



BLOOD AND MARROW
TRANSPLANT
CLINICAL TRIALS NETWORK

**Fludarabine-based Conditioning for Allogeneic Marrow
Transplantation From HLA-compatible Unrelated Donors in Severe
Aplastic Anemia**

(Posted on clinicaltrials.gov as NCT00326417)

**BMT CTN PROTOCOL 0301
VERSION 9.0**

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**Sponsored by the National Institutes of Health
National Heart, Lung, and Blood Institute
National Cancer Institute**

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PROTOCOL SYNOPSIS - BMT CTN PROTOCOL #0301**Fludarabine-based Conditioning for Allogeneic Marrow Transplantation from HLA-compatible Unrelated Donors in Severe Aplastic Anemia**

- Study Chairperson:** Paolo Anderlini, M.D.
- Primary Objective:** The primary objective of this study is to determine the feasibility and toxicity of employing fludarabine-based conditioning to reduce transplant-related toxicity while maintaining (or ideally improving) engraftment in allogeneic donor marrow transplantation from matched (and mismatched) unrelated donors (MUD) in patients with severe aplastic anemia (SAA). The combination of reduced transplant-related toxicity and preserved engraftment should translate into improvement in long-term survival, which is the ultimate goal of the study. More specifically, the study will determine the degree of cyclophosphamide (CY) dose reduction achievable with the introduction of fludarabine in the preparative regimen, with the goal of maintaining (or improving) engraftment, reducing major transplant-related toxicity and early deaths, and thereby ultimately improving long-term survival. The primary endpoint is selection of the optimal CY dose based on Day 100 assessments of graft failure (primary and secondary), regimen-related toxicity and early death.
- Secondary Objectives:** Secondary endpoints of clinical interest include post-transplant survival, graft failure, and acute and chronic GVHD.
- Study Design:** The study is a prospective Phase I/II dose optimization study. All patients are given a fixed dose of ATG (either thymoglobulin: 3 mg/kg IV daily x 3 or ATGAM 30 mg/kg IV daily x 3, on Days -4 to -2), Fludarabine (30 mg/m² IV daily x 4, on Days -5 to -2), and TBI (200 cGy from a linear accelerator at ≤ 20 cGy/min on Day -1). The starting CY dose will be 150 mg/kg (50 mg/kg intravenously daily, Days -4 to -2), and will be de-escalated depending on engraftment and toxicity. The Phase I portion of the trial (maximum of 24-27 patients) tests each of four dose levels of CY for adequate safety and graft retention. The Phase II portion of the trial refines the dose selection and allocates an additional 70 patients to the optimal dose, at which two-year post-transplant survival will be assessed. The combined enrollment in Phase I and II will total 94 patients.

Dosage Levels for CY		
Days	Dose	Total Dose
3 (Day –4, –3, –2)	50 mg/kg/day	150 mg/kg
2 (Day –3, –2)	50 mg/kg/day	100 mg/kg
1 (Day –2)	50 mg/kg/day	50 mg/kg
0 (None)	None	0 mg/kg

Dose Finding Plan

The first patients treated will receive a dose of 50 mg/kg intravenously daily, on Days –4 to –2 (total dose of 150 mg/kg). The doses to be considered range from 150 mg/kg to 0 mg/kg, in 50 mg/kg decrements. At each dose level, a minimum of six patients are initially evaluated before de-escalating to the next lower dose.

The study design uses an adaptive Bayesian method for dose-finding in Phase I/II clinical trials based on trade-offs between the true (i.e., population) rates of engraftment and toxicity (i.e., severe clinical toxicity, early death or both)¹.

Patients will be enrolled sequentially. There may be wait periods between enrollment of successive patients for endpoint assessments. Doses for subsequent patients are based on the engraftment and toxicity experience in preceding patients. The total sample size of 94 patients enrolled in Phases I and II of the trial contribute to selection of the optimal dose. For a complete description of the Study Design refer to Chapter 5 of the Protocol.

- Accrual Objective:** A maximum of 94 patients will be enrolled and followed for 24 months post-transplant.
- Accrual Period:** The estimated accrual period is eight years.
- Eligibility Criteria:** Patients up to 65 years of age with a diagnosis of SAA and an available unrelated donor with a 7/8 or 8/8 match for HLA-A, B, C (intermediate resolution) and DRB1 (high resolution) antigen and willing to provide a marrow allograft.

Treatment Description:

The preparative regimen will consist of:

- Fludarabine: 30 mg/m² IV daily x 4, on Days –5 to –2
- Cyclophosphamide (CY): the CY dose will be de-escalated (see Study Design above). Full details on the de-escalation scheme are provided in the Statistical Section (Chapter 5) of the protocol.
- Antithymocyte globulin (ATG; Thymoglobulin): 3 mg/kg IV daily x 3, on Days –4 to –2. A biologically equivalent dose of ATGAM (horse ATG; conversion ratio 10:1) is recommended.
- TBI: 200 cGy from a linear accelerator at ≤ 20 cGy/min on Day –1.
- Day 0 will be the day of infusion. GVHD prophylaxis will consist of cyclosporine (to be administered for no less than nine months after transplant) in combination with methotrexate at Days 1, 3, 6 and 11. Tacrolimus may be substituted for cyclosporine intolerance.

Study Duration:

Patients will be followed for 24 months post-transplant.

TREATMENT SCHEMA

ATG 3 mg/kg Day -4 to -2
Fludarabine 30 mg/m² Day -5 to -2
TBI 200 cGy Day -1

Phase I
Test Each Dose for Safety
and
Adequate Graft Retention

Phase II
Refine Dose Selection
and
Allocate Patients to Optimal Dose

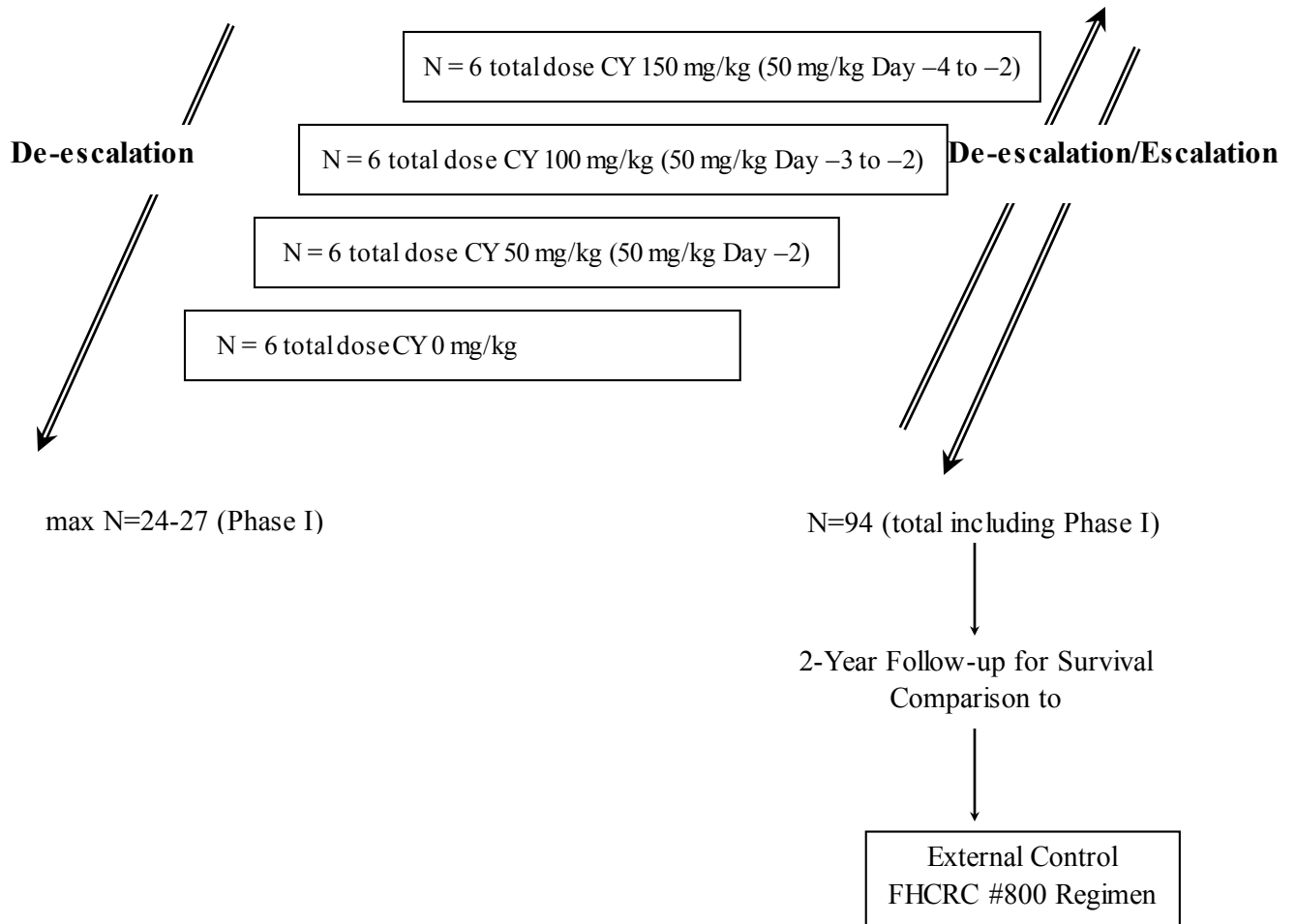


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CHAPTER 1

1. BACKGROUND AND STUDY RATIONALE

1.1. Objectives

The primary objective of this study is to determine the feasibility and toxicity of employing fludarabine-based conditioning to reduce transplant-related toxicity while maintaining (or ideally improving) engraftment in allogeneic donor stem cell transplantation from matched (and mismatched) unrelated donors (MUD) in patients with severe aplastic anemia (SAA). The combination of reduced transplant-related toxicity and preserved engraftment should translate into improvement in long-term survival, which is the ultimate goal of the study.

More specifically, the study will determine the degree of cyclophosphamide (CY) dose reduction achievable with the introduction of fludarabine in the preparative regimen, with the goal of maintaining (or improving) engraftment, reducing major transplant-related toxicity and early deaths, and thereby ultimately improving long-term survival. The primary endpoint of the study is selection of the optimal CY dose based on Day 100 assessments of graft failure (primary and secondary), major regimen-related toxicity and early deaths. The primary endpoint of the Phase II portion of the study is the two-year post-transplant survival achieved with the level of CY dose reduction selected in the dose-finding portion of the study.

1.2. Background

Aplastic anemia (AA) remains a life-threatening illness. Treatment options include supportive care (transfusions, growth factors, etc.), immunosuppression therapy and stem cell transplantation^{2, 3}. Only the latter two have favorably impacted the natural history of the disease. The prognosis of AA patients, particularly SAA, as defined by Camitta et al.⁴, who fail to respond to immunosuppressive therapy (IS) or who relapse after an initial response to IS is poor. Although many of these patients can be supported in the short term with growth factors, transfusions and possibly rechallenged successfully with IS, the cumulative morbidity and mortality from infection, hemorrhage or transfusion-related complications is substantial.

While allogeneic bone marrow transplantation is potentially curative in AA, no more than 25% of patients have an HLA-identical sibling donor⁵. Cyclophosphamide (CY)-ATG has been recommended as the preparative regimen of choice in sibling donor transplants⁶. Results of bone marrow transplantation from alternative donors, such as matched unrelated donors and mismatched related donors in AA patients who have failed IS, have largely been unsatisfactory⁵. The cyclophosphamide-ATG conditioning regimen has proved inadequate in ensuring engraftment in allogeneic transplants from matched, unrelated donors for AA⁷. This was the major reason why total body radiation (TBI) has been added to the conditioning regimen.

