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PROTOCOL TITLE: Pilot and Feasibility Study of Reduced-Intensity Hematopoietic Stem Cell Transplant for Patients with GATA2 Mutations

Abbreviated Title: HSCT for GATA2 Mutation

Principal Investigator: Dennis D. Hickstein M.D. [1]
Telephone: 301-594-1718
Email: hicksted@mail.nih.gov

Lead Associate Investigator: Mark Parta, M.D.[9]
Telephone: 301-451-4955
Email: mark.parta@nih.gov

Medical Advisory Investigator: Juan Gea-Banacloche, M.D.[1]
Telephone: 301-675-9512
Email: banacloj@mail.nih.gov

Associate Investigators:

Terry Fry, M.D.[7]	Steve Holland, M.D.[2]
Ronald Gress, M.D. [1]	#Brenna Hansen, R.N. [10]
Steven Pavletic, M.D. [1]	#Constance Yuan, MD, PhD[9]
Daniel Fowler, M.D.[1]	James Kochenderfer, M.D.[1]
#Charles Bolan, M.D. [6]	#Jennifer Wilder, R.N.[8]
#Daniele Avila, C.R.N.P. [1]	#Seth Steinberg, Ph.D. [4]
#Fran Hakim, Ph.D.[1]	#Cynthia Delbrook, R.N.[10]
#Bazetta Blacklock-Schuver, R.N.[10]	#Thomas Hughes, Pharm.D.[3]
#Maryalice Stetler-Stevenson, MD, PhD[9]	#Hanh Khuu, M.D. [5]
#David Stroncek, M.D. [5]	#Jennifer Mann, C.R.N.P.[1]
#Sharon Adams, M.T. [5]	#Stephanie Cotton, R.N. [10]
#Kristen Cole, R.N. [10]	

[1] Experimental Transplantation and Immunology Branch, CCR, NCI, NIH
[2] Clinical Infectious Diseases, NIAID, NIH
[3] Pharmacy Department, Clinical Center, NIH
[4] Biostatistics and Data Management Section, National Cancer Institute
[5] Department of Transfusion Medicine, Clinical Center, NIH
[6] Hematology Branch, NHLBI, NIH
[7] Pediatric Oncology Branch, CCR, NCI, NIH
[8] Leidos Biomedical Research, Inc. (NCI ETIB Contractor)
[9] Laboratory of Pathology, CCR, NCI, NIH
[10] Office of Clinical Director, CCR, NCI, NIH

Referral contact: Dennis D. Hickstein M.D
#Denotes individuals who will not make any clinical protocol decisions
Study coordinator: Kristen Cole, R.N

Email: colekr@mail.nih.gov
Telephone: 301-827-0374

Précis

Background

- Mutations in GATA2 lead to an immunodeficiency disease that transforms into myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML). This syndrome, previously known as MonoMAC, has 4 clinical features: 1) infections with Mycobacterium Avium Complex (MAC) and other opportunistic infections as a teenager or young adult, 2) a peripheral blood leukocyte flow cytometry profile with T-lymphocytes, but a severe deficiency of monocytes, B-lymphocytes, and Natural Killer (NK) cells, 3) the propensity to progress to MDS/AML, and 4) mutations in the gene GATA2. In this pilot study we propose to evaluate the efficacy and safety of a reduced-intensity allogeneic hematopoietic stem cell transplantation (HSCT) regimen for patients with mutations in GATA2. We are particularly interested in determining whether allogeneic HSCT using this regimen reconstitutes normal hematopoiesis in patients with mutations in GATA2.

Objectives

- Primary Objective:
  1) To determine efficacy, namely whether reduced-intensity allogeneic HSCT results in engraftment and restores normal hematopoiesis by day +100 in patients with mutations in GATA2.
  2) To determine the safety of this HSCT regimen in patients with mutations in GATA2, including transplant related toxicity, the incidence of acute and chronic graft-versus-host disease, immune reconstitution, overall survival, and disease-free survival

Eligibility

Eligibility includes patients 12-60 years old with mutations in GATA2 who have a life-expectancy of > 3 months but < 24 months, and who have a 10/10 matched related donor, a 10/10 or 9/10 matched unrelated donor (HLA -A, -B, -C, DRB1, DQB1 by high resolution typing identified through the National Marrow Donor Program), a 4/6 (or greater HLA -A, -B, DRB1) matched unrelated umbilical cord donor, or a haploidentical donor. Patients with GATA2 mutations who are 12-17 years of age are required to have MDS with chromosomal abnormalities to be eligible for this protocol.

Design

- Patients with mutations in GATA2 with a 10/10 matched related or 10/10 matched unrelated donor, will receive a reduced-intensity pre-transplant conditioning regimen consisting of fludarabine 30 mg/m^2/day on days -4, -3, and -2, 200 cGy total body irradiation (TBI) on day -1, and HSCT on day 0. Patients with mutations in GATA2 and a 9/10 matched unrelated donor will receive a reduced-intensity pre-transplant conditioning regimen consisting of fludarabine 30 mg/m^2/day on days -4, -3, and -2, 300 cGy total body irradiation (TBI) on day -1, and HSCT on day 0. Patients with mutations in GATA2 with umbilical cord blood units will receive a reduced-intensity conditioning regimen with cyclophosphamide 50 mg/kg on day -6, fludarabine 40 mg/m^2 on days -6 to -2, equine ATG 30 mg/kg IV on days -6, -5 and -4, 200 cGy TBI on day -1, and HSCT on day 0. Patients with a haploidentical donor will receive a reduced intensity-conditioning regimen with cyclophosphamide 14.5 mg/kg on days -6 and -5, fludarabine 30 mg/m^2 on

days -6 to -2, and 200 cGy TBI on day -1. Donor bone marrow cells will be infused on day 0.

- Post-transplant immunosuppression for graft-versus-host-disease prophylaxis will consist of sirolimus (Rapamycin) and tacrolimus until day +180, provided that there is no evidence of graft-versus-host disease. Post-transplant immunosuppression for graft-versus-host-disease prophylaxis for recipients of haploidentical donors will consist of cyclophosphamide 50 mg/kg on days +3 and +4, along with sirolimus from day +5 to day 180, and tacrolimus from day +5 to day 180, providing that there is no GVHD.

