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CHAPTER 1

BACKGROUND AND RATIONALE

CHAPTER 1

BACKGROUND AND RATIONALE

1.1 ALLOGENEIC UMBILICAL CORD BLOOD BANKING AND TRANSPLANTATION

1.1.1 Overview

Transplantation of hematopoietic stem cells (HSC) from HLA-identical sibling bone marrow donors has been successfully utilized in the treatment of patients with high-risk or recurrent hematological malignancies, bone marrow failure syndromes, selected hereditary immunodeficiency states and metabolic disorders. However, use of HSC transplant therapy has been limited by a lack of HLA matched donors. While there are currently more than 4 million potential donors in marrow donor registries around the world (approximately 2 million are HLA, B, and DR typed), more than 30% of patients requiring transplant therapy are still unable to find an HLA 0-1 antigen disparate marrow donor, with even less chance of a successful search in patients of non-Northern European descent. Even if a marrow donor is identified and the transplant performed, severe acute and extensive chronic GVHD and increased risk of opportunistic infection limit transplant outcome. The presence of one (or more) HLA antigen mismatches clearly increases the risk of graft-versus-host disease (GVHD) and adversely affects survival.

In an attempt to increase the availability of suitable donors and reduce the morbidity and mortality associated with allogeneic bone marrow transplantation, clinical investigators worldwide have evaluated umbilical cord blood (UCB) as an alternate source of hematopoietic stem and progenitor cells for transplantation (1-18). Laboratory investigators have confirmed the high frequency of primitive hematopoietic stem and progenitor cells in umbilical cord blood (19).

1.1.2 Unrelated Donor UCB Transplantation

As a result of the early successes with umbilical cord blood from sibling donors, pilot programs for the banking of unrelated donor umbilical cord blood began New York, Milan, Dusseldorf, Paris and London. Benefits of using banked umbilical cord blood include: 1) availability of unit on demand, 2) receipt of the unit at the transplant center prior to the start of conditioning, and 3) minimal risk or inconvenience to the donor. Additional advantages which remain to be proven include 1) lower risk of transmissible infectious diseases, such as cytomegalovirus (21) and Epstein-Barr virus (22), 2) a lower risk of acute and chronic GVHD as compared to unrelated-donor marrow transplants, 3) the ability to tolerate HLA mismatched transplants, and 4) shorter interval between search initiation and transplant.

There are also potential disadvantages of umbilical cord blood. Unlike marrow donors who are screened for major medical problems at the time of collection, the newborn cord blood donor does not have a medical history. The newborn's mother is a surrogate for information pertaining to the family medical history and high-risk behaviors. There is often no opportunity to gather additional information about the newborn donor after the cord blood unit (CBU) has been banked and stored. Therefore, it is possible that banked CBUs may be obtained from newborns who subsequently

develop hematopoietic or immune system disorders and could transmit the disorder to the recipient. In addition, the limited volume of each CBU results in a nucleated cell dose that is typically 10-fold lower than a bone marrow cell dose. The lower cell dose may account for the delayed neutrophil and platelet engraftment that has been observed in CBU recipients compared to what has been reported for marrow recipients. Although marrow has been used successfully for transplant more than10 years after cryopreservation, the length of time a frozen cord blood unit retains its engraftment potential is unknown. There are also ethical and legal issues regarding the timing of donor informed consent, subsequent additional testing performed on CBUs, and the ownership of CBUs that remain unresolved (20).

1.1.2.1 The New York Blood Center Placental Blood Program

In 1993 the first unrelated placental cord blood bank was established at the New York Blood Center (NYBC) with grant support from NHLBI. In 1998, Rubinstein et al reported the collection of 7,705 CBUs during a five year period and the performance of 6,497 searches for potential recipients from 290 transplant centers (23). This report described the outcomes for the first 562 consecutive NYBC CBU transplants performed at 98 transplant centers in the United States and abroad. Transplants were performed as part of the treatment of malignant (n=378) and non-malignant disorders (n=184). The majority of patients reported in the study were children and young adults.

The report suggested that the time to myeloid engraftment was associated with the CBU nucleated cell dose. Of 562 patients, myeloid engraftment, defined as reaching an ANC $\geq 5 \times 10^8$/L of donor origin, did not occur in 160; 102 died before engrafting, 13 had autologous reconstitution, 29 received a second transplant, and 16 relapsed. The median time to neutrophil recovery was 28 days for all patients and 25 days for those who engrafted. The median time to platelet recovery (platelet count $\geq 5 \times 10^8$/L without transfusion for 7 days) was 90 days for all patients and 71 days for patients who reached this endpoint.

Severe acute GVHD (grade III-IV) occurred in 23% of patients and chronic GVHD occurred in 25%. Notably, the incidence of grade III-IV acute GVHD was lower in recipients of six of six antigen matched grafts, but did not otherwise correlate with the number of mismatches. Transplant-related events (death, autologous recovery and second transplant) were associated with the patient's underlying disease, age, CBU cell dose, HLA disparity, and location of transplant center (U.S. versus foreign).

Although the reports by Rubinstein et al demonstrated the feasibility of cord blood transplants, many questions remain unanswered. Specifically, what is the lowest cell dose that reliably results in engraftment? What is the maximal degree of HLA disparity that can be tolerated without GVHD or compromised immune function? Do the outcomes differ in adult recipients compared to pediatric patients? Are there specific graft, demographic or treatment parameters that predict a good or poor outcome? What is the composition of the optimal graft? Are there specific groups of patients who should not be treated with UCB transplants either because of increased risk of graft failure or disease relapse (e.g. Fanconi Anemia and CML).

1.1.2.2 Combined Clinical Results from Duke University and the University of Minnesota

Nearly 375 unrelated donor UCB transplants have been performed at Duke University and University of Minnesota. In July 2000, a detailed analysis of their combined data sets was performed to determine the potential influence of various factors (e.g., graft cell dose and donor/recipient HLA disparity) on rate of hematopoietic recovery and probabilities of engraftment, acute GVHD, chronic GVHD, non relapse mortality, relapse and overall survival. In comparison to prior reports on unrelated donor UCB transplantation, the present study benefits from standardized HLA typing with high resolution typing of HLA-DR, greater homogeneity in supportive care treatments between two centers, and the ability to internally verify data accuracy.

At these centers, patients with acute leukemia, bone marrow failure syndromes, immunodeficiency states or inborn errors of metabolism were eligible for unrelated donor UCB transplantation if: 1) an HLA-compatible related or unrelated bone marrow donor was not immediately available at the time needed, and 2) the subject/parent(s) consented to the transplant procedure. At the University of Minnesota, patients were preferentially offered BMT. Protocols for myeloablative therapy and use of unrelated donor UCB for transplantation were reviewed and approved by the respective institutional review boards.

Patients

Between August 1993 and April 15, 2000, 312 patients were transplanted with unmanipulated, banked unrelated donor UCB at Duke University and the University of Minnesota (excluding COBLT study transplants). Cord blood units were primarily obtained from the Placental Blood Program of the New York Blood Center and St. Louis Cord Blood Bank. For this analysis, patients with <100 days follow-up post transplant (n=27) or who had a history of prior allogeneic HSC transplantation (n=24), an HLA 4 antigen mismatched UCB donor (n=2), or who required less than conventional myeloablative therapy (n=2) were excluded. Therefore, 257 patients treated for various malignant and non-malignant disorders were evaluable. The univariate analyses described below refer to the sample of 257 patients. The multivariate results are from an analysis of the first 146 patients performed in July 1999. Multivariate analyses are pending for this group of 257 patients. Median age of the patients was 8.1 years (range, 0.2-58) and median weight was 24.5 kg (range, 3.9-102.8).

Preparative regimen and GVHD prophylaxis

Pre-transplant conditioning varied according to the patient's disease, disease status and institution. At the University of Minnesota, 94% patients received a total body irradiation (TBI)-containing regimen and at Duke University, 56% patients received TBI. Most patients received anti-thymocyte globulin (ATG) prior to unrelated donor UCB transplantation. Prophylaxis for acute GVHD primarily consisted of cyclosporine A (CsA) or CsA and methylprednisolone (MP). MP dosing and CsA taper varied slightly between institutions. Of note, some early Duke patients were treated on a protocol that prescribed a higher dose of MP.

Graft Characteristics

HLA-matching: 6/6 loci in 18 patients, 5/6 loci in 90 patients, 4/6 loci in 123 patients, and 3/6 loci in 15 patients. High resolution DRB1 typing was not available for 11 patients. The median nucleated cell dose (pre-cryopreservation) was 3.7×10^7/kg with a range of $0.7 - 57.9 \times 10^7$/kg. The

median CD34 cell dose (post-thaw) was 3.3 x 10^5/kg with a range of 0.2 - 105 x 10^5/kg. The median CD3 cell dose (post-thaw) was 7.0 x 10^6/kg with a range of 0.0 - 101 x 10^6/kg.

Neutrophil Recovery

The probability of neutrophil recovery (ANC $\geq$5 x 10^8/L) by day 42 was 0.87 (0.83-0.92). In univariate analysis, younger recipient age, lower recipient weight, diagnosis of malignant disease, non-TBI containing preparative regimen, higher UCB unit cell dose, and use of G-CSF correlated with faster neutrophil recovery and superior engraftment. At the University of Minnesota, CD34 cell dose also strongly correlated with neutrophil recovery. (Changes in methodology precluded this analysis at Duke.) Notably, HLA disparity had no demonstrable effect on rate of neutrophil recovery or probability of engraftment (p=0.62).

In a multivariate analysis of the first 146 patients, only higher cell dose and diagnosis of malignant disease were identified as significant factors associated with superior neutrophil recovery and engraftment. Recipient age and weight interact with cell dose making it difficult to separate the effects of these variables. A randomized trial would be required to determine if there is any true beneficial effect on the use of G-CSF. Notably, patients undergoing a second transplant using UCB had poorer engraftment; however, reasons for second transplant included graft rejection which may explain this observation.

Platelet Recovery

The cumulative incidence of platelet recovery by 6 months was 0.51 (0.44-0.58). In univariate analysis, younger recipient age, lower recipient weight, diagnosis of malignant disease, standard risk malignancy, non-TBI containing preparative regimen, CMV negative serostatus, and higher UCB unit cell dose were correlated with faster platelet recovery and superior engraftment. Notably, HLA disparity had no demonstrable effect on rate of platelet recovery or probability of engraftment.

On the basis of a prior analysis of the first 146 patients, only higher cell dose and CD34 cell dose were significant factors associated with superior platelet recovery and engraftment in multivariate analysis.

Acute Graft-versus-Host Disease

The overall probabilities of grade II-IV and grade III-IV acute GVHD for the entire group of patients were 0.30 (0.24-0.36) and 0.12 (0.08-0.16) by day 100 post transplant, respectively. In univariate analysis, no factor was associated with risk of acute GVHD, including degree of HLA disparity. Higher CD3 cell dose was associated with less GVHD; however, this is confounded with younger patient age and thus hard to interpret. Notably, no difference in the probability of grade II-IV acute GVHD could be discerned between patients treated with CsA plus high dose MP, versus lower dose MP versus other regimens.

A multivariate analysis of the first 146 patients found that no factor was associated with acute GVHD. Younger recipient age, HLA match and lower CD3 cell dose are known to be associated with lower GVHD in recipients of unrelated donor marrow but cannot be discerned in these analyses.

Chronic Graft-versus-Host Disease

The overall probability of chronic GVHD for the entire group of patients was 0.07 (0.04-0.10) at 1 year after transplant. In univariate analysis, recipient age, recipient weight, use of high dose

methylprednisolone and other GVHD prophylaxis, diagnosis of non malignant disease, higher CD3 cell dose, and use of a non-TBI containing regimen were associated with lower risk of chronic GVHD. Also, use of high dose melphalan (MEL) was associated with a higher risk of chronic GVHD.

Results of unrelated donor marrow transplants suggest that younger recipient age is associated with less chronic GVHD and that greater HLA disparity and higher CD3 graft content is associated with more chronic GVHD. However, these findings were not observed in this study of unrelated cord blood transplantation. Multivariate analysis was not previously performed for this endpoint.

Survival

With a median follow up of 1.7 years, the probabilities of survival at 2 years and 4 years after unrelated donor UCB transplantation were 0.45 (0.39-0.52) and 0.41 (0.33-0.48), respectively. In univariate analysis, younger recipient age, lower recipient weight, diagnosis of non-malignant disease, standard risk malignancy, recipient CMV negative serostatus, higher graft nucleated cell dose, absence of acute GVHD, use of UCB for primary transplant and Caucasian race were associated with improved survival. Increased degree of HLA disparity did not significantly alter the probability of survival post transplant. Notably, the effect of recipient age and cell dose was preserved even when evaluating only those patients that engrafted. In the multivariate analysis of the first 146 patients, only recipient age and higher cell dose were identified as significant factors associated with superior survival.

