

CORD BLOOD TRANSPLANTATION STUDY PROTOCOL

CHAPTER 2

STUDY DESIGN

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2.1 STUDY ENDPOINTS AND DEFINITIONS

The primary endpoint for the study of umbilical cord stem and progenitor cell transplantation is 180 day survival .

The secondary endpoints are:

1. Disease-free survival (DFS)
2. Long-term patient survival
3. Incidence of neutrophil engraftment
4. Incidence of both primary and secondary graft failure
5. Incidence of platelet engraftment
6. Incidence of RBC engraftment
7. Incidence and severity of acute and chronic graft-versus-host disease (GVHD)
8. Incidence of complications, including infection, veno-occlusive disease, and interstitial pneumonitis
9. Incidence of relapse
10. Incidence of other malignancies, lymphoproliferative disorders, and post-transplant myelodysplasia
11. Immune reconstitution

2.1.1 Primary and Secondary Graft Failure

Primary Graft Failure: Failure to engraft where engraftment is defined as achieving ANC \geq 500/mm³ for three consecutive measurements on different days by Day 42. The first of the three measurements may occur on Day 42. The ANC recovery must be of donor origin documented by either bone marrow or peripheral blood chimerism assays indicating at least 90% of cells of donor origin. Infusion of stem cells prior to Day 42 will be considered primary graft failure.

Secondary Graft Failure: Documented engraftment as defined above followed by:

1. severe neutropenia (ANC < 500/mm³) or
2. absence of donor cells in the marrow or blood as demonstrated by a chimerism assay

without subsequent improvement occurring either spontaneously or after growth factor treatment. Improvement is defined as ANC \geq 500/mm³ consistently. Severe neutropenia with marrow cellularity \geq 25% is not secondary graft failure.

Aplasia is defined as less than 5% cellularity in marrow as measured from either particle section or biopsy.

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Should a patient suffer graft failure, an attempt will be made to determine the cause of failure. Evaluation will include:

1. Bone marrow analysis for residual or recurrent leukemia and cellularity
2. Chimerism studies of residual circulating lymphocytes and bone marrow
3. Bacterial and viral cultures and/or DNA studies of peripheral blood or marrow

2.1.2 Neutrophil Engraftment

Neutrophil engraftment is defined as achieving $ANC \geq 500/mm^3$ for three consecutive measurements on different days by Day 42. The first of the three measurements may occur on Day 42. The ANC recovery must be of donor origin documented by either bone marrow or peripheral blood chimerism assays indicating at least 90% of cells of donor origin. A patient receiving a stem cell infusion prior to Day 42 will be considered a graft failure.

2.1.3 Platelet Engraftment

Platelet engraftment will be defined as the first day of a minimum of three consecutive measurements on different days such that the patient:

1. Has achieved a platelet count $> 50,000/mm^3$, and
2. Is platelet transfusion independent for a minimum of seven days.

2.1.4 RBC Engraftment

Time to red cell engraftment is defined as the first day of two consecutive measurements on different days such that the patient has achieved an absolute reticulocyte count $> 30,000/mm^3$. Measurements should be made weekly starting on Day 28 and may be stopped following RBC engraftment.

2.1.5 Acute and Chronic GVHD

Acute GVHD usually develops within the first three months after transplantation and appears as a characteristic dermatitis often accompanied by cholestasis and enteritis. Initial symptoms of chronic GVHD frequently include nausea and anorexia with ocular and oral sicca. Rash characteristically appears with pigmentary changes progressing to sclerosis and contractures. Other organs may be involved. Symptoms may mimic those seen in patients with scleroderma and other autoimmune disorders.

The staging of acute GVHD will follow NMDP guidelines but will include weekly capture of symptoms and characterization of alternative causes.

Chronic GVHD typically does not occur until three or more months after transplantation. Details regarding the definition and diagnosis are listed in Appendix C and Section 2.4 - Treatment Plan, respectively.

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2.1.6 Venous-Occlusive Disease

Veno-occlusive disease is defined by the occurrence of two of the following within 30 days of transplantation with no other explanation for these signs and symptoms present at time of diagnosis: hyperbilirubinemia (total serum bilirubin > 34.2 $\mu\text{mol/L}$ [2 mg/dL]), hepatomegaly or right upper quadrant pain of liver origin, and sudden weight gain (> 5% of baseline body weight) because of fluid accumulation. Reversal of hepatic blood flow can frequently be demonstrated on doppler ultrasonography.

2.1.7 Interstitial Pneumonitis

Interstitial pneumonitis is defined by diffuse interstitial infiltrates on chest x-ray not caused by fluid overload. It may be caused by a virus, bacteria, fungus, or may be of unknown etiology.

2.1.8 Infection Grading

Infections will be graded according to the following severity scale:

1. Mild, no active treatment (e.g., viral syndromes)
2. Moderate, requires outpatient PO antibiotic
3. Severe, requires IV antibiotic or antifungal or hospitalization
4. Life-threatening (e.g., septic shock)
5. Caused or contributed to death

For infection as a secondary endpoint, only grades 3-5 infections will be considered.

2.1.9 Relapse and Residual Disease

The term relapse is used to describe the recurrence of disease after transplantation. For the purposes of this study, relapse will be defined separately for each disease eligible for transplantation. The time to relapse is the time to the first observation of hematologic or cytogenetic changes which result in characterization as relapse. Treatment given for relapse reversal will be considered indicative of relapse even in the absence of the characteristics described below.

Acute Leukemia: Relapse will be diagnosed when leukemic blasts (>25%) are documented in the blood or bone marrow after transplantation, **or** leukemic blasts >5% are documented and supported by reappearance of cytogenetic abnormality, **or** leukemic blasts >5% are documented on multiple occasions, **or** there is disease detected at an extramedullary site.

Lymphoblastic Lymphoma: Relapse will be diagnosed when lymphoma cells are documented in the blood or bone marrow, **and/or** new extramedullary mass is documented by radiographic techniques or physical examination, **or** previous masses demonstrate an increase in size as documented by radiographic techniques, or by physical examination, **or** by the presence of blasts and a white cell blood count > 5/mm³ in the cerebral spinal fluid. The diagnosis of hematologic relapse must be supported by the reappearance of host cells and confirmed by the appearance of

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cytogenetic abnormalities previously documented before transplantation (if applicable). Reappearance of cytogenetic markers cannot be documented by use of amplification methods alone. Non-hematologic relapse will be confirmed by histologic evaluation of biopsied or resected extramedullary mass.

Non-Lymphoblastic Non-Hodgkin's Lymphoma: Relapse will be diagnosed when one or more of the following criteria apply: 1) any progression more than 25% in the product of the two largest diameters of any measurable lesion, 2) the appearance of new definitive lesions confirmed by biopsy, 3) the appearance of blasts > 25% in any one bone marrow aspirate or the appearance of lymphoma within a bone marrow biopsy, 4) the appearance of blasts > 5% are documented and supported by the reappearance of cytogenetic abnormality, 5) the appearance of blasts > 5% are documented on multiple occasions, 6) the presence of blasts and a white cell blood count > 5/mm³ in the cerebral spinal fluid.

Hodgkin's Disease: Relapse will be diagnosed when one or more of the following criteria apply: 1) any progression more than 25% in the product of the two largest diameters of any measurable lesion, 2) the appearance of any new definitive lesions confirmed by biopsy, 3) the presence of Hodgkin's Disease in any bone marrow specimens.

Chronic Myelogenous Leukemia (CML): Hematologic relapse will be diagnosed when immature hematopoietic cells are persistently documented in the peripheral blood **or** there is myeloid hyperplasia in the bone marrow in the absence of infection or hematopoietic growth factor therapy. The diagnosis of hematologic relapse will be supported by the reappearance of host cells (except by amplification methods alone) and confirmed by the reappearance of the 9;22 translocation. In the absence of hematologic abnormality, a cytogenetic relapse will be diagnosed when 1) 50% of metaphases exhibit the characteristic 9;22 translocation with at least ten metaphases analyzed, **or** 2) one to five metaphases exhibit the 9;22 translocation on each of two separate consecutive examinations at least one month apart, regardless of number of metaphases analyzed.

Juvenile Myelomonocytic Leukemia (JMML): Relapse will be diagnosed when there is reappearance of host cells (except by amplification methods alone) **and** clinical and laboratory features consistent with the patient's original disease. The diagnosis of relapse is further supported by the return of an abnormal cytogenetic marker (if present at diagnosis) **and/or** GM-CSF hypersensitivity or spontaneous growth of CFU-GMs in peripheral blood.

Myelodysplastic Syndrome: Relapse will be diagnosed when there is reappearance of morphologic abnormalities associated with MDS detected in two consecutive bone marrow specimens taken at least one month apart **and** documentation of > 10% of the cells being of recipient origin. If a cytogenetic abnormality associated with the MDS was present prior to transplant, then the diagnosis of hematologic relapse will be supported by the reappearance of the abnormality.

Familial Erythrophagocytic Lymphohistiocytosis (FEL) and Langerhans Cell Histiocytosis (LCH): Biopsy evidence of erythrophagocytosis or infiltrative disease consistent with FEL or LCH, with or without evidence of reappearance of host hematopoiesis.

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2.1.10 Disease-Free Survival

Disease-free survival is defined as the minimum time interval of the times to relapse/recurrence, to death, or to last follow up. Disease-free survival will only be evaluated in patients with malignant disease, as listed in Section 2.1.8.

2.2 ELIGIBILITY AND EXCLUSION CRITERIA

2.2.1 Eligibility Criteria

Patients fulfilling the following criteria will be eligible for this study.

Malignant Disease

1. Patients with AML, with or without history of myelodysplastic syndrome, **excluding:**
 - a) Patients in first complete remission ($\leq 5\%$ blasts in marrow) with translocations t(8;21) and inv (16) unless failed first-line induction therapy

OR

 - b) Patients in first complete remission ($\leq 5\%$ blasts in marrow) with translocations t(15;17) abnormality unless:
 - i) failed first-line induction therapy OR
 - ii) patient has molecular evidence of persistent disease

OR

 - c) Patients in first complete remission with Down's Syndrome.