Graft failure is a very serious and frequently life-threatening or fatal event following MUD allografts in aplastic anemia. It is an immunologically mediated event. Risk factors for graft

failure include the use of HLA nonidentical or unrelated donors, a poor marrow nucleated cell dose as well as prolonged transfusional support prior to BMT (which increases the probability of patient sensitization to multiple antigens). While some patients may achieve autologous hematopoietic recovery, prolonged pancytopenia is common and infection-related morbidity and mortality are very substantial⁵. Reconditioning for a second allograft from the same or a different donor is frequently not successful. While the addition of TBI and intensive pre-transplant conditioning has led to a sizable improvement in engraftment rates, this has come with a price, particularly in adult patients. Transplant-related toxicity has been a major and frequent problem. Radiation-induced pulmonary toxicity in particular has been common, usually in the form of diffuse alveolar damage or diffuse interstitial pneumonitis^{5, 8, 9}. In addition, GVHD-related morbidity and mortality in these patients have also been substantial.

A recent update from the National Marrow Donor Program on 141 patients showed that only 51 (36%) were surviving with a median follow-up of 36 months. Morbidity and mortality was very high, largely because of graft failure (11%) and transplant-related toxicities⁸. As stated previously, toxicities are thought to be related to the administration of standard-dose TBI and, to a lesser extent, intensive chemotherapy as part of the conditioning regimen, deemed necessary to maximize engraftment rates.

More recently, Deeg et al. investigated the option of reducing the intensity of TBI in the preparative regimen to minimize TBI-related complications while attempting to preserve the beneficial effect of TBI on engraftment. A TBI de-escalation trial was conducted in matched, unrelated donors, with TBI doses as low as 200 cGy (TBI 200). All patients received CY (200 mg/kg) and ATG (90 mg/kg). In an initial cohort of 13 patients treated with TBI 200, the graft failure rate was 8% with a 61% survival¹⁰. Largely based on these data, it was concluded that TBI 200 in combination with CY and ATG was sufficient to allow for engraftment without inducing prohibitive toxicity. However, among 45 patients there still were 12 deaths (including two early ones), mainly transplant-related (31%) and major regimen-related toxicities plus early deaths in nine patients (20%). Thirty-three of 45 patients (73%) survived with a variable follow-up (Deeg J, personal communication, 2004).

Purine-analogs, in particular fludarabine, have emerged as powerful immunosuppressive agents with minimal systemic toxicities¹¹. Fludarabine-based, reduced-intensity preparative regimens have been shown to allow alloengraftment in the related and unrelated donor setting with acceptable systemic toxicity in patients with a variety of hematologic malignancies^{12, 13, 14, 15, 16}. Encouraging data on the fludarabine-cyclophosphamide regimen have been reported^{14, 17}. A fludarabine-based, less intensive, conditioning regimen, with adequate immunosuppressive activity could conceivably allow engraftment of stem cells from alternative donors in AA patients with acceptable engraftment rates and low transplant-related mortality. Regimen-related toxicity is believed to be a major contributing factor to GVHD^{18, 19}. Therefore this approach may also lead to reduced GVHD, as some investigators have suggested^{20, 21}. Anecdotal reports^{22, 23} suggested the feasibility of this approach, and a recent update has confirmed these data. In a cohort of 38 patients (median age 14 years) with aplastic anemia transplanted with a fludarabine-cyclophosphamide-ATG conditioning regimen, seven patients (18%) experienced graft failure, with two cases of autologous reconstitution. Six of these seven cases were older

patients (i.e., > 15 years of age). Thirty-one patients (82%) engrafted²⁴. High-dose CY (200 mg/kg) may lead to substantial morbidity and mortality (cardiotoxicity, hepatotoxicity, pulmonary toxicity, hemorrhagic cystitis, mucositis^{25, 26}), and the replacement of at least part (and possibly all) of CY by fludarabine in the Deeg regimen would be expected to maintain immunosuppression (and therefore engraftment) while reducing transplant-related complications (i.e., regimen-related toxicity and possibly GVHD), and thereby improve survival.

Conditioning regimens, including high-dose ATG (particularly thymoglobulin) employed in the context of matched, unrelated donor marrow transplant, have been associated with Epstein-Barr virus (EBV) reactivation and disease, such as post-transplant lymphoproliferative disorders (PTLPD)^{27, 28}. Molecular monitoring for EBV reactivation in peripheral blood with pre-emptive rituximab treatment has been recommended to manage this complication^{29, 30}. This approach is particularly appealing, as established PTLPD carries a high mortality^{27, 28}.

The role of filgrastim-mobilized peripheral blood stem cells (PBSC) in matched, unrelated donor transplantation is not well defined, although preliminary data suggest that they can be employed safely^{31, 32}. In the context of aplastic anemia, however, a recent analysis from the Center for International Blood and Marrow Transplant Research/European Blood and Marrow Transplant Group (CIBMTR/EBMT) has suggested that while after HLA-identical siblings transplants, recipients of PBSC transplants have faster engraftment, they also have a poorer long-term outcome than bone marrow recipients, largely because of higher incidence of chronic graft-vs-host disease (GVHD)-related mortality³³. Similar data for MUD allografts are lacking at the moment, but at the present time unrelated donor bone marrow remains the preferred stem cell source for allografting in AA patients. In view of these data this study will focus on bone marrow as the stem cell source.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

The purpose of the current study is to continue to optimize conditioning regimens in high-risk patients with severe aplastic anemia transplanted with marrow from HLA-compatible unrelated donors. Specifically, the study will determine whether the addition of fludarabine to the conditioning regimen previously described by Deeg et al. will permit a reduction in the CY dose, to a point where sustained hematopoietic engraftment and survival are maintained (or improved), while the frequency of major regimen-related toxicity (RRT) and early deaths is reduced.

2.2. Patient Eligibility

2.2.1. Inclusion Criteria

Patients fulfilling the following criteria will be eligible for entry into this study:

1. Patients up to 65 years of age at time of registration with a diagnosis of severe aplastic anemia (SAA)⁴. SAA is defined as follows:
 - Bone marrow cellularity < 25%, or marrow cellularity < 50% but with < 30% residual hematopoietic cells.
 - Two out of three of the following (in peripheral blood): neutrophils < 0.5 x 10⁹/L; platelets < 20 x 10⁹/L; reticulocytes < 20 x 10⁹/L.

SAA diagnostic criteria may be applied to assessment at initial diagnosis or follow-up assessments.

2. Patient must have an available unrelated donor with a 7/8 or 8/8 match for HLA-A, B, C and DRB1 antigen. Typing is by DNA techniques: intermediate resolution for A, B and C, and high resolution for DRB1. HLA-DQ typing is recommended but will not count in the match.
3. Patient and/or legal guardian able to provide signed informed consent.
4. Matched unrelated donor must consent to provide a marrow allograft.
5. Patients with adequate organ function as measured by:
 - a) Cardiac: left ventricular ejection fraction at rest > 40% or shortening fraction > 20%
 - b) Hepatic: serum total bilirubin < 2x upper limit of normal for age as per local laboratory (with the exception of isolated hyperbilirubinemia due to Gilbert's syndrome); ALT and AST < 4x upper limit of normal for age as per local laboratory
 - c) Renal: serum creatinine < 2x upper limit of normal for age (as per local laboratory). For patients with serum creatinine above the normal range, a glomerular filtration rate

(measured as per institutional practice, typically creatinine clearance) equal to or greater than 60 mL/min (corrected to 1.73m² body surface area) is required. For pediatric patients, other comparable methods for renal function estimation in keeping with local institutional practice are permitted.

- d) Pulmonary: FEV₁, FVC and DLCO (corrected for Hb) > 50% predicted. For patients where pulse oxymetry is performed, O₂ saturation > 92%
6. The diagnosis of Fanconi anemia must be excluded in patients younger than 18 years of age by diepoxybutane (DEB) testing on peripheral blood or comparable testing on marrow.

2.2.2. Exclusion Criteria

1. Clonal cytogenetic abnormalities associated with MDS or AML on marrow examination.
2. Diagnosis of other “congenital” aplastic anemias such as: Diamond-Blackfan; Shwachman-Diamond; congenital amegakaryocytosis.
3. Symptomatic or uncontrolled cardiac failure or coronary artery disease.
4. Karnofsky performance status < 60% or Lansky < 40% for patients < 16 years old.
5. Uncontrolled bacterial, viral or fungal infections (currently taking medication and progression of clinical symptoms). Patients with fever despite broad-spectrum antimicrobials but no clinical or hemodynamic evidence of sepsis will be allowed.
6. Seropositive for the human immunodeficiency virus (HIV).
7. Pregnancy (positive \exists -HCG) or breastfeeding.
8. Presence of large accumulation of ascites or pleural effusions, which would be a contraindication to the administration of methotrexate for GVHD prophylaxis.
9. Known severe or life-threatening allergy or intolerance to ATG or cyclosporine/tacrolimus.
10. Planned administration of alemtuzumab (Campath-1H) or other investigational agents as alternative agent for GVHD prophylaxis.
11. Concomitant enrollment in a Phase I study.
12. Positive patient anti-donor lymphocyte crossmatch in HLA-A or B mismatched transplants (test recommended but not mandatory). The definition of match is in Section 2.2.1.
13. Prior allogeneic marrow or stem cell transplantation.
14. Patients with prior malignancies except resected basal cell carcinoma or treated carcinoma in-situ. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or Protocol Chair. Cancer treated with curative intent > 5 years previously will be allowed.

2.3. Treatment/Study Plan**2.3.1. Preparative Regimen**

The preparative regimen will be as follows:

- Fludarabine: 30 mg/m² IV over no less than 30 min daily x 4, Days –5 to –2. For patients weighing more than 100% of their ideal body weight (IBW), fludarabine will be dosed based on their adjusted ideal body weight (AIBW). For patients weighing less than 100% of their IBW, it is recommended, but not required, that fludarabine be dosed based on their IBW.

Recommended Ideal Body Weight (IBW) Formulas :**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

For patients less than 5 feet, subtract 2.3 kg/inch

Patients less than or equal to 18 Years**Less than 60 inches**

IBW = (ht² x 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

Recommended Adjusted Ideal Body Weight (AIBW) Formula:

AIBW = IBW + [(0.25) x (actual body weight - IBW)]

- Cyclophosphamide (CY): the CY dose, to be given intravenously (IV) over no less than 2 hours, will be de-escalated as follows:

Dosage Levels for CY		
Days	Dose	Total Dose
3 (Day –4, –3, –2)	50 mg/kg/day	150 mg/kg
2 (Day –3, –2)	50 mg/kg/day	100 mg/kg
1 (Day –2)	50 mg/kg/day	50 mg/kg
0 (None)	None	0 mg/kg

The initial CY dose will be 50 mg/kg intravenously x 3 days (Day –4, –3, and –2). Full details on the de-escalation scheme are provided in the Statistical Section (Chapter 5) of the protocol. Doses and schedule for uroprotective agents (i.e. mesna) should follow

local institutional practice. For patients weighing more than 100% of their ideal body weight (IBW), cyclophosphamide will be dosed based on their adjusted ideal body weight (AIBW). For patients weighing less than 100% of their IBW, it is recommended, but not required, that cyclophosphamide be dosed based on their IBW.

It is recommended that fludarabine be administered prior to cyclophosphamide.