Summary

These results demonstrate that cryopreserved UCB from HLA 0-3 antigen mismatched unrelated donors contains sufficient numbers of transplantable hematopoietic stem and progenitor cells for most small patients. The data presented indicate that the probabilities of grade III-IV acute GVHD and extensive chronic GVHD are low. Moreover, the results of this statistical analysis demonstrated the importance of graft cell dose in determining outcome after unrelated donor UCB transplantation. Within the group of patients with either an HLA-1 or HLA-2 antigen disparate donor, graft cell dose rather than degree of HLA disparity had the most significant impact upon the probabilities of engraftment, non-relapse mortality and survival.

Therefore, these data suggest that cell dose rather than degree of HLA disparity should determine the choice of UCB graft when a patient has more than one HLA-mismatched CBU. The importance of cell dose on transplant outcomes also provides a compelling argument for focusing on the collection of larger UCB grafts and for investigating ex vivo HSC expansion for future clinical trials.

1.1.2.3 Unrelated UCB Transplantation in Adult Recipients-Duke University

Twenty-four adult patients $\geq$ 18 years of age, the majority of whom had high risk hematologic malignancies, were consecutively transplanted at Duke University from February 1995 through September 1997 with partially HLA-matched UCB. These patients are a subset of those reported in Section 1.1.2.2. The median weight of these patients was 68.8 kg (range 43 to 91.7 kg), and the median age was 30 years (range 18 to 58 years). Six patients underwent UCB transplantation as a second transplant following relapse after a prior transplant.

Graft Characteristics

HLA-matching: 6/6 loci in one patient, 5/6 loci in five patients, 4/6 loci in 16 patients, and 3/6 loci in two patients. The median nucleated cell dose (pre-cryopreservation) was $2.1x10^7$/kg (range 1.1 to 6.3 x 10^7/kg). CD34+ progenitor cells (post-thaw) were 3.6 x 10^5/kg (0.7 to16.7 x 10^5/kg), total colony-forming units (CFU, post-thaw) were 1.0 x 10^4/kg (0 to 7.3 x 10^4/kg), and CD3+ cells (post-thaw) were $4.2x10^6$/kg (2.4 to 8.8 x 10^6/kg).

Hematopoietic Recovery

There was evidence of myeloid engraftment in 19 of 24 patients, with a median of 24 days to attain an ANC of 500/mL (range 13 to 37 days). Two patients experienced primary graft failure. Three patients died before day 34 without evidence of engraftment. There was evidence of platelet recovery in 14 patients, with a median recovery time of 58 days (range 35 to 142 days). Platelet counts of 50 and 100K/mL in surviving patients were attained at median day +110 (range 42 to 188 days) and +134 days (range 88 to 176 days), respectively. Twelve patients attained RBC recovery independent of transfusion support at median day +80 post transplant (range 27 to 160 days). All patients who had hematologic recovery showed >98% donor engraftment by chimerism analyses, and no late graft failures have been observed.

Time to attain neutrophil recovery (ANC $\geq$ 500/mL), but not RBC or platelet recovery, correlated with number of infused nucleated cells (p=.02) and CFU (p=.003). Although there was a trend toward improved survival in patients whose graft contained $\geq 2x10^7$/kg cryopreserved cell dose, this trend did not attain statistical significance (p=0.08).

Graft versus Host Disease

19 patients were evaluable for GVHD. The estimate of the actuarial probability of developing grade III-IV acute GVHD up to 100 days post transplant was .26 (6/19 patients) with a 95% confidence interval of .13-.57. Three patients died of complications of acute GVHD. The estimate of the actuarial probability of chronic GVHD was .38 (.14-.68). The five patients who developed chronic GVHD involving the gastrointestinal tract and liver previously had grade I-III acute GVHD.

Immune Reconstitution

Immune function of patients was analyzed at 3 month intervals during the first year post transplant, and at six month intervals during the second year post transplant. Recovery of circulating naive T lymphocytes and evident *in vitro* proliferative responses to plant mitogens and recall antigens were detected generally 100-180 days after transplantation. Natural killer cells emerged as the predominate lymphocyte population at 100 days post transplant.

Survival

As of June 24, 1998, 8 patients survived in unmaintained remission for 9 to 40 months after UCB transplantation, rendering an event-free survival rate of 33 percent. The probability of survival at 3 months was 54% (95% CI: .36 -.72) and at 6 months was 50% (.32-.68). There were five deaths after day +100. There were no long-term survivors of patients undergoing UCB transplantation after failing a previous autologous or allogeneic transplant.

Summary
The rate of engraftment in this series of adult recipients was similar to that observed in children. The infused cell dose was the most consistent predictive value for time to myeloid engraftment. Although only 1 of the 24 patients in this study had a 6/6 HLA antigen matched graft, the estimate of the probability of severe acute GVHD was only 32%, and 38% for chronic GVHD. Although this is somewhat higher than the results in children receiving unrelated UCB grafts, it compares favorably with that observed in adults undergoing unrelated-donor marrow transplantation. This analysis demonstrates that partially HLA-matched UCB transplants from unrelated donors are feasible for adults. Additional studies in a larger series of patients are warranted.

1.2 COBLT STUDY BACKGROUND

Despite the apparent clinical success of cord blood transplants, many scientific and clinical questions remain to be answered. Areas in need of active investigation include the identification of surrogates predictive of successful engraftment; the degree of acceptable HLA disparity and its impact on GVHD and engraftment; optimization of UCB transplantation in adults and larger children; and optimal collection, storage and CBU characterization methods.

In 1996, NHLBI initiated the prospective, multi-center Cord Blood Transplantation study (COBLT). The COBLT study was designed to address these questions, and to obtain uniform data on cord blood collection, graft characterization and transplantation in order to assist in development of UCB graft product standards. The original goal of the study was to build a bank of 15,000 racially/ethnically diverse UCB units and perform transplants with units from the bank to determine if UCB cells are a suitable alternative for transplantation of patients with malignant and non-malignant blood diseases who do not have a matched unrelated marrow donor.

Accrual of transplant patients to the study was slower than expected because additional time was needed for the bank to build a critical mass of UCB units. Even when the bank reached 3000 units, recruitment lagged because many potential recipients had a better-matched or larger UCB unit in another bank or an unrelated marrow donor, and most transplant physicians prefer marrow to less-well-studied UCB. The study's Data and Safety Monitoring Board (DSMB) determined that the study was too far behind schedule to meet its accrual goals as originally stated. Because new clinical transplant data were available, the DSMB recommended that NHLBI convene an Ad Hoc Advisory Group composed of experts in marrow and cord blood transplantation to review the study design and recommend changes to the protocol and/or study goals. In their January 2000 report, the Advisory Group recommended that the study be redesigned to emphasize HLA mismatching in pediatric patients. They also recommended that the relationship between cell dose and engraftment in adult patients be examined separately. Offers to participate in the study were extended to new centers with an interest in UCB transplantation. In addition, patients who receive transplants with units from other banks now are eligible for enrollment provided the transplant center agrees to follow the COBLT treatment protocol and the stored units meet quality assurance criteria. Patients receiving transplants with units from the UCB banks in Table 1.1 are currently eligible for enrollment in the study.

Table 1.1
Transplants with Units from UCB Banks

Bank Name	Searchable Units	Number of Transplants
NYBC	9,000	900
NMDP	5,200	151
COBLT	4,300	40

1.3 TRANSPLANT STUDY OVERVIEW

Previous studies suggest that UCB is a useful source of stem cells for hematopoietic reconstitution but this has not been evaluated in a prospective multi-center study with a standard therapy and supportive care plan and with standard reporting of engraftment, GVHD, and other complications. The purpose of the COBLT study is to accurately describe180-day survival and other events after UCB transplantation.

Patients will be enrolled under the current transplant community standard for HLA matching which is low resolution typing for HLA-A and HLA-B loci and high resolution typing for HLA-DRB1. Retrospectively, patients and units will be high resolution typed for HLA-A, -B, -DRB1 and classified into one of the eight strata that comprise the study. Tables 1.2 and 1.3 illustrate the eight strata.

Table 1.2
Patients with Malignant Disease ≤ 18 Years of Age

5/6 or 6/6	High Resolution HLA Match
4/6	High Resolution HLA Match
3/6	High Resolution HLA Match
<3/6	High Resolution HLA Match

Table 1.3
Other Strata

Patients with Inborn Errors of Metabolism/Storage Diseases
Patients with Severe Aplastic Anemia/Fanconi Anemia/Other Marrow Failure Syndromes
Patients with Malignant Disease non-TBI/Alternative Conditioning Regimen
Patients > 18 Years

In accordance with the Ad Hoc Advisory Group recommendation, a sample size of up to 300 pediatric patients with malignant disease is sufficient to obtain 75 patients in the 3/6 and 4/6 high resolution strata. This sample size will allow accurate estimates of 180-day survival within each of these strata. In addition, approximately 30 patients are anticipated in each of the strata displayed in Table 1.3.

1.4 CORD BLOOD BANK STUDY OVERVIEW

The National Heart, Lung and Blood Institute (NHLBI) has funded two UCB banks as part of this project to collect approximately 15,000 UCB units. The banked units will have a diverse ethnic/minority representation such that patients in various population groups will have similar chances of finding a suitable unit for transplant.

The UCB banks work closely with obstetricians to obtain informed consent from expectant mothers. Potential participants are told about the study by their doctors, and are provided a written brochure that describes the study, a list of medical history questions, and a sample informed consent document. When possible the consent document is signed before the onset of labor (and reaffirmed following delivery), but especially at the outset, the consent document sometimes was signed at an appropriate time following delivery while the mother remained in the hospital. If consent is not obtained or rescinded, a unit is discarded. The link between the donor's identity and the unit is maintained, but confidentiality is protected carefully. Mothers are contacted approximately 6 months post-delivery to check on the health of the infant. The model informed consent document for cord blood donors is found in Appendix B of the Standard Operating Procedures manual (SOP).

Units are collected in a separate room near the delivery room by dedicated bank staff following delivery and the usual approach to clamping and tying the umbilical cord by the obstetrician. Units must contain at least 60 mL or 600 million nucleated cells of to be considered for processing. A detailed medical history is obtained from the mother, screening for infectious and genetic diseases is performed, and the unit is HLA typed using DNA-based technology. Flow cytometry and an assay for colony forming units are performed on each unit before freezing. Units are quarantined either in a separate freezer or in the vapor phase of liquid nitrogen until results of testing indicate the unit is fit for permanent storage in the liquid phase. A bar code-based labeling system facilitates unit identification, record keeping, and unit inventory. Additional details of the collection, testing, processing, and freezing procedures can be found in the SOP.

Before the start of patient conditioning, units are shipped to transplant center stem cell processing laboratories in liquid nitrogen dry shippers at temperatures less than -120°C. Information including the intended patient, the HLA type of the unit, the pre-freeze cell dose, and the results of all tests performed are provided with the unit. Standard instructions for storage and thawing are provided. Laboratories confirm to the coordinating center that the unit arrived in good condition. Details are provided in the Investigator's Brochure, Chapter 4 of the SOP and Chapters 8 and 9 of the Transplant Center Manual of Procedures (MOP).

When this study began in 1996, only patients receiving CBUs from COBLT banks were to be enrolled. However, in order to increase enrollment, a decision was made subsequently (February 2000) to enroll patients who receive CBUs from other U.S. banks provided the banks can certify that they follow the NetCord-FAHCT, AABB, or NMDP standards.

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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/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. 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/mm^3) 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/mm^3 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.

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.

2.1.6 **Veno-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 µmo1/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/mm^3 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

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 > $5/mm^3$ 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.

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 (≤ 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 (≤ 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 ≥ 3rd 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 ≤ 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

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/mm^3 6-12 months of age
b) > 200,000/mm^3 ≥ 10 and < 18 years old
c) > 20,000/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 ≥ 3rd 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 ≥ 3rd medullary relapse or refractory disease (other than primary induction failures) will receive the busulfan/melphalan conditioning regimen. (Closed to accrual.) Infant leukemia may receive bulsulfan/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)

5. Patients with Myelodysplastic Syndrome(s) defined by the following:

   a) Refractory Anemia: Anemia with ≤ 1% blasts in peripheral blood and dyserythropoiesis

   b) Refractory Anemia with Ringed Sideroblasts: Refractory anemia defined above, including the presence of ringed sideroblasts ≥ 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 (> $1x10^3$/liter) with < 5% blasts in the peripheral blood and ≤ 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/μL, platelet count < 20,000/μL, 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,

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/mm^3, 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 phosphoylase (PNP) deficiency, X-linked SCID, common variable immune deficiency (VID), Nezeloff's syndrome, and cartilage hair hypoplasia, reticular dygenesis

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 .