AML patients ≥ 3 rd medullary relapse or refractory disease (other than primary induction failures) will receive the busulfan/melphalan conditioning regimen. (Closed to accrual.)

2. Patients with ALL, in either of the following categories:
 - a) Not in first complete remission (complete remission is defined as $\leq 5\%$ blasts in marrow)

OR

 - b) High-risk ALL patients in first complete remission where high-risk is defined as:
 - i) hypoploidy (≤ 44 chromosomes)

OR

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- ii) pseudodiploidy with translocations or molecular evidence of t(9;22), 11q23, or t(8;14) (excluding B-ALL) or + MLL gene rearrangement

OR

- iii) elevated WBC at presentation as follows:
 - a) $> 100,000/\text{mm}^3$ 6-12 months of age
 - b) $> 200,000/\text{mm}^3$ ≥ 10 and < 18 years old
 - c) $> 20,000/\text{mm}^3$ ≥ 18 years old

OR

- iv) failed to achieve complete remission after four weeks of induction therapy

OR

- c) If patient has B-ALL, they must either not be in 1st complete remission or must meet at least one of the high risk criteria specified in 2(b) or the following must all be no
 - i) Patient has translocation t(8;14)
 - ii) Blasts have surface immunoglobulins
 - iii) Patient is CD10+

OR

- d) ALL patients ≥ 3 rd medullary relapse or refractory disease (other than primary induction failures). These patients will receive the busulfan/melphalan conditioning regimen. (Closed to accrual.)

- 3. Patients with undifferentiated leukemia (AUL), infant leukemia or bi-phenotypic leukemia. Patients ≥ 3 rd medullary relapse or refractory disease (other than primary induction failures) will receive the busulfan/melphalan conditioning regimen. (Closed to accrual.) Infant leukemia may receive busulfan/melphalan. (Closed to accrual.)

- 4. Patients with CML

- a) Patients with accelerated phase CML

OR

- b) Patients in chronic phase if ≥ 1 year from diagnosis without an identified matched unrelated bone marrow donor AND if unresponsive to interferon or unable to tolerate interferon

OR

- c) Patients in blast crisis. Blast crisis is defined as $>30\%$ promyelocytes plus blasts in the marrow. These patients will receive the busulfan/melphalan conditioning regimen. (Closed to accrual)

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5. Patients with Myelodysplastic Syndrome(s) defined by the following:
 - a) Refractory Anemia: Anemia with $\leq 1\%$ blasts in peripheral blood and dyserythropoiesis
 - b) Refractory Anemia with Ringed Sideroblasts: Refractory anemia defined above, including the presence of ringed sideroblasts $\geq 15\%$ of all nucleated cells in the marrow
 - c) Refractory Anemia with Excess Blasts: Refractory anemia as defined above with 5-20% myeloblasts in the marrow and $< 5\%$ blasts in the peripheral blood, as well as abnormalities in erythroid, megakaryocytic, and granulocytic maturation
 - d) Refractory Anemia with Excess Blasts in Transformation: Refractory anemia as described above with
 - i) $> 5\%$ blasts in the peripheral blood OR
 - ii) 21-30% myeloblasts in the marrow OR
 - iii) Auer rods in granulocytic precursors in the marrow or blood and myeloblasts in the marrow
 - e) Chronic Myelomonocytic Leukemia: Absolute monocytosis ($> 1 \times 10^3$ /liter) with $< 5\%$ blasts in the peripheral blood and $\leq 20\%$ blasts in the marrow
6. Patients with Paroxysmal Nocturnal Hemoglobinuria (PNH)
7. Patients with lymphomas:
 - a) Patients with Hodgkins and non-Hodgkins lymphoma beyond first complete remission or primary induction failures AND
 - b) Tumors have demonstrated chemosensitivity defined as $> 50\%$ reduction in mass size after the most recent therapy

Non-Malignant Disease

8. Closed to accrual. Acquired severe aplastic anemia (SAA) (defined as at least 2 of the following: granulocyte count < 500 cell/ μ L, platelet count $< 20,000$ / μ L, or an absolute reticulocyte count $< 20,000$ after correction for hematocrit) that is unresponsive to medical therapy with anti-thymocyte globulin and/or cyclosporine
9. Closed to accrual. Inborn errors of metabolism including, but not limited to, Hurler's syndrome, adrenoleukodystrophy (ALD), Maroteaux-Lamy syndrome,

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globoid cell leukodystrophy, metachromatic leukodystrophy, fucosidosis and mannosidosis.

The patient's developmental quotient, IQ, or clinical neurodevelopmental examination should demonstrate potential for stabilization at a level of functioning where continuous life support (e.g. mechanical ventilation) would not be predicted to be required in the year following transplantation.

10. Closed to accrual. Fanconi anemia documented by increased chromosomal fragility assays AND:
 - a) Severe pancytopenia as demonstrated by ANC < 500/mm³, platelets < 20,000 and hemoglobin < 8gm/dL
 - OR
 - b) Morphologic evidence of myelodysplastic syndrome with clonal chromosomal abnormalities
 - OR
 - c) Leukemic transformation
11. Closed to accrual. Other marrow failure syndromes including:
 - a) Blackfan-Diamond (congenital pure red cell aplasia) unresponsive to medical therapy
 - b) Kostmann's congenital agranulocytosis unresponsive to medical therapy
 - c) Congenital amegakaryocytic thrombocytopenia
 - d) TAR
12. Combined immune deficiencies including, but not limited to, severe combined immunodeficiency (SCID), Wiskott-Aldrich syndrome, leukocyte adhesion defect (LAD), Chediak-Higashi disease, X-linked lymphoproliferative disease, adenosine deaminase (ADA) deficiency, purine nucleoside phosphorylase (PNP) deficiency, X-linked SCID, common variable immune deficiency (VID), Nezeloff's syndrome, and cartilage hair hypoplasia, reticular dysgenesis
13. Patients may be enrolled on study only once.

HLA Typing

14. a) These criteria apply to all patients and COBLT cord blood units. HLA-A, B, and DRB1 loci will be examined for histocompatibility matching. HLA-A, B and DRB1 typing will be performed using DNA technology .

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If a single type is detected for a particular locus, the HLA type will be classified as homozygous. Homozygotes are treated as if the single type detected is present twice for that locus.

For patients enrolling with 4 of 6, 5 of 6, or 6 of 6 match, the acceptable level of disparity is defined by the match criteria below:

Match Criteria:

- 1) At HLA-DRB1, a match is HLA DRB1 identity as determined by high resolution DNA typing.

AND

- 2) At HLA-A and HLA-B, a match is HLA-A or HLA-B identity as determined by DNA typing at the “serologic level.”

For patients enrolling with 3 of 6 match, HLA-A, HLA-B, HLA-DRB1 must be matched at high resolution DNA typing.

The “serologic level” equivalent for each allele designation is listed in the COBLT Manual of Procedures. This table is derived from the WHO definitions and is maintained by the COBLT Histocompatibility Subcommittee.

- b) Units obtained from non-COBLT banks may only provide typing by serology for Class I. In this circumstance, the matching criteria shown in #14 still apply, with the removal of the DNA typing requirement for Class I. The serologic match must be at the split level. In addition, a sample of the unit must be available for retrospective DNA HLA typing.

15. Patients with adequate physical function as measured by:

- a) Cardiac: Asymptomatic or, if symptomatic, then left ventricular ejection fraction at rest must be > 40% and must improve with exercise, or shortening fraction > 26%
- b) Hepatic: < 5 x ULN SGOT and < 2.5 mg/dL total serum bilirubin or cleared by the Steering Committee Chairperson
- c) Renal: Serum creatinine within normal range for age or if serum creatinine outside normal range for age then renal function (creatinine clearance or gfr) > 50% LLN for age
- d) Pulmonary: Asymptomatic or, if symptomatic, DLCO, FEV1, FEC (diffusion capacity) > 45% of predicted (corrected for

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hemoglobin); if unable to obtain PFT, O₂ saturation > 85% on room air

16. Nucleated cell dose: Cord blood unit must provide a minimum of 1×10^7 nucleated cells per kilogram of recipient weight based on nucleated cell count of the unit post-processing/pre-cryopreservation.
17. Cord blood units may be obtained from COBLT cord blood banks, the New York Blood Center, NMDP-approved cord blood banks or U.S. banks meeting Netcord-FAHCT standards.

2.2.2 Exclusion Criteria

Patients with the following will be ineligible for registration onto this study:

1. Active CNS leukemia involvement at the time of study enrollment (cerebrospinal fluid with > 5 WBC/mm³ AND malignant cells on cytopspin)
2. Female patients who are pregnant (positive β -HCG) or breastfeeding
3. Karnofsky performance status $< 70\%$ or Lansky $< 50\%$ for patients < 16 years old
4. Age > 55 years old
5. Prior allogeneic stem cell transplant with cytoreductive preparative therapy within 12 months of enrollment
6. Prior autologous stem cell transplant within 6 months of enrollment
7. Uncontrolled viral, bacterial, or fungal infection at the time of study enrollment
8. Seropositive for HIV
9. Consenting 5 of 6 or 6 of 6 HLA-matched related donor available
10. Primary myelofibrosis
11. Greater than or equal to Grade 3 myelofibrosis
12. Unable to provide informed consent
13. Immune deficiency patients who do not require cytoreduction
14. Patients who have a diagnosis of dyskeratosis congenita
15. FEL patients with at least one of the following:
 - a) abnormal brain MRI, or
 - b) neurologic symptoms, or
 - c) $> 7/\text{mm}^3$ lymphocytes plus monocytes in the cerebrospinal fluid.