- Thymoglobulin: 3 mg/kg IV over no less than four (and preferably six) hours daily x 3, Days -4 to -2. The thymoglobulin dose should ordinarily be calculated based on actual body weight. In the event of institutional preference or thymoglobulin intolerance, patients may receive a biologically equivalent dose of horse ATG (ATGAM or comparable product). A 10:1 conversion ratio is recommended (i.e., 1 mg thymoglobulin = 10 mg ATGAM) with regard to dosage calculations. Thymoglobulin premedication should follow local institutional practice, but should include a minimum of 100 mg methylprednisolone (or equivalent steroid) IV (preferably repeated in 2-3 hours during the infusion). For pediatric patients the methylprednisolone dose should be a minimum of 1 mg/kg prior to ATG infusion, preferably repeated in three hours. A 24-hour delay in ATG administration to allow for the resolution of ATG-related toxicities is permitted.
- All three agents (fludarabine, cyclophosphamide and thymoglobulin) are commercially available and will not be provided for the study. Please refer to the agents' package inserts for additional information.
- TBI: 200 cGy from a linear accelerator at ≤ 20 cGy/min on Day -1 (single dose). Lung shielding is strongly recommended, but not mandatory.

2.3.2. Marrow Processing and Handling

Processing of Bone Marrow Products - No processing of bone marrow, other than anticoagulation, filtration, packaging, and labeling in preparation for transportation, shall be performed by the collection center. Processing of bone marrow for reduction of volume, plasma, red blood cells, or fat, including labeling or re-labeling, may be performed by the transplant center. No additional product manipulation (i.e., T cell depletion, CD34+ cell selection, etc.) is allowed at the transplant center.

Infusion of Bone Marrow - Day 0 will be the day of marrow infusion. Premedications for marrow infusion, as well as infusion rates and use of diuretics to prevent fluid overload will be dictated by local institutional practice. The marrow should be infused as soon as possible after arrival at the Transplant Center. If infusion occurs over 2 days, Day 0 will be the day infusion is completed.

2.3.3. GVHD Prophylaxis

GVHD prophylaxis will consist of cyclosporine (CSA) 3 mg/kg/day by continuous infusion beginning at least 12 hours prior to marrow infusion (to be switched to oral administration as soon as clinically feasible). Oral loading of CSA will be allowed. CSA dose adjustments for

pediatric patients are allowed as per local institutional practice, provided a therapeutic serum level is achieved. An intermittent CSA infusion schedule is also acceptable if dictated by local institutional practice.

The intravenous and oral dose should be titrated to maintain a serum level of 200-400 ng/mL. Oral CSA should be continued for a minimum of nine months after transplant and then tapered as per institutional practice. Patients will also receive methotrexate (MTX) at the dose of 10 mg/m² IV on Day 1, 3, 6 and 11. MTX dose reductions should be made for renal, hepatic and mucosal toxicity, and leucovorin rescue is allowed according to local institutional practice. MTX levels should preferably be monitored 24-72 hours after administration in patients with renal dysfunction. In the event of CSA intolerance or local institutional preference, tacrolimus may be substituted for CSA, with a recommended starting dose of 0.015-0.030 mg/kg/day intravenously and targeting a serum level of 5-15 ng/mL. Alemtuzumab (Campath-1H) is not allowed for GVHD prophylaxis, in view of its known impact on engraftment and post-transplant chimerism.

2.3.4. EBV Monitoring

Patients will have EBV DNA quantitative PCR testing on peripheral blood at least every two to four weeks beginning at engraftment through Day 90-100. Rituximab (375 mg/m² intravenously x 1) will be administered whenever an EBV DNA level of 1000 copies/mL or higher is detected on two consecutive occasions. In the event of persistent EBV viremia or signs/symptoms consistent with EBV-related PTLPD (adenopathy, fever, etc.) despite rituximab administration, patients will be treated according to institutional protocols. Institutions employing real-time PCR for EBV DNA monitoring will determine the threshold for rituximab treatment according to the local sensitivity and specificity of the test.

2.4. Supportive Care

All patients will receive supportive care (prophylactic antibiotics/antifungals, menstrual suppression, empiric antibiotics, intravenous immunoglobulin, red cell and/or platelet transfusions, hyperalimentation, CMV monitoring, etc.) according to local institutional practices.

Transfusion thresholds and practice for blood product support will be in keeping with local institutional practice. All blood products will be irradiated in accordance with local institutional practice.

Growth factor (rhG-CSF, rhGM-CSF) administration will be allowed according to local institutional practice.

2.5. Management of Graft Failure

Patients experiencing primary or secondary graft failure (see Sections 3.1 and 3.2 for definitions) will be managed according to local institutional practices and will be eligible to receive a second

allograft from the original or a different donor. Preparative regimen and stem cell source will be dictated by local institutional practice.

2.6. Management of ATG Intolerance

Patients experiencing a new, severe or life-threatening reaction to ATG and therefore unable or unwilling to receive the full planned cumulative dose of ATG (rabbit or horse product) will continue to be evaluated for the Phase I and II portion of the study (see below). Their conditioning regimen will then be altered as per local institutional preference or practice.

2.7. Therapy Toxicities

Toxicities will be graded using the NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 as well as the Bearman toxicity criteria. The Bearman toxicity grading scales are included in the protocol as Appendix A. All of the agents employed by this study are commercially available.

CHAPTER 3

3. STUDY ENDPOINTS AND DEFINITIONS

3.1. Primary Endpoint

The primary endpoint is selection of the optimal CY dose based on Day 100 assessments of graft failure (primary and secondary), regimen-related toxicity and early death.

3.1.1. Graft Failure

Neutrophil engraftment is defined as the achievement of an ANC $\geq 0.5 \times 10^9/L$ for three consecutive measurements on different days. Primary graft failure is defined by the lack of neutrophil engraftment; i.e., ANC $< 0.5 \times 10^9/L$ measured for three consecutive measurements on different days by 100 days post-transplant. Secondary graft failure, as defined in Section 3.2.1, prior to Day 100 post-transplant will count towards the graft failure endpoint.

3.1.2. Regimen-related Toxicity (RRT)

RRT will be scored according to the Bearman scale³⁴ (see Appendix A). Major RRT is defined as severity of grade 4 in any organ system or grade 3 for pulmonary, cardiac, renal, oral mucosal or hepatic, in keeping with the approach adopted in FHCRC Protocol #800. The assessment for RRT will be carried out weekly until Day 100 post-transplant. The NCI's CTCAE version 3.0 will be used to supplement the Bearman toxicity criteria.

3.1.3. Early Death

This endpoint is defined as death prior to Day 100 post-transplant.

3.2. Secondary Endpoints

3.2.1. Post-transplant Survival

Post-transplant survival is defined as time from transplant to death from any cause. Patients alive at the time of last observation, for statistical purposes, will have a survival time which is censored.

3.2.2. Secondary Graft Failure

Secondary graft failure is defined (in patients surviving at least 100 days) by initial neutrophil engraftment followed by subsequent decline in the ANC to $< 0.5 \times 10^9/L$ for 3 consecutive measurements on different days, unresponsive to growth factor therapy.

3.2.3. Acute GVHD of Grades 2-4 and 3-4

Acute GVHD is graded according to the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Manual of Procedures (MOP). The first day of acute GVHD onset at a certain grade will be used to calculate cumulative incidence curves for that GVHD grade (e.g., if the onset of grade 1 acute GVHD is on Day 19 post-transplant and onset of grade 3 is on Day 70 post-transplant, time to grade 3 is Day 70). This endpoint will be evaluated through 100 days.

3.2.4. Chronic GVHD

Chronic GVHD is scored according to the BMT CTN MOP. The first day of chronic GVHD onset will be used to calculate cumulative incidence curves.

CHAPTER 4

4. EVALUATION AND ENROLLMENT

4.1. Enrollment Procedures

Patients will be registered using the BMT CTN Electronic Data Capture System (AdvantageEDCSM). The following procedures should be followed:

1. The coordinator must contact the DCC/EMMES as soon as a potential patient is identified. Enrollment may be on hold until the evaluation of the 100-Day outcomes for regimen-related toxicities, early deaths and graft retention of prior patients is complete. However, it may be possible to enroll the patient on the lowest of other available previously tested doses in Phase I or the best of other available doses in Phase II. The final decision about waiting versus treating the patient on another dose or off study will be made at the local transplant center level.
2. All enrollments must be pre-approved by the DCC/EMMES. Prior to initiation of the conditioning regimen, an authorized user at the transplant center completes the Demographics Form and the Segment A Enrollment Form in AdvantageEDC. The eligibility screening (Segment A) includes a question confirming that the patient signed the informed consent form.
3. If the patient is eligible, a study number and treatment dose assignment are generated on the “Enrollment Successful” page. This page should be printed immediately as it is the only opportunity to do such. A copy should be forwarded to the pharmacy to ensure the appropriate regimen is employed and a copy should be filed in the patient’s research file.
4. A visit schedule based on transplant date is displayed for printing and is referred to as ‘Segment A Follow-up.’

4.2. Study Monitoring

4.2.1. Follow-up Schedule

The Follow-up Schedule for scheduled study visits is outlined in Table 4.2.1. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User’s Guide.

Table 4.2.1: Follow-up Schedule

Study Visit	Target Day ¹ Post-Transplant
1 week	7
2 week	14
3 week	21
4 week	28
5 week	35
6 week	42
7 week	49
8 week	56
9 week	63
10 week	70
11 week	77
12 week	84
13 week	91
14 week	98
100 day	100
4 month	120
6 month	180
12 months	365
18 month	540
24 month	730

¹ Target day range = ± 2 days up to Day 100, ± 14 days for Day 120 and ± 28 days for Day 180, 365, 540 and 730 post-transplant unless otherwise specified.

4.2.2. Case Report Forms

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered into AdvantageEDC within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the AdvantageEDC and integrated into the Data Coordinating Center's (DCC) master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

Reporting Patient Deaths: Recipient death information must be entered into AdvantageEDC within 24 business hours of knowledge of the patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated in AdvantageEDC.

CIBMTR Data Reporting: Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem cell transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem

Cell Transplant Outcomes Database (SCTOD). (Note: Federal legislation requires submission of these forms for all US allotransplant recipients.) Enrollment on BMT CTN #0602 must be indicated on the SCTOD pretransplant registration form, if applicable. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule. Patients not undergoing HCT are not required to have their information reported to the CIBMTR.

Weekly GVHD Monitoring: GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day 100 post-transplant for GVHD. After Day 100 patients will be assessed at each follow-up visit (Day 120, 180, 365 and 730) for the presence of GVHD. For scheduling, a target day range has been provided in Table 4.2.1.

4.2.3. Adverse Event Reporting

Unexpected, grade 3-5 adverse events (AE) will be reported through an expedited AE reporting system via AdvantageEDC. Unexpected, grade 4-5 AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. NCI's CTCAE Version 3.0 should be used to describe events. Expected adverse events will be reported using NCI's CTCAE Version 3.0 at regular intervals as defined on the Form Submission Schedule.

4.2.4. Patient Evaluations

4.2.4.1. Pre-transplant evaluations

The following observations should be determined within six weeks prior to the initiation of the preparative regimen, unless otherwise specified:

1. History, physical examination, height and weight upon admission.
2. Karnofsky/Lansky performance status upon admission.
3. CBC with differential and platelet count, creatinine, bilirubin, alkaline phosphatase, AST and ALT.
4. CMV antibody test, hepatitis panel (HBs Ab, HBs Ag, HBcAb, Hepatitis C Ab), syphilis, HIV1, HIV2 and HTLV1/2 antibody.
5. EKG, LVEF measurement by echocardiogram, cardiac scan or shortening fraction.
6. DLCO (corrected for Hb), FEV1, and FVC. For pediatric patients and patients unable to fully cooperate with the execution of the pulmonary function tests, pulse oxymetry is an acceptable alternative.
7. Bone marrow aspirate and biopsy for pathology and cytogenetics within 12 weeks prior to initiation of conditioning regimen (unless approved by the Protocol Officer or Protocol Chair).