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1 INTRODUCTION

1.1 PRIMARY STUDY OBJECTIVES

1) In a pilot fashion, to determine efficacy, namely whether reduced-intensity allogeneic hematopoietic stem cell transplant (HSCT) results in engraftment and restores normal hematopoiesis by day +100 in patients with mutations in GATA2.
2) To determine the safety of reduced-intensity allogeneic HSCT regimen in patients with mutations in GATA2 including transplant related toxicity, the incidence of acute and chronic graft-versus-host disease, immune reconstitution, overall survival, and disease-free survival.

1.2 BACKGROUND AND RATIONALE

1.2.1 Introduction

Since its first description 50 years ago, the number of hematopoietic stem cell transplants (HSCT) has increased steadily; 50,000 patients worldwide received a HSCT in 2006 (Thomas et al. 1957; Appelbaum 2007). Although allogeneic HSCT can cure hematological malignancies, bone marrow failure syndromes, and genetic hematological and immunodeficiency diseases, the success of therapy depends upon the prompt identification of a suitable donor. Because matched related donors were required in the initial HSCT, this limited the number of HSCT performed; only 30 percent of otherwise eligible patients with leukemia in the United States have a related histocompatible donor. A major factor in the increase in HSCT over the preceding 20 years has resulted from the use of alternative donors.

1.2.2 Reduced-Intensity Conditioning Regimens for Matched Related Donors

Allogeneic hematopoietic stem cell transplantation with a myeloablative-conditioning regimen represents a potentially curative therapy for hematological malignancies, however this treatment approach has not been pursued in many instances due to the high regimen-related toxicity with age greater than 60 years, or with concomitant co-morbidities. The unacceptable risk of death from conventional myeloablative transplantation has also precluded this curative therapy for patients with other non-malignant hematological diseases. However, it has recently been shown that these high intensity regimens may not be required for engraftment. As a result of these findings, many centers are now exploring reduced-intensity conditioning regimens in order to reduce the toxicity associated with HSCT in both malignant and non-malignant hematological diseases. The evolution in thinking regarding myeloablative versus reduced-intensity conditioning regimens is described in the ensuing paragraphs.

The use of conditioning regimens containing high doses of alkylating agents, often in combination with total body irradiation, are considered “myeloablative;” these regimens were perceived to be essential both for eradication of malignant disease and for suppression of the host-versus-graft response to the donor stem cells (i.e. rejection) (Copelan 2006). Myeloablative conditioning regimens caused severe damage to normal organs within the body resulting in a high incidence of morbidity and mortality (Bearman et al. 1988). This limited the application of allogeneic HSCT to younger patients (< 60 years of age) who could tolerate these toxicities. Subsequent studies and observations challenged the assumption that myeloablative conditioning was necessary to eliminate malignancy and permit the engraftment of the donor hematopoietic and immune systems.

The high frequency of relapse observed in the setting of T-cell-depleted allogeneic HSCT suggested that a significant proportion of the curative potential of allogeneic HSCT resulted from a T-cell-mediated graft-versus-tumor (GVT) effect (Champlin 1990). It was also recognized that conventional myeloablative regimens do not completely eliminate the host response to the allograft, even in recipients of HLA-matched allografts. For example, one study indicated that 30 to 40% of patients will reject a T-cell-depleted, HLA-matched allogeneic stem cell graft after myeloablative conditioning (Martin et al. 1988). In addition, there are certain clinical situations where the malignancy is very susceptible to the allogeneic GVT effect (e.g. AML in complete remission, chronic leukemia or low-grade lymphoma. In these clinical situations, the use of a myeloablative-conditioning regimen may be adding toxicity without eradicating the tumor.

These limitations of myeloablative conditioning regimens, combined with the high levels of morbidity and mortality associated with myeloablation, result in a compromised therapeutic index for this form of allogeneic HSCT. Reducing treatment-related toxicity would improve the therapeutic index of allogeneic HSCT, permitting the broader application and potential benefit of this treatment to more patients. This principle led to the development of less intensive "nonmyeloablative" and "reduced-intensity" conditioning regimens for allogeneic HSCT, which were designed with the purpose of allowing engraftment of donor cells to exploit the potential allogeneic GVT effect (Giralt et al. 1997; Slavin et al. 1998; Childs et al. 1999; Spitzer et al. 2000; Khouri et al. 2001).

The early reports of studies employing nonmyeloablative and reduced-intensity conditioning regimens prior to allogeneic HSCT demonstrated that early engraftment occurred in the majority of patients, and early transplant-related toxicities (first 100 days after transplantation) were reduced. This indicated that reduced-intensity regimens could be used for older patients and patients with co-morbidities. However, it was also observed that both nonmyeloablative and reduced-intensity conditioning were associated with high incidences of incomplete donor chimerism, higher incidences of graft rejection/failure, and increased relapse rates, as compared to results with myeloablative conditioning regimens (Khouri et al. 1998; Aoudjhane et al. 2005). Mixed chimerism and graft rejection were also associated with HLA-disparity and the use of unrelated donors, and correlated inversely with the amount of therapy prior to transplant (McSweeney et al. 2001; Maris et al. 2003; Carvallo et al. 2004). It was also observed that a GVT effect was not observed until full donor chimerism was achieved (Truitt and Atasoylu 1991; Mackinnon et al. 1994; Childs et al. 2000; Orsini et al. 2000; Petrus et al. 2000).

To decrease toxicity, and to develop immunosuppressive as opposed to myeloablative regimens, many centers explored combinations of regimens including the use of low dose radiation. Low dose radiation alone has been shown to have anti-leukemia properties. A dose of 200 cGy total body irradiation (TBI) was adequate for inducing remission, albeit short lived, in refractory patients ineligible for other standard treatments (Shulman et al. 1998). Doses of 100 to 500 cGy have been shown to have very little toxicity in both murine and rhesus transplant models (Huhn et al. 1999; Kang et al. 2001). Even patients with Fanconi anemia, who would be considered high risk for radiation induced toxicity, tolerate moderate dose (500 cGy) radiation based regimens (Dufour et al. 2001). While regarded as a method designed for the creation of "space" within the marrow in bone marrow transplantation (BMT) regimens, radiation has long been the basis of the establishment of immunosuppressive protocols for both BMT and solid organ transplantation in animal models (Myburgh et al. 1980; Myburgh et al. 1984). There is extensive clinical experience demonstrating stable mixed chimerism with the use of 200 cGy total body

irradiation and fludarabine, followed by GVHD prophylaxis with Cyclosporine (CSP) and mycophenolic acid (MMF) (Hegenbart et al. 2006).