2.3 REGISTRATION PROCEDURES

2.3.1 Registration Procedures

To enter a patient on this study, the following procedure should be followed:

1. FAX the completed Eligibility Form to the Medical Coordinating Center (MCC) at 301-251-1355.

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2. The MCC will fax the Confirmation of Registration/CBU Release Request to the transplant center to confirm the registration and patient identification number.

If the Eligibility Form is received outside regular business hours (9:00 am - 5:00 pm Eastern Time, Monday - Friday), then the MCC will perform registration at the start of the next business day. The day of registration cannot be more than 14 days prior to initiation of conditioning therapy. In addition, conditioning therapy cannot be initiated prior to registration.

2.3.2 Stratification Variables

As described in the Eligibility Section, patients with malignant and non-malignant hematologic disorders will be entered into the study. Patients will be retrospectively HLA-typed by DNA high resolution methods within 1 month of enrollment. After high resolution typing is completed, patients will be stratified as follows:

1. Malignant disease, 5/6 or 6/6 high resolution HLA match, \leq 18 years of age
2. Malignant disease, 4/6 high resolution HLA match, \leq 18 years of age
3. Malignant disease, 3/6 high resolution HLA match, \leq 18 years of age
4. Malignant disease, 2/6 or 1/6 high resolution HLA match, \leq 18 years of age
5. Severe aplastic anemia, Fanconi anemia and other marrow failure syndromes (Closed to accrual)
- 6A. Inborn errors of metabolism/storage diseases (Closed to accrual)
- 6B. Combined immune deficiencies
- 6C. Other non-malignant diseases not described above (Closed to accrual)
7. Malignant disease alternative conditioning regimen (busulfan and melphalan) (Closed to accrual)
8. Adult patients ($>$ 18 years of age) (Closed to accrual)

2.4 TREATMENT PLAN

The immediate pre-transplant evaluation will be carried out according to the operating procedures of the participating institutions and should be in keeping with the data reporting requirements of this study. Similarly, special orders and procedures will be those defined by the operations manuals of the Clinical Centers. All patients enrolled on this protocol will be hospitalized in accordance with isolation procedures for recipients of unrelated-donor marrow transplants as defined by the given institution.

2.4.1 Conditioning Regimens for Patients with Malignant Diseases or Severe Aplastic Anemia

| <u>Day</u> | <u>Treatment</u> |
|-----------------|-------------------|
| -8 ^a | TBI (150 cGy x 1) |
| -7 | TBI (150 cGy x 2) |
| -6 | TBI (150 cGy x 2) |
| -5 | TBI (150 cGy x 2) |
| -4 | TBI (150 cGy x 2) |

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| | |
|----|---|
| -3 | Cyclophosphamide 60 mg/kg (see Section 2.4.6 for dose adjustment for patients > 125% IBW) Methylprednisolone 2-2.5 mg/kg/day IV in divided doses Antithymocyte globulin (equine) 30 mg/kg IV QD |
| -2 | Cyclophosphamide 60 mg/kg Methylprednisolone 2-2.5 mg/kg/day IV in divided doses Antithymocyte globulin (equine) 30 mg/kg IV QD |
| -1 | Methylprednisolone 2-2.5 mg/kg/day IV in divided doses Antithymocyte globulin (equine) 30 mg/kg IV QD |
| 0 | Cord blood transplant Methylprednisolone 2 mg/kg/day IV in divided doses with 1 mg/kg of the total given just prior to infusion |

^a. Two fractions of TBI may be given on Day -8 and one fraction on a subsequent day, but the total number of fractions will remain unchanged at 9 fractions and total dose at 1350 cGy.

Methylprednisolone should be given within 2 hours before ATG.

Antithymocyte globulin (equine) may also be given as 15 mg/kg IV BID. Rabbit ATG may be substituted at a dose of 3 mg/kg QD or 1.5 mg/kg BID. Either form of ATG dose may be rounded to the nearest vial quantity. If neither form of ATG is tolerated, then substitute total dose of 1 gm/m² of methylprednisolone (q 12 hours divided doses for each ATG day). This means a total daily dose of 1 gm/m². The methylprednisolone specified as 2 - 2.5 mg/kg/day, which is given on ATG days -3, -2, and -1, is administered for pre-medication of ATG, and for patients unable to tolerate ATG, it should NOT be given in addition to the 1 gm/m²/day.

TBI Principles

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 1350 cGy in 9 fractions over 5 days. Dose will be prescribed at the level of the umbilicus at midplane.

The dose along the central axis of the patient should be kept to within 10% of the prescription dose. Compensators, bolus, or transmission blocks may be placed in an effort to accomplish this.

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 chestwall

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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 chestwall. Regardless of the partial blocking used, the lung may receive an estimated maximum of the prescription dose (1350 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 1350 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 9 fractions are given over 5 days (Days -8, -7, -6, -5, and -4). On 4 of these days, 2 fractions are given at a minimum of 6 hours apart from beam on to beam on, and on 1 of these days a single fraction is given. On the day of the single fraction, if treating AP and PA, one-half of the prescribed fraction will be given to each of the treatment fields.

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 $\geq 90\%$ 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 is given in a single 400 cGy fraction 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.

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2.4.2 Conditioning Regimen for Patients with Fanconi Anemia - Closed

| <u>Day</u> | <u>Treatment</u> |
|------------|---|
| -6 | TBI 450 cGy |
| -5 | Cyclophosphamide 10 mg/kg IV Fludarabine 35 mg/m ² IV Methylprednisolone 2 mg/kg IV Antithymocyte globulin (equine) 30 mg/kg/day IV |
| -4 | Cyclophosphamide 10 mg/kg IV Fludarabine 35 mg/m ² IV Methylprednisolone 2 mg/kg IV Antithymocyte globulin (equine) 30 mg/kg/day IV |
| -3 | Cyclophosphamide 10 mg/kg IV Fludarabine 35 mg/m ² IV Methylprednisolone 2 mg/kg IV Antithymocyte globulin (equine) 30 mg/kg/day IV |
| -2 | Cyclophosphamide 10 mg/kg IV Fludarabine 35 mg/m ² IV Methylprednisolone 2 mg/kg IV Antithymocyte globulin (equine) 30 mg/kg/day IV |
| -1 | Methylprednisolone 2 mg/kg IV Antithymocyte globulin (equine) 30 mg/kg/day IV |
| 0 | Cord blood transplant |
| +1 | Initiate G-CSF 5 mcg/kg per day IV (continue until ANC \geq 2.5 x 10 ⁹ /L) |

Methylprednisolone should be given within 2 hours before ATG.

Rabbit ATG may be substituted at a dose of 3 mg/kg QD. Either form of ATG dose may be rounded to the nearest vial quantity. If neither form of ATG is tolerated, then substitute total dose of 1 gm/m² of methylprednisolone for each dose of ATG. The methylprednisolone specified as 2 - 2.5 mg/kg/day, which is given on ATG days -3, -2, and -1, is administered for pre-medication of ATG, and for patients unable to tolerate ATG, it should NOT be given in addition to the 1 gm/m²/day.

Patients may be treated either in the AP PA position 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).

The treatment dose will be a total of 450 cGy given to the total body in a single fraction on Day - 6 prescribed to the mid-pelvis at midplane. Appropriate compensation devices may be used to ensure homogeneity of dose throughout the entire body of +/- 10% of the prescription point dose, but blocks are not allowed.

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.

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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 $\geq 90\%$ of the prescribed dose.

Testicular boosts will not be given.

2.4.3 Conditioning Regimen for Patients with Inborn Errors of Metabolism/Storage Disease - Closed

| <u>Day</u> | <u>Agents</u> |
|------------|--|
| -9 | Busulfan ^a or Busulfex ^b |
| -8 | Busulfan ^a or Busulfex ^b |
| -7 | Busulfan ^a or Busulfex ^b |
| -6 | Busulfan ^a or Busulfex ^b |
| -5 | Cyclophosphamide 50 mg/kg/day IV (see Section 2.4.6 for dose adjustment) |
| -4 | Cyclophosphamide 50mg/kg/day IV (see Section 2.4.6 for dose adjustment) |
| -3 | Cyclophosphamide 50mg/kg/day IV Methylprednisolone 2-2.5 mg/kg/day IV in divided doses ATG (equine) 30 mg/kg/day IV |
| -2 | Cyclophosphamide 50 mg/kg day IV Methylprednisolone 2-2.5 mg/kg/day IV in divided doses ATG (equine) 30 mg/kg/day IV |
| -1 | Methylprednisolone 2-2.5 mg/kg/day IV in divided doses ATG (equine) 30 mg/kg/day IV |
| 0 | Cord blood transplant Methylprednisolone 2 mg/kg/day IV in divided doses with 1 mg/kg of the total given just prior to infusion |

^a Busulfan doses are as follows:

| | |
|--------------------|---|
| < 3 months | 20 mg/m ² /dose q 6 hours PO |
| 3 months - 6 years | 40 mg/m ² /dose q 6 hours PO |
| ≥ 6 years | 1 mg/kg PO q 6 hours |

Dose should be repeated if vomiting occurs within 30 minutes

^b Busulfex doses are as follows:

| | |
|----------------|--|
| ≤ 4 years | Initial dose at 1.0 mg/kg actual body weight |
| > 4 years | Initial dose at 0.8 mg/kg actual body weight |

Example of dose calculation for 30 kg patient (age > 4 years):

$$(30 \text{ kg} \times 0.8 \text{ mg/kg}) / (6 \text{ mg/mL}) = 4.0 \text{ mL Busulfex} = 24 \text{ mg dose}$$

Add 4.0 mL Busulfex to 40 mL diluent = 44 mL for infusion

$$(4.0 \text{ mL} \times 6 \text{ mg/mL}) / (44.0 \text{ mL}) = 0.54 \text{ mg/mL Busulfex}$$

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Blood is to be drawn and busulfan/busulfex levels obtained with dose #1 or dose #2, according to the schedule specified in Section 2.4.6. Busulfan/Busulfex dose adjustments are required only for patients < 6 years of age or for patients receiving a second transplant with cytoreduction. Maintain concentration at steady state (CSS) levels busulfan: 600 ng/mL to 900 ng/mL or busulfex AUC: 900 ng/mL to 1300 ng/mL. See Section 2.4.6 for busulfan/busulfex dose adjustment guidelines.

