	❑ Polymyositis ___
❑ Cardiomyopathy ___	❑ Myasthenia Gravis ___	❑ Peripheral neuropathy ___	❑ Progressive onset___
❑ Coronary artery involvement ___		❑ Platelets < 100,000/µl ___	
❑ Other(s): Specify & score_____			

Based on observations checked in the above table, select the severity of chronic GvHD for this assessment (Check only one)

☐ **None**

☐ **Mild chronic GVHD** involves only 1 or 2 organs or sites (except the lung: see below ‡), with no clinically significant functional impairment (maximum of score 1 in all affected organs or sites)

☐ **Moderate chronic GVHD** involves: (1) at least 1 organ or site with clinically significant but no major disability (maximum score of 2 in any affected organ or site) or (2) 3 or more organs or sites with no clinically significant functional impairment (maximum score of 1 in all affected organs or sites). ‡*A lung score of 1 will also be considered moderate chronic GVHD.*

☐ **Severe chronic GVHD** indicates major disability caused by chronic GVHD (score of 3 in any organ or site). ‡*A lung score of 2 or greater will also be considered severe chronic GVHD.*

Appendix H IMMUNE RECONSTITUTION AND VACCINATION SCHEDULE

1) Immune Reconstitution

The immune reconstitution studies listed below will be performed to assess humoral immunity (total immunoglobulin levels) and cellular immune recovery (lymphocyte subset analysis to quantify numbers and proportions of different lymphocyte subpopulations).

	< 6 Months	**6 Months**	**12 Months**	**18 months**	**24 Months**
Immunoglobulin levels: IgG, IgA, IgM, IgE	Day 30, 60, 90-100	X	X	X	X
Lymphoid Phenotyping: Lymphocyte subsets CD3, CD4, CD8, CD19, CD16/56	Day 60 and 100	X	X	X	X

Data forms will capture the use of IVIG or other immune globulin products (e.g. Cytogam) in the previous two months and the last day of administration of IVIg. IVIg supplementation should be given if the IgG level is less than 400.

2) Vaccination Response

This will be measured for infections of particular clinical relevance. All patients will be vaccinated at 12 months unless severe GVHD is present. Assessment of severe GVHD is dictated by treating physician.

Vaccine	**12 months**	**14 months**	**16 months**	**20 months**
Tetanus, H.Influenzae B, Prevnar	Draw Titers then Vaccine 1	Vaccine 2	Vaccine 3	Response Titers

Procedure should be as follows:

- Titers must be drawn pre-vaccination. Patients will be vaccinated (series of 3) for tetanus, pneumococcus (Prevnar), and Hemophilus influenza type B.
- Titers must be re-measured post vaccination. Vaccine response will be defined as seroconversion in a seronegative patient or $\geq$3-fold increase in titer following immunization. For pneumococcus, response will be either seroconversion in a seronegative patient or a >3 fold rise in titer against measured serotypes contained in the vaccine. For Prevnar, serotypes included in response will include 14, 19, and 23. Partial response will be defined as 1.5-2 fold increase over baseline titers, or response to some but not all measured serotypes contained in the vaccine (pneumococcus). Failure to respond will be defined as lack of seroconversion in seronegative patients or <1.5 rise in titer.
- Response titers may be drawn at the Month 18 follow-up visit if patient not scheduled for a Month 20 visit.
- Patients with response to Prevnar can be boosted with Pneumovax to increase coverage against additional serotypes.

- Also, provided patients are at least 6 months post transplant, seasonal Influenza A virus vaccine should be given regardless of GVHD status and response documented by checking titers at 6 weeks. If no response should re-vaccinate once.

Additional vaccines may be given per local institution policy.

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