This protocol aims to take advantage of the immunodeficiency state in patients with mutations in GATA2 to achieve a high level of donor chimerism following HSCT using a reduced intensity conditioning regimen with low-dose TBI and fludarabine. Because opportunistic infections are present in many of these patients, along with MDS, both high levels of chimerism and low regimen related toxicity are important considerations in designing a transplant regimen for this cohort.

1.2.3 Reduced-Intensity Conditioning Regimens for Matched Unrelated Donors

Allogeneic HSCT is potentially curative for many hematological malignancies, however, as previously noted, stem cell donor availability limits the broader application of this treatment. Only 25% to 30% of patients have a sibling donor who is fully matched at the class I and II major histocompatibility complex (MHC) loci that encode for the human leukocyte antigens (HLA-A, -B, -C, DRB1, and DQB1) (Appelbaum 2007). This limitation prompted the exploration of alternative stem cell sources for allogeneic HSCT including the use HLA-matched unrelated donors (Kernan et al. 1993). Enrollment of volunteer donors in national and international registries, such as National Marrow Donor Program (NMDP) in the United States, has facilitated the identification of unrelated stem cell donors for patients without HLA-matched sibling donors. However, transplants from unrelated donors are complicated by higher rates of graft rejection, acute and chronic GVHD, and treatment-related mortality. These complications are partially balanced by lower relapse rates attributed to increased histocompatibility differences. These complications have limited matched unrelated donor myeloablative HSCT to patients less than 55 years of age and without any significant co-morbidity. Within these limitations, matched unrelated donor transplant has become widely utilized to treat hematological malignancies.

In light of the high treatment-related morbidity and mortality associated with myeloablative allogeneic HSCT from unrelated donors, clinical studies utilizing nonmyeloablative and reduced-intensity conditioning regimens were initiated after encouraging results with these regimens for HLA-matched siblings had been reported. However, because of greater genetic disparity between the donors and recipients, it was hypothesized that nonmyeloablative conditioning regimens for unrelated donor transplantation might require more intense immunosuppression than a transplant from an HLA-matched sibling. Since 2001, there have been a number of relatively large single-institution and multi-institutional studies on nonmyeloablative and reduced-intensity allogeneic HSCT utilizing unrelated donors (Tables 1A and 1B) (Giralt et al. 2001; Chakraverty et al. 2002; Kroger et al. 2002; Maris et al. 2003; Niederwieser et al. 2003; Wong et al. 2003). Of note, the median ages of recipients has ranged from 44 to 59 years with patients as old as 72 years successfully undergoing this procedure.

The incidence of graft rejection in these HSCT ranged from 0 to 21%. The primary adverse factors related to graft rejection were: use of nonmyeloablative conditioning regimens, HLA-mismatching, transplant with bone marrow rather than mobilized peripheral blood, and a minimal amount of prior therapy prior to transplant. Using a variety of GVHD prophylaxis regimens, the reported incidences of Grade II-IV and Grade III-IV acute GVHD have been 21-63% and 6-39%, respectively. Early ($\leq$ 100 days post-transplant) treatment-related mortality

rates have varied from 10 to 48%; one-year treatment-related mortality rates have varied from 17 to 55%. One-year overall survival rates have been reported to be 32 to 75% (Tables 1A and 1B).

The majority of the reduced-intensity regimens are based on chemotherapy, however a considerable experience, primarily from the Fred Hutchinson Cancer Research Center, has accumulated using 200 cGy low-dose TBI in the conditioning regimen along with fludarabine (Maris et al. 2003; Niederwieser et al. 2003). This work was first described in the 1990's in the canine model and demonstrated that 200 cGy TBI pre-transplant combined with post-transplant immunosuppression with Cyclosporine (CSP) and Mycophenolate (MMF) resulted in stable engraftment of dog leukocyte antigen (DLA)-matched littermate recipients (Storb et al. 1999). When these results were translated to humans it was shown that the addition of fludarabine significantly increased the rate of engraftment of donor hematopoietic stem cells (Mielcarek et al. 2007). Using this regimen the Seattle group recently reported comparable engraftment between matched unrelated and matched related recipient.

This protocol uses a reduced-intensity regimen to treat patients with mutations in GATA2 who have a 10/10 matched related donor, a 10/10 matched unrelated donor, or a 9/10 matched unrelated donor. This regimen carries one of the lowest incidences of transplant related mortalities (Maris et al. 2003). This factor is important since patients with mutations in GATA2 are frequently debilitated from infections with opportunistic organisms such as mycobacterium avium complex (MAC).

Table 1A - Patient and Treatment Characteristics of Reported Trials of Non-myeloablative and Reduced-intensity Allogeneic HSCT Utilizing HLA-matched Unrelated Donors