8. Patient anti-donor lymphocyte cross match is recommended in HLA-A or B mismatched transplants to rule out donor-directed desensitization.
9. Chest X-ray.
10. β -HCG for serum pregnancy test within two weeks of admission (females only).
11. Heparinized blood sample for post-transplant chimerism assay.
12. Heparinized blood sample for retrospective HLA typing to the NMDP repository. A separate NMDP consent form is used for blood sample acquisition for the NMDP repository and HLA typing.
13. Serum for quantification of IgG, IgM and IgA (recommended but not mandatory).
14. Sample from the marrow allograft for graft characterization.
15. Diepoxybutane (DEB) testing on peripheral blood or comparable testing on marrow for Fanconi anemia in patients younger than 18 years of age.

4.2.4.2. Post-transplant evaluations

The following post-transplant evaluations should be performed.

1. History and physical exam to assess GVHD and other morbidity weekly until Day 100 post-transplant, then at four months, six months, one year, 18 months and two years post-transplant. GVHD evaluation and grading to be in keeping with BMT CTN MOP. For scheduling purposes, a target day range has been provided in Table 4.2.1.
2. CBC at least three times a week from Day 0 until ANC $> 0.5 \times 10^9/L$ for 3 consecutive measurements over 3 or more days. Thereafter CBC at least twice per week until Day 28, then preferably weekly until Day 100, then at 12 months, 18 months and two years post-transplant.
3. Creatinine, bilirubin, alkaline phosphatase, ALT, AST, twice a week until Day 28 and then preferably weekly until 12 weeks, then at four months, six months, one year, eighteen months and two years post-transplant. Cyclosporine or tacrolimus levels will be measured at least once weekly until Day 100, and then at each follow-up visit until the drug is tapered off.
4. Bone marrow aspirate and biopsy at Day 100 ± 30 days on all patients. Bone marrow aspirate and biopsy at 12 ± 3 months post-transplant is recommended, but not required, for all patients. Cytogenetics or FISH (X/Y probe) on marrow or peripheral blood is required at Day 28, Day 100 and 12 months post-transplant if patient donor sex mismatch is present.
5. Quantification of peripheral blood or marrow chimerism (including whenever possible lineage-specific, myeloid and T cell chimerism) at Day 28 ± 1 week, Day 100 ± 30 days and 12 ± 3 months post-transplant. Chimerism will be measured by PCR-based methods (e.g., microsatellite polymorphism analysis or real-time PCR).

6. EBV DNA quantitative PCR testing on peripheral blood at least every two to four weeks beginning at engraftment through Day 90-100. Institutions employing real-time PCR for EBV DNA monitoring will determine the threshold for rituximab treatment (see Section 2.3.4) according to the local sensitivity and specificity of the test.
7. Regimen-related toxicity (RRT) assessments will be conducted weekly until Day 100 and additional toxicity assessments will be conducted on Day 100, 180, 365 and 730 post-transplant.

4.2.5. Donor Evaluations

4.2.5.1. Pre-transplant evaluations

1. Heparinized blood sample for post-transplant chimerism assay prior to collection.
2. Heparinized blood sample for retrospective HLA typing to the NMDP repository. A separate NMDP consent form is used for blood sample acquisition for the NMDP repository and HLA typing.

Table 4.2.2: Patient Clinical Assessments

Study Assessments / Testing	Base-line	Days Post-Transplant																		
		7	14	21	28	35	42	49	56	63	70	77	84	90	100	120	180	365	540	730
History, Physical Exam, Weight, Height, and Karnofsky/Lansky Performance Status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC ¹ , Differential, Platelet Count, and Blood Chemistries ²	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Infectious Disease Titers ³	X																			
EKG, LVEF or Shortening Fraction	X																			
Pulmonary Function Tests ⁴	X																			
Peripheral Blood Chimerism	X				X									X			X			
EBV DNA Quantitative PCR Testing ⁵			X		X		X		X		X		X		X					
Chest X-ray	X																			
Sample from marrow allograft for graft characterization	X																			
Serum Quantification of IgG, IgM, and IgA (recommended)	X																			
Bone Marrow Aspirate for Pathology and Cytogenetics ⁶	X													X				X		
β-HCG Serum Pregnancy Test (females only)	X																			
Testing on Marrow for Fanconi Anemia ⁷	X																			
Regimen-related and Other Toxicity Assessments ⁸		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X
Acute GVHD		X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Acute/Chronic GVHD														X	X	X	X	X	X	X

Notes:

- ¹ CBC performed three times weekly from Day 0 until ANC > 0.5 x 10⁹/L for three consecutive measurements on different days. CBC then performed twice per week until Day 28, then weekly until 100 days, then at one year, 18 months and two years post-transplant.
- ² Blood chemistries include: creatinine, bilirubin, alkaline phosphatase, AST, ALT and cyclosporine or tacrolimus. Cyclosporine or tacrolimus levels will be measured at least once weekly until Day 100 and then at each follow-up visit until the drug is tapered off. Blood chemistries performed twice weekly until Day 30 and then weekly until Day 100, four months, six months, one year, eighteen months, and two years post-transplant.
- ³ Infectious disease titers include: CMV, hepatitis panel (HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), syphilis, HIV and HTLV 1/2 antibody.
- ⁴ DLCO (corrected for Hb), FEV1, FVC or pulse oxymetry.
- ⁵ EBV testing will be conducted at least every 2 to 4 weeks.
- ⁶ Bone marrow aspirate and biopsy at Day 100 (± 30 days); bone marrow aspirate and biopsy at 12 months (± 3 months) post-transplant is optional. Cytogenetics or FISH (X/Y probe) on marrow or peripheral blood will be done at Day 28, Day 100 and 12 months post-transplant only if patient-donor sex mismatch is present.
- ⁷ Results of Diepoxybutane (DEB) testing on peripheral blood or comparable testing on marrow for Fanconi anemia patients less than 18 years old.
- ⁸ Regimen-related toxicities assessed weekly until Day 100, then general toxicities assessed at Day 100, 180, 365 and 730.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Rationale

A TBI de-escalation trial, FCHRC #800, conducted by Deeg et al.¹⁰ recently showed that reducing the intensity of TBI to 200 cGy in a preparative regimen which included cyclophosphamide (CY) (200 mg/kg) and ATG (90 mg/kg) minimized TBI-related complications while preserving the beneficial effect of TBI on engraftment. However, high dose CY (200 mg/kg) leads to substantial morbidity and mortality. The current study will modify the regimen used in the Deeg et al.¹⁰ study by adding fludarabine for four days and searching for the optimal dose of CY in a range from 0 mg/kg to 150 mg/kg.

5.2. Objectives

The purpose of this study is to continue to optimize conditioning regimens in high-risk patients with severe aplastic anemia transplanted with marrow from HLA-compatible unrelated donors. Specifically, the study will address whether the addition of fludarabine to the conditioning regimen of Deeg et al.¹⁰ will permit reduction in the CY dose to a point where survival is maintained or improved and sustained hematopoietic engraftment is maintained or improved with reduced major regimen-related toxicity (RRT) and/or early death.

5.3. Study Design Synopsis

The study is a prospective single-arm Phase I/II dose-selection and evaluation study. The study will seek the optimal dose level of CY from among those shown in Table 5.3 based on assessments of graft failure, toxicity and early death during 100 days of follow-up post-transplant. Graft failure is defined in Section 3.1.1, regimen-related toxicity in Section 3.1.2, and early death in Section 3.1.3. Details of the dose selection algorithm are given in the technical appendix (Section 5.11) below. A brief synopsis is given below.

Phase I – Test Each Dose for Adequate Safety and Graft Retention

1. Proceed from the highest dose (150 mg/kg CY) to the lowest dose (0 mg/kg CY), treating a minimum of six patients at each dose.
2. Evaluate the 100-Day outcomes for toxicity, death and graft failure on each patient enrolled at the current dose, or until stopping criteria are met.
3. If there are three or more graft failures at the current dose, the current dose and all lower doses are closed to further enrollment.
4. If there are five or more severe regimen-related toxicities and/or early deaths at the current dose, the current dose is closed to further enrollment, and the next lower dose is tested.

5. Dose de-escalation ceases once all four doses are tested or closed to further enrollment.

Phase II – Refine Dose Selection and Allocate Patients to the Optimal Dose

1. Treat each newly enrolled patient at the most desirable of the dose levels remaining open to enrollment. This can involve de-escalation, escalation, or no change in dose.
2. As each patient completes the observation period, evaluate the 100-Day outcomes for graft failure, toxicity and/or early death for this patient, or until stopping criteria are met.
3. If there are excess (according to the criteria in Table 5.8) graft failures, that patient's dose and all lower doses are closed to further enrollment.
4. If there are excess (according to the criteria in Table 5.8) toxicities and/or early deaths, that patient's dose is closed to further enrollment.
5. Re-evaluate the desirability of the current dose level based on the 100-Day outcomes for toxicity and/or early death and graft failure.
6. Repeat steps 1-5 until 54 patients are enrolled in Phase II, or all dose levels are closed to further enrollment.

Phase I consists of de-escalating through the doses, testing each one to ensure that toxicity and/or early death and graft failure rates are within acceptable parameters. The patient can be put on the currently tested dose level even if some patients previously enrolled to that dose level have not completed their 100-Day observation period, as long as their unobserved outcomes cannot trigger the 100-day safety stopping rule. If the new patient cannot be enrolled to the currently tested dose, the patient may be treated at the lowest of other available previously tested doses. Note that since the study de-escalates through dose levels, previously tested dose levels are higher, and presumably have less risk of graft failure. It is anticipated that Phase I will have between 24-27 subjects enrolled, assuming that the lowest dose level is reached, and an additional 3 subjects are enrolled to a previous dose level.

Phase II allocates 70 additional patients to the optimal dose, bringing the maximum sample size to 94 in total. Adaptive Bayesian criteria described in the technical appendix (Section 5.11) are used to rank the desirability of doses, and rankings can change as data accumulates, refining the selection of optimal dose made at the end of the Phase I portion of the study. A new patient can be put on the best dose even if some patients previously enrolled to that dose level have not completed their 100-day observation period, as long as their unobserved outcomes cannot trigger the 42-day safety stopping rule. If the number of graft failure in the cohort before Day 42 exceeds the Day 42 boundary, enrollment to the cohort is temporarily suspended while waiting for Day 100 results. If the number of graft failures exceeds the 100 Day boundary, enrollment to the cohort is permanently halted.

Table 5.3 Dosage Levels for CY

Days	Dose	Total Dose	Dose Level
3 (Day -4, -3, -2)	50 mg/kg/day	150 mg/kg	3
2 (Day -3, -2)	50 mg/kg/day	100 mg/kg	2
1 (Day -2)	50 mg/kg/day	50 mg/kg	1
0 (None)	None	0 mg/kg	0

5.4. Accrual

A maximum of 94 patients, up to 65 years of age with severe aplastic anemia who do not have an HLA-matched related donor, will be enrolled and followed for 24 months post-transplant. Recent NMDP data (Confer D, personal communication, 2004) indicate that in 2002, 2003 and 2004 the NMDP has facilitated a total of 30, 44 and 48 matched, unrelated donor transplants for aplastic anemia, respectively. While not all such patients would be captured in this study, these figures suggest that the enrollment portion of the study could be completed in eight years.

5.5. Primary Endpoint

The primary endpoint is the selection of the optimal CY dose based on assessments of graft failure (primary or secondary), toxicity and early death during 100 days of follow-up post-transplant.

5.6. Primary Objective

The primary objective is that with high probability, the optimal CY dose group will be selected by the dose-finding algorithm.

5.7. Power Considerations

The power for selecting the optimal dose, as computed via simulation, is given in Table 5.7.B corresponding to hypothetical scenarios in Table 5.7.A. Simulations are based on the maximum sample size of 81 subjects.