Methylprednisolone should be given within 2 hours before ATG.

Antithymocyte globulin (horse) may also be given as 15 mg/kg IV BID. Rabbit ATG may be substituted at a dose of 3 mg/kg QD or 1.5 mg/kg IV BID. Either form of ATG dose may be rounded to the nearest vial quantity. If neither form of ATG is tolerated, then substitute total dose of 1 gm/m² of methylprednisolone for each dose of ATG. This means a total daily dose of 1 gm/m². The methylprednisolone specified as 2 - 2.5 mg/kg/day, which is given on ATG days -3, -2, and -1, is administered for pre-medication of ATG, and for patients unable to tolerate ATG, it should NOT be given in addition to the 1 gm/m²/day.

2.4.4 Conditioning Regimen for Patients with Other Non-Malignant Diseases

| <u>Day</u> | <u>Treatment</u> |
|-----------------|--|
| -9 | Busulfan ^b or Busulfex ^c |
| -8 | Busulfan ^b or Busulfex ^c |
| -7 | Busulfan ^b or Busulfex ^c |
| -6 | Busulfan ^b or Busulfex ^c |
| -5 ^a | Cyclophosphamide 50 mg/kg IV (see Section 2.4.6 for dose adjustments) |
| -4 ^a | Cyclophosphamide 50 mg/kg IV |
| -3 ^a | Cyclophosphamide 50 mg/kg IV Methylprednisolone 2-2.5 mg/kg/day IV in divided doses ATG (equine) 30 mg/kg IV QD |
| -2 | Cyclophosphamide 50 mg/kg IV Methylprednisolone 2-2.5 mg/kg/day IV in divided doses ATG (equine) 30 mg/kg IV QD |
| -1 | Methylprednisolone 2-2.5 mg/kg/day IV in divided doses ATG (equine) 30 mg/kg IV QD |
| 0 | Cord blood transplant Methylprednisolone 2 mg/kg/day IV in divided doses with 1 mg/kg of the total given just prior to infusion |

^a For patients with FEL or LCH, VP-16 should be given on days -5, -4, and -3 at a dose of 300 mg/m².

^b Busulfan doses are as follows:

| | |
|--------------------|---|
| < 3 months | 20 mg/m ² /dose q 6 hours PO |
| 3 months - 6 years | 40 mg/m ² /dose q 6 hours PO |
| ≥ 6 years | 1 mg/kg PO q 6 hours |

Dose should be repeated if vomiting occurs within 30 minutes

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^c Busulfex doses are as follows:

| | |
|-----------|--|
| ≤ 4 years | Initial dose at 1.0 mg/kg actual body weight |
| > 4 years | Initial dose at 0.8 mg/kg actual body weight |

Example of dose calculation for 30 kg patient (age > 4 years):

$$(30 \text{ kg} \times 0.8 \text{ mg/kg}) / (6 \text{ mg/mL}) = 4.0 \text{ mL Busulfex} = 24 \text{ mg dose}$$

Add 4.0 mL Busulfex to 40 mL diluent = 44 mL for infusion

$$(4.0 \text{ mL} \times 6 \text{ mg/mL}) / (44.0 \text{ mL}) = 0.54 \text{ mg/mL Busulfan}$$

Blood is to be drawn and busulfan/busulfex levels obtained with dose #1 or dose #2, according to the schedule specified in Section 2.4.6. Busulfan/Busulfex dose adjustments are required only for patients < 6 years of age or for patients receiving a second transplant with cytoreduction. Maintain busulfan/busulfex concentration at steady state (CSS) levels between busulfan: 600 ng/mL to 900 ng/mL or busulfex AUC: 900 ng/mL to 1300 ng/mL. See Section 2.4.6 for busulfan/busulfex dose adjustment guidelines.

Methylprednisolone should be given within 2 hours before ATG.

ATG (equine) may be given 15 mg/kg IV BID. Rabbit ATG may be substituted at a dose of 3 mg/kg QD or 1.5 mg/kg BID. Either form of ATG dose may be rounded to the nearest vial quantity. If neither form of ATG is tolerated, then substitute total dose 1 gm/m² of methylprednisolone (q 12 hours divided dose for each ATG day). This means a total daily dose of 1 gm/m². The methylprednisolone specified as 2 - 2.5 mg/kg/day, which is given on ATG days -3, -2, and -1, is administered for pre-medication of ATG, and for patients unable to tolerate ATG, it should NOT be given in addition to the 1 gm/m²/day. For patients greater than 125% IBW, see Section 2.4.6.3 for ATG dose adjustment.

2.4.5 Non-TBI-Containing Conditioning Regimen for Patients with Malignant Diseases - Closed

This conditioning regimen may be used for patients diagnosed with infant acute leukemia when less than 2 years old. Infant leukemia is defined as any acute leukemia with morphology consistent with ALL or AML diagnosed in an infant (<12 months of age). Also includes acute leukemia diagnosed in a child < 2 years of age which contains the cytogenetic markers t(4;11) or t(9;11), 11q23 and/or MLL gene rearrangement 19.

| <u>Day</u> | <u>Treatment</u> |
|------------|---|
| -8 | Busulfan ^a or Busulfex ^b |
| -7 | Busulfan ^a or Busulfex ^b |
| -6 | Busulfan ^a or Busulfex ^b |
| -5 | Busulfan ^a or Busulfex ^b |
| -4 | Melphalan 45 mg/m ² IV |
| -3 | Melphalan 45 mg/m ² IV Methylprednisolone 2-2.5 mg/kg/day IV in divided doses |

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| | |
|----|---|
| -2 | ATG (equine) 30 mg/kg/day IV QD Melphalan 45 mg/m ² IV Methylprednisolone 2-2.5 mg/kg/day IV in divided doses |
| -1 | ATG (equine) 30 mg/kg/day IV QD Methylprednisolone 2-2.5 mg/kg/day IV in divided doses |
| 0 | ATG (equine) 30 mg/kg/day IV QD Cord blood transplant Methylprednisolone 2 mg/kg/day IV in divided doses with 1 mg/kg of the total given just prior to infusion |

^a Busulfan doses are as follows:

| | |
|--------------------|---|
| < 3 months | 20 mg/m ² /dose q 6 hours PO |
| 3 months - 6 years | 40 mg/m ² /dose q 6 hours PO |
| ≥ 6 years | 1 mg/kg PO q 6 hours |

Dose should be repeated if vomiting occurs within 30 minutes

^b Busulfex doses are as follows:

| | |
|-----------|--|
| ≤ 4 years | Initial dose at 1.0 mg/kg actual body weight |
| > 4 years | Initial dose at 0.8 mg/kg actual body weight |

Example of dose calculation for 30 kg patient (age > 4 years):

$$(30 \text{ kg} \times 0.8 \text{ mg/kg}) / (6 \text{ mg/mL}) = 4.0 \text{ mL Busulfex} = 24 \text{ mg dose}$$

Add 4.0 mL Busulfex to 40 mL diluent = 44 mL for infusion

$$(4.0 \text{ mL} \times 6 \text{ mg/mL}) / (44.0 \text{ mL}) = 0.54 \text{ mg/mL Busulfan}$$

Blood is to be drawn and busulfan/busulfex levels obtained with dose #1 or dose #2, according to the schedule specified in Section 2.4.6. Busulfan/Busulfex dose adjustments are required only for patients < 6 years of age or for patients receiving a second transplant with cytoreduction. Maintain busulfan/busulfex concentration at steady state (CSS) levels between busulfan: 600 ng/mL to 900 ng/mL or busulfex AUC: 900 ng/mL to 1300 ng/mL. See Section 2.4.6 for busulfan/busulfex dose adjustment guidelines.

Methylprednisolone should be given within 2 hours before ATG.

ATG (equine) may be given as 15 mg/kg IV BID. Rabbit ATG may be substituted at a dose of 3 mg/kg QD or 1.5 mg/kg BID. Either form of ATG dose may be rounded to the nearest vial quantity. If neither form of ATG is tolerated, then substitute total dose 1 gm/m² of methylprednisolone (q 12 hours divided dose for each ATG day). This means a total daily dose of 1 gm/m². The methylprednisolone specified as 2 - 2.5 mg/kg/day, which is given on ATG days -3, -2, and -1, is administered for pre-medication of ATG, and for patients unable to tolerate ATG, it should NOT be given in addition to the 1 gm/m²/day.

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2.4.6 Dose Adjustments

2.4.6.1 Cyclophosphamide Ideal Body Weight Dose Adjustment. If a patient's weight is \geq 125% of ideal body weight (IBW), then calculate the dose of cyclophosphamide according to adjusted IBW. A suggested method of estimation is as follows:

1. Estimation of IBW. Body weight and height are measured directly. An approximate weight for height would be calculated from standard table or equations which reflect ideal "values."

Patients Over 18 Years

Males IBW = 50 kg + 2.3 kg/inch over 5 feet

Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Patients 1 to 18 Years of Age

Less than 60 inches

IBW = $(ht^2 \times 1.65)/1000$ where ht = cm, IBW = kg

More than 60 inches

Males IBW = $39.0 + [2.27 \times (ht - 60)]$ where ht = inches, IBW = kg

Females IBW = $42.2 + [2.27 \times (ht - 60)]$ where ht = inches, IBW = kg

2. If the patient's weight is greater than 125% of IBW, then the following adjusted weight should be used to calculate the cyclophosphamide dose:

Method: Step 1 TBW
 -IBW
 Excess Weight

Step 2 Excess Weight
 x 40%
 Weight Adjustment

Step 3 Body Weight Used to Calculate Cyclophosphamide Dose =
 IBW + Weight Adjustment

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2.4.6.2 Busulfan and Busulfex Dose Adjustments.