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, FEVI, FEC (diffusion capacity) > 45% of predicted (corrected for

hemoglobin); if unable to obtain PFT, O_2 saturation > 85% on room air

16. Nucleated cell dose: Cord blood unit must provide a minimum of $1x10^7$ 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/mm^3 AND malignant cells on cytospin)
2. Female patients who are pregnant (positive ß-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/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.

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, ≤ 18 years of age
2. Malignant disease, 4/6 high resolution HLA match, ≤ 18 years of age
3. Malignant disease, 3/6 high resolution HLA match, ≤ 18 years of age
4. Malignant disease, 2/6 or 1/6 high resolution HLA match, ≤ 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**

Day	Treatment
-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)

-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/m^2 of methylprednisolone (q 12 hours divided doses for each ATG day). This means a total daily dose of 1 gm/m^2. 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/m^2/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

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 ≥ 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.

2.4.2 **Conditioning Regimen for Patients with Fanconi Anemia - Closed**

Day	Treatment
-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/m^2 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/m^2/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.

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 will not be given.

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

Day	Agents
-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 kg x 0.8 mg/kg)/(6 mg/mL) = 4.0 mL Busulfex = 24 mg dose

Add 4.0 mL Busulfex to 40 mL diluent = 44 mL for infusion
(4.0 mL x 6 mg/mL)/(44.0 mL) = 44 mL for infusion
= 0.54 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 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/m^2 of methylprednisolone for each dose of ATG. This means a total daily dose of 1 gm/m^2. 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/m^2/day.

2.4.4 **Conditioning Regimen for Patients with Other Non-Malignant Diseases**

Day	Treatment
-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/m^2.

[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

[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 kg x 0.8 mg/kg)/(6 mg/mL) = 4.0 mL Busulfex = 24 mg dose

Add 4.0 mL Busulfex to 40 mL diluent = 44 mL for infusion
(4.0 mL x 6 mg/mL)/(44.0 mL) = 44 mL for infusion
= 0.54 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/m^2 of methylprednisolone (q 12 hours divided dose for each ATG day). This means a total daily dose of 1 gm/m^2. 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/m^2/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.

Day	Treatment
-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

	ATG (equine) 30 mg/kg/day IV QD
-2	Melphalan 45 mg/m^2 IV
	Methylprednisolone 2-2.5 mg/kg/day IV in divided doses
	ATG (equine) 30 mg/kg/day IV QD
-1	Methylprednisolone 2-2.5 mg/kg/day IV in divided doses
	ATG (equine) 30 mg/kg/day 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] 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 kg x 0.8 mg/kg)/(6 mg/mL) = 4.0 mL Busulfex = 24 mg dose

Add 4.0 mL Busulfex to 40 mL diluent = 44 mL for infusion
(4.0 mL x 6 mg/mL)/(44.0 mL) = 44 mL for infusion
= 0.54 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/m^2 of methylprednisolone (q 12 hours divided dose for each ATG day). This means a total daily dose of 1 gm/m^2. 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/m^2/day.

2.4.6 **Dose Adjustments**

2.4.6.1 Cyclophosphamide Ideal Body Weight Dose Adjustment. If a patient's weight is ≥ 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 x (ht - 60)] where ht = inches, IBW = kg
   Females IBW = 42.2 + [2.27 x (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

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

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 ANCs reach $\geq$ 500/mm^3. After ANCs have reached $\geq$ 500/mm^3, steroids should be tapered by 0.2 mg/kg/week.

If a patient experiences fever > 103°F and erythroderma between Days 5 and 9, it is recommended that the patient be treated with Solumedrol at a dose of 500 mg/m^2.

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.

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 μg/kg/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 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 μg/kg/day. See Section 2.4.2 for G-CSF treatment of Fanconi Anemia patients.

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 $\geq$ 750 for two consecutive days. Prophylaxis should continue until Day +100. Ganciclovir should not be given from Day -2 until ANC $\geq$ 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.

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/mm^3 at all times and greater than 50,000/mm^3 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

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.

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

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**,

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

		Day						Month Post-Transplant						
	Baseline	**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	X[2]	X	X[2]	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 / mm^3.
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/mm^3 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/mm^3 for two consecutive weekly measurements.

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.

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

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

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%.

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

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

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

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 $E(N \mid \theta_i)$. 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.

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.

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.

CHAPTER 3

LABORATORY PROCEDURES

CHAPTER 3

LABORATORY PROCEDURES

3.1 HLA TYPING

Molecular HLA typing will be performed for all donor cord blood units and patients in the three reference laboratories identified for the COBLT study. The laboratories are led by Dr. LeeAnn Baxter-Lowe, University of California, San Francisco, Dr. Elaine Reed, University of California - Los Angeles, and Dr. Jennifer Ng, Navy Medical Research Institute.

Initial HLA typing will be done at low resolution for class I, HLA-A, -B, and at intermediate to high resolution for HLA-DRB1. Retrospective typing will be done for all donor-patient pairs. The retrospective typing will be performed at high resolution (corresponding to a single allele for most samples) for HLA-A, -B, -C, DRB1, and DQB1. The types used will be defined according to the current WHO Nomenclature Committee for Factors of the HLA System. These will be updated as necessary. Supplemental typing of HLA-DQA, DPB, and DPA may be determined at a later date.

The specimens for HLA typing will be: a) for the cord blood unit - frozen aliquots from the granulocyte/red cell-enriched pellets that remain after preparation of the cord blood unit, or appropriate sample if unit is obtained from a non-COBLT cord blood bank, b) patient - blood samples from the recipient.

Appropriate HLA typing reagents are described in the detailed protocols of each reference Laboratory including oligonucleotide sequences of PCR and sequencing primers. A list of these reagents will be maintained by the Laboratory in compliance with ASHI regulations. Reagents may be revised during the project as techniques and knowledge of HLA polymorphism improves. Historic records regarding reagents are maintained in accordance with ASHI regulations.

Samples will be typed according to the appropriate SOPs within the reference Laboratory. The final data will be interpreted with respect to a list of recognized HLA alleles that is maintained by the Medical Coordinating Center (MCC).

Each reference laboratory will send to the MCC a report and an electronic data file containing the following information for typings completed during the week:

a. Specimen identification, including bar code label number
b. Assigned type
c. HLA alleles that are potentially present in the specimen
d. Special notation for samples with unusual linkage
e. Typing method
f. Data required by the MCC to update typing assignments as knowledge of HLA polymorphism improves

3.2 □□A□A□□□□□□A□□□ □F □□AF□

A list of the variables proposed for characterizing the cells present in umbilical cord blood units banked for transplant is shown in the table below. With the exception of CD3 each of these measures is in widespread use for characterization of both autologous and allogeneic hematopoietic grafts. CD34-positive cell assay and the colony forming cell assays (CFU-GM and BFU-E) are included as they have the potential to provide a measure of the hematopoietic engraftment potential of the umbilical cord blood unit. In addition, the colony assays provide a direct measure of the viability of hematopoietic progenitor cells. This parameter would otherwise be extrapolated from the viability of the population as a whole as determined by trypan blue dye exclusion. CD3 is included in this panel because the content of T-lymphocytes might have an impact on the incidence and severity of GVHD following transplant.

Variables Used in Graft Characterization

Following red cell and plasma depletion, but prior to addition of cryoprotectant:	
nucleated cell count	automated cell counter e.g.: Coulter MD II 8
leukocyte count	manual differential count
mononuclear cell count	manual differential count
viability	trypan blue dye exclusion
CD34-positive cell count	flow cytometry
CD3-positive cells count	flow cytometry
CFU-GM	colony-forming cell assay
BFU-E	colony-forming cell assay
At the time of thaw/transplant:	
nucleated cell count	automated cell counter e.g.: Coulter MD II 8
viability	trypan blue dye exclusion

To be defined as CD34-positive in this study a cell must exhibit each of the following properties.

- low to intermediate side scatter
  CD34-bright (high fluorescence intensity)
  CD 45-dim (clearly CD45-positive, but not as intensely positive as CD34-negative cells)

To be defined as CD3-positive in this study a cell must exhibit each of the following properties.

- low forward and side scatter
  CD3-bright (high fluorescence intensity)
  CD 45- bright (high fluorescence intensity)

The flow cytometric panel performed at the COBLT Cord Blood Banks for graft characterization prior to cryopreservation is shown in the table below. The $CB34^+$ subsets ($34^+/61^+$, $34^+/90^+$, and $34^+/38^-$) will be discontinued when sufficient numbers of units have been evaluated as determined by the COBLT Bank Subcommittee.

Tube	Marker	Stains
1	Procount (or equivalent)	Nucleic Acid Dye/34/45
2	Procount Control (or equivalent)	Nucleic Acid Dye/Gamma 1/45
3	34+/61+	CD61 FITC / CD34 PE / CD45 (Per-CP or equivalent)
4	34+/90+	CD90 FITC / CD34 PE / CD45 (Per-CP or equivalent)
5	34+/38-	CD38 FITC / CD34 PE / CD45 (Per-CP or equivalent)
6	34+/Control	IgG1 FITC Control / CD34 PE / CD45 (Per-CP or equivalent)
7	19+/16+ & 56+	CD19 FITC / CD16 & 56 PE / CD45 (Per-CP or equivalent)
8	Tri-test or equivalent	CD4 FITC / CD8 PE / CD3 (Per-CP or equivalent)

3.3 IMMUNE RECONSTITUTION

3.3.1 Introduction and Objectives

Two of the principal problems that occur following allogeneic transplantation are graft versus host disease (GVHD) and post-transplant immunodeficiency. After marrow transplantation, the severity of acute GVHD increases as the difference in HLA antigens between donors and recipients increases. Recipients of unrelated-donor marrow have an immunodeficiency of greater severity compared to recipients of related marrow than can be explained by the increased severity and frequency of their acute GVHD. Thus, other factors beside GVHD may contribute to the prolonged immunodeficiency observed in recipients of unrelated marrow. Among the reasons hypothesized for the increased immunodeficiency are the effects of GVHD on the thymus (4), defects in differentiation of donor T cells in the recipient, and histo-incompatibility between donor-derived T cells and patient antigen presenting cells. It is of interest to determine the extent to which this prolonged immunodeficiency occurs following cord blood transplantation.

There is no permanent carryover of antigen specific T lymphocyte or B lymphocyte functions. Antigen specific immune function after transplantation depends on the development of new antigen specific T cells that differentiate through the recipient thymus, and on subsequent development and maturation of new functional B cells. Most of the patients in the post-transplant period are receiving IVIG. Therefore, development of B lymphocyte function can only be assessed by immunization of the patients with neo-antigens. For this reason, the objectives of this study are to characterize the regulation of the production of new T cells in the recipient, evaluate the antigen specific T cell response to infectious antigens (herpes simplex, HSV; varicella, VZV; cytomegalovirus, CMV) and following immunization (tetanus toxoid, TT), determine the role of cytokines (IL-2 and IL-7) in antigen specific function, and to investigate the capacity of recipient T cells to interact with B cells to induce specific antibody production. Data will be analyzed considering patient age at transplant, primary disease, and degree of HLA mismatch. Each assay will be performed in a single laboratory to eliminate inter-site variation.

3.3.2 Specific Aims

The three specific aims of the laboratory studies of immune reconstitution are listed below.

Specific Aim 1 - Production of New Lymphocytes:

The hypothesis for this aim is that following cord blood transplant, the patients without GVHD will develop antigen specific T lymphocytes within 9 months, and that the development is delayed in patients with GVHD.

Peripheral blood samples will be obtained to evaluate immunological responses according to the schedule in Table 3.3.3.1.

Specific Aim 2 - Antigen Specific T Cell Function

The hypothesis for this aim is that cord blood transplant patients will develop a proliferative response to the mitogen PHA by three months post transplant, a response to tetanus toxoid immunization by six months, and a response to herpes viral antigens by 6 months. The secondary hypotheses that exogenous IL-2 and IL-7 will hasten the antigen specific responses and occurrence of GVHD will delay the antigen specific responses will be evaluated. The antigen specific response will be correlated with the appearance of T cells as determined in specific aim 1.

Specific Aim 3 - T and B Cell Cooperation:

The hypothesis for this aim is that cord blood transplant recipients produce antibodies to ΦX 174 antigen synchronously with development of expression of CD40 ligand.