In scenarios A through I in Table 5.7.A, different probabilities of major toxicity (including early death) and engraftment, respectively, were assumed for various CY dose levels. The optimal dose level probabilities are given in bold italics. Refer to the technical appendix (Section 5.11) for the optimality criteria. These scenarios were then used to estimate the probabilities with which a certain dose level would be chosen by the CY dose finding scheme (Table 5.7.B). The operating characteristics of the design shown in Table 5.7.B were obtained from 10,000 simulated trials.

In scenarios A-D in Table 5.7.B, the algorithm selects the optimal dose with probability ranging from 0.76 to 0.80. If we define an “acceptable dose” as one with engraftment probability at least 0.80 and toxicity probability less than 0.40, the probability of selecting an acceptable dose ranges from 0.84 to 0.95. Scenarios E through I correspond to situations in which the trial should stop early. In scenario E there is no acceptable dose, and the algorithm almost always stops without finding a dose, on average after treating only 21 patients. In scenario F, the top three doses increasingly exceed the acceptable toxicity level of 0.30. However, given the increasing probabilities of engraftment at the higher doses, the dose-finding algorithm preferentially selects the higher doses. Scenario G is the most challenging to handle since the toxicity probabilities are all well below the 0.40 toxicity threshold, and the third dose’s engraftment probability is quite close to the 0.20 graft failure threshold. Nonetheless, the trial is stopped early almost 73% of the time. In the remaining 27% of trials that are not stopped early, the third dose is almost always selected. Scenarios H and I are similar to G; however, the lower efficacy probabilities result in earlier trial stopping.

Table 5.7.A Hypothetical Settings used in the Simulation Study for the CY Dose Finding Scheme

Scenario	CY Dose Level			
	Probability of Toxicity or Early Death / Probability of Engraftment			
	0	1	2	3
A	0.30/0.60	0.30/0.70	0.30/0.80	0.30/0.90
B	0.30/0.70	0.30/0.80	0.30/0.90	0.50/0.90
C	0.30/0.80	0.30/0.90	0.50/0.90	0.70/0.90
D	0.30/0.90	0.50/0.90	0.70/0.90	0.90/0.90
E	0.30/0.30	0.50/0.50	0.70/0.70	0.90/0.90
F	0.30/0.80	0.35/0.85	0.40/0.90	0.45/0.95
G	0.05/0.30	0.10/0.45	0.15/0.60	0.20/0.75
H	0.05/0.25	0.10/0.40	0.15/0.55	0.20/0.70
I	0.05/0.20	0.10/0.35	0.15/0.50	0.20/0.65

Table 5.7.B Statistical Properties of the CY Dose Finding Scheme

Scenario	Probability of Each Dose Being the Final Selection					Average Number of Patients Treated at Each Dose			
	Stop*	0	1	2	3	0	1	2	3
A	0.034	0.001	0.014	0.155	0.799	4	7	18	51
B	0.043	0.007	0.112	0.756	0.082	6	13	46	14
C	0.059	0.115	0.721	0.101	0.004	12	41	17	7
D	0.092	0.803	0.101	0.004	0.001	45	19	8	6
E	1.000	0.000	0.000	0.000	0.000	1	5	9	6
F	0.026	0.076	0.215	0.364	0.319	10	17	26	28
G	0.730	0.000	0.000	0.005	0.266	0	2	8	36
H	0.933	0.000	0.000	0.001	0.066	0	2	6	24
I	0.989	0.000	0.000	0.000	0.011	0	1	4	17

* Stops and fails to find a dose.

5.8. Stopping Guidelines for Safety Monitoring

Since this is a single-arm study (i.e., there is no patient randomization involved), the study will not stop early for efficacy. Safety monitoring has two objectives:

1. Minimize the number of patients who are put on a dose whose probability of graft failure and/or fatality without graft failure is too high.
2. Maximize the number of patients who are treated at the acceptable dose with the best levels of toxicity and efficacy (criteria are detailed in Section 5.11).

Objective 1 is accomplished through the stopping guideline in Table 5.8.A. Table 5.8.A specifies the guideline by which a dose would be declared “unacceptable” with respect to the composite endpoint or graft failure and/or fatality without graft failure by Day 100. Objective 2 is accomplished by evaluating dose desirability using engraftment-toxicity trade-off contours. The motivation for the guidelines is Bayesian and is discussed further in the technical appendix.

A new patient can be put on the optimal dose even if some patients already enrolled to that dose have not completed their 100-day observation period, as long as their unobserved outcomes cannot trigger the 42-day pausing guideline in Table 5.8.B.

After 29 subjects had been enrolled to the current trial, both the 150 mg/kg and the 0 mg/kg doses were closed due to excess toxicity and excess graft failure, respectively. It was determined

that the next cohort of six subjects will be allocated to the 100 mg/kg dose level. The stopping guideline for the composite endpoint was not met after six subjects were enrolled to the 100 mg/kg dose level, the DSMB was consulted to discuss how additional subjects should be allocated (i.e., to the 100 mg/kg or the 50 mg/kg dose). To aid this decision making, the Bayesian posterior probability calculations of graft failures and fatalities without graft failure were provided. While the 100 mg/kg dose level is currently the most optimal dose, the 50 mg/kg dose is very close to the 100 mg/kg dose in desirability. The DSMB endorsed the proposal to allocate additional subjects in parallel to both the 100 mg/kg and 50 mg/kg dose levels under separate but parallel tracks covered by the current stopping guideline for the composite endpoint. Accrual at these two dose levels will be allowed to continue until the stopping guidelines are individually met or the data as a whole will trigger a formal DSMB review.

Monitoring of key safety endpoints will be conducted weekly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures.

Table 5.8.A Stopping Guidelines for Closing a Dose to Accrual

Number of Subjects Assigned to the Dose	Number of Graft Failures or Fatalities without Graft Failure by Day 100
1-6	3
7-12	5
13-18	6
19-24	7
25-30	9
31-36	10
37-42	11
43-48	12
49-54	14
55-60	15

Table 5.8.B Guidelines for Pausing Accrual While Waiting for 100-Day Outcomes

Number of Subjects Assigned to the Dose	Number of Graft Failures or Fatalities without Graft Failure by Day 42
1-6	2
7-12	3
13-18	4
19-24	4
25-30	5
31-36	6
37-42	6
43-48	7
49-54	8
55-60	8

5.9. Demographics and Baseline Characteristics

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

5.10. Secondary Endpoints and Analysis Plan

The secondary endpoints discussed in this section will be analyzed for the optimal CY dose group.

Post-Transplant Survival

The event analyzed is the time from transplant to death from any cause. Patients alive at the time of last observation will, for statistical purposes, have a survival time, which is censored. The 24-month probability of survival and corresponding 95% confidence interval will be calculated using the Kaplan-Meier product limit estimator.

Graft Failure

Secondary graft failure will be analyzed. In addition, the cumulative incidence of patients with primary or secondary graft failure, with death as a competing risk, will be estimated.

Time to Acute GVHD

To assess the incidence and severity of grades 2-4 and 3-4 acute GVHD from day of transplant, the first day of acute GVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that acute GVHD grade. An overall cumulative incidence curve will be computed along with a 95% confidence interval at 100 days post-transplant with death considered as a competing risk.

Time to Chronic GVHD

To assess the incidence and severity of chronic GVHD from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at two years post-transplant. Death will be considered a competing risk.

5.11. Technical Appendix – Bayesian Dose-selection Methodology

Overview

The dose selection method is motivated by the approach of Thall and Cook (2004, *Biometrics* **60**, 684-693). The dose-finding algorithm is initialized by specifying beta prior distributions for the respective engraftment and fatality without graft failure rates at each CY dose level. After each cohort of patients is enrolled and followed, the observed endpoint data from the cohort is used to update the posterior distributions for the engraftment and fatality without graft failure rates of the dose that the cohort received.

The stopping rule for the composite endpoint of graft failure and/or fatality without graft failure (Table 5.8A) defines a candidate set of doses that are still open for enrollment. If there are untried doses in the candidate set, the next cohort will receive the dose level immediately below the current dose. If all of the candidate doses have been tested, the next cohort's dose will be the "most desirable" dose as defined below.

Bayesian Motivation for the Stopping Guideline in Table 5.8A

The 100-day stopping rule for the composite endpoint of graft failure and/or fatality without graft failure states that a dose is unacceptable if there is sufficient evidence to suggest a composite event rate (i.e., graft failure rate or fatality without graft failure rate) greater than 20%.

To construct the guideline, we computed the minimum number of events in, respectively, $x = 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78$ patients which would correspond to $\Pr\{\pi_F > 0.2 \mid \text{number of events in } x \text{ patients}\} > 0.7$

Here, π_F is assumed to have a beta (0.6, 5.4) prior distribution. This distribution has mean 0.1 and has the weight of six "prior" patients.

The Day 42 pausing guideline (see Table 5.8.B) was motivated by the fact that we anticipate that roughly half of the events in the first 100 days will occur before Day 42 and half after. This assumption is supported by the experience in the Phase I portion of this protocol, and is consistent with CIBMTR data.

Thall’s and Cook’s Dose-desirability Method

We use Thall’s and Cook’s dose-desirability method which is based on Bayesian and geometric methodology. There are three steps needed to implement this method:

1. Specify the maximum number of patients to be enrolled (78) and the cohort size (6).
2. Specify dose-specific prior distributions for engraftment and fatality without graft failure, respectively.
3. Construct a dose-desirability engraftment-fatality without graft failure trade-off contour.

For Step 1, the cohort size refers to the number of patients we plan to put on a dose before possibly changing the dose. Note that enrollment to a cohort will be stopped prematurely if the stopping guideline for the composite endpoint is met. The total sample size and cohort size were dictated in part by the logistical constraints of operating a multi-center study in a rare disease, and in part by the statistical power to select the optimal dose, as shown in Tables 5.7.A and 5.7.B.

For Step 2, we hypothesized for the four CY doses prior mean probabilities of engraftment of (0.70, 0.80, 0.90 and 0.95) and for fatality without graft failure of (0.05, 0.10, 0.20, and 0.30). See Table 5.11. Note that the distribution is symmetric, giving each outcome equal impact on dose desirability calculations. Assigning the weight of three “prior” patients to each prior mean, we obtained the following beta prior distributions:

Table 5.11: Prior Distributions for the Four CY Doses

Dose	Prior Mean for Engraftment	Beta Prior Distribution for Engraftment	Prior Mean for Fatalities without Graft Failure	Beta Prior Distribution for Fatality without Graft Failure
0 = 0 mg/kg CY	0.70	(2.1, 0.9)	0.05	(0.15, 2.85)
1 = 50 mg/kg CY	0.80	(2.4, 0.6)	0.10	(0.3, 2.7)
2 = 100 mg/kg CY	0.90	(2.7, 0.3)	0.20	(0.6, 2.4)
3 = 150 mg/kg CY	0.95	(2.85, 0.15)	0.30	(0.9, 2.1)

For Step 3, we specified the engraftment-fatality without graft failure trade-off as follows: First, we selected 0.80 as the smallest engraftment rate that would be considered acceptable if the fatality without graft failure rate were 0. Next, we selected 0.20 as the largest acceptable fatality without graft failure rate if the engraftment rate were 1.0 (i.e., engraftment always occurs).

Lastly, we selected (0.90, 0.10) as an intermediate point on the acceptable engraftment-fatality without graft failure trade-off contour. Using Thall’s and Cook’s EffTox Version 2.10 software, we obtained the trade-off contour pictured in Figure 5.11.