Busulfan Pharmacokinetic Targeting

Analysis of busulfan/busulfex, pharmacokinetic parameter fitting and dose adjustment recommendation are done at the Fred Hutchinson Cancer Research Center (FHCRC) in the Pharmacokinetic Laboratory or at individual institutions. The concentration at steady state (CSS) is determined for each dose measured. This concentration (ng/mL) is compared to a target (busulfan CSS: 600 ng/mL - 900 ng/mL; busulfex AUC: 900 microM.min to 1300 microM.min) and a dose adjustment (mg q 6 hr) recommendation is given.

Information for sending samples to the FHCRC laboratory are provided in Chapter 9 of the Manual of Procedures.

Dose Measurements

Busulfan/busulfex concentrations are determined in plasma (sodium heparin) taken during times specified below. The following is the blood draw schedule:

Busulfan draw schedule for Dose 1 or Dose 2: (all times post dose): 30 minutes, 1, 1.5, 2, 3, 4, 5, and 6 hours.

Busulfex draw schedule for Dose 1 or Dose 2: end of infusion (2 hours), 2 hours 15 minutes, 2 hours 30 minutes and 3, 4, 5 and 6 hours.

Plasma busulfan/busulfex concentrations are measured using gas chromatography with mass spectrophotometry or electron capture detection. The plasma concentrations are then used to calculate AUC and CSS (AUC/dose interval). Clearance (mg/mL *min) is also determined for each dose measured.

Reporting of Data

For each dose, the final CSS result and dose recommendation are given to the Physician via verbal phone communication followed by a faxed confirmation. The confirmation will include patient identifiers, dose number, date of dose, amount of busulfan given, CSS, target CSS, and recommended dose.

2.4.7 GVHD Prophylaxis

A standardized regimen of cyclosporine and corticosteroids will be used for GVHD prophylaxis in all patients. The dose of cyclosporine will be based on actual body weight.

2.4.7.1 Cyclosporine Prophylaxis. The prophylactic IV cyclosporine administration will begin between Day -3 to -1 with at least a dose of 3.0 mg/kg/day i.v. in two divided doses (1.5 mg/kg each) 12 hours apart and infused over a period of one-four hours or given by continuous infusion. Trough levels of 200 ng/mL by TDX (or equivalent with other measurement approaches), if

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given by bolus, or levels of 400 ng/mL by TDX if given by continuous infusion, should be present on Day 0 and thereafter until a taper is initiated. Cyclosporine trough levels should be measured weekly during the first 100 days post-transplant. Pediatric patients may require higher dosages and/or frequency. Unless toxicities are encountered, cyclosporine will be continued for a minimum of six months after transplantation. Thereafter, if there are no signs or symptoms of GVHD and the patient is not receiving corticosteroids, the dose of cyclosporine may be gradually reduced by 5% per week, and the drug will be discontinued at approximately one year after transplantation. Intravenous cyclosporine will be discontinued once the patient starts eating, and the drug will be given orally in two divided doses to maintain desired trough levels. Cyclosporine may be administered orally in capsule form or as a liquid mixed with a suitable fluid such as milk, chocolate milk, or juice.

2.4.7.2 Corticosteroids. Solumedrol will be given at a dose of 1 mg/kg (0.5 mg/kg BID) on Day +1 to Day +4 and 2 mg/kg (1 mg/kg BID) beginning on Day +5 until Day +19 or until the first day ANC's reach $\geq 500/\text{mm}^3$. After ANC's have reached $\geq 500/\text{mm}^3$, steroids should be tapered by 0.2 mg/kg/week.

If a patient experiences fever $> 103^\circ\text{F}$ and erythroderma between Days 5 and 9, it is recommended that the patient be treated with Solumedrol at a dose of 500 mg/m².

2.4.8 Cord Blood Infusion

Procedures detailed in the Manual of Procedures should be followed for requesting, receiving and characterizing the cord blood unit for infusion. Contingency plans for cord blood units which can not be infused will be made according to institutional policies. These plans may consist of autologous marrow back-up, obtaining marrow from a haploidentical relative, supportive care, or acquisition of another compatible cord blood unit, following local institutional practices.

The cord blood should be thawed and washed as described in the Investigators Brochure contained in the Manual of Procedures. Infusion should begin within 1 hour of washing. The infusion should take no longer than 30 minutes. Pre-medications (if any) prior to cord blood infusion will be at the discretion of the center. All transplant centers must be certified on the thawing procedures as detailed in the Manual of Procedures.

Under no circumstances is the cord blood to be irradiated. No in-line leukocyte filter should be used and no medications or fluids should be given piggyback through the catheter lumen that is being used for cord blood infusion. Vital signs should be monitored before beginning the infusion and periodically during administration.

Benadryl, epinephrine, and hydrocortisone should be available at the bedside for emergency use if necessary. Oxygen with nasal prongs for standby use should be present in the room.

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2.4.9 Diagnosis of Acute GVHD

Acute GVHD generally develops within the first three months after transplantation and appears as a characteristic dermatitis often accompanied by hepatic cholestasis and enteritis. The clinical appearance of skin GVHD can be mimicked by toxicity of the transplant conditioning regimen and by drug reactions. Therefore, documentation of the diagnosis by skin biopsy is recommended. Severity of liver GVHD is usually described according to the serum bilirubin level. Hepatic GVHD cannot be assessed solely on clinical grounds in patients who have concurrent drug toxicity, viral hepatitis, or toxicity caused by the pre-transplant chemotherapy and irradiation. Liver biopsy can be helpful but often cannot be done because of clinical contraindications such as thrombocytopenia. Gastrointestinal GVHD is characterized by watery diarrhea with anorexia, nausea and vomiting accompanied in more severe cases by abdominal cramps, gastrointestinal hemorrhage, and ileus. Symptoms are often exacerbated by eating. The volume of diarrhea has been used as an indicator of the severity of gut GVHD, but this can be inaccurate and highly variable from day to day. In many cases, it can be difficult to distinguish GVHD from infectious enteritis, and endoscopic biopsy is often helpful and should be done wherever possible. If a liver biopsy is performed, if possible, tissue should be sent to Dr. LeeAnn Baxter-Lowe's laboratory for testing for presence of maternal (umbilical cord blood) donor cells.

2.4.10 Diagnosis of Chronic GVHD

Manifestations of chronic GVHD typically do not occur until three to twelve months after transplantation. Initial symptoms frequently include nausea, anorexia and weight loss, ocular and oral sicca, and skin changes. Rash characteristically appears with pigmentary changes, vitiligo, mottling, erythema, plaques, papules, nodules, poikiloderma, or exfoliation progressing to sclerosis and contractures. Hair loss and onychodystrophy may also indicate chronic GVHD. Pulmonary involvement may be indicated by cough and dyspnea with wheezing, rales, and abnormal PFTS. Diarrhea and abdominal pain may occur but are relatively infrequent. Liver involvement may be indicated by increased bilirubin and alkaline phosphatase and less frequently by increased transaminase levels.

2.4.11 Use of Growth Factor

G-CSF will be administered beginning four hours post-infusion on Day 0 at the dose of 5 - 10 $\mu\text{g}/\text{kg}/\text{day}$ rounding to the nearest vial dose of 300 or 480 μg (except for Fanconi Anemia patients). G-CSF may be given by IV or subcutaneously. This dose will be maintained until $\text{ANC} \geq 2,000$ for three days, following which it will be tapered 50% minimum every other day, then stopped when dose is reduced to 1 $\mu\text{g}/\text{kg}/\text{day}$. See Section 2.4.2 for G-CSF treatment of Fanconi Anemia patients.

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2.4.12 Prophylaxis Against Infections

1. Patient should be given prophylaxis for:
 - a) *Pneumocystis carinii*: Prophylaxis should be given starting on the first day of conditioning until Day -2. Prophylaxis will be restarted at the time of engraftment or on Day 30 according to institutional preference. Prophylaxis should be continued until one year post-transplant or until 3 months past discontinuation of immunosuppression.
 - b) Herpes simplex According to institutional practice until Day 30 for HSV+ recipients.
 - c) Fungal infections: According to institutional practice.
2. Cytomegalovirus (CMV) Infections:
 - a) Patients who are CMV antibody negative pre-transplant should receive CMV seronegative blood products and/or leukocyte-depleted products. Patients should be screened weekly for CMV using culture or CMV antigen test.
 - b) If the patient has a positive antibody titer to CMV, the patient may begin CMV prophylaxis post-transplant when the ANC is ≥ 750 for two consecutive days. Prophylaxis should continue until Day +100. Ganciclovir should not be given from Day -2 until ANC ≥ 750 .
 - c) Surveillance for CMV will be done for all patients according to institutional policy.
3. Intravenous Immunoglobulin: Should be given according to institutional practice.

2.4.12.1 Identification of Opportunistic Infections. In the event that a patient develops fever, sinusitis, interstitial pneumonia, diarrhea, or hepatitis, all efforts will be made to identify the responsible organism. Cultures will include routine bacterial, fungal, mycobacterial, and viral cultures. Bronchial lavages and open lung biopsies will also be evaluated for pneumocystis carinii. If possible, these samples will also be evaluated for RSV and legionella. Stool samples will also be evaluated for *C. difficile* toxin, cryptosporidium, and rotavirus. Samples will not be routinely sent for EM studies. If a GI biopsy is performed, evaluation for CMV with immunofluorescence and PCR should be considered.

A Post-Transplant Infection Report Form should be completed for each infectious episode with a severity grade assigned for each known agent contributing to the episode.

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2.4.12.2 Post-Transplant Immunization Schedule. Once a patient is off all immunosuppressive therapy or has evidence of T cell function (approximately one-year post-transplant), immunizations may be given according to institutional practice. For patients with malignant diseases, tetanus immunizations must be given at 3 months, 6 months, and 12 months, then according to institutional practice.