Author/Institute Journal/Date	Patient # Median Age	HLA Loci	Degree match	Level of HLA Match		Conditioning Regimen	GVHD Prophylaxis	Stem Cell Source	CD34 Dose (10^6/kg)	CD3 Dose (10^8/kg)
				Class I	Class II					
Giralt et al. MDACC Blood 2001	N = 86 (URD = 40) 52 years (22-70)	6	6/6	Serology	Molecular	Flu (25 mg/m^2/d) x 5d Mel 90–140 mg/m^2 Clad (12 mg/m^2/d) x 5d Mel 90–140 mg/m^2	FK506 x 6 months MTX (5 mg/m^2) +1,+3,+6,+11	NA	NA	NA
Chakraverty et al. UK multi-institute Blood 2002	N = 47 44 years (18–62)	10	9-10/10 (s) 8-10/10 (m)	serologic – A,B mole – C	Molecular	Flu (30 mg/m^2/d) x 5d Mel 140 mg/m^2 CAMPATH (20 mg/kg) -8,-7,-6,-5,-4,-3	CsA x 3 months [CAMPATH]	BM = 46 PB = 1	NA	NA
Kroger et al. German multi-institute Blood 2002	N = 21 (all multiple myeloma) 50 years (32-61)	8 HLA-A,B, DRB1,DQB1	7-8/8	Serology c	Molecular	Flu (30 mg/m^2/d) x 5d Mel 100 mg/m^2 ATG (10 mg/kg) -3,-2,-1	CsA x 3 months MTX (10 mg/m^2) +1,+3,+6	BM = 6 PB = 15	3.9 (0.4 - 12.5)	NA
Niederwieser et al. FHCRC Consortium Blood 2003	N = 52 48 years (6–65)	8 (10) HLA-A,B,C, DRB1, (DQB1)	7-8/8 (s) 6-8/8 (m)	Serologi c	Molecular	Flu (30 mg/m^2/d) x 3d TBI 200 cGy	CsA x 2-3 months, taper until +180) MMF x 40d,taper	BM = 13 PB = 39	6.0 (1.5 – 21.6) (BM = 2.7)	3.4 (0.8 – 10.0) (BM = 0.3)
Maris et al. FHCRC Consortium Blood 2003	N = 85 53 years (5–69)	10 HLA-A,B,C, DRB1,DQB1	10/10	serologic (81% molecula r at Class I)	Molecular	Flu (30 mg/m^2/d) x 3d TBI 200 cGy	CsA x 3 months, taper until +180 MMF x 40d,taper	BM = 18 PB = 71	6.99 (1.26 -16.4)	2.61 (0.8 -37.7)
Wong et al. MDACC Blood 2003	N = 29 59 years (55-69)	6	6/6 5/6 (n = 2)	Serologi c	Molecular	Flu (25 mg/m^2/d) x 5d Mel 90–140 mg/m^2 +/- ATG (n = 17)	FK506 x 6 - 8 months MTX (5 mg/m^2) +1,+3,+6,+11	BM = 28 PB = 1	4.46 (0.37 – 8.6)	NA

Table 1B - Clinical Outcomes of Reported Trials of Non-myeloablative and Reduced-intensity Allogeneic HSCT Utilizing HLA-matched Unrelated Donors

Author/Institute Journal/Date	Graft Rejection and Failure	Acute GVHD		Chronic GVHD	TRM			OAS	
		II-IV	III-IV		Day 100	1 year	2 year	1 year	2 year
Giralt et al. MDACC Blood 2001	5% (n =2)	62% (11 deaths)	**39%**	**68%**	37.4%	NA	**45%** (all patients)	32% (URD)	28% (all patients)
Chakraverty et al. UK multi-institute Blood 2002	3% (n= 2)	**21.3%**	**6.4%**	8% (all limited)	15%	**20%**	**(~25%)**	75%	NA
Kroger et al. German multi-institute Blood 2002	0	38%	19%	37% Limited = 25% Extensive = 12%	**10%**	26%	NA	74%	74%
Niederwieser et al. FHCRC Consortium Blood 2003	12% (low CD3; low CD34)	**63%**	21%	60% Limited = 30% Extensive = 30%	11%	29%	**40%**	44%	35% (19 mo)
Maris et al. FHCRC Consortium Blood 2003	**21% (PB vs. BM: 15% vs. 44%)**	52%	10%	45% (85) Extensive = 37%	11%	17%	NA	52%	(~42%)
Wong MDACC Blood 2003	14% (n= 4)	41%	20%	62.5%	**48%**	**55%**	NA	44%	(~44%)

1.2.4 Effect of HLA on Transplant Outcomes*:*

Since its inception, the NMDP has required evaluation of donor-recipient histocompatibility matching (HLA-A, -B, and -DR) prior to unrelated donor HSCT (Bray et al. 2008). The minimum acceptable match was originally defined by serologic testing at these 3 loci (6 possible antigens) and required at least 5 matches, that is, a "5 of 6 match". This requirement has changed little over the years. Currently, to request a donor for transplantation, the minimal acceptable level of matching remains a 5 of 6 match for HLA-A, -B, and -DRB1. Although only evaluated at antigen level of resolution ("low resolution") for donor release, each of these 3 loci must now be typed at high-resolution by DNA-based methods. High-resolution typing is defined as the identification of alleles based on differences in the antigen recognition site (ARS) domains (Exons 2 and 3 of Class I and exon 2 of Class II genes). In 2005, a requirement for HLA-C typing was added. The most recent studies have clearly shown that transplant outcomes can be improved by matching strategies that increase the overall degree of HLA compatibility above the minimum accepted level by matching also for HLA-C, -DP, -DQ, and haplotypes (Petersdorf 2006).

As data on high resolution typing accumulated, the NMDP performed an analysis on the effects of HLA matching (low or high resolution or both) on engraftment, graft-versus-host disease (GVHD), and mortality in 1874 donor-recipient pairs that were retrospectively typed using high resolution techniques for HLA-A, -B, -C, -DRB1, -DQ, and -DP (Flomenberg et al. 2004). There was no effect of any mismatch on engraftment, and only a mismatch at HLA-A affected whether the patients experienced Grade II-IV acute GVHD or chronic GVHD. Single mismatches at HLA-A, -B, -C, and -DRB1 each had similar adverse effects on mortality (relative risk 1.23 - 1.33), as compared to individuals matched at all four loci. The 5-year overall survival rates for a match at all four loci ("8 of 8" as compared to a single mismatch ("7 of 8") was 39%

and 31%, respectively. In this analysis, mismatches at HLA-DQ or -DP did not appear to exert any significant effect on survival (5-year overall survival = 39%) or any other outcome. The observed adverse effects on outcome were more evident in transplants with low-resolution versus only high-resolution mismatches. When high-resolution mismatches at HLA-A, -B, -C, and -DRB1 were considered together, adverse effects on survival and GVHD were observed. The authors concluded that matching for HLA-C should be incorporated into algorithms for unrelated donor selection, along with HLA-A, -B, and -DRB1 (i.e. "8 of 8").