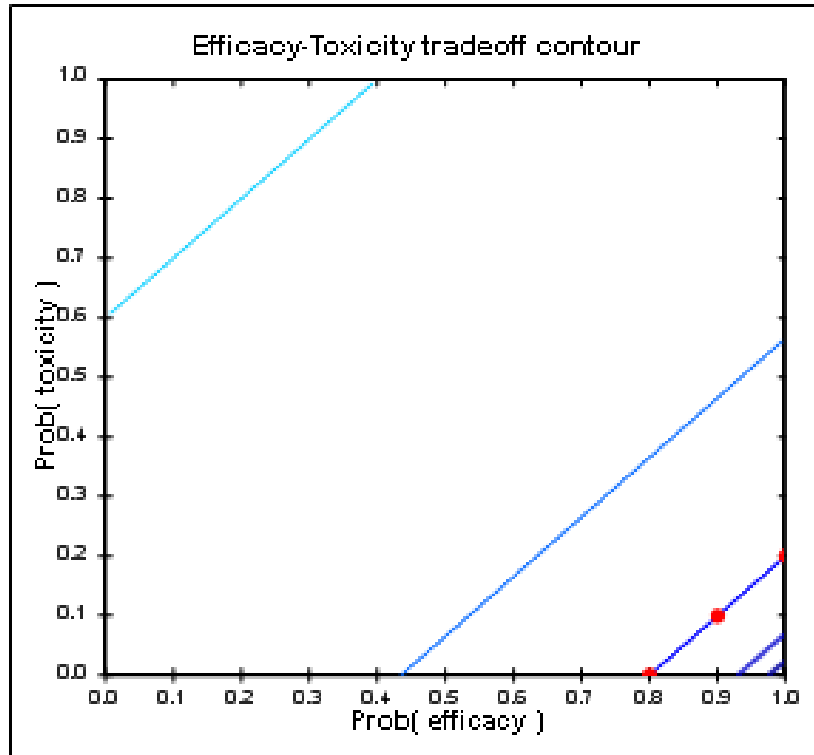


Figure 5.11 – Efficacy-Toxicity Trade-off Contours for Trial

The “desirability” of a dose is defined on p.687 of Thall and Cook (2004) in terms of the dose’s posterior means of engraftment and fatality without graft failure corresponding to the prior distributions from Step 2 and the trade-off contour from Step 3. Specifically, let:

\mathbf{q} = the ordered pair of posterior means of engraftment and toxicity conditional on the observed data and prior distributions

\mathbf{C} = trade-off contour

$\mathbf{p} = \mathbf{C} _ \{ \text{the straight line through } \mathbf{q} \text{ and } (1,0) \}$

$\rho(\mathbf{q})$ = Euclidean distance from \mathbf{q} to (1,0)

$\rho(\mathbf{p})$ = Euclidean distance from \mathbf{p} to (1,0)

Then the *desirability* of dose x is $\{ \rho(\mathbf{p}) / \rho(\mathbf{q}) \} - 1$. Note that all doses on the same contour line in Figure 5.12 have the same desirability, and desirability of a dose increases as we move towards the (1,0) corner of the graph depicting perfect engraftment and no fatalities without graft failure.

APPENDIX A
BEARMAN SCALE

APPENDIX A

BEARMAN TOXICITY GRADING SCALES
Regimen-Related Toxicity According to Organ System

	Grade I	Grade II	Grade III
Cardiac toxicity	Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on chest x-ray 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 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days 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 chest x-ray changes not caused by infection or congestive heart failure; or chest x-ray showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	Chest x-ray 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 bilirubin \geq 2.0 mg/dL and \leq 6.0 mg/dL or weight gain > 2.5% and < 5% from baseline, of non-cardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning	Moderate hepatic dysfunction with bilirubin > 6.0 mg/dL and < 20 mg/dL; or SGOT increase > 5-fold from preconditioning; or clinical ascitis or image documented ascitis > 100 mL; or weight gain > 5% from baseline of non-cardiac origin	Severe hepatic dysfunction with bilirubin > 20 mg/dL; or hepatic encephalopathy; or ascitis compromising respiratory function
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

	Grade I	Grade II	Grade III
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 day not related to infection	Watery stools > 2,000 mL every day 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

Note: Grade IV regimen-related toxicity is defined as fatal toxicity

Abbreviation: IV = intravenous

APPENDIX B
CONSENT FORM

PATIENT CONSENT

IRB #

Informed Consent to Participate in Research



Principal Investigator Contact Information

(INSERT CONTACT INFORMATION FOR PI AT YOUR SITE.)

Study Sponsor

This study is sponsored by the National Institutes of Health (NIH) by providing financial support through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

Introduction

This is a clinical trial, which is a research study designed to answer specific medical questions. The information from this study may or may not help you to overcome your disease. It may help future patients. The investigator/physician responsible for the study at your institution will explain the clinical trial to you and will answer the questions you may have. Clinical trials include only people who choose to join the study.

Please take your time to decide if you want to join this study. Some people find it helpful to talk about the study with their family and friends before they make a decision. It may also be useful to talk with your doctor and other people on your health care team about the study. If you have questions or want to know more about the study, you can ask them for more information.

You are being asked to take part in this study because you have Severe Aplastic Anemia (SAA). An allogeneic bone marrow transplant can be used to treat SAA. An allogeneic marrow transplant is when marrow cells from another person are collected and are infused in your body. The donated cells can come from a related or unrelated donor. Donated cells should match your cells as closely as possible. You do not have a matched related donor therefore you will need to receive cells from a matched, unrelated donor.

Before you decide whether or not to join the study, please read the information below. Feel free to ask questions to understand your rights and protections. Participating in this study is your choice. If you decide not to be in this study, you and your doctor will discuss other treatment options.

PATIENT CONSENT**IRB #****Why is this study being done?**

Your bone marrow produces white cells, red cells and platelets. The cells produced in the bone marrow move into your blood stream. Aplastic anemia is a blood disorder where the bone marrow has stopped working and produces very few or no cells to be released into the bloodstream. If not successfully treated, this condition will almost always lead to death, primarily because of infection or bleeding. A bone marrow transplant is an option for you because your doctors believe it may cure your aplastic anemia. As you do not have a matched, related donor, a matched, unrelated donor (i.e., not a blood relative) has been selected for you. Before you receive a matched, unrelated donor marrow transplant, you will receive medications and radiation to kill immune cells in your body that might reject the cells from your donor. This will allow your body to accept the donor marrow. This is called engraftment. The purpose of this study is to determine what dose of cyclophosphamide (a medication used to reduce your diseased cells and lower your immune system to allow your body to accept the donor marrow cells) should be used along with other pre transplant medications to improve safety and outcomes of transplantation. The purpose includes trying to reduce the risks and complications of the transplant while still allowing the donor marrow cells to replace the diseased marrow cells.

How many people will take part in the study?

As many as 94 patients will take part in this study at different hospitals in the United States.

What will happen if I take part in this research study?

Before you begin the study — You will need to have the following exams, tests or procedures to find out if you can be in the study. These tests are a normal part of transplantation procedure and would be done even if you did not join the study. If you have had some of them recently, they may not need to be done again. This will be up to your study doctor. The tests include:

- Medical history
- Physical examination, including height and weight
- Blood tests
- Urine tests
- Heart function tests
- Lung tests, including a Pulmonary Function Test (PFT) or pulse oxymetry for pediatric patients
- Bone marrow biopsies and aspirates
- If you are a woman able to have children, a serum pregnancy test will also be performed. If you are pregnant, you will not be able to take part in this study.

During the study — If the exams, tests and procedures show that you can be in the study, you will receive a matched, unrelated bone marrow transplant. Before the transplant, you will be given medications and radiation to allow your body to accept your donor's stem cells. This is called the conditioning regimen. Prior to your transplant a catheter (i.e., a soft plastic tube) will be inserted into a vein under the collarbone to allow for medications and fluids to be given.

PATIENT CONSENT**IRB #**

Conditioning regimen — The conditioning regimen is used to kill the cells in your body that may reject the donor cells. The drugs to be used in this clinical trial are fludarabine, cyclophosphamide, and antithymocyte globulin (ATG), as well as low-dose total body radiation. All patients in this study will receive the same amount of fludarabine, ATG and low-dose total body radiation. You will receive fludarabine on the fifth, fourth, third and second days before your transplant by intravenous infusion (through your vein). You will receive ATG on the fourth, third, and second days before your transplant. ATG is an animal serum, and your study doctor may decide to employ a horse or a rabbit product. Both products have similar side effects and efficacy. You will receive total body radiation on the day before your transplant. Different groups of patients will get different amounts of cyclophosphamide. You will receive cyclophosphamide for a total of either one or two days before your transplant. The number of days depends on your group. Patients will be placed in the group that is being tested at that time. Your doctor will tell you which group you are in. Neither you nor your doctor can choose the group.

The main purpose of this study is to determine the best dose of cyclophosphamide. This means that small groups of patients are treated with a given dose of cyclophosphamide and followed closely until it can be determined that the new marrow has taken and the treatment has not caused any unacceptable harm. The doctors who are conducting the study have by now gained some experience with this conditioning regimen, and this has helped them to narrow down the number of possible doses of cyclophosphamide that you, as a new patient, may receive. You should be aware that some patients who received three days of cyclophosphamide (i.e., the highest dose) suffered severe side effects (including death), and therefore, new patients are no longer being placed in this group. You should also be aware that patients who received zero days of cyclophosphamide (i.e. the lowest dose) experienced graft rejection (i.e., their body did not accept the new marrow, and the new cells failed to grow), therefore, new patients are no longer being assigned to this group. Patients who now come to transplantation will be treated with either one or two days of cyclophosphamide.

Reinfusion of stem cells (transplantation) — After the conditioning regimen, the donor marrow cells will be given to you through your catheter. The cells will travel into the bloodstream to reach your bone marrow where they are expected to make healthy, new blood cells. This step is necessary to replace your diseased marrow and because the high dosages of drugs given to you during the conditioning regimen may also damage or destroy healthy cells in your bone marrow. Until the new cells begin producing healthy blood cells, you will be at an increased risk of bleeding or developing an infection.

Following the transplant, you will have the following standard tests and evaluations:

- Medical history
- Physical examination, including height and weight
- Blood tests
- Urine tests

PATIENT CONSENT**IRB #**

You will be expected to stay at the transplant center for at least three months after your transplant. You will be asked to return to the transplant center for regular follow-up care. The standard tests will be done at that time.

How long will I be in the study?

You will be in the study for up to two years. Follow-up for transplant will last as long as you require care. However, we would like to keep track of your medical condition for the rest of your life by contacting you and the doctor providing your regular medical care by phone or mail once a year. Keeping in touch with you and checking on your condition every year helps us look at the long-term effects of the study and transplantation in general. Many transplant centers include this type of long-term follow-up as part of their regular medical care. It is not necessary for you to agree to follow-up for longer than two years to participate in this study.

Can I stop being in the study?

Yes. You can decide to stop at any time. Tell your doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely. If you withdraw from the study, you will continue to have access to healthcare at [participating institution]. If you decide to withdraw, you should inform [the Principal Investigator] in writing. It is important to tell your doctor if you are thinking about stopping so any risks from the medications can be evaluated. Another reason to tell your doctor that you are thinking about stopping is to discuss what follow-up care and testing could be most helpful to you.

If you withdraw, there will be no penalty and you will not lose any benefits to which you are otherwise entitled. You will be asked to return for a checkup.

Can the Principal Investigator withdraw me from the study?

You can be taken off the study (with or without your consent) for any of the following reasons:

- You do not qualify to be in the study because you do not meet the study requirements. Ask your doctor if you would like more information about this.
- You need a medical treatment not allowed in this study.
- Sometimes there may be a wait period for a new patient to be enrolled. This may happen to you.
- The investigator decides that continuing in the study would be harmful to you.
- The study treatments have a bad effect on you.
- You become pregnant and the study treatment could be harmful to the fetus.
- You are unable to keep appointments or take study drugs as directed.
- The study is cancelled by the Food and Drug Administration (FDA) or the National Institutes of Health (NIH).