2.4.13 Blood Product Support

Following initiation of the pre-transplant cytoreduction, all blood products, with the exception of the cord blood graft, will be irradiated to approximately 2500 cGy to the midplane of the bag with a minimum of 1500 cGy to other points before transfusion to inactivate lymphocytes capable of initiating lethal GVHD. Use of dosimeters is recommended. Patients who are CMV-seronegative pre-transplant should receive CMV-seronegative blood products and/or leukocyte-depleted products. Platelets should be administered when there is clinical evidence of active hemorrhage. To minimize bleeding, platelets may be transfused prophylactically in order to maintain a platelet count greater than 10,000/mm³ at all times and greater than 50,000/mm³ in the event of active bleeding. Packed irradiated red blood cells will be administered as clinically indicated.

2.4.14 CNS Prophylaxis

Intrathecal therapy or cranio-spinal radiation may be administered for patients with prior CNS involvement. Prophylaxis should be given prior to Day 0 or after Day 42.

2.4.15 Miscellaneous Support Measures

1. Prophylaxis Against Menorrhagia: All menstruating females will receive prophylaxis for menorrhagia.
2. Nutritional Support: Nutritional status should be carefully monitored, and high-calorie parenteral alimentation should be introduced as needed. Vitamin supplements should be administered as clinically indicated.
3. Prophylaxis Against Hemorrhagic Cystitis: Either hydration, MESNA, or bladder irrigation.

2.5 STUDY MONITORING AND PARTICIPANT RISKS

2.5.1 Follow-Up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 2.5.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the MOP, Chapter 10.

Follow-Up Visits: The timing of follow-up visits is based on the date of cord blood infusion. Following notification of the date of infusion, the MCC will send the Clinical Center a Patient

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Visit Schedule listing target dates for the acute GVHD assessments and all the follow-up visits. Week 1-14, Day 120, and Day 150 visits are primarily for GVHD assessments. The subsequent visits are for follow-up reports, i.e., NMDP 130 and 140.

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Manual of Procedures. Forms that are not received at the MCC within the specified time will be considered delinquent. Clinical Centers will receive a listing of delinquent forms twice monthly. A missing form will continue to be requested either until the form is submitted and integrated into the MCC's master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Manual of Procedures.

Reporting Patient Deaths: The NMDP Form 190 (Recipient Death Information) must be faxed to the Medical Coordinating Center (MCC) within 24 hours of the patient's death. If the cause of death is unknown at that time, it need not be recorded on the NMDP 190 Form. However, once the cause of death is determined, an updated NMDP 190 should be sent to the MCC.

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**Table 2.5.1
FOLLOW-UP SCHEDULE**

| Study Visit | Target Day (Days Post-UCBT) |
|--------------------|--|
| 1 week | 7 days |
| 2 week | 14 days |
| 3 week | 21 days |
| 4 week | 28 days |
| 5 week | 35 days |
| 6 week | 42 days |
| 7 week | 49 days |
| 8 week | 56 days |
| 9 week | 63 days |
| 10 week | 70 days |
| 11 week | 77 days |
| 12 week | 84 days |
| 13 week | 91 days |
| 14 week | 98 days |
| 100 day | 100 days |
| 120 day | 120 days |
| 150 day | 150 days |
| 6 month | 182 days |
| 9 month | 270 days (optional) |
| 12 month | 365 days |
| 18 month | 547 days (optional) |
| 24 month | 730 days |
| 36 month | 1,095 days |

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2.5.2 Required Observations

Pre-Transplant

1. History, physical examination, weight, height, BSA, and head circumference if < 2 years
2. CBC, differential, platelet count
3. ABO and Rh typing
4. Liver function tests (bilirubin, ALT)
5. Renal function tests (creatinine, BUN)
6. Bone marrow aspirate within 14 days of the start of the preparative regimen for patients with ALL, AML, JMML, and MDS, if peripheral blasts are not present on smear. Bone marrow aspirate within 30 days of the start of the preparative regimen for patients with CML, aplastic anemia, Fanconi anemia, NHL, or Hodgkin's Disease if prior history of marrow involvement
7. Lumbar puncture within 14 days of the start of the preparative regimen for cell count, cytospin differential, and/or cytology for all patients with ALL, FEL, lymphoblastic lymphoma, or Burkits lymphoma, and for patients with AML if clinically appropriate
8. Cardiac evaluation: echocardiogram or MUGA with ejection fraction (or shortening fraction is appropriate)
9. Pulmonary function evaluation: CXR, pulmonary function tests (if age appropriate and feasible)
10. Serology for CMV, HSV, HIV, toxoplasmosis, varicella, hepatitis B-surface antigen, hepatitis B-core antibody, hepatitis C
11. Karnofsky or Lansky score (age appropriate)
12. 5 mL of serum to be cryopreserved at the transplant center for future testing (e.g., infectious disease antibodies). This should be collected in a red top tube.
13. 3 mL sample of recipient peripheral blood to be stored for chimerism studies. These samples must be stored for future centralized testing. Each patient must also have a chimerism test done by Day 42 to assess engraftment.
14. **For patients with normal WBC**, 7 mL of peripheral blood should be obtained for retrospective HLA typing. Note that in smaller patients, 2 mL of peripheral blood is usually sufficient if acquisition of 7 mL is problematic. **For patients with low WBC**,

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20 mL of peripheral blood or 5 mL of peripheral blood PLUS 2 buccal swabs should be obtained. Note that only the buccal swab kits obtained through the MCC can be used.

Post-Transplant

1. Daily CBC, differential (once $WBC \geq 500/mm^3$) until $ANC \geq 500/mm^3$ for 3 consecutive days; Post-engraftment: CBC and platelet count 3 times a week until discharge; Post-discharge: CBC and platelet count weekly until PRBC and platelet transfusion independent, and at Days 100, 180, 270, and 360. Differential must be done if $WBC < 1500/mm^3$ at any time post-engraftment and at Days 100, 180, 270 and 360
2. Reticulocyte count at 4 weeks post-transplant, then weekly until reticulocyte count $> 30,000/mm^3$ for two consecutive weekly measurements
3. Bone marrow aspirate on Day 42 for patients who do not have an $ANC \geq 500/mm^3$ by Day 42
4. CMV surveillance should be performed according to institutional policy
5. For patients with CML, cytogenetic tests should be performed on bone marrow specimens at 3, 6, and 12 months
6. For patients with lymphoma, radiologic studies which were positive prior to transplantation should be repeated at 3, 6, and 12 months
7. IgG, IgA, and IgM immunoglobulin levels at 6, 12, 18, and 24 months
8. 3 mL of peripheral blood between Days 28 and 42, Day 100, and 1 year to be stored for future chimerism studies
9. Peripheral blood samples collected Monday through Thursday for immune reconstitution at 1, 2, 3, 6, 9 (optional), 12, 18 (optional), 24, 36, and 48 months
10. Karnofsky/Lansky history and physical examination, CBC, renal and liver function tests, cardiac function tests (echocardiogram or MUGA scan), pulmonary function tests, thyroid function tests yearly for 4 years then as clinically indicated, height, weight, head circumference, if age appropriate

Table 2.5.2.1
REQUIRED OBSERVATIONS

| | Baseline | Day | | | | | | Month Post-Transplant | | | | | | |
|---|----------------|--|----|----|----------------|----|-----|-----------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| | | 14 | 28 | 30 | 42 | 60 | 100 | 6 | 9 | 12 | 18 | 24 | 36 | 48 |
| History, physical exam, weight, height, BSA and head circumference ¹ | X | | | | | | | | | X | | X | X | X |
| CBC, differential ⁶ | X | X-Refer to Section 2.5.2 for frequency | | | | | X | X | X ² | X | | X | X | X |
| Reticulocyte count ⁷ | | | X | | | | | | | | | | | |
| Platelet count ² | X | X-Refer to Section 2.5.2 for frequency | | | | | X | X | X ² | X | | X | X | X |
| CMV surveillance | X | Per institutional policy | | | | | | | | | | | | |
| ABO and RH typing | X | | | | | | | | | | | | | |
| IgG, IgA, IgM immunoglobulin levels | | | | | | | | X | | X | X ² | X | | |
| Bilirubin, ALT | X | Per inst. policy but at least once per | | | | | X | | | X | | X | X | X |
| Creatinine, BUN | X | Per inst. policy but at least once per | | | | | X | | | X | | X | X | X |
| Bone marrow aspirate | X ³ | | | | X ⁴ | | | | | | | | | |
| Lumbar puncture | X ³ | | | | | | | | | | | | | |
| Echocardiogram or MUGA with ejection fraction | X | | | | | | | | | X ² | | X ² | X ² | X ² |
| CXR, pulmonary function tests ¹ | X | | | | | | | | | X ² | | X ² | X ² | X ² |
| Thyroid function tests | | | | | | | | | | X ² | | X ² | X ² | X ² |
| Serology for CMV, HSV, HIV, toxoplasmosis, varicella, hepatitis B- | X | | | | | | | | | | | | | |
| Karnofsky or Lansky performance status | X | | | | | | | | | X | | X | X | X |
| Cytogenetic tests on bone marrow specimens (CML patients only) | X | | | | | | X | X | | X | | | | |
| Radiologic studies (Lymphoma patients) | X | | | | | | X | X | | X | | | | |
| Tetanus Immunization | | | | | | | X | X | | X | | | | |
| Chimerism Studies | X | | | | X | | X | | | X | | | | |
| Serum sample: 5 mL | X | | | | | | | | | | | | | |
| Peripheral blood samples ⁵ | X | | | X | | X | X | X ² | X | X ² | X | X | X | X |

1. As age appropriate.
2. Optional.
3. Within 14 days of start of preparative therapy for patients as specified in Section 2.5.2.
4. If ANC < 500 / mm³.
5. Peripheral blood sample to be used for HLA and immune reconstitution studies as specified in Section 2.5.2.
6. Differential must be done if WBC < 1500/mm³ at any time post-engraftment and at Days 100, 180, 270, and 360.
7. Collect reticulocyte count at Day 28 post-transplant, then weekly until reticulocyte count > 30,000/mm³ for two consecutive weekly measurements.