In a subsequent analysis, the NMDP looked at data from 3857 transplantations. Patient-donor pairs were fully typed for HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1, and -DPA1 alleles (Lee et al. 2007). High-resolution DNA matching for HLA-A, -B, -C, and -DRB1 (8 of 8 match) was the minimum level of matching associated with the highest survival. A single mismatch detected by low- or high-resolution DNA testing at HLA-A, -B, -C or -DRB1 (7/8 match) was associated with higher mortality (relative risk = 1.25; 95% CI, 1.13-1.38; $P < 0.001$) and 1-year survival of 43% compared with 52% for 8 of 8 matched pairs. Single mismatches at HLA-B or HLA-C appeared to be better tolerated than mismatches at HLA-A or HLA-DRB1. Mismatching at 2 or more loci compounded the risk. Mismatching at HLA-DP or -DQ loci and donor factors other than HLA type were not associated with survival. In multivariate modeling, patient age, race, disease stage, and cytomegalovirus status were as predictive of survival as donor HLA matching. The authors concluded that high-resolution DNA matching for HLA-A, -B, -C, and -DRB1 alleles is associated with higher rates of survival.

Based on these results, the NMDP has established guidelines relative to donor selection based on HLA-typing (Bray et al. 2008). The optimal donor should be matched at HLA-A, -B, -C, and -DRB1 by high-resolution typing. When there are multiple donors with this degree of matching, further matching at HLA-DQ may be utilized; however, the clinical utility does not reach statistical significance. If a mismatch is unavoidable, a single mismatch at HLA-A, -B, -C, and -DRB1 should be sought. In the NMDP data, mismatches at HLA-B and -C may be less detrimental than those at HLA-A and -DRB1.

1.2.5 Reduced Intensity Umbilical Cord Blood Transplantation

Umbilical cord blood (UCB) represents an alternative to bone marrow as a source of HSC for transplantation in individuals who lack an HLA-matched donor, and has been used as a source of donor hematopoietic stem cells HSC to treat a variety of malignant and non-malignant hematological disorders (Kurtzberg et al. 1996; Wagner et al. 2002). The advantages of UCB include the ease of procurement, lack of donor attrition, absence of maternal risk, reduced risk of GVHD, and less stringent criteria for HLA matching (Wagner and Kurtzberg 1997). The major limitations in UCB are the relatively decreased cell dose leading to delayed engraftment, extended post-transplant neutropenia and thrombocytopenia, and an increased incidence of infection and bleeding. The developments since the first HSCT using UCB in 1989 are described in the ensuing paragraphs.

Unrelated Donor UCB HSCT in Pediatrics

The initial descriptions of significant cohorts of patients treated using UCB transplant involved the use of single UCB units in children and myeloablative conditioning regimens (Kurtzberg et

al. 1996; Gluckman et al. 1997). Both studies confirmed the potential of UCB transplant to induce remission in patients with refractory hematological malignancies. The two studies also reported a lower than predicted frequency of GVHD.

These early studies with UCB HSCT from sibling donors led to the banking of unrelated donor UCB. Using banked UCB (from the New York Blood Center) in 167 children with hematological malignancies, congenital / acquired bone marrow failure syndromes, and genetic diseases, 93% of the patients engrafted. Engraftment was subsequently found to correlate with the number of CD34+ cells transplanted, rather than the degree of HLA matching. Retrospective analysis indicated that a dose of CD34+ cells <2-3 x 10^5 CD34+ cells/kg resulted in diminished and delayed engraftment of platelets and neutrophils. Multivariate analysis revealed that any dose >3 x 10^5 CD34+ cells/kg results in fairly consistent engraftment.

Unrelated Donor UCB HSCT in Adults

Three studies specifically addressed the use of UCB grafts in adults (Gluckman 2000; Laughlin et al. 2001; Sanz et al. 2001). These studies consisted of adult patients with hematological diseases who underwent myeloablative conditioning followed by HLA-matched or mismatched UCB infusion. The probability of myeloid engraftment and the probability of developing acute GVHD grades II-IV ranged from 81% to 100% and 38% to 60%, respectively. The event free survival (EFS) (with at least 1 year of follow up) ranged from 21% to 53%. These data indicated that UCB SCT led to lower than expected rates of acute GVHD, especially in the setting of mismatched HLA grafts, and encouraged further study using UCB.

Two recent reports specifically compared the outcomes of adult patients with acute leukemia or myelodysplastic syndrome (MDS) transplanted with either HLA-mismatched UCB or HLA-matched and mismatched BM grafts (Laughlin et al. 2004; Rocha et al. 2004). Time to neutrophil and platelet engraftment and recovery were significantly longer after UCB SCT than BMT. Acute GVHD was more likely after mismatched marrow transplant, however chronic GVHD was more likely after UCB transplant. Treatment-related mortality (TRM), treatment failure (i.e. relapse or death), and overall mortality were lowest in patients receiving HLA-matched BM grafts. There was no difference in the rate of recurrence of leukemia among the groups. The adjusted probability of three-year survival was 26% for patients receiving UCB grafts; this was similar to patients receiving HLA-mismatched BM.

The conclusion from these studies was that UCB represented a curative alternative donor source for patients with hematological malignancies who lacked a bone marrow donor. When compared with URD BMT, there is a delay in neutrophil and platelet engraftment, a higher risk of graft failure, and an increase in TRM during the first 100 days. This is balanced in part by a decrease in acute GVHD seen in recipients of UCB.

Reduced Intensity Unrelated Donor UCB HSCT

The use of UCB SCT in the adult population has been tempered by the increased risk of graft failure and decreased survival associated with the small cell dose in physically larger patients. Investigators at Case Western Reserve University found that a higher nucleated cell dose and higher CD34+ cell dose correlated with more rapid neutrophil recovery and improved survival, respectively in adult patients undergoing UCB HSCT (Laughlin et al. 2001). The importance of cell dose was further supported by data which showed that UCB recipients infused with < 1.8 x

10^7 NC/kg or < 1.7 x 10^5 CD34+ cells/kg body weight had significantly decreased engraftment and survival (Wagner et al. 2002).