3.3.3 **Schedule and Samples**

Immunizations will be performed and peripheral blood mononuclear cells isolated from transplant patients will be tested according to the schedule in Table 3.3.3.1. Procedures for immunization, antibody analysis and CD40 ligand induction are specified in chapter 9 of the MOP. Use of the phage antigen ΦX174 allows assessment of a specific antibody response in patients receiving immune globulin. Antibody isotype, avidity, and class switching will be studied.

For immunophenotyping, specimens will be prepared as described in MOP chapter 9 and analyzed by two color immunofluorescence using the monoclonal antibody combinations displayed in Table 3.3.3.2. Three color immunofluorescence will be used for CD4/CD45RA/CD45RO.

Table 3.3.3.1
SCHEDULE OF IMMUNE EVALUATION

Forms	Months Post-Transplant																			
	1	2	3	4	5	6	7	8	9	10	11	12	15	18	21	24	30	36	42	48
Immunophenotyping [1]	X	X	X			X			X			X		X		X		X		X
PHA Response [2]	X		X			X			X			X		X		X		X		X
Antigen Specific Blastogenesis [3]			X			X			X			X		X		X		X		X
Tetanus Toxoid Immunization			X			X						X								
ΦX174 Stimulation												X				X		X		X
CD40 Ligand Expression [4]												X				X		X		X

[1] CD3, 4, 8, 56, 19/20, 4/8, RA/RO/4
[2] 3 doses of PHA ± IL-2
[3] Tetanus toxoid, varicella zoster, CMV, herpes simplex, ± IL-2, IL-7
[4] After PHA/PMA stimulation

Table 3.3.3.2
MONOCLONAL ANTIBODY PANEL FOR IMMUNOPHENOTYPING-
IMMUNE RECONSTITUTION

FITC Antibody	PE Antibody	Detects
IgG1	IgG2a	Non-specific Binding
IgG2a	IgG1[1]	Non-specific Binding
Anti-CD3[2]	Anti-CD4	Helper T Cell Subset
Anti-CD3	Anti-CD8	Cytotoxic T Cell Subset
Anti-CD4	Anti-CD8	Double Positive T Cells
Anti-CD45RA[3]	Anti-CD45RO	Naive vs Memory T Cells
Anti-CD16	Anti-CD3	NK Cells
Anti-CD3	Anti-CD19	B Cells

[1] Plus additional isotype controls as required
[2] CD3+ cells will be characterized as $CD3^{dim}$ or $CD3^{bright}$
[3] Three-color immunofluorescence using CD4

3.4 CHIMERISM

TO BE COMPLETED

APPENDICES - CORD BLOOD TRANSPLANTATION STUDY PROTOCOL

APPENDIX A

Sample Informed Consents

A.1 SAMPLE CONSENT FORM FOR PATIENTS WITH MALIGNANT DISEASES

Written Summary of Informed Consent

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH

You (your child) are being asked to take part in a clinical research study. Clinical research tries to find better ways to diagnose and treat disease. Taking part in any clinical research involves risks and benefits. You need to understand these risks and benefits to make an informed decision about whether or not to join the study. This process is known as informed consent.

This consent form gives detailed information about the research study which your doctor will discuss with you. Once you understand the study, you will be asked to sign this form if you wish to take part. You will have a copy to keep as a record.

The research study you are being asked to join is:

UNRELATED DONOR UMBILICAL CORD BLOOD AS AN ALTERNATE SOURCE OF STEM CELLS FOR TRANSPLANTATION

PURPOSE OF THE RESEARCH STUDY

You (your child) have been asked to participate in a research study to look at the ability of umbilical cord blood cells from an unrelated donor to serve as a source of stem cells for patients undergoing bone marrow replacement. Stem cells can be thought of as "parent cells" of bone marrow. These "parent cells" make new bone marrow which in turn makes red blood cells, white blood cells, and platelets. These cells are absolutely necessary to live. The main purpose of this study is to determine how well cord blood cells from unrelated donors will grow after transplantation into the patient. This study will also determine the side effects that may result from a transplant using unrelated donor cord blood . You (your child) will be one of approximately 350 patients enrolled in this study.

You (your child) have a disease of the bone marrow which is unlikely to be cured with conventional non-transplant treatment. The best results with bone marrow transplantation are obtained when the donor is a relative who has the same tissue type (HLA-type) as the patient. You (your child) do not have such a donor available. This study will investigate whether or not umbilical cord blood may be used instead of marrow cells.

Umbilical cord blood cells are a readily available source of donor stem cells. These cells are obtained by taking blood from the placenta and umbilical cord of the donor at birth and freezing them for future use. This umbilical cord blood has the potential to replace the diseased cells in your(your child)'s bone marrow that normally make white blood cells, red blood cells and platelets.

To date, approximately 1000 patients worldwide have been transplanted with umbilical cord blood from unrelated donors. There is no evidence of a difference in long-term bone marrow function between patients who have received umbilical cord blood cells as compared to patients who have received bone marrow. However, the first unrelated cord blood transplant was performed in 1993 and average follow-up is limited.

DESCRIPTION OF THE RESEARCH PROCEDURES

You (your child) will undergo an intensive treatment before transplantation of the unrelated donor umbilical cord blood cells. The intensive treatment is designed to kill any abnormal cells in your (your child's) bone marrow. The treatment will also kill all the normal cells in your (your child's) bone marrow. The treatment will include drugs and may include total body irradiation .

Starting about nine days before transplant, you (your child) will receive total body irradiation and three additional drugs. You (your child) will receive total body irradiation twice per day for four days and once on the fifth day. **If you (your child) are a male and have been diagnosed with Acute Lymphoblastic Leukemia (ALL), you may also receive testicular radiation during this time.** Then, you (your child) will receive the chemotherapy drug cyclophosphamide. The second drug you (your child) will receive is methylprednisolone. This drug will be given by vein for 3 days to suppress your (your child's) immune system. The third drug you (your child) will receive is antithymocyte globulin (ATG). You (your child) will receive this drug by vein for 3 days to further suppress your (your child's) immune system. None of these drugs are considered to be experimental. The risks of this intensive treatment are given below.

During the period of this intensive treatment and until your immune system shows signs of recovery, you (your child) will be isolated in a protective environment. As part of your (your child's) transplant procedure, you (your child) will have a central venous (e.g. Hickman) catheter inserted. This will allow blood to be drawn daily. This will also allow medicine and nutrition to be given daily without having to do several skin punctures. This common procedure is also used for patients receiving marrow transplants.

The umbilical cord blood cells will be given to you (your child) by vein the day following the intensive treatment. These cells will be given to restore normal bone marrow function. The cord blood will be administered in a small transfusion bag through your (your child's) central venous (Hickman) catheter.

Following transplantation, you (your child) will receive two medications to decrease the chance of graft versus host disease (GVHD). GVHD is the reaction of the donor cells against your (your child's) body. On the day before the cord blood transplant, you (your child) will receive the drug cyclosporine to suppress your (your child's) immune system. This will be given in your (your child's) catheter until you (your child) is again able to take medications by mouth. Beginning five days after the transplant, you (your child) will also receive more methylprednisolone. This will also be given through your (your child's) catheter until you (your child) are able to take medications by mouth. Both of these drugs will be reduced over a 3-6 month period after transplantation.

You (your child) will receive a drug called granulocyte-colony stimulating factor (G-CSF) starting the day of the transplant. G-CSF is believed to help your (your child's) new "parent cells" grow.

After the transplant, it will be necessary to monitor the ability of cord blood cells to grow in your (your child's) body. Your (your child's) doctors will examine your (your child's) blood (2 teaspoons) and bone marrow (1 teaspoon) on days 30, 60, 90, at 6 months, and yearly after transplantation. This is necessary to make sure that the cord blood cells are growing and making new bone marrow.

Your (your child's) doctors will also evaluate the recovery of your (your child's) immune system. This will require a blood sample (2 teaspoons) on days 30, 60, 90, at months 6, 9, 12, 18, 24, and yearly until your (your child's) immune system is normal. Two-three teaspoonful of additional blood may also be taken and used for HLA typing, chimerism assays and storage for future studies. These studies will not directly benefit you (your child) but will hopefully benefit patients in the future.

The blood will be obtained from your (your child's) catheter until the catheter is removed. Afterwards, it will be collected through your (your child's) vein. Bone marrow samples will be removed from your (your child's) hip bones using a needle and syringe. These procedures may cause brief pain to you (your child).

You (your child) will be followed very closely by the transplant doctors for the first year after transplantation. Your (your child's) doctors will then examine you (your child) at least once a year or more often if there are continuing medical problems.

You (your child) may also be asked to give informed consent for participation in other studies. These studies typically deal with prevention and treatment of transplant problems. You (your child) will only be asked to take part if you (your child) are at risk for developing the particular problem related to the study.

DESCRIPTION OF SIDE EFFECTS AND TREATMENT - RISKS

The side effects associated with transplantation can be uncomfortable and in some cases are potentially dangerous, life-threatening, or fatal. Because this is a research study and the treatments are relatively new, there may be additional side effects which are not known or predictable at this time. Both radiation and drugs may lead to an increased chance of malignancy later in life. **As is true with all medication and treatment, other unexpected side effects may occur.** The known or possible side effects of the pre and post-transplant treatments you may receive as part of this study are listed below:

1. **Total body irradiation.** Total body irradiation (TBI) may cause transient nausea and vomiting. Anti-vomiting medications can be given as a treatment. TBI may also cause mucositis, an irritation of the lining of the mouth, esophagus, bowel, and rectum. This can lead to mouth pain and ulcers, difficulty swallowing and eating, cramps, and diarrhea. Swelling of the salivary glands may be another problem. Low blood counts, hair loss, and temporary redness of the skin, with later darkening of the skin may also occur.

Late effects of TBI include cataracts. Cataracts are cloudiness in the lens of the eye. They can be surgically corrected. There can be interference with normal growth which can sometimes be corrected with hormone replacement. There can be thyroid and adrenal gland insufficiency. This can be treated with hormone replacement. Permanent sterility may occur. A secondary cancer can also occur. Younger children may be at risk for permanent interference with mental functioning.

2. **Cyclophosphamide.** The high doses of cyclophosphamide may cause low blood counts, hair loss, mucositis and darkening of the skin, and transient nausea and vomiting. Anti-vomiting medications can be given to treat the transient nausea and vomiting.

   Less common side effects include water retention which can be treated with medicines; potentially fatal damage to the lungs or liver or weakening of the heart muscle; bloody urine, for which precautions will be taken but which is occasionally very severe.

3. **Antithymocyte globulin (ATG).** Antithymocyte globulin (ATG) has been associated with fever, feeling weak, joint pains, rash, low blood counts, hemolysis, and breakdown of red cells in the blood. More severe toxicities include hives, difficulty breathing, and low blood pressure (hypotension.)

4. **Cyclosporine.** The possible side effects of cyclosporine include growth of excessive body hair, reddened gums, increased blood pressure, and liver and kidney damage. In rare instances, central nervous system toxicity with tremor, somnolence (sleepiness), confusion, and seizures may occur. These are reversible after stopping the medication. The amount of cyclosporine in the blood will be monitored to keep these problems to a minimum.

5. **Corticosteroids.** Corticosteroids are prednisone (when taken by mouth) and methylprednisolone (when taken by vein). The possible side effects include weight gain and water retention, puffiness of the face, high blood pressure, high blood glucose, bleeding from the stomach and intestines, and personality changes including depression and psychosis. High blood pressure can be treated with medications. High blood glucose can be corrected with insulin.

   Although long term corticosteroid administration is not proposed in this study, corticosteroids can be used for treatment of acute and chronic GVHD. Long-term use has been associated with muscle weakness and wasting, cataracts, and suppression of growth in children. It has also been associated with bone thinning. This has sometimes progressed to areas of bone death especially at the knees and hips. In addition, steroids may make you (your child) prone to infections.

6. **Granulocyte-Colony Stimulating Factor (G-CSF).** G-CSF will be used to help regrow the new bone marrow. In general, this drug has few serious side effects. Side effects which have been reported include fever, feeling tired, bone pain, and enlargement of the spleen. Additionally, there is a rare risk of allergic reaction to this drug.

Busulfan and Melphalan only used on specific patients who do not receive TBI and cyclophosphamide

7. **Busulfan.** Busulfan may cause vomiting, diarrhea, and seizures. Medication will be given to minimize or prevent some of these side effects. Late effects, which are usually temporary, may include hair loss and increased color of the skin (hyper pigmentation.) Some patients may develop a rash. Some patients may develop mouth sores (mucositis). Some patients may develop abnormal function of the liver or lungs which may be mild, moderate, or potentially fatal if severe.