PATIENT CONSENT

IRB #

If you withdraw, can information about you still be used and/or collected?

If you withdraw from the study or your doctor withdraws you from the study, we ask that you agree that we can continue using all information about you that has already been collected as part of the study prior to your withdrawal and to continue to allow your doctor to tell us about your progress until 24 months after your transplant. You may, of course, say “no.”

What side effects or risks can I expect from being in the study?

Risks and toxicities related to bone marrow transplantation are described in detail below. They include low blood counts, bleeding, infection and graft-vs-host disease (GVHD). The risk of dying as a result of bone marrow transplantation is no less than 30%. Everyone taking part in the study will be watched carefully for any side effects. However, doctors don’t know all the side effects that may happen. Your health care team may give you medicines to help lessen side effects. In some cases, side effects can be serious, long-lasting, or may never go away.

You should talk to your study doctor about any side effects that you have while taking part in the study.

The following are side effects from the medications administered to help your body accept your donor’s marrow. These side effects usually get better completely after you stop taking the drugs, but some permanent or long-term problems, such as organ damage, may occur.

PATIENT CONSENT**IRB #** _____**POTENTIAL SIDE EFFECTS and RISKS from STUDY DRUGS**

Study Drug	Likely (10-25%)	Less Likely but Serious (< 10%)
Fludarabine	<ul style="list-style-type: none"> • Numbness/tingling palms and soles* 	<ul style="list-style-type: none"> • Confusion* • Tremors* • Seizures • Coma • Cancers of the immune system (i.e., lymphomas) • Lung damage
Cyclophosphamide	<ul style="list-style-type: none"> • Nausea/vomiting* • Diarrhea* • Sore mouth/throat* 	<ul style="list-style-type: none"> • Heart damage • Bladder bleeding • Lung damage
ATG (Antithymocyte globulin) Horse or Rabbit Product	<ul style="list-style-type: none"> • Fever/chills* • Hives* • Nausea* • Headache* • Body swelling* • Skin rash, joint aches and pain 	<ul style="list-style-type: none"> • Severe or life-threatening allergic reaction • Lymphomas (i.e., cancers of the immune system)
Total Body Radiation	<ul style="list-style-type: none"> • Fever* • Nausea/vomiting* • Diarrhea* • Skin redness* • Headache* • Hypothyroidism • Infertility 	<ul style="list-style-type: none"> • Lung or heart damage with scarring • Lung or heart failure • Cataract

* All of these side effects are temporary.

Risks, side effects and toxicities are described in greater detail on pages 12-15.

Are there benefits to taking part in the study?

There may or may not be direct benefits to you from participating in this study. We hope that information gathered in this trial will help future transplant patients.

What other choices do I have if I do not take part in the study?

There is no agreement among doctors on which medications to give patients prior to a matched unrelated donor marrow transplant to allow the donor cells to replace the diseased marrow. Patients who do not receive a transplant are usually treated with a variety of immunosuppressive drugs (drugs aimed at weakening their immune system). However, this care is not known to provide long-term control or cure of the disease. Most patients preparing for transplantation

PATIENT CONSENT**IRB #**

have already received immunosuppressive therapy without success. If you do not want to join this study, you should know your other options. These options may include:

- Treatment with other immunosuppressive drugs or combination of drugs.
- Treatment with drugs aimed at temporarily boosting your blood counts.
- Treatment with blood transfusions only.
- No therapy for the aplastic anemia at this time, with care to help you feel more comfortable.

You should know about your treatment choices before you decide if you will take part in this study.

What are the costs of taking part in this study?

You and/or your insurance company will pay all standard care relating to your transplant.

You will not be billed for any tests or procedures that are only for research.

You will not be paid to be in this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at <http://cancer.gov/clinicaltrials/understanding/insurance-coverage>. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

What if I am injured as a result of being in this study?

In the event that this research activity results in an injury, treatment will be available including first aid, emergency treatment and follow-up care as needed. Care for such injuries will be billed in the ordinary manner, to your insurance company. If you think you have suffered a research-related injury, let the study doctors know right away. Unexpected side effects or accidents might result in you getting sicker than anticipated in the course of this treatment. All available medical care will be provided to you, but you and your insurance company (3rd party payer) are responsible for the costs of all such care. If you have any question about study-related injuries, you may call [insert person's name at institution] at [insert phone #].

What are my rights if I take part in this study?

Taking part in this study is your choice. You may choose either to take part or not to take part in the study. If you decide to take part in this study, you may leave the study at any time. No matter what decision you make, there will be no penalty to you and you will not lose any of your regular

PATIENT CONSENT**IRB #**

benefits. Leaving the study will not affect your medical care. You can still get your medical care from our institution.

We will tell you about new information or changes in the study that may affect your health or your willingness to continue in this study.

In the case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

If you have any questions about your rights as a study patient, you may call the Institutional Review Board (IRB) office at [insert phone number].

Will my medical information be kept private?

Study records that have your name will be kept private as required by law. You will not be identified by name in the central study records. Your records will be given a unique code number. The key to the code will be kept in a locked file in the Principal Investigator's office.

All necessary steps will be undertaken to avoid you being identified in any public presentations. However, the results of this study treatment may be published in scientific journals in the future, but no one patient (including you) will be identified. Information concerning your transplant course may be reviewed or transmitted to national and international transplant registries, including the Center for International Blood and Marrow Transplant Research (CIBMTR), the National Marrow Donor Program (NMDP), the Food and Drug Administration (FDA), Data Coordinating Center of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), the EMMES Corporation (which is helping to coordinate this study) and other authorized study organizations. However, you will not be identified by name in publications or reports coming from such groups or review.

Expiration date for retention of records

Information about the study results will stay in your research file at [insert institution] for at least six years or until after the study is completed, whichever is longer. At that time either the research information not already in your medical record will be destroyed or your name and other identifying information will be removed from such study results. Research information in your medical record will be kept indefinitely.

PATIENT CONSENT

IRB #

HIPAA¹ authorization to use and disclose individual health information for research purposes

- a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher’s staff to use and disclose my individual health information for the purpose of conducting the research study
- b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work up and after transplantation (e.g., CT scan, blood tests, biopsy results).
- c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher’s staff may obtain my individual health information from:
(list hospitals, clinics or providers from which health care information can be requested)

- d. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item c and information disclosed by me during the course of the research may be received and used by the following parties:
 - Principal Investigator and the researcher’s staff, including Dr. Paolo Anderlini, Study Chairperson at MD Anderson Cancer Care Center
 - National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
 - Blood and Marrow Transplant Clinical Trials Network (BMT CTN), Data Coordinating Center
 - U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
 - U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments

¹ HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

PATIENT CONSENT**IRB #**

- e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.
- f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.
- g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.
- h. This authorization does not have an expiration date.

Who can I contact if I have questions or problems?

If you think you have suffered an injury as a result of this study, or have any other problems, you may contact (*INSERT CONTACT INFORMATION*). If it is after normal business hours or a weekend, you may contact (*INSERT CONTACT INFORMATION*).

If you have any questions about this study, you may contact the study Principal Investigator listed on the first page of this form.

If you have any questions about your rights as a research participant, you may contact (*INSERT CONTACT INFORMATION*).

PATIENT CONSENT

IRB #

CONSENT AND ASSENT INSTRUCTIONS

CONSENT: Patients 18 years and older must sign on the subject line below. For patients under 18, consent must be provided by the Legally Authorized Representative.

ASSENT: Is required for patients under the age of 18, using the Assent Section on the following page.

I have been informed about this study’s purpose, procedures, possible benefits and risks. I have been given the chance to ask questions. My questions have all been answered satisfactorily. I understand that I can ask other questions at any time.

I voluntarily agree to take part, or to allow my child to take part, in this study.

By signing this consent form, I have not given up any of the legal rights that I (my child) otherwise would have as a patient in a research study.

Patient’s Signature

Date

If you are not the patient, please print your name _____
and indicate one of the following:

The patient’s parent
A surrogate
A proxy

The patient’s guardian
A durable power of attorney
Other, please explain:

Legally Authorized Representative Signature

Date

As a representative of this study, I have explained the purpose, the procedures, the benefits, and the risks that are involved in this research study:

Signature of person conducting informed consent

Date

PATIENT CONSENT

IRB #

ASSENT SIGNATURES: For patients under the age of 18 years.

Assent of Minor

I have been told what I will be asked to do if I am in this study. I have been told that I don't have to be in this study. I may quit the study at any time, and no one will be mad at me. I have had a chance to discuss the study and ask questions. My questions have been answered. I agree to be in the study and do what I am asked to do so long as I continue in the study.

Signature of Minor
Study Personnel

Date

Age (years)

I have explained the purposes, procedures, and risks involved in this research study in detail to:

Print name(s) of Parents/Authorized Consenting Party, and

who in my opinion ____ IS/ ____ IS NOT capable of assenting to participate in this study.

Print child's name

Signature of Person Conducting Assent

Date

PATIENT CONSENT**IRB #****ATTACHMENT A****RISKS AND TOXICITIES RELATED TO BONE MARROW TRANSPLANT**

There are certain risks related to a marrow transplant. There are risks from the medications and irradiation therapy you will receive as part of the conditioning for the transplant and risks related to the transplant itself. Most of these risks and side effects are listed below, but they will vary from person to person.

Risks Related to the Transplant Procedure

Cyclophosphamide is a common medication used to treat cancer. This medication kills cancer cells by stopping them from growing. Cyclophosphamide may cause you to have diarrhea (loose stools), nausea (feeling sick to your stomach), vomiting (throwing up), short-term hair loss, short-term bladder problems, and, at times, bleeding from the bladder (blood in your urine). A few patients may have bladder damage and bleeding for a longer time. You will be given large amounts of a sterile solution through your central line to protect your bladder. A bladder catheter (thin plastic tube) may be inserted into your bladder, if your physician thinks that it can help you. Cyclophosphamide slows the making of new blood cells. This causes a risk of infection and/or severe bleeding until the transplanted donor cells begin to work in you. You will get blood transfusions as needed. Cyclophosphamide also lowers your immune (defense) system and as a result you may have more infections. In a small number of patients, cyclophosphamide can damage the heart muscle causing the heart not to pump as well (heart failure). If this occurs you may have shortness of breath and have fluids build-up in your body. Cyclophosphamide can damage the male (testes) or female (ovaries) sex glands. In men, the number of sperm may be reduced but you would still be able to have intercourse. Women who are still menstruating may have irregular periods or may no longer have any periods. Whether you are a man or woman, this medication may decrease your chances of being able to have a child.

Fludarabine may cause confusion, seizures, coma, or peripheral nerve damage with pain. It may also cause kidney, liver, and/or central nervous system dysfunction.

Antithymocyte globulin (ATG) may cause skin rash, skin itching, joint pain, swelling, and/or fever. Severe or life-threatening allergic reactions, including severe shortness of breath, throat swelling, and/or diarrhea may also occur. Some of these reactions can be life threatening or even fatal. Antithymocyte globulin can cause bleeding. ATG is an animal serum. Your doctor may decide to employ a rabbit or a horse product. The development of aggressive lymphomas (i.e., cancers of the immune system) has been reported in patients receiving ATG.

Total body radiation can cause skin redness, fever, nausea, vomiting, diarrhea, heart or lung damage with scarring, or low blood counts. If the bone marrow transplant that follows total body radiation is not successful, it will cause death.