Anti-thymocyte globulin (ATG) has been used as part of the preparative regimen prior to the infusion of donor cells to decrease the incidence of graft rejection and to decrease the incidence of GVHD by eliminating donor T-cells in the graft. Animal experiments originally demonstrated the synergistic immunosuppressive effects between ATG and alkylating agents. The combination of ATG and cyclophosphamide was subsequently used widely in HSCT.

In a non-malignant disease setting, substitution of cyclophosphamide by fludarabine and rabbit ATG in a busulfan based regimen of matched related donor transplant in 5 children and adults with beta-thalassemia, all patients engrafted with 2 of the 5 maintaining 100% donor chimerism (Sauer et al. 2005).

Additional support for the use of ATG in UCB appeared in late 2007 with the description of 110 adult patients who received UCB following a nonmyeloablative-conditioning regimen (Brunstein et al. 2007). This large study of 110 patients utilized a conditioning regimen consisting of Fludarabine 200 mg/m2, Cytoxan 50 mg/kg, and 200 cGy TBI, and Equine ATG (ATGAM) 15 mg/kg IV every 12 hours on day -6, -5, and -4. The investigators aimed to transplant at least 3 x 10^7 total nucleated cells (TNC)/kg in each patient, and if the cord blood unit contained less than that amount, two UCB units were infused. This resulted in 85% of patients receiving two UCB units. With the double UCB units neutrophils recovered to 500/µl at a median of 12 days and platelets required a mean of 49 days to achieve a level of 20,000/µl. Grade 3 and 4 GVHD occurred in 22% and 23% of patients, respectively. Interestingly, by 21 days post-transplant one of the units prevailed in over 80% of the patients. Survival was 45% at 3 years with an event free survival of 38%.

Advantages of UCB

The reasons for the expanded use of UCB as a donor source are many. The first advantage lies in the immediate availability of cells. The median time from request to acquisition of the UCB is 13.5 days (Barker et al. 2002). Since there is no risk to the donor, there is no donor unavailability, such as that which occurs with unrelated donors in which an estimated 25 percent of donors who are identified are subsequently unable to donate HSC (Wagner et al. 2002). Second, acceptable cord-blood units can be identified for most patients because less stringent HLA-compatibility with the recipient is required (Rocha et al. 2000). Third, the incidence and severity of GVHD are not excessive with UCB, even with cord-blood units mismatched for more than one HLA antigen (Brunstein et al. 2007). Moreover, the graft-versus-leukemia effects are maintained with UCB grafts (Laughlin et al. 2004).

UCB banks have been developed worldwide and standardized banking procedures have been implemented (Rubinstein et al. 1998). The low rate of viral infection at birth and the collection of cord blood from ethnic groups not well represented in bone marrow donor registries are additional advantages of UCB. There are an estimated 170,000 units in 37 cord blood registries in 21 countries (Bone Marrow Donors Worldwide Homepage (www.bmdw.org).

The main obstacle for use of cord blood transplant in adults has been the low number of stem cells in cord blood units leading to delayed engraftment and graft failure. The number of HSC in a unit of cord blood is approximately one tenth the number of HSC in a marrow harvest (Laughlin et al. 2001). The low number of HSC in UCB results in more gradual reconstitution of hematopoiesis following UCB grafts compared to bone marrow or peripheral blood stem cell

grafts, and this contributes to the increased rates of post-transplant infection and early death (Rocha et al. 2000). This limitation in UCB SCT has been decreasing with the increasing use of double cord units.

We propose to consider the use UCB as the source of donor HSC for patients with mutations in GATA2 who lack a 10/10 matched related or unrelated donor. The ability to acquire UCB within two weeks of the request represents a potential advantage for patients with mutations in GATA2 who may have a narrow window of opportunity for transplant between severe infections.

1.2.6 HLA-Haploidentical Hematopoietic Stem Cell Transplantation Using Nonmyeloablative Conditioning and High-Dose, Post-Transplantation Cyclophosphamide

During the previous decade there has been an increasing body of evidence that high-dose cyclophosphamide given post-transplant prevents both graft rejection and graft-versus-host disease. Cyclophosphamide has a long history as an immunosuppressive agent that is selectively toxic to lymphocytes that are proliferating in response to recent antigen stimulation. Cyclophosphamide can be safely administered in high doses after allogeneic HSCT with a favorable safety profile, especially a lack of toxicity to hematopoietic stem cells. In animal models both graft rejection and GVHD were inhibited by administration of high-dose cyclophosphamide after allogeneic bone marrow transplantation. When given in high doses after allogeneic HSCT cyclophosphamide targets proliferating, alloreactive T-cells and prevents GVHD (Luznik et al. 2002).

In 2002 O'Donnell and colleagues at Johns Hopkins described results using a nonmyeloablative bone marrow transplant from partially HLA-mismatched related donors using post-transplant cyclophosphamide (O'Donnell et al. 2002). Donors were partially HLA-matched (up to 3 HLA antigens) from first-degree relatives. They demonstrated comparable levels of engraftment and GVHD to that seen with matched unrelated donor transplants. The regimen is shown (Figure 1).

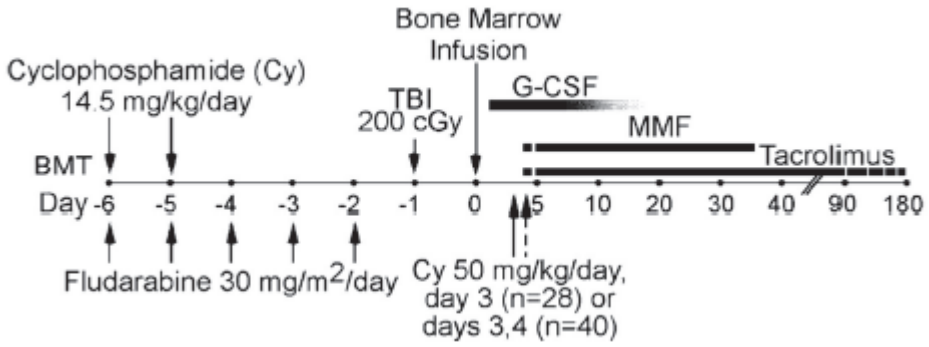


Figure 1. Nonmyeloablative haploidentical BMT conditioning and post grafting immunosuppressive regimen.