8. **Melphalan.** The major side effects of melphalan include severe suppression of blood counts, nausea, vomiting, mouth and throat sores (mucositis) and diarrhea. Scarring of the lungs (pulmonary fibrosis) has been reported to occur after the use of melphalan. Serious allergic reactions including low blood pressure (hypotension) and heart stoppage (cardiac arrest) following use of this drug also have been reported.

In addition to the intensive pre/post-transplant treatment side effects, the following risks may occur:

1. **Bone marrow depression.** Bone marrow depression means decreased blood counts, including red blood cells, white blood cells, and platelets. Until the new cord blood cells begin to grow, you (your child) are at risk of developing infections or bleeding. Infections can be treated with antibiotics. Bleeding can be corrected, at least in part, by transfusions. However, there are risks associated with the transfusions of red blood cells and platelets during the post-transplantation period. These risks include fluid overload; serious allergic reactions; and infections, including hepatitis, cytomegalovirus (CMV), and human immunodeficiency virus (HIV), the virus that causes AIDS. All blood products will be screened for these infections in order to reduce the chance that the blood contains these viruses.

2. **Graft Failure.** The cord blood may fail to "take" or engraft. This may occur in a significant number of patients, depending on the disease for which you (your child) are being transplanted. It is possible that the cord blood will grow, but not work normally. This will result in low blood counts for a long period of time. Graft failure is typically fatal. Should the graft fail, you (your child) will not have access to additional stem cells from the donor.

3. **Graft-versus-host-disease (GVHD).** This condition results from a reaction of the transplanted cord blood cells against your (your child's) body and organs. This reaction ranges from a mild skin disorder to severe involvement of the skin, liver, and/or gut. It may be fatal in some patients. You (your child) will be monitored for this complication and given specific treatment to prevent and treat it. There are two forms: acute (early) and chronic (late).

   Acute GVHD may produce skin rashes, liver disease, diarrhea, and an increased risk of infection. All of these can range in severity from mild to fatal. To confirm the diagnosis of acute GVHD, you (your child) may be required to have a skin biopsy and possibly a liver or

gut biopsy. The treatment of acute GVHD requires you (your child) to take high doses of corticosteroids. Occasionally, other drugs such as antithymocyte globulin (ATG) are given.

Acute GVHD can persist and become chronic GVHD. Chronic GVHD can also appear in patients without prior acute GVHD. Chronic GVHD may also produce skin rashes, liver disease, diarrhea and an increased risk of infection. Chronic GVHD may be mild and respond to agents which suppress the immune system, or it could be very severe. It may also last for over a year.

4. **Veno-occlusive disease of the liver.** This is a complication that results from high doses of chemotherapy, or radiation, or both. Patients who suffer this develop jaundice (yellowish skin), liver function abnormalities, abdominal swelling, and abdominal or shoulder pain. These usually occur in the first month after transplant. Although most patients recover completely, veno-occlusive disease can be fatal.

5. **Interstitial pneumonia.** Some patients suffer severe lung problems from either a viral infection called cytomegalovirus (CMV) or a reaction to the chemotherapy given. Although treatments are available, this form of pneumonia can be fatal.

6. **Recurrence of disease.** It is possible that your (your child's) disease may recur even if the transplant is successful. Patients transplanted in relapse have a higher risk of disease recurrence than patients not in relapse at the time of transplant.

7. **Risk of a secondary malignancy.** A second malignancy different from the primary disease may occur following chemotherapy and irradiation.

8. **Central nervous system damage.** Patients with certain kinds of leukemia are at increased risk for central nervous system involvement. Frequently, such patients may have previously received radiation treatments to the head and spine, and/or chemotherapy treatment to the spinal cord fluid. These types of previous treatments may increase the risk of damage to the central nervous system when such patients then receive TBI before the transplant.

   Central nervous system damage may include difficulty with thinking and poor ability to concentrate. Subsequent mild learning disability in children may result. There may also be forgetfulness, personality changes, and weakness or paralysis in very unusual cases.

9. **Serious infections.** Full and complete recovery of your (your child's) immune system may take several months following successful marrow engraftment. During this time, there is an increased risk of infections. You (your child) will be prescribed certain medications to reduce the chance of those infections. Preventive treatment is not always effective. If you (your child) have an infection, you (your child) may have to be re-hospitalized after your transplant. Infections may be fatal. Fatal complications of infections include life-threatening pneumonia, liver disease, and/or loss of your (your child's) new bone marrow.

10. **Organ damage.** In addition to the complications listed above, it is possible that the transplant procedure will result in damage to your (your child's) heart, lungs, kidneys and/or

liver. This damage may be mild, moderate or severe. Severe damage may be fatal. Long-term complications from the transplant procedure include the potential for growth problems, hormonal and learning difficulties, and infertility.

11. **Genetic Disease Transmission**. There is the potential that certain genetic diseases (such as thalassemia or adrenoleukodystrophy) may be passed through the cord blood transplant. These diseases are very rare. Each cord blood is carefully screened to further reduce the possibility that these genetic diseases are present.

ALTERNATIVE TREATMENTS

The other options potentially available to you are autologous (self) transplantation, a bone marrow transplant from an unrelated donor, a bone marrow transplant from a family member who has a different tissue type (HLA- type), chemotherapy, or no therapy other than supportive care. Each option will be fully explained to you.

You will be informed of the progress of this research study. During the time you are part of it, you will be informed of any new findings which might affect your willingness to continue.

BENEFITS

When compared to unrelated bone marrow transplants, cord blood transplants may have some benefits. These include immediate availability, reduced risk of viral contamination and absence of risk to the cord blood donor. Although it is our hope that this research study will be of benefit to you (your child), and that it will help other patients, we cannot say that it will be directly beneficial to you (your child).

FINANCIAL COST

If you receive a cord blood unit from the COBLT cord blood banks, you will not be responsible for any costs associated with the shipping or testing of the cord blood unit. You will be responsible for the costs of hospitalization, physician's visits, and established diagnostic laboratory tests and the chemotherapy drugs, radiation therapy, and other medicines used in your care. These costs will be the same as for any other bone marrow transplant patient. Further, your (your child's) financial responsibility for this treatment will not be different from that of other patients treated at _____.

If you (your child) are injured as a result of taking part in this research study, emergency care, hospitalization and outpatient care will be made available by the hospital. This will be billed to you as part of your medical expenses. No money will be provided by the hospital as compensation for a research-related injury.

PRIVACY

We request that you permit _____ to use the clinical data included in your treatment records for reporting the results of this program. The results will be reported to the

National Heart, Lung and Blood Institute, the EMMES Corporation (the Medical Coordinating Center), the Food and Drug Administration, the National Cancer Institute or drug sponsor, and the scientific community. No mention of your (your child's) name or any identifying information will appear in any of these reports. Data will be collected until April 2004 and may continue indefinitely after that date.

Your (your child's) research and hospital records are confidential. Your (your child's) name or any other information which can identify you (your child) will not be used in study reports or publications. It is to be understood, however, that representatives from the Medical Coordinating Center, the National, Heart, Lung and Blood Institute, and the Food and Drug Administration or other authorized agencies may inspect your clinical records without removal of such identifying information.

RIGHT TO REFUSE OR WITHDRAW

The choice to enter or not enter this study is yours. You are in a position to make a decision if you understand what the doctor has explained and what you have read about the research study and possible forms of care. If you decide not to take part, the other choices are available to you (your child) without prejudice. If you (your child) begin the study, you still have the right to withdraw at any time. If you (your child) should withdraw, you (your child) will be offered other available care which suits your (your child's) needs and medical condition. In either case, there will be no penalty or loss of benefits to which you (your child) are entitled.

If you decide to withdraw from this proposed treatment **before** receiving the high doses of drugs, we will continue to offer you (your child) the best available alternative care according to your (your child's) needs and physical condition. Please submit in writing to _____(*Name of IRB Contact Person*) of your (your child's) decision to withdraw from the study. However, you should understand that if you withdraw from this treatment plan **after** administration of TBI and high doses of chemotherapy, but before infusion of the umbilical cord blood cells, you (your child) might die. The reason is that you (your child) would be left without enough cells in the marrow to produce the white blood cells, platelets and red cells necessary to sustain you (your child).

INSTITUTIONAL REVIEW

_____ (*Name of IRB*) is legally responsible for making sure that research with patients is appropriate and that the patient's rights and welfare are protected. It has reviewed and approved this study.

The physicians in charge of this study are _____ (*Names of Physicians*). If you need more information about this study before you decide to join, or at any other time, you may wish to contact one of them. A non-physician whom you may call for information about the consent process, research patient's rights, or research-related injury is _____ (*Name of Contact Person*) at _____ (*Phone Number*).

PATIENT INFORMED CONSENT
FOR CLINICAL RESEARCH

TITLE: UNRELATED DONOR UMBILICAL CORD BLOOD AS AN ALTERNATE SOURCE OF STEM CELLS FOR TRANSPLANTATION

PURPOSE: The primary purpose of this study is to determine how well cord blood cells from unrelated donors will grow in the new patients' bodies. This study will also determine the side effects that may result from a transplant using unrelated donor cord blood "parent cells".

STATEMENT OF PHYSICIAN OBTAINING INFORMED CONSENT:

I have fully explained this research study to the patient or guardian of patient. In my judgement, and the patient's or guardian's, there was sufficient access to information, including risks and benefits, to make an informed decision.

DATE: **PHYSICIAN'S SIGNATURE:** _____

PHYSICIAN'S NAME: _____

PATIENT'S (OR GUARDIAN'S) STATEMENT:

I have read the description of the clinical research study or have had it translated into a language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my/the patient's participation is voluntary. I know enough about the purpose, methods, risks, and benefits of the research study to judge that I want (the patient) to take part in it.

PATIENT NUMBER: _____ **PATIENT'S SIGNATURE:**_____
(Guardian)

PATIENT'S NAME: _____
(Print)

DATE: _____

ASSENT: _____

If the patient is a minor, I have obtained his/her assent to participate in the study to the best of his/her ability to understand.

DATE: _____ **PHYSICIAN'S SIGNATURE:** _____

A.2 SAMPLE CONSENT FORM FOR PATIENTS WITH NON MALIGNANT DISEASES

Written Summary of Informed Consent

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH

You (your child) are being asked to take part in a clinical research study. Clinical research tries to find better ways to diagnose and treat disease. Taking part in any clinical research involves risks and benefits. You need to understand these risks and benefits to make an informed decision about whether or not to join the study. This process is known as informed consent.

This consent form gives detailed information about the research study which your doctor will discuss with you. Once you understand the study, you will be asked to sign this form if you wish to take part. You will have a copy to keep as a record.

The research study you are being asked to join is:

UNRELATED DONOR UMBILICAL CORD BLOOD AS AN ALTERNATE SOURCE OF STEM CELLS FOR TRANSPLANTATION

PURPOSE OF THE RESEARCH STUDY

You (your child) have been asked to participate in a research study to look at the ability of umbilical cord blood cells from an unrelated donor to serve as a source of stem cells for patients undergoing bone marrow replacement. Stem cells can be thought of as "parent cells" of bone marrow. These "parent cells" make new bone marrow which in turn makes red blood cells, white blood cells, and platelets. These cells are absolutely necessary to live. The main purpose of this study is to determine how well cord blood cells from unrelated donors will grow after transplantation into the patient. This study will also determine the side effects that may result from a transplant using unrelated donor cord blood. You (your child) will be one of approximately 350 patients enrolled in this study.

You (your child) have a disease of the bone marrow which is unlikely to be cured with conventional non-transplant treatment. The best results with bone marrow transplantation are obtained when the donor is a relative who has the same tissue type (HLA-type) as the patient. You (your child) do not have such a donor available. This study will investigate whether or not umbilical cord blood may be used instead of marrow cells.

Umbilical cord blood cells are a readily available source of donor stem cells. These cells are obtained by taking blood from the placenta and umbilical cord of the donor at birth and freezing them for future use. This umbilical cord blood has the potential to replace the diseased cells in your (your child)'s bone marrow that normally make white blood cells, red blood cells and platelets.

To date, approximately 1,000 patients worldwide have been transplanted with umbilical cord blood from unrelated donors. There is no evidence of a difference in long-term bone marrow function between patients who have received umbilical cord blood cells as compared to patients who have received bone marrow. However, the first unrelated cord blood transplant was performed in 1993 and follow-up is limited.

DESCRIPTION OF THE RESEARCH PROCEDURES

You (your child) will undergo an intensive treatment before transplantation of the unrelated donor umbilical cord blood cells. The intensive treatment is designed to kill any abnormal cells in your (your child's) bone marrow. The treatment will also kill all the normal cells in your (your child's) bone marrow.

For patients with Fanconi anemia:

The treatment will include drugs and total body irradiation.

You (your child) will receive cyclophosphamide (Cytoxan) and fludarabine for four days intravenously and total body irradiation once.