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2.5.3 Weekly GVHD Monitoring

To determine the severity of acute GVHD, data will be collected weekly to characterize the severity of symptoms and signs caused by GVHD and to evaluate possible confounding factors. Real time data collection will include descriptive characteristics of rash and estimates of body surface area involved; extent of any wet dermal/epidermal separation; identification of concomitant causes of rash other than GVHD; peak serum bilirubin; concomitant causes of increased bilirubin other than GVHD; presence or absence of nausea, vomiting, or anorexia persistent after engraftment; peak diarrhea volume with annotations concerning the presence or absence of urinary mixing and estimates of the true diarrhea volume; presence or absence of abdominal cramps; presence or absence of frank stool blood or melena; concomitant causes of gastrointestinal symptoms other than GVHD; biopsy results; identification of any agents used for treatment; and autopsy results. A mannequin to aid in estimating the percentage of body surface involved will be included on each weekly assessment form. A grading system for GVHD is included in Appendix B.

2.5.4 Risks and Toxicities

Recipients of cord blood transplants incur risks from pre-transplant conditioning and post-transplant therapy which must be weighed against the risk of the disease for which the transplant is prescribed. Major risks following transplantation include: 1) Infection which can be of a bacterial, viral, parasitic, or fungal nature. Often, these infections are life-threatening, particularly when caused by viral or fungal agents, and are associated with a high mortality rate in the transplant population; 2) GVHD, either acute or chronic in nature, may occur following cord blood transplants. The degree of GVHD varies from mild cutaneous reactions to extensive widespread and systemic involvement of skin, liver, and gastrointestinal tract. Probably due to a direct association, the incidence of fatal infection is greater in patients developing GVHD; 3) Graft Failure can occur and is associated with a high risk of mortality; 4) End Organ Damage of all or any of the major organs may occur as a result of reactions to drugs (e.g., antibiotics, antifungal medications, etc.), and as a result of destructive processes (e.g., infection, GVHD, etc.), and may have a fatal outcome; 5) for patients transplanted for malignant disorders, Relapse of the underlying disease may occur, especially in patients with far advanced disease status at time of transplant; 6) Unknown Toxicities may occur in any individual patient due to multiple events and cumulative effects which may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function; and 7) Death.

Cord blood transplantation has many physical and psychological effects. The patient may be in an isolation room for approximately 30 to 40 days or longer. The patient will be required to care for a central line catheter and to use the medications and dressings for this procedure. There will be strict guidelines for hygiene and care necessary to prevent infection. For most patients, modifications in lifestyle will occur for at least the first year following transplant and may extend beyond that time.

Damage to major body organs may include the brain, eyes, heart, lung, liver, and kidneys. Possible late effects may include growth retardation deformities, cataracts, changes in endocrine function, sterility, learning disabilities or brain damage, and secondary malignancy.

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Specific possible side effects of the constituent therapies are:

Radiation Therapy

Nausea and vomiting, diarrhea
Parotiditis causing jaw pain and swelling
Fever
Erythema
Hyperpigmentation
Mucositis
Alopecia

Cyclophosphamide

Nausea and vomiting, diarrhea
Edema with increased weight
Cardiomyopathy
Stomatitis
Hemorrhagic cystitis
Hemolytic anemia
Sterility
Alopecia
Skin rash

Busulfan

Nausea, vomiting, diarrhea
Stomatitis
Skin rash, discoloring
Seizures
Veno-occlusive disease
Alopecia
Pulmonary fibrosis
Bone marrow failure
Sterility
Swelling

ATG

Chills
Fever
Rashes
Joint pain
Allergic reaction
Low blood pressure
Fast heart rate
Fast breathing rate
Hives
Respiratory distress, anaphylaxis

Cord Blood Infusion

Allergic reactions
Emboli to lungs
Fever
Passage of genetic and infectious disease

Methylprednisolone

Edema and increased weight
Appetite stimulation
Elevated sugar in blood and urine
Peptic ulceration
Increased risk for infection
Muscle weakness
Osteoporosis
Growth retardation
Decreased vision or cataract formation
Hypertension
Mood swings

Cyclosporine

Renal dysfunction
Hepatic dysfunction
Tremor or seizures
Hirsutism
Hypertension
Gingival Hyperplasia
Hypomagnesemia
Transient blindness

Etoposide (VP-16)

Pancytopenia
Neuropathy
Elevated liver function tests
High fever
Hypotension with rapid infusion
Anaphylaxis
Secondary leukemia

Melphalan

Pancytopenia
Nausea, vomiting
Allergic reaction
Pulmonary fibrosis
Seizures
Mucositis
VOD

G-CSF

Fever
Fatigue
Bone pain
Splenomegaly
Allergic reaction

Fludarabine

Nausea, vomiting
Diarrhea
Confusion or Coma
Kidney problems
Pancytopenia
Mouth sores

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All toxicities will be graded using the criteria outlined in Appendix D.

2.5.4.1 Reporting. All unexpected fatal or life-threatening adverse experiences will be reported by the MCC to the FDA by telephone or fax within seven calendar days after receipt of the information following FDA guidelines (21 CFR 312.32). All other unexpected serious adverse experiences should be reported by the MCC to the FDA within fifteen calendar days of receipt of the information. All expected adverse experiences (i.e., those listed in the informed consent, product inserts, or study materials) not covered under the above requirements which are reported elsewhere need not be reported to the MCC using an Adverse Experience Form. Although death and graft failures are not considered unexpected experiences, they will be reported to the FDA via annual reports submitted according to FDA guidelines (21 CFR 312.33).

Transplant centers will report to the MCC adverse experiences according to the above guidelines and according to the Manual of Procedures. A medical monitor associated with the MCC is responsible for reviewing all adverse experience reports and assisting the MCC in reporting these events to the FDA. The Data and Safety Monitoring Board will receive summary reports of all adverse experiences on at least an annual basis.

2.6 STATISTICAL CONSIDERATIONS

2.6.1 Sample Size and Power Considerations

The primary focus of the study is to assess 180 day post-transplant survival. Up to 300 pediatric patients with malignant disease will be enrolled. This sample size will achieve the secondary goal of providing 75 patients in the 3/6 and 4/6 strata where match is defined as high resolution for HLA-A, -B, -DRB1. In the other strata, cell size is likely to be smaller, perhaps 30 per cell. Patients are registered based on the current community standard for HLA matching. Retrospectively, all patients and cord blood units will be high resolution DNA HLA typed. Because of the difficulty of high resolution HLA typing, requiring allele level typing before enrollment could delay time to transplant, add expense, and reduce patient enrollment. After high resolution typing is performed, malignant disease pediatric patients will be stratified into strata 1 - 4 according to degree of match at high resolution. The sample size, power considerations and stopping guidelines that follow relate to the final stratification of patients.

Survival probability will be estimated in each cell separately because survival probability may vary with degree of HLA match and type of disease. The per cell sample sizes can be interpreted in two different ways. First, the sample size determines the length of the confidence interval for the survival probability. Table 2.6.1.1 provides confidence interval lengths for a variety of true underlying proportions. Of particular interest is where $n = 75$ and the survival probability is 60%, which is the anticipated 180 day survival rate. For this setting, the confidence interval length is 22.2%. The proportions below and above 60% are meant to represent other plausible survival proportions. The other values of n , 30, 150 and 300 are for the non-malignant cells and for the case where all the malignant cells are aggregated.

The precision of the estimates alternatively could be viewed as a lower bound on the survival rate. Each sample size provides a specific “power,” that is, the probability to rule out survival

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proportions of a certain size. Table 2.6.1.2 provides the probability (or power) that the lower bound of a 95% two-sided confidence interval for the survival probability will be greater than a threshold of 50%, 45% or 40%.

When n is 75 and the true proportion is 60%, there is about 94% power to rule out a survival probability of 40%. With 30 patients per cell, there is 82% power to rule out a survival probability of 35% if the true probability is 60%.

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**Table 2.6.1.1
CONFIDENCE INTERVAL LENGTHS AND A POSSIBLE CONFIDENCE INTERVAL
FOR VARIOUS CELL SIZES AND UNDERLYING SURVIVAL PROBABILITIES**

| N | Survival % | Length of 95% Confidence Interval | Possible Confidence Interval |
|----------|-------------------|--|---|
| 75 | 65 | 21.6 | 54.2, 75.8 |
| | 60 | 22.2 | 48.9, 71.1 |
| | 50 | 22.6 | 38.7, 61.3 |
| | 40 | 22.2 | 28.9, 51.1 |
| 30 | 65 | 34.2 | 47.9, 82.1 |
| | 60 | 35.0 | 42.5, 77.5 |
| | 50 | 35.8 | 32.1, 67.9 |
| | 40 | 35.0 | 22.5, 57.5 |
| 150 | 65 | 15.2 | 57.4, 72.6 |
| | 60 | 15.6 | 52.2, 67.8 |
| | 50 | 16.0 | 42.0, 58.0 |
| | 40 | 15.6 | 32.2, 47.8 |
| 300 | 65 | 10.8 | 59.6, 70.4 |
| | 60 | 11.0 | 54.5, 65.5 |
| | 50 | 11.4 | 44.3, 55.7 |
| | 40 | 11.0 | 34.5, 45.5 |