Non-malignant diseases were also amenable to the nonmyeloablative regimen described. In 2008 Brodsky described HSCT for thrombotic paroxysmal nocturnal hemoglobinuria using the regimen described (228, Brodsky, BMT). Two of three patients were disease free with full donor

chimerism and on no immunosuppressive therapy and nonmyeloablative HLA-haploidentical HSCT using post-transplant cyclophosphamide.

The largest series of HLA-haploidentical nonmyeloablative HSCT was reported from Johns Hopkins and the Fred Hutchinson Cancer research Center by Luznik in 2008 (Luznik et al. 2008). A total of 67 patients were transplanted using the regimen shown in Figure 1 only with either one day of cyclophosphamide post-transplant on day +3 or two days of cyclophosphamide on days + 3 and +4. There were 9 graft failures (13%). There was no difference in GVHD with one or two days of cyclophosphamide post-transplant. The overall survival at one year was 46% with an event-free survival of 34% in a high risk population of patients with leukemia.

When this data was summarized recently, there was a total of 210 recipients of mini-Haplo HSCT at Johns Hopkins with 87% sustained donor engraftment(Munchel et al.). The cumulative incidence of grades II-IV acute GVHD and chronic GVHD were 27% and 13%, respectively. The 5-year cumulative incidence of non-relapse mortality was 18%; relapse was 55%, actuarial overall survival 35%, event-free survival 27%. Thus, mini-Haplo HSCT with post-transplant cyclophosphamide was associated with favorable toxicity and overall survival for patients without matched related or unrelated donors.

1.2.7 Hematopoietic Stem Cell Transplant for Primary Immunodeficiency Disease

Most primary immunodeficiency diseases (PID) consist of intrinsic genetic defects of hematopoietic lineage-derived cells. Therefore, replacement of genetically impaired cells by normal HSC represents a logical therapeutic approach. It has now been demonstrated that HSCT can reverse the phenotype in a variety of PID. The initial reports of successful HSCT in PID included severe combined immunodeficiency disease (SCID) and Wiskott-Alrich Syndrome (WASP) (Bach et al. 1968; Gatti et al. 1968). By 2003, over 1200 patients with PID had undergone allogeneic HSCT (Buckley 2003). In all, 26 different inherited immune deficiencies had been corrected by HSCT, including a number of SCID variants, WASP, and various phagocytic disorders such as chronic granulomatous disease (CGD), and leukocyte adhesion deficiency (LAD) (Le Deist et al. 1989; Buckley 2003).

In the largest cohort of patients with SCID receiving HSCT, Buckley reported on 89 infants transplanted at Duke between 1992 and 1998 (Buckley et al. 1999). The majority of patients with SCID were boys with X-linked common ∂-chain SCID. However, six had Janus kinase 3 (Jak3) mutations, 13 had adenosine deaminase deficiency (ADA)-SCID, three had IL-7R alpha-chain defects, and 21 infants had unclassified autosomal recessive SCID. A total of 77 infants received T-cell depleted, HLA haploidentical parental marrow and 12 received HLA-identical marrow from a related donor. Just over 80 percent of the patients were alive 3 months to 16.5 years post-transplant, including all 12 patients who received HLA-identical related donor marrow. None of these patients received chemotherapy pre-transplant or GVHD prophylaxis. T-cell function became normal within two weeks of transplant in the patients who received unfractionated HLA-identical marrow. B-cell function was reconstituted in less than 50 per cent of the long-term survivors necessitating life-long immunoglobulin replacement in those patients without B-cell reconstitution. Although GVHD developed in 28 of the 77 infants who received T-cell depleted haploidentical marrow, in most cases the GVHD was mild and did not require treatment.

Although most of the initial studies in PID used related donors, matched unrelated donor HSCT has been successfully employed in a number of patients with PID (Ash et al. 1990; Filipovich et al. 1992; Kernan et al. 1993). Kernan reported a 50 percent success rate using matched URD HSCT to treat PID (Kernan et al. 1993).

In primary immune deficiency diseases (PID) donor engraftment may be achieved following allogeneic HSCT with no or reduced-intensity conditioning due to the inability of the host immune system to mediate graft rejection (Buckley et al. 1999; Rao et al. 2005). Thus, PID constitutes an experiment of nature in which a nonmyeloablative-conditioning regimen may result in sufficient levels of donor engraftment to mediate a graft-versus-leukemia effect without the toxicity of a myeloablative-conditioning regimen. Reduced intensity conditioning regimens are particularly well suited for allogeneic stem cell transplantation in patients with non-malignant stem cell disorders where tumor kill is not required and co-morbid conditions are frequent. Also, since a reduced-intensity conditioning regimen allows autologous recovery if the graft should fail, a second transplant remains a possibility.

The optimal nonmyeloablative-conditioning regimen for HSCT in PID has yet to be defined. However, a number of non-malignant diseases have been successfully treated using reduced-intensity conditioning regimens in stem cell transplantation, often leading to a state of mixed chimerism. Immunodeficiency diseases treated with non-myeloablative transplantation include hyper-IgM syndrome (Kato et al. 1999), adenosine deaminase deficiency (Honig et al. 2007), chronic granulomatous disease (Horwitz et al. 2001), Wiskott-Aldrich syndrome (Kapoor et al. 1981; Longhurst et al. 2002), and leukocyte adhesion deficiency–I (LAD-I) (Engel et al. 2006).

In a study of 14 patients with PID receiving either no conditioning or conditioning with 200 cGy TBI +/- fludarbine prior to matched related or unrelated donor HSCT, event-free survival was 62 per cent (Burroughs et al. 2007). This was a heterogeneous group with six patients receiving matched related donor HSCT, and 8 receiving matched unrelated donor HSCT. Correction of the immune dysfunction was achieved in 8 of 10 patients with stable donor engraftment. There was a transplant-related mortality of 23 percent.