In addition, you (your child) will receive two drugs to suppress the immune system. The first drug is methylprednisolone. This drug will be given by vein for 5 days before transplant. The second is antithymocyte globulin (ATG). This drug will be given by vein for 5 days before transplant and on day 5, 7, 9, 11, and 13 after transplant. None of these drugs are considered to be experimental. The risks of this intensive treatment are given below.

For patients with severe aplastic anemia:

The treatment will include drugs and total body irradiation.

You (your child) will receive cyclophosphamide (Cytoxan) intravenously for two days and 9 doses of total body irradiation.

In addition, you (your child) will receive two drugs to suppress the immune system. The first drug is methylprednisolone. This drug will be given by vein for 3 days before transplant. The second drug is antithymocyte globulin (ATG). This drug will be given by vein for 3 days before transplant. None of these drugs are considered to be experimental. The risks of this intensive treatment are given below.

For patients with a storage disease (e.g. Hurler syndrome, Maroteaux-Lamy syndrome, adrenoleukodystrophy, metachromatic leukodystrophy, globoid cell leukodystrophy):

The treatment will include two chemotherapy drugs and two drugs to suppress the immune system.

The chemotherapy drugs you (your child) will receive are busulfan orally for four days, and cyclophosphamide (Cytoxan) intravenously for four days.

In addition, you (your child) will receive two drugs to suppress the immune system. The first drug is methylprednisolone. This drug will be given by vein for 3 days before transplant. The second drug you (your child) will receive is antithymocyte globulin (ATG). You (your child) will receive this drug by vein for 3 days before transplant. None of these drugs are considered to be experimental. The risks of this intensive treatment are given below.

For patients with an immunodeficiency state (e.g. severe combined immune deficiency (SCID), Wiskott-Aldrich syndrome) or other bone marrow failure syndrome:

The treatment will include drugs.

You (your child) will receive busulfan orally for four days, and cyclophosphamide (Cytoxan) intravenously for four days.

In addition, you (your child) will receive two drugs to suppress the immune system. The first drug is methylprednisolone. This drug will be given by vein for 3 days before transplant. The second drug is antithymocyte globulin (ATG). This drug will be given by vein for 3 days before transplant. None of these drugs are considered to be experimental. The risks of this intensive treatment are given below.

For patients with a histiocytosis syndrome (e.g. HLH, FEL):

The treatment will include drugs.

You (your child) will receive busulfan orally for four days, etoposide (VP-16) intravenously for three days, and cyclophosphamide (Cytoxan) intravenously for four days.

In addition, you (your child) will receive two drugs to suppress the immune system. The first drug is methylprednisolone. This drug will be given by vein for 3 days before transplant. The second drug is antithymocyte globulin (ATG). This drug will be given by vein for 3 days before transplant. None of these drugs are considered to be experimental. The risks of this intensive treatment are given below.

During the period of this intensive treatment and until your immune system shows signs of recovery, you (your child) will be isolated in a protective environment. As part of your (your child's) transplant procedure, you (your child) will have a central venous (Hickman) catheter inserted. This will allow blood to be drawn daily. This will also allow medicine and nutrition to be given daily without having to do several skin punctures. This common procedure is also used for patients receiving marrow transplants.

The umbilical cord blood cells will be given to you (your child) by vein the day following the intensive treatment. These cells will be given back to you (your child) to restore normal bone marrow function. The cord blood will be administered in a small transfusion bag through your (your child's) central venous (Hickman) catheter.

Following transplantation, you (your child) will receive two medications to decrease the chance of graft versus host disease (GVHD). GVHD is the reaction of the donor cells against your (your child's) body. On the day before the cord blood transplant, you (your child) will receive the drug cyclosporine to suppress your (your child's) immune system. This will be given in your (your child's) catheter until you (your child) is again able to take medications by mouth. Beginning five days after the transplant, you (your child) will also receive more methylprednisolone. This will also be given through your (your child's) catheter until you (your child) are able to take medications by mouth. Both of these drugs will be reduced over a 3-6 month period after transplantation.

You (your child) will receive a drug called granulocyte-colony stimulating factor (G-CSF) starting the day of the transplant. G-CSF will help your (your child's) new "parent cells" grow.

After the transplant, it will be necessary to monitor the ability of cord blood cells to grow in your (your child's) body. Your (your child's) doctors will examine your (your child's) blood (2 teaspoons) and bone marrow (1 teaspoon) on days 30, 60, 90, at 6 months, and yearly after transplantation. This is necessary to make sure that the cord blood cells are growing and making new bone marrow.

Your (your child's) doctors will also evaluate the recovery of your (your child's) immune system. This will require a blood sample (2 teaspoons) on days 30, 60, 90, at months 6, 9, 12, 18, 24, and yearly until your (your child's) immune system is normal. Two-three teaspoonful of additional blood may also be taken and used for HLA typing, chimerism assays and storage for future studies. These studies will not directly benefit you (your child) but will hopefully benefit patients in the future.

The blood will be obtained from your (your child's) catheter until the catheter is removed. Afterwards, it will be collected through your (your child's) vein. Bone marrow samples will be removed from your (your child's) hip bones using a needle and syringe. These procedures may cause brief pain to you (your child).

You (your child) will be followed very closely by the transplant doctors for the first year after transplantation. Your (your child's) doctors will then examine you (your child) at least once a year or more often if there are continuing medical problems.

You (your child) may also be asked to give informed consent for participation in other studies. These studies typically deal with prevention and treatment of transplant problems. You (your child) will only be asked to take part if you (your child) are at risk for developing the particular problem related to the study.

DESCRIPTION OF SIDE EFFECTS AND TREATMENT - RISKS

The side effects associated with transplantation can be uncomfortable and in some cases are potentially dangerous, life-threatening, or fatal. Because this is a research study and the treatments are relatively new, there may be additional side effects which are not known or predictable at this time. Both radiation and drugs may lead to an increased chance of malignancy later in life. **As is true with all medication and treatment, other unexpected side effects may occur.** The known or possible side effects of the pre and post-transplant treatments you may receive as part of this study are listed below:

The following therapy is used for patients with Fanconi anemia, severe aplastic anemia, and storage disease:

- **Total body irradiation.** Total body irradiation (TBI) may cause transient nausea and vomiting. Anti-vomiting medications can be given as a treatment. TBI may also cause mucositis, an irritation of the lining of the mouth, esophagus, bowel, and rectum. This can lead to mouth pain and ulcers, difficulty swallowing and eating, cramps, and diarrhea. Swelling of the salivary glands may be another problem. Low blood counts, hair loss, and temporary redness of the skin, with later darkening of the skin may also occur.

  Late effects of TBI include cataracts. Cataracts are cloudiness in the lens of the eye. They can be surgically corrected. There can be interference with normal growth which can sometimes be corrected with hormone replacement. There can be thyroid and adrenal gland insufficiency. This can be treated with hormone replacement. Permanent sterility may occur. A secondary cancer can also occur. Younger children may be at risk for permanent interference with mental functioning.

The following therapy is used for patients with a storage disease, an immunodeficiency, or a histiocytosis syndrome.

- **Busulfan.** Busulfan may cause vomiting, diarrhea, and seizures. Medication will be given to minimize or prevent some of these side effects. Late effects, which are usually temporary, may include hair loss and increased color of the skin (hyper pigmentation.) Some patients may develop a rash. Some patients may develop mouth sores (mucositis). Some patients may develop abnormal function of the liver or lungs which may be mild, moderate, or potentially fatal if severe.

The following therapy is used for patients with a histiocytosis syndrome:

- **Etoposide (VP-16).** Etoposide (VP-16) has been associated with fever, abnormal depression of all cellular elements of the blood, nerve damage, and elevated liver function tests. More severe toxicities include severe allergic reactions , low blood pressure (hypotension), and secondary leukemia.

The following therapies are used for all patients:

- **Cyclophosphamide.** The high doses of cyclophosphamide may cause low blood counts, hair loss, mucositis and darkening of the skin, and transient nausea and vomiting. Anti-vomiting medications can be given to treat the transient nausea and vomiting.

  Less common side effects include water retention which can be treated with medicines; potentially fatal damage to the lungs or liver or weakening of the heart muscle; bloody urine, for which precautions will be taken but which is occasionally very severe.

- **Antithymocyte globulin (ATG).** Antithymocyte globulin (ATG) has been associated with fever, feeling weak, joint pains, rash, low blood counts, hemolysis, and breakdown of red cells in the blood. More severe toxicities include hives, difficulty breathing, and low blood pressure (hypotension.)

- **Cyclosporine.** The possible side effects of cyclosporine include growth of excessive body hair, reddened gums, increased blood pressure, and liver and kidney damage. In rare instances, central nervous system toxicity with tremor, somnolence (sleepiness), confusion, and seizures may occur. These are reversible after stopping the medication. The amount of cyclosporine in the blood will be monitored to keep these problems to a minimum.

- **Corticosteroids.** Corticosteroids are prednisone (when taken by mouth) and methylprednisolone (when taken by vein). The possible side effects include weight gain and water retention, puffiness of the face, high blood pressure, high blood glucose, bleeding from the stomach and intestines, and personality changes including depression and psychosis. High blood pressure can be treated with medications. High blood glucose can be corrected with insulin.

  Although long term corticosteroid administration is not proposed in this study, corticosteroids can be used for treatment of acute and chronic GVHD. Long-term use has been associated with muscle weakness and wasting, cataracts, and suppression of growth in children. It has also been associated with bone thinning. This has sometimes progressed to areas of bone death especially at the knees and hips. In addition, steroids may make you (your child) prone to infections.

- **Granulocyte-Colony Stimulating Factor (G-CSF).** G-CSF will be used to help regrow the new bone marrow. In general, this drug has few serious side effects. Side effects which have been reported include fever, feeling tired, bone pain, and enlargement of the spleen. Additionally, there is a rare risk of allergic reaction to this drug.

In addition to the intensive pre/post-transplant treatment side effects, the following risks may occur:

1. **Bone marrow depression.** Bone marrow depression means decreased blood counts, including red blood cells, white blood cells, and platelets. Until the new cord blood cells begin to grow, you (your child) are at risk of developing infections or bleeding. Infections can be treated with antibiotics. Bleeding can be corrected, at least in part, by transfusions. However, there are risks associated with the transfusions of red blood cells and platelets during the post-transplantation period. These risks include fluid overload; serious allergic reactions; and infections, including hepatitis, cytomegalovirus (CMV), and human immunodeficiency virus (HIV), the virus that causes AIDS. All blood products will be screened for these infections in order to reduce the chance that the blood contains these viruses.

2. **Graft Failure.** The cord blood may fail to "take" or engraft. This may occur in a significant number of patients, depending on the disease for which you (your child) are being transplanted. It is possible that the cord blood will grow, but not work normally. This will

result in low blood counts for a long period of time. Graft failure is typically fatal. Should the graft fail, you (your child) will not have access to additional stem cells from the donor.

3. **Graft-versus-host-disease (GVHD).** This condition results from a reaction of the transplanted cord blood cells against your (your child's) body and organs. This reaction ranges from a mild skin disorder to severe involvement of the skin, liver, and/or gut. It may be fatal in some patients. You (your child) will be monitored for this complication and given specific treatment to prevent and treat it. There are two forms: acute (early) and chronic (late).

   Acute GVHD may produce skin rashes, liver disease, diarrhea, and an increased risk of infection. All of these can range in severity from mild to fatal. To confirm the diagnosis of acute GVHD, you (your child) may be required to have a skin biopsy and possibly a liver or gut biopsy. The treatment of acute GVHD requires you (your child) to take high doses of corticosteroids. Occasionally, other drugs such as antithymocyte globulin (ATG) are given.

   Acute GVHD can persist and become chronic GVHD. Chronic GVHD can also appear in patients without prior acute GVHD. Chronic GVHD may also produce skin rashes, liver disease, diarrhea and increased risk of infection. Chronic GVHD may be mild and respond to agents which suppress the immune system, or it could be very severe. It may also last for over a year.

4. **Veno-occlusive disease of the liver.** This is a complication that results from high doses of chemotherapy, or radiation, or both. Patients who suffer this develop jaundice (yellowish skin), liver function abnormalities, abdominal swelling, and abdominal or shoulder pain. These usually occur in the first month after transplant. Although most patients recover completely, veno-occlusive disease can be fatal.

5. **Interstitial pneumonia.** Some patients suffer severe lung problems from either a viral infection called cytomegalovirus (CMV) or a reaction to the chemotherapy given. Although treatments are available, this form of pneumonia can be fatal.

6. **Recurrence of disease.** It is possible that your (your child's) disease may recur even if the transplant is successful. Patients transplanted in relapse have a higher risk of disease recurrence than patients not in relapse at the time of transplant.