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**Table 2.6.1.2a
PROBABILITY OF RULING OUT A THRESHOLD OF SIZE T OR LARGER FOR
VARIOUS SAMPLE SIZES AND TRUE UNDERLYING
SURVIVAL PROPORTIONS**

| N | True Survival % | Probability of Ruling Out Survival Proportions of Size T or Smaller | | |
|-----|-----------------|---|---------|---------|
| | | T = 50% | T = 45% | T = 40% |
| 75 | 65 | .78 | .93 | .99 |
| | 60 | .46 | .73 | .94 |
| | 55 | .18 | .38 | .74 |
| | 50 | | .13 | .40 |
| 150 | 65 | .97 | 1.00 | 1.00 |
| | 60 | .73 | .95 | 1.00 |
| | 55 | .27 | .68 | .96 |
| | 50 | | .22 | .73 |
| 300 | 65 | 1.00 | 1.00 | 1.00 |
| | 60 | .94 | 1.00 | 1.00 |
| | 55 | .42 | .95 | 1.00 |
| | 50 | | .42 | .94 |

**Table 2.6.1.2b
PROBABILITY OF RULING OUT A THRESHOLD OF SIZE T OR LARGER FOR
VARIOUS SAMPLE SIZES AND TRUE UNDERLYING
SURVIVAL PROPORTIONS**

| N | True Survival % | Probability of Ruling Out Survival Proportions of Size T or Smaller | |
|----|-----------------|---|---------|
| | | T = 40% | T = 35% |
| 30 | 65 | .77 | .94 |
| | 60 | .59 | .82 |
| | 55 | .38 | .65 |
| | 50 | .17 | .40 |

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2.6.2 Accrual Objectives

Accrual of up to 360 pediatric patients is anticipated. 300 pediatric patients (age ≤ 18 years) with malignant disease will be enrolled to accurately determine 180-day survival. Based on current trends, approximately 120 patients with 5/6 or 6/6 HLA matches (using low resolution typing for HLA A and B, allele level for DRB1), and 180 patients with 4/6 or 3/6 matches are expected. These patients will be stratified retrospectively by allele level HLA typing for HLA A, B, and DRB1.

It is difficult to estimate the number of donor/patient pairs that will appear matched at entry but will be found to be mismatched at the allele level. The transition rate is dependent on the level of HLA typing at registration, the percentages of recipients with common haplotypes and the racial composition of recipients. The majority of COBLT units are DNA typed for Class I at low resolution which should reduce the transition rate from matched to mismatched, however, units from other banks may have serologic Class I typing at the time of patient registration which will increase the transition rate.

Extrapolating from published studies ^(1,2) and 20 COBLT donor/recipient pairs for which allele level typing is available, the transition from a low resolution HLA match to an allele level mismatch is expected to affect approximately half the pairs. Strata of at least 75 patients, the minimum number required to accurately determine 180-day survival for 3/6 and 4/6 HLA matches, should be achievable within 300 patients.

Approximately 60 patients with non-malignant disease and 30 patients with malignant disease receiving the alternative busulfan/melphalan conditioning regimen will be entered in separate strata to calculate the 180 day survival and engraftment. Approximately 30 adult patients will also be registered. In addition, approximately 60 patients who do not meet study eligibility criteria but need a transplant and have a matched unit in the COBLT bank are expected to be entered. These off-study patients will also be analyzed for 180-day survival, engraftment and GVHD.

2.6.3 Early Stopping Guidelines

Day 180 survival will be monitored by early stopping guidelines. These guidelines are intended to cause closer evaluation of relevant data and will not necessarily close accrual to a particular stratum.

Once a month, for the duration of the study, a test will be performed to compare the null hypothesis that six month survival is greater than or equal to 60%, against an alternative hypothesis that survival is less than 60%. The sequential testing procedure which will be used is an extension of the Sequential Probability Ratio Test (SPRT). A description of the procedure is provided below with further details given in Appendix E.

The extended SPRT test can be represented graphically. At each monthly interim analysis, the total time on study is plotted against the total number of observed deaths. If the graph falls outside of a continuation region defined by two parallel lines (with common slope of .72 and

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intercepts of -3.4 and 1.9), or a total of 75 patients are put on trial, the trial is stopped. Only the lower boundary will be used for monitoring each stratum to protect against poor 180 day survival. When the graph crosses the lower bound, it indicates that there are more deaths than predicted by the observed time on study, and the SPRT rejects the null hypothesis in favor of the alternative.

In stem cell transplantation, the hazard or rate of failure is relatively constant during the first six months on study, and then drops substantially. This procedure assumes an exponential distribution for the time until failure during the first six months, but censors follow up time after six months. Only deaths that occur before the patient has been followed for six months on study are counted. Total time on study is computed as time from entry to death, or six months, whichever comes first, summed over all individuals on study.

The usual measures of performance of a SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N | \theta_i)$. The operating characteristics of the test to be used in this protocol were developed to contrast a 60% versus 40% six month survival rate and are shown in Table 2.6.3.1 below. These operating characteristics were determined in a simulation study which assumed exponential time to failure and uniform accrual at the rate of 75 individuals over a three year period. Since 100,000 replications were used, the estimates have two digits of precision.

Table 2.6.3.1. Operating Characteristics of Sequential Testing Procedure from a Simulation Study with 100,000 Replications

| True 6 Month Survival | 60% | 50% | 40% | 30% |
|---------------------------------|------------|------------|------------|------------|
| Probability Reject Null | 0.04 | 0.38 | 0.91 | 1.00 |
| Mean Month Stopped | 41.1 | 33.8 | 19.3 | 11.3 |
| Mean # Deaths in 6 Mo. | 29.3 | 30.5 | 21.1 | 13.4 |
| Mean # Patients Enrolled | 73.5 | 62.6 | 39.2 | 23.7 |

The procedure rejects the null hypothesis in favor of the alternative 3.7% of the time when the true survival rate is 60%, and 91% if the time when the true survival rate is 40%. This corresponds to a type I error rate $\alpha = .038$ and a type II error rate of $\beta = .09$. When the true six month survival rate is 30%, the procedure is almost certain (100%) to reject the null hypothesis in favor of the alternative. In this situation, on average, the study will be halted 11.3 months after opening, when 13 deaths have been observed in 23 patients.

Primary graft failure, incidence of grades III-IV GVHD and Day 100 survival will be regularly monitored, but formal stopping guidelines will not be used for these secondary endpoints.

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2.6.4 Primary Analyses

The primary analysis will consist of estimating the Day 180 survival probability based on the Kaplan-Meier product limit estimator for Strata 1 to 4 combined and each strata separately. The Day 180 survival probabilities and confidence intervals will be calculated for each of these cells. All transplanted patients will be used in the analysis. Similar calculations will be performed for the secondary endpoints, e.g. neutrophil engraftment, red cell engraftment, platelet engraftment, overall survival, disease-free survival, acute GVHD, etc. The primary analysis of neutrophil graft failure will be conducted conditional on patients surviving at least 14 days.

Factors influencing time to event endpoints such as time to death or time to engraftment will be evaluated using Cox regression. Factors influencing binary endpoints such as engraftment will be evaluated using logistic regression. Factors to be evaluated include cell dose, degree of mismatch, age of recipient, race of donor and recipient, disease of recipient, and graft characteristics (e.g. number of T cells). Separate analyses will be performed by gender for each of these endpoints.

Methods for repeated measures data analysis, such as random effects models and GEE, will be used to describe the “natural history” of repeated cell counts, e.g. neutrophil counts, following transplantation.

2.6.5 Secondary Analysis

Overall relapse rates will be estimated by Kaplan-Meier product limit curves using log-rank tests to compare strata. Adjustments will be made as necessary for covariates including age of recipient, disease risk status, interval between diagnosis and transplant, disease type, gender of donor, post-transplant chimerism, pre-transplant Karnofsky score, or other measure of performance status by use of proportional hazard or other multivariate models as appropriate.

A secondary analysis of neutrophil graft failure will be conducted conditional on patients surviving at least 28 days.

A secondary analysis will be performed on patients who fail to engraft. Incidence rates of both acute and chronic GVHD will be estimated using Kaplan-Meier product limit curves. Multivariate models will be employed to adjust for covariates.

The interaction of cell dose and degree of HLA mismatch on transplant outcomes will be examined using appropriate statistical models.

The secondary endpoint of infectious complications will be analyzed with respect to the number, the severity, and the subsequent complications of infectious episodes while controlling for important prognostic factors as previously described. Rates of other complications such as veno-occlusive disease and interstitial pneumonitis will be examined. Type and severity of adverse events will also be analyzed, including incidence of other malignancies, lymphoproliferative disorders, and post-transplant myelodysplasia.

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2.6.6 References

1. Prasad VK, Kernan NA, Heller G. DNA typing for HLA-A and HLA-B identifies disparities between patients and unrelated donors matched by HLA-A and HLA-B serology and HLA-DRB1. *Blood* 1999; 93(1): 399-409.
2. Petersdorf EW, Goole TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood* 1998; 92(10): 3515-3520.

2.7 OFF-STUDY CORD BLOOD TRANSPLANTS

Potential cord blood transplant recipients not meeting all the eligibility criteria in Section 2.2 may be registered to receive a COBLT cord blood unit. These patients will be considered off-study and on the Expanded Access Protocol.

Before a COBLT unit will be released for transplant, the following requirements must be met.

1. The registration procedure detailed in the COBLT Protocol Section 2.3 must be completed.
2. Confirmatory HLA typing of the recipient and the cord blood unit by a COBLT HLA lab must be completed.
3. IRB-approval for the Expanded Access Protocol.
4. Documentation of an IRB-approved alternative cord blood transplant protocol and evidence that the subject has provided informed consent for treatment on the approved protocol must be submitted to the MCC.
5. The transplant center must complete all COBLT forms as detailed in the COBLT Expanded Access Protocol Section 2.4.2, Follow-up Schedule. Required observations as described in the COBLT Protocol Section 2.5.2 must also be obtained. Note that blood samples for immune reconstitution studies, blood samples for chimerism assays and reticulocyte counts will not be required for these patients.

Chimerism assays conducted at the institution still must be performed by approximately Day 42 to document engraftment.