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Cyclosporine and tacrolimus may cause rash and/or liver and/or kidney damage. These effects are temporary, and in most cases reversible. Any or all of these complications can be severe, irreversible, and may lead to death. They may cause tremors, confusion and seizures, and/or other blood-related effects that are reversible upon stopping the drug. Rare, fatal cases of severe allergic reactions or the development of aggressive lymphomas have been reported in patients receiving cyclosporine and/or ATG or tacrolimus.

Methotrexate may cause mouth sores, rash, liver damage or kidney damage.

Graft rejection may occur. Graft rejection means the infused donor cells fail to grow in the patient's body and are unable to produce new blood cells. This can be a serious or life-threatening complication.

Graft versus host disease (GVHD) is due to immune cells (i.e. lymphocytes) from the donor attacking the patient's organs. The acute form of GVHD may cause redness of the skin, nausea, vomiting, diarrhea, and/or liver problems. The chronic form of GVHD may cause dryness of the eyes and/or mouth, and/or breathing problems. It may cause tightness and/or scarring of the skin, weight loss, diarrhea, and/or difficulty swallowing. Either one of the forms of GVHD can range from mild to severe to life-threatening.

Acute GVHD usually occurs within the first 100 days post-transplant. Chronic GVHD usually occurs between 100 days and 1 year post-transplant and occasionally greater than 1 year post-transplant. Using these drugs with other drugs could cause other side effects that are not seen when each drug is given alone. If any doctor other than the Study doctor prescribes other drugs, the patient must tell the study nurse or doctor right away.

The immune system is severely weakened for the first 6-12 months after transplantation and slowly improves over several years. Unusual or late infections may occur because of this problem. These infections occasionally lead to death.

This research study may involve unpredictable risks to the participants.

Central venous catheter is a flexible sterile tube that can be placed into a large vein either under the collar bone or in your groin area so that blood can be withdrawn. This tube is placed under local anesthesia. Complications may include blood clots and infection. Clotting may necessitate removal of the catheter or treatment of the clot by injecting a medicine that dissolves blood clots. If you develop an infection, you will require treatment with antibiotics. If the catheter is placed under the collarbone, other uncommon side effects may include swelling of the face and arm and/or lung collapse. If the lung collapses, it may be necessary to place a tube between the ribs to allow the lung to re-expand.

Low White Cell Count: Your white cell count will remain low for at least 3-4 weeks after receiving your new marrow. Infections (for example, pneumonia) can occur and they can be severe or life-threatening. They can be caused by bacteria, viruses or fungi.

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Bleeding: Platelets help your blood to clot. Your platelets will be low until the new bone marrow grows and, as a result, bleeding may occur. This can be minor bleeding, such as nosebleeds or bruising, but more serious, life-threatening bleeding in the lungs, brain and other organs can occur if the platelet count remains low. Usually, there is success in preventing major bleeding problems with transfusions of platelets. However, some patients may not respond well to transfused platelets and may be at serious risk for bleeding.

Red Cell and/or Platelet Transfusions: As part of this treatment you are expected to need red cell and/or platelet transfusions.

Veno-Occlusive Disease (VOD): This can occur as a result of high dose chemotherapy, radiation therapy, or both. Veno-occlusive causes severe damage to the liver. Symptoms include jaundice (yellowing of the skin and eyes), weight gain, and extra fluid build-up in the abdominal cavity and other parts of the body. It can often be managed successfully, and completely resolved, but can potentially cause death.

Mouth Sores and Diarrhea: The chemotherapy and radiation cause irritation in the lining of the mouth and intestines. This can result in painful mouth sores and diarrhea and you may need medication to help control the pain. If your mouth sores are severe you may not be able to eat normally until the sores are healed. Mouth sores get better when the white blood count starts to rise.

Capillary Leak Syndrome: This may occur as a result of chemotherapy and radiation therapy. The blood vessels may become ‘leaky’ and fluid enters the abdominal cavity, lungs, and other tissues. You may gain water weight and not go to the bathroom as often as you normally do. Capillary leak syndrome can be difficult to manage if extra fluid enters the lungs and causes difficulty breathing. You may die if there is continued fluid collection in the lungs.

Unexpected Organ Damage and Other Side Effects: Although your major organs function well, it is possible you may experience unexpected, life-threatening heart, lung, kidney, or liver damage as a result of the transplant. Occasionally, the high doses of chemotherapy and radiation cause severe lung damage that cannot always be treated. If this happens, you may need to use oxygen or even a respirator. The lung damage can be life threatening. Rarely, multi-organ failure (such as lung and kidney failure) may occur, which is often fatal.

Thrombotic Thrombocytopenic Purpura (Thrombotic Microangiopathy): In this post-transplant complication, chemotherapy and/or radiation produce damage to the lining of the blood vessels causing generalized clotting in the bloodstream. These clots can choke the blood supply to some of the body organs and this can lead to brain and kidney damage, a drop in the platelet count, as well as high blood pressure. This complication may be severe or life threatening and plasma exchange may be required.

Late Effects: You may experience side effects that occur several months to many years after your transplant. You may experience poor function of the thyroid gland, requiring you to take thyroid medication. Symptoms of poor thyroid function include dry skin, constipation, fatigue,

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cold intolerance, weight gain and poor appetite. As a result of radiation, cataracts may occur earlier in life compared to a person who had not had a transplant. If you develop cataracts they may require treatment. It is rare, but your kidneys could be affected, causing anemia or high blood pressure. There is also a risk you may develop cancer including leukemia as a result of the chemotherapy. If secondary cancers occur they generally do not occur until 10 to 15 years after the transplant but can occur sometimes within five years after transplant. The long-term effects upon heart, lung, and brain are unknown.

Fluid Build-up: You will receive intravenous fluids during the transplant process and you may have difficulty eliminating this fluid. Your doctor will use standard drugs to remove this fluid. This drug may cause hearing loss and loss of body chemicals such as potassium and sodium. This loss is temporary and reversible.

Risk to the Unborn

The treatment that you are undertaking has not been proven to be safe at any stage of pregnancy. Therefore, if you are pregnant or nursing, you are not eligible for this study. Women who have the potential of becoming pregnant must use some form of effective birth control.

Sterility and Future Childbearing Potential for Men and Women

Chemotherapy and/or irradiation can impair or decrease fertility and may cause lasting effects on the reproductive potential of both men and women treated in this manner. The transplantation procedure may cause a decrease in sexual desire and function. It should be emphasized that your cancer treatment/therapy may cause your menstrual periods to become irregular or cease altogether. However, this DOES NOT MEAN THAT YOU CANNOT BECOME PREGNANT, as the effect on fertility is usually not permanent or irreversible. You must use birth control if you want to avoid becoming pregnant.

Risks Related to the Infusion of Bone Marrow

The stem cell infusion is given similar to a blood transfusion. The infusion of stem cells usually has few side effects. Rarely the infusion may cause a headache, chest pain or trouble breathing, a slight fever, or blood in the urine. You will be given pre-medications just prior to the infusion to decrease the risk of a reaction. In rare instances, a severe allergic reaction can occur called anaphylaxis, which could cause a drop in blood pressure or extreme difficulty in breathing. You will be monitored very closely.

APPENDIX C
LABORATORY PROCEDURES

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LABORATORY PROCEDURES

1. HLA TYPING

Before Transplantation: HLA typing will be performed for all patients and donors in American Society of Histocompatibility and Immunogenetics (ASHI)-approved laboratories designated by the transplant centers. HLA typing must be performed by DNA methods for HLA-A, -B, and -C at intermediate resolution, and DRB1 at high resolution, consistent with NMDP standard procedures. HLA typing for DQ is recommended but not mandatory.

After Transplantation: High resolution HLA typing of cryopreserved patient and donor samples is conducted as an ongoing research study by the NMDP. Data will be shared with the BMT CTN.

2. CHIMERISM

Samples of peripheral blood or marrow are collected from patient and donor pre-transplant for chimerism studies according to institutional standards. Patient samples are also collected on Day 28, 100 and 12 months post-transplant. Chimerism results will be reported as “percent donor DNA.” Chimerism should be determined by PCR-based methods (e.g., microsatellite polymorphism analysis or real-time PCR).

3. PATHOLOGY/CYTOGENETICS STUDIES

A bone marrow biopsy/aspirate is required within 12 weeks prior to the initiation of conditioning therapy, and at Day 100 \pm 30 days for all patients. A bone marrow biopsy/aspirate is recommended but not required at 12 months \pm 3 months post-transplant for all patients. Cytogenetics is required at Day 28, Day 100 and 12 months post transplant if patient-donor sex mismatch present. Cytogenetics may be replaced by FISH (X/Y probe) on marrow or peripheral blood at similar timepoints. Pathology and cytogenetic studies will be conducted per institutional guidelines.

4. GRAFT CHARACTERIZATION

Graft processing and characterization will be performed in keeping with the BMT CTN MOP and local institutional practice.

5. IMMUNOGLOBULIN MONITORING

Quantitative immunoglobulin levels including IgG, IgA and IgM are recommended, but not required, within six weeks prior to the initiation of the preparative regimen. Testing will be done in keeping with the BMT CTN MOP and local institutional practice.

6. EPSTEIN-BARR VIRUS (EBV) SURVEILLANCE

Epstein-Barr Virus (EBV) surveillance using a real time quantitative EBV DNA PCR plasma-based assay will be performed at least every two to four weeks beginning at engraftment through Day 90-100, according to institutional standards. Institutions employing real-time PCR for EBV DNA monitoring will determine the threshold for rituximab treatment according to the local sensitivity and specificity of the test.

SCHEDULE OF LABORATORY EVALUATIONS

Type of Sample	Method	Type of Storage	Dates Samples Obtained	Shipping Specifications	Test Location
HLA Typing	According to institutional practice	According to institutional practice	<i>Patient:</i> Prior to the initiation of conditioning therapy. <i>Donor:</i> Prior to the day of transplantation.	N/A	Transplant Center
Chimerism	PCR-based methods (e.g., microsatellite polymorphism analysis or real-time PCR)	According to institutional practice	<i>Patient:</i> Prior to the initiation of conditioning therapy and on Day 28, 100, 365 post-transplant. <i>Donor:</i> Prior to collection.	N/A	Transplant Center
Pathology/ Cytogenetic Studies	According to institutional practice	According to institutional practice	Within 12 weeks prior to the initiation of conditioning therapy and on Day 100 ±30 days for all patients (12 months ± 3 months post-transplant is optional for all patients). Cytogenetics or FISH (X/Y probe) on the marrow or peripheral blood required for patient-donor sex mismatch at Day 28, Day 100 and 12 months.	N/A	Transplant Center
Graft Characterization	According to BMT CTN MOP and institutional practice	According to BMT CTN MOP and institutional practice	Day of collection.	N/A	Transplant Center
Immunoglobulin Monitoring ¹	According to institutional practice	According to institutional practice	Within six weeks prior to the initiation of conditioning therapy.	N/A	Transplant Center
Epstein-Barr Virus (EBV) Surveillance	Quantitative PCR	According to institutional practice	At least every 2 to 4 weeks beginning at engraftment through Day 90-100.	N/A	Transplant Center

¹Recommended but not mandatory

APPENDIX D
HUMAN SUBJECTS

APPENDIX D

HUMAN SUBJECTS

1. Patient Consent

A conference will be held with the patient and family to discuss this study and alternative treatments available for the treatment of the underlying disease. The Principal Investigator or another designated physician will conduct the conference. All potential risks associated with the treatment should be discussed as objectively as possible.

The consent document should be reviewed with the patient and family prior to proceeding to transplant.

Informed consent from the patient will be obtained using a form approved by the Institutional Review Board of the institution enrolling the patient.

2. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relating the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the BMT CTN Data Coordinating Center upon enrollment.

3. Participation of Women and Minorities and Other Populations

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on incidence of aplastic anemia in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment strategies.

APPENDIX E
REFERENCES

APPENDIX E**REFERENCES**

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