7. **Risk of a secondary malignancy.** A second malignancy different from the primary disease may occur following chemotherapy and irradiation.

8. **Central nervous system damage.** Patients with certain kinds of leukemia are at increased risk for central nervous system involvement. Frequently, such patients may have previously received radiation treatments to the head and spine, and/or chemotherapy treatment to the spinal cord fluid. These types of previous treatments may increase the risk of damage to the central nervous system when such patients then receive TBI before the transplant.

Central nervous system damage may include difficulty with thinking and poor ability to concentrate. Subsequent mild learning disability in children may result. There may also be forgetfulness, personality changes, and weakness or paralysis in very unusual cases.

9. **Serious infections.** Full and complete recovery of your (your child's) immune system may take several months following successful marrow engraftment. During this time, there is an increased risk of infections. You (your child) will be prescribed certain medications to reduce the chance of those infections. Preventive treatment is not always effective. If you (your child) have an infection, you (your child) may have to be re-hospitalized after your transplant. Infections may be fatal. Fatal complications of infections include life-threatening pneumonia, liver disease, and/or loss of your (your child's) new bone marrow.

10. **Organ damage.** In addition to the complications listed above, it is possible that the transplant procedure will result in damage to your (your child's) heart, lungs, kidneys and/or liver. This damage may be mild, moderate or severe. Severe damage may be fatal. Long-term complications from the transplant procedure include the potential for growth problems, hormonal and learning difficulties, and infertility.

11. **Genetic Disease Transmission**. There is the potential that certain genetic diseases (such as thalassemia or adrenoleukodystrophy) may be passed through the cord blood transplant. These diseases are very rare. Each cord blood is carefully screened to further reduce the possibility that these genetic diseases are present.

ALTERNATIVE TREATMENTS

The other options potentially available to you are autologous (self) transplantation, a bone marrow transplant from an unrelated donor, a bone marrow transplant from a family member who has a different tissue type (HLA- type), chemotherapy, or no therapy other than supportive care. Each option will be fully explained to you.

You will be informed of the progress of this research study. During the time you are part of it, you will be informed of any new findings which might affect your willingness to continue.

BENEFITS

When compared to unrelated bone marrow transplants, cord blood transplants may have some benefits. These include immediate availability, reduced risk of viral contamination and absence of risk to the cord blood donor. Although it is our hope that this research study will be of benefit to you (your child), and that it will help other patients, we cannot say that it will be directly beneficial to you (your child).

FINANCIAL COST

If you receive a cord blood unit from the COBLT cord blood banks, you will not be responsible for any costs associated with the shipping or testing of the cord blood unit. You will be responsible for the costs of hospitalization, physician's visits, and established diagnostic laboratory tests and the chemotherapy drugs, radiation therapy, and other medicines used in your care. These costs will be the same as for any other bone marrow transplant patient. Further, your (your child's) financial responsibility for this treatment will not be different from that of other patients treated at _____.

If you (your child) are injured as a result of taking part in this research study, emergency care, hospitalization and outpatient care will be made available by the hospital. This will be billed to you as part of your medical expenses. No money will be provided by the hospital as compensation for a research-related injury.

PRIVACY

We request that you permit _____ to use the clinical data included in your treatment records for reporting the results of this program. The results will be reported to the National Heart, Lung and Blood Institute, the EMMES Corporation (the Medical Coordinating Center), the Food and Drug Administration, the National Cancer Institute or drug sponsor, and the scientific community. No mention of your (your child's) name or any identifying information will appear in any of these reports. Data will be collected until April 2004 and may continue indefinitely after that date.

Your (your child's) research and hospital records are confidential. Your (your child's) name or any other information which can identify you (your child) will not be used in study reports or publications. It is to be understood, however, that representatives from the Medical Coordinating Center, the National, Heart, Lung and Blood Institute, and the Food and Drug Administration or other authorized agencies may inspect your clinical records without removal of such identifying information.

RIGHT TO REFUSE OR WITHDRAW

The choice to enter or not enter this study is yours. You are in a position to make a decision if you understand what the doctor has explained and what you have read about the research study and possible forms of care. If you decide not to take part, the other choices are available to you (your child) without prejudice. If you (your child) begin the study, you still have the right to withdraw at any time. If you (your child) should withdraw, you (your child) will be offered other available care which suits your (your child's) needs and medical condition. In either case, there will be no penalty or loss of benefits to which you (your child) are entitled.

If you decide to withdraw from this proposed treatment **before** receiving the high doses of drugs, we will continue to offer you (your child) the best available alternative care according to your (your child's) needs and physical condition. **Please submit in writing to _____(*Name of IRB Contact Person*) of your (your child's) decision to withdraw from the study.** However,

you should understand that if you withdraw from this treatment plan **after** administration of TBI or high doses of chemotherapy, but before infusion of the umbilical cord blood cells, you (your child) might die. The reason is that you (your child) would be left without enough cells in the marrow to produce the white blood cells, platelets and red cells necessary to sustain you (your child).

INSTITUTIONAL REVIEW

_____ (*Name of IRB*) is legally responsible for making sure that research with patients is appropriate and that the patient's rights and welfare are protected. It has reviewed and approved this study.

The physicians in charge of this study are _____ (*Names of Physicians*). If you need more information about this study before you decide to join, or at any other time, you may wish to contact one of them. A non-physician whom you may call for information about the consent process, research patient's rights, or research-related injury is _____ (*Name of Contact Person*) at _____ (*Phone Number*).

PATIENT INFORMED CONSENT FOR CLINICAL RESEARCH

TITLE: UNRELATED DONOR UMBILICAL CORD BLOOD AS AN ALTERNATE SOURCE OF STEM CELLS FOR TRANSPLANTATION

PURPOSE: The primary purpose of this study is to determine how well cord blood cells from unrelated donors will grow in the new patients' bodies. This study will also determine the side effects that may result from a transplant using unrelated donor cord blood "parent cells".

STATEMENT OF PHYSICIAN OBTAINING INFORMED CONSENT:

I have fully explained this research study to the patient or guardian of patient. In my judgement, and the patient's or guardian's, there was sufficient access to information, including risks and benefits, to make an informed decision.

DATE: **PHYSICIAN'S SIGNATURE:** _____

PHYSICIAN'S NAME: _____

PATIENT'S (OR GUARDIAN'S) STATEMENT:

I have read the description of the clinical research study or have had it translated into a language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my/the patient's participation is voluntary. I know enough about the purpose, methods, risks, and benefits of the research study to judge that I want (the patient) to take part in it.

PATIENT NUMBER: _____ **PATIENT'S SIGNATURE:**_____
(Guardian)

PATIENT'S NAME: _____
(Print)

DATE: _____

ASSENT: _____

If the patient is a minor, I have obtained his/her assent to participate in the study to the best of his/her ability to understand.

DATE: _____ **PHYSICIAN'S SIGNATURE:** _____

USE OF UMBILICAL CORD AND PLACENTAL BLOOD FROM UNRELATED DONORS AS A SOURCE OF HEMATOPOIETIC STEM CELLS

ASSENT (AGES 8-17 YEARS)

BACKGROUND INFORMATION

After you are treated with chemotherapy and irradiation, your bone marrow no longer produces red blood cells which carry oxygen around the body, white blood cells which fight infection, and platelets which cause your blood to clot when you are cut. Because we all need these cells to live, it is necessary to put back some of the bone marrow's parent cells which will make the bone marrow grow back.

Doctors have learned that these parent cells of the bone marrow can be found in a newborn baby's blood. While we never take blood from a baby, there is some blood left in the umbilical cord and placenta which helps nourish the baby before it is born. We get this blood and save it. It happens that we have found cord blood that matches you. We will give this blood to you after you have completed your chemotherapy and irradiation so that your new bone marrow will grow back.

PROCEDURE

The cord blood will be given through your Hickman catheter either in small syringes or bags. When the blood gets into your body, you may feel sick to your stomach but that will go away quickly. This happens because there are certain drugs in the blood to help keep it safe while it is frozen.

Before the transplant, you will be given a drug called "ATG" and a drug called methylprednisolone. After the transplant, you will also be given a drug called cyclosporine and continued on methylprednisolone. All these drugs can be given through your Hickman.

It will be necessary to check your blood and bone marrow after the transplant to make sure your new bone marrow is growing. Your doctors will do a bone marrow test 21 days, 60 days, and 90 days after the transplant. Blood tests will also be done by taking blood through your Hickman.

RISKS/DISCOMFORTS

It is possible that your new bone marrow will not grow back. If the bone marrow does not grow after the cord blood transplantation, then you will need to get antibiotics since you will not be able to fight infections, and you will need to get blood transfusions since your new bone marrow will not be making new blood cells. There are several possible treatments for this that may help it grow; it may even be necessary to do a second transplant using your own or someone else's bone marrow.

Cyclosporine is a drug that can cause high blood pressure, headaches, and kidney problems. Your doctors will check your blood to make sure that you are getting the right dose of Cyclosporine and that your kidneys are working OK. ATG may cause fevers, rash, joint pain, tiredness, and difficulty breathing. Methylprednisolone may cause nausea, vomiting, sleeplessness, weight gain, and

personality changes. G-CSF usually causes few side effects, but may cause fevers, skin rashes, bone aches, or headaches.

QUESTIONS

If you do not want to participate in this study, you don't have to. There are other types of treatment for your disease. If you have any questions about these treatments, please ask the doctors. The doctors want to help you understand what this is all about. If you want to, you can call _____ (*Name of Physician*) at _____ (*phone number*); this is the doctor in charge of this study. The nurses and doctors can help you call.

We want you to understand as much about your treatment with cord blood as possible. If you have asked all your questions and you feel OK about going ahead with this treatment, please sign your name on the first line below. Do not sign your name if you do not want this treatment with cord blood.

_____ _____

Subject's Signature Date

_____ _____

Parent's Signature Date

I have explained fully to the patient the above objective of this study, what is to be expected, and the possible complications.

_____ _____

Counseling Physician's Signature Date

THE PARENTS MUST SIGN A CONSENT FORM.

APPENDIX B

Staging and Grading of Acute GVHD

APPENDIX B

STAGING AND GRADING OF ACUTE GVHD

	Extent of Organ Involvement		
	Skin	Liver	Gut
Stage[x]			
1	Rash on < 25% of skin[a]	Bilirubin 2-3 mg/dl[b]	Diarrhea > 500 mL/day[c] (> 280 ml/m^2 in children) or persistent nausea[d]
2	Rash on 25-50% of skin	Bilirubin 3.1-6 mg/dl	Diarrhea > 1,000 ml/day (> 555 ml/m^2 in children)
3	Rash on > 50% of skin	Bilirubin 6.1-15 mg/dl	Diarrhea > 1,500 ml/day (> 833 ml/m^2 in children)
4	Generalized erythroderma with bullous formation	Bilirubin > 15 mg/dl	Severe abdominal pain with or without ileus
Grade[e]			
0	None and	None and	None
I	Stage 1-2 and	None and	None
II	Stage 3 and/or	Stage 1 and/or	Stage 1
III	None - Stage 3 with	Stage 2-3 or	Stage 2-4
IV	Stage 4 or	Stage 4	N/A

[a] Use 'Rule of Nines' or burn chart to determine extent of rash.
[b] Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
[c] Downgrade one stage if an additional cause of diarrhea has been documented.
[d] Persistent nausea, vomiting, or anorexia in the absence of other known cause, unless negative histology is present.
[e] Criteria for grading given as minimum degree of organ involvement required to confer that grade.

[x] Actual symptom/sign collected but stage assignment occurs only if GVHD is indicated as a cause of at least one organ's symptomatology.

APPENDIX C

Chronic GVHD: Clinical and Pathological Manifestations

APPENDIX C

CHRONIC GVHD: CLINICAL AND PATHOLOGICAL MANIFESTATIONS

CGVHD (Chronic Graft-Versus-Host-Disease) is typically a late complication of BMT characterized by a connective-tissue-like syndrome and usually, but not always, occurring greater than 100 days following transplantation. Occasionally, its onset may closely follow acute GVHD (known as progressive presentation of CGVHD). The pathogenesis of CGVHD is believed related to immunological reactivity between host and donor cells, and is probably mediated by allo- and/or auto-reactive T-lymphocytes. Clinically, CGVHD may be manifested by involvement of the following organ systems:

Skin

Skin involvement is usual in CGVHD, occurring in approximately 95% of patients. Manifestations may include an initial, possibly erythematous rash, with or without macules, plaques and/or desquamation. The onset may be acute or insidious. Later, hair follicle atrophy, sclerodermatous changes with skin thickening, progressive epidermal atrophy and fibrosis and hyper- or hypo-pigmentation may occur. Lichen planus-like lesions may be present. Photoactivation of skin CGVHD may occur. Early histological changes include hyperkeratosis and epidermal hypertrophy. Later changes are epidermal atrophy and dermal fibrosis. Localized lesions may show lichenoid reactions, epidermal atrophy or dense focal fibrosis.

Eye

Symptoms (a sicca syndrome) occur in 80% of patients with CGVHD. Dry, gritty eyes are common and are occasionally associated with photophobia.

Mucosa

The oral mucosa is involved in 80% of cases. Symptoms include oral pain, intolerance of foods and dryness. Signs include diffuse erythema of the oral mucosa, white, lacy mucosal thickening, discrete ulceration and/or lichenoid changes. Oral changes may contribute to inadequate nutrition in some patients. Histological features include labial mucosal atrophy, squamous cell necrosis and mononuclear cell infiltration and minor salivary gland epithelial cytolysis. Vaginal mucosal involvement may lead to vaginal dryness or dyspareunia. Vaginal stenosis has been described.

Gastrointestinal

Anorexia, nausea, vomiting, abdominal pain, and/or weight loss may be manifest with GI involvement. Esophageal involvement may occur in some patients with anorexia and/or dysphagia. An esophageal dysmotility syndrome may also occur. Esophageal biopsy may show mucosal inflammation with desquamation or submucosal fibrosis, though submucosal involvement throughout the GI tract may not be evident on superficial endoscopic mucosal biopsies. 25% of patients overall have significant weight loss and 5% may develop malabsorption with or without chronic diarrhea.

Liver

90% of patients have abnormalities of liver function. This is usually a cholestatic pattern, with elevations of bilirubin, alkaline phosphatase, and 5' nucleotidase. Progression to liver failure is rare. Pathological changes include lobular hepatitis with small or absent bile canaliculi.

Joints and soft tissue

Arthritis or systemic lupus erythematosis-like manifestations may occur. Periarticular dermal fibrosis may lead to loss of joint mobility. Contractures may develop as CGVHD progresses. Muscle cramps, usually without myositis, may develop. Polyserositis may occasionally occur.

Pulmonary

Bronchiolitis obliterans with bronchodilator-resistant obstructive lung disease complicates CGVHD in 5% of cases. Diagnosis is established by open lung biopsy, which shows lymphocytic bronchitis, lymphoid interstitial pneumonitis or obliterative bronchiolitis.

Immunological abnormalities and immunodeficiency

Immunological abnormalities include the development of autoantibodies (e.g., FANA, Rheumatoid Factor, positive Coomb's test). Hypogammaglobulinemia, anergy, lymphopenia, functional hypo- or asplenia and immune dysregulation all may lead to increased risk of bacterial, fungal and viral infection.

Other manifestations

Suppression of blood counts may complicate CGVHD. Thrombocytopenia is especially associated with a poor prognosis.

APPENDIX D

Toxicity Grading Scale

APPENDIX D

TOXICITY GRADING SCALE

Toxicity Grading

	GRADE I	GRADE II	GRADE III
Cardiac toxicity	Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms	Moderate EKG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe EKG abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention; or decrease in voltage by more than 50%
Bladder toxicity	Macroscopic hematuria after 2 d from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 d from last chemotherapy dose not caused by infection; or hematuria after 2 d with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure
Renal toxicity	Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning)	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Pulmonary toxicity	Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF, or decrease of PO_2 (> 10% from baseline but not requiring mechanical ventilation or > 50% O_2 on mask and not caused by infection or CHF	Interstitial changes requiring mechanical ventilatory support or > 50% oxygen on mask and not caused by infection or CHF
Hepatic toxicity	Mild hepatic dysfunction with 2.0 mg% ≤ bilirubin ≤ 6.0 mg%; or weight gain > 2.5% and < 5% from baseline, of noncardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest pre-conditioning	Moderate hepatic dysfunction bilirubin > 6 mg% < 20 mg%, or SGOT increase > 5-fold from pre-conditioning; or clinical ascites or image documented ascites > 100ml; or weight gain > 5% from baseline of noncardiac origin	Severe hepatic dysfunction with bilirubin > 20mg%; or hepatic encephalopathy; or ascites compromising respiratory function

Toxicity Grading

	GRADE I	GRADE II	GRADE III
CNS toxicity	Somnolence but the patient is easily arousable and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection	Seizures or coma not explained (documented) by other medication, CNS infection, or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous IV narcotic drug	Pain and/or ulceration requiring a continuous IV narcotic drug (morphine drip)	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI toxicity	Watery stools > 500 ml but < 2,000 ml every d not related to infection	Watery stools > 2,000 ml every d not related to infection, or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion

Notes: Grade IV regimen-related toxicity is defined as fatal toxicity.
Abbreviations: CXR, chest x-ray; IV, intravenous
Reference: Bearman SI, Appelbaum FR, Bucker CD, Peterson FB, Fisher LD, Clift RA, Thomas ED. (1988). Regimen-related toxicity in patients undergoing bone marrow transplantation. *Journal of Clinical Oncology* **6(10)**:1562-1568.

APPENDIX E

Derivation of a Sequential Test Statistic for Censored Exponential Data

APPENDIX E

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

Background - The Sequential Probability Ratio Test

Let $f(.,\theta)$ be the density function for a random variable X. According to Neyman and Pearson, the most powerful test of H_0: $\theta = \theta_0$ versus H_1: $\theta = \theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n = \Pi_i^n f(x_i;\theta_1)/f(x_i;\theta_0)$ is the likelihood ratio, and c_α is determined to have the size α. When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B<L_n<A$ for some constant $B<1<A$, stops sampling and decides in favor of H_1 as soon as $L_n>A$, and stops sampling and decides in favor of H_0 as soon as $L_n<B$.

The usual measures of performance of such a procedures 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 $E(N \mid \theta_i)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $Pr_0(reject\ H_0)\le\alpha$ and $Pr_1(reject\ H_1)\le\beta$, and for which $E_j(N)$ are finite, $j=0,1$, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the $X_1,X_2,...$ are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $T(x)$, then any SPRT for testing θ_0 against $\theta_1(>\theta_0)$ has a nondecreasing power function.

For the SPRT with error probabilities α and β, the SPRT boundaries are given approximately by $A=(1-\beta)/\alpha$ and $B=\beta/(1-\alpha)$. The operating characteristics of the SPRT are given by $O(\theta;\alpha,\beta,\theta_0,\theta_1)=(A^{h(\theta)}-1)/(A^{h(\theta)}-B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to $\int(f(x;\theta_1)/f(x;\theta_0))^{h(\theta)}f(x;\theta)dx=1$. The average sample number for an arbitrary θ is given by $E(N;\theta)=[\{1-O(\theta)\}\log A+O(\theta)\log B]/E(Z;\theta)$. The sample size distribution is very highly skewed, $Var(N)\sim[E(N)]^2$. Thus we will consider a truncated test with maximum sample size of N_0, and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Uncensored Exponential Survival Times

We wish to construct a sequential test for the composite null hypothesis that survival at six months is greater or equal to 60% versus the composite alternative hypothesis that it is less than or equal to 60%. For the derivation of the uncensored SPRT, we will require that the type I error of the test be less than 5%, and that the test provide 80% power to reject the null hypothesis under a specified alternative that the true survival rate is 40%. A maximum sample size of 75 patients will be permitted.

Let us assume that the survival times, $T_1,T_2,...,T_n$, are completely observed (uncensored) and are i.i.d. with exponential density function $f(T,\theta)=\theta e^{-\theta T}$. These assumptions will be relaxed to incompletely observed data subsequently. In the exponential parameterization, six month survival of 60% translates into a mean survival of 0.979 years ($\theta_0 = 1.021$) and 40% translates into a mean survival of 0.546 years ($\theta_1 = 1.832$).

The SPRT is derived with reference to a simple null and alternative hypothesis, in this case, $H_0{:}\theta=\theta_0=1.021$ versus $H_1{:}\theta=\theta_1=1.832$. However, since the log-likelihood ratio for the exponential, $\log\Pi_i^n f(x_i;\theta_1)-\log\Pi_i^n f(x_i;\theta_0)=n(\log(\theta_1)-\log(\theta_0))-(\theta_1-\theta_0)\sum_i^n T_i$, is a monotone function of $\sum_i^n T_i$, the power of the test is non-decreasing in θ. Thus the SPRT is a one-sided level .05 test of a composite null ($H_0{:}\theta\geq\theta_0=1.021$) versus a composite alternative ($H_0{:}\theta\leq\theta_0=1.021$), with power of $1-\beta=0.80$ at the selected alternative $\theta=\theta_1=1.832$.

The SPRT can be represented graphically. The continuation region is bounded by two parallel lines with common slope $(\log\theta_0-\log\theta_1)/(\theta_0-\theta_1)=0.721$, and intercepts $\log A/(\theta_0-\theta_1)=-3.42$ and $\log B/(\theta_0-\theta_1)=1.92$, for the lower and upper bounds, respectively. As each individual unit is put on trial and observed to fail, the cumulative sum of failure times $\sum_i^n T_i$ is recomputed, and plotted against the current sample size n. When this graph crosses the lower boundary, the null hypothesis is rejected.

The maximum sample size of 75 patients requires that the SPRT be truncated. We choose to truncate the SPRT by declaring that if the test has failed to terminate after 75 patients, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at a sample size of 75 is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity.

Derivation of a Modified SPRT Test for Censored Exponential Data

The assumption of uncensored exponential survival times is flawed. We believe that the hazard is reasonably constant over the first six months on trial, and is likely to decrease substantially thereafter. Furthermore, it is not practical to conduct a clinical study by putting each individual on trial, and waiting until that individual is observed to fail. We relax our assumptions as follows. Firstly, each individual's time on study will be computed as time from entry to failure, or to the six month time point, whichever comes first. Secondly, we will put individuals on trial as soon as they become available, without waiting for the previous individual to fail.

Let us consider the impact of relaxing these assumptions one at a time. In a fixed sample size trial with uncensored exponential failure times, mean survival time is estimated by the sample mean of the failure times, or total time on study divided by the number individuals enrolled. When censoring is introduced, the estimate becomes the total time on study divided by the number of observed (non-censored) failures. This suggests that in an exponential SPRT test modified to incorporate censoring, we replace the observed failure times $T_1,T_2,...,T_n$ with censored failure times $X_1,X_2,...,X_n$, and the current sample size n with the number of observed failures d.

Now we relax the second assumption, and put individuals on trial as soon as they become available, without waiting for the previous individual to fail. Assume that three years are required for accrual of 75 patients to the study, and that the final analysis takes place six months after the last patient is entered. Putting this all together, we propose a modified truncated SPRT, where at any interim time point, s, ranging from 0 to 3.5 years, the sum of observed time on study, $\sum_i^n X_i(s)$, is plotted against the number of observed failures, $d(s)$.

A further modification made to the SPRT was to only use the lower boundary for stopping since the primary focus of the monitoring is to protect against unacceptable 180 day survival.

Operating Characteristics of the Modified SPRT Test for Censored Exponential Data

The modified SPRT for censored exponential survival times targeted a drop in survival from 60% to 40%, rather than from 60% to 45%, as was used for the derivation of the uncensored test. Requiring type I and type II error rates of 0.05 and 0.20, and solving for the parameters of the SPRT, we obtain A = 16, and B = 0.21. Since only the lower boundary is used for monitoring, the continuation region of the test is bounded below by a line with slope of 0.72 and intercept of -3.4. Under the further assumption of uniform accrual over a three year period, and monthly interim analyses over the course of the study, the operating characteristics of the modified SPRT were obtained from a simulation study.

Table E.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

While the motivation for this testing procedure is largely heuristic rather than theoretical, the simulation results validate the approach. When the true survival rate at six months was 60%, the test crossed the lower boundary in 3759 of 100,000 replications, for an estimated type I error rate of 0.038. When the true survival rate at six months was 40%, the test failed to cross the lower boundary (either crossed the upper boundary or remained in the continuation region) in 9214 of 100,000 replications, for an estimated type II error rate of 0.09. The test is almost certain (100%) to reject the null hypothesis when the true survival rate at six months is 30%.

It is interesting to note that the SPRT derived above for exponential failure times with censoring at six months, has operating characteristics which are similar to those of a more traditional SPRT, derived for binomial variates with success probability equal to the six month failure rate. Using time to failure rather than a simple binary indicator of failure, leads to little improvement in power when failure times are censored relatively soon after entry on study. We speculate that if the constant hazard rate over the first six month period is high, the exponential test will reject faster than the binomial test, but have not conducted simulation studies to demonstrate this.