The use of unrelated donors for HSCT in PID has been successful in a several other studies. In the most extensive use of reduced-intensity conditioning in patients with PID receiving unrelated donor HSCT, Veys and colleagues demonstrated a 94 percent survival in 33 patients treated with a reduced-intensity regimen consisting of Melphalan 140 mg/m^2, fludarabine 150 mg/m^2, and alemtuzumab (Campath 1H 1 mg/kg) (Veys et al. 2005). All HSCT were T-cell replete. They observed rapid engraftment in 32 of the 33 patients with neutrophil recovery at 13 days and platelet recovery at 16 days. Of note, at one-year post-transplant only 55 percent of the patients had 100 percent donor chimerism. Possibly as a result of the low level of complete donor chimerism, only 9 percent of the patients in the RIC group had acute GVHD more than grade II. There was also a very high rate of viral reactivation associated with the use of Campath.

The lower regimen-related toxicity with low-dose TBI plus fludarabine 30 mg/m^2/day for 3 days supports the use of this regimen in patients with mutations in GATA2 who have co-morbidities related to their underlying immunodeficiency. The use of Campath, although an effective immunoablative agent, poses an unacceptable risk in patients who are already at high risk for opportunistic infections.

1.2.8 GATA2 Mutation Syndrome

Nearly three years ago Dr. Steve Holland of the NIAID Clinical Infectious Diseases Branch at the NIH identified a unique immunodeficiency disease syndrome, subsequently named MonoMAC, in which patients have a severe deficiency of monocytes (Mono) in the peripheral blood, and the propensity to develop Mycobacterium Avium Complex (MAC) infections (Vinh et al. 2010). These patients have now been shown to harbor mutations in GATA2 (Hsu et al.). The important differences between patients with mutations in GATA2 and previously described primary immunodeficiency diseases are that patients with mutations in GATA2 have: 1) onset of disease beyond infancy, frequently in late adolescence or early adulthood, 2) a clinical history of life-threatening opportunistic infections, primarily MAC, 3) a peripheral blood leukocyte subset profile with the presence of T-lymphocytes, but a severe deficiency of B-lymphocytes, NK cells, and monocytes, 4) a genetic component suggestive of a dominant pattern of inheritance, and 5) frequent progression to MDS. This contrasts with the vast majority of genetic PID which manifest in infancy, rarely have both disseminated MAC and disseminated fungal infections, lack T-lymphocytes, and do not progress to MDS/AML.

Previously described PID are characterized by loss of the T-lymphocyte compartment. The first category, recognized over 50 years ago, consists of T-, B+, and Natural Killer (NK)- (severe combined immunodeficiency disease (SCID). The largest group of these patients is X-SCID due to mutations in the common gamma chain of the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. Autosomal recessive forms of T-,B+, NK- SCID are due to mutations in the Jak3 kinase. The second group of PID consists of T-, B-, NK+ SCID. These patients have defects in the variable, diversity, joining (VDJ) recombination machinery. Examples of this form of PID include Rag (Recombination activating gene) defects, Omenn syndrome, and Artemis deficiency. Both these forms of PID manifest at birth with severe infections; these infections commonly lead to death by 6 months of age.

Patients with mutations in GATA2 present in their late teens or early twenties, and ultimately succumb to either disseminated infection or MDS/AML over 2-10 years. It appears that the loss of monocytes and NK cells accounts for the propensity to develop opportunistic infections with MAC and fungal organisms, including histoplasmosis and aspergillus. The onset of MDS is variable, but usually develops within several years of the onset of the disease. Current management of the GATA2 mutation syndrome is palliative and revolves around treatment with antibiotics, anti-viral, anti-tuberculous, and anti-fungal agents. However, relapses with additional opportunistic infections invariably occur leading to progressive disability. The lung is the organ system most frequently affected. Total lung pulmonary alveolar lavage has been used to transiently improve pulmonary function. HSCT has been used successfully to treat both genetic immunodeficiency diseases and MDS/AML, the two characteristics of the patients with mutations in GATA2, and therefore represents a potential therapy to reverse the inevitably fatal course in these patients.

A brief case history of a patient with a GATA2 mutation illustrates the natural history of the disease.

JD: 33 yr old man presented in November of 2006 with disseminated histoplasmosis. He had a history of pneumonias every few months for the preceding year, and a history of severe warts. During his 16-month hospitalization he suffered multiple pulmonary infections including pulmonary histoplasmosis, Neosartorya utagawa, and disseminated Mycobacterium avium. He

was subsequently found to have severely reduced B-lymphocytes, natural killer (NK) cells, and monocytes with CD4 496, CD8 150, NK cells 0, B-cells 0, monocytes 0. In February of 2007 he developed myelodysplasia with 5-10 % blasts and monosomy 7 in 4-5% of metaphase spreads. By August 1997 95% of metaphase spreads displayed monosomy 7. While hospitalized he developed cerebral aspergillus and died in March of 2008. His family history was significant for an older sister who developed an immunodeficiency at age 14 and died from disseminated zoster at age 17 awaiting HSCT for MDS. The patient JD was 13 yr old at the time of his sister's death.

Thus, patients with mutations in GATA2 succumb to either from opportunistic infection or bone marrow failure from MDS/AML. Six of the 20 patients with mutations in GATA2 have died either from infection or progression to MDS and/or leukemia. Patient JD described above died at the Clinical Center in March of 2008 awaiting unrelated donor transplant for MDS. Several of the current patients with mutations in GATA2 have had first-degree relatives die from infections or MDS/AML. The biological question for patients with mutations in GATA2 is whether the individual leukocyte compartments that are lost in this syndrome (B-lymphocytes, NK cells, and monocytes) will be constituted with HSCT. ,

Both autosomal dominant and sporadic cases of GATA2 mutation occurs in this syndrome. To date, we have identified 12 distinct mutations in *GATA2* affecting 20 patients and relatives with this syndrome, including recurrent missense mutations affecting the zinc finger-2 domain (R398W and T354M) (Hsu et al.). Four discrete insertion/deletion mutations leading to frame shifts and premature termination implicate haploinsufficiency as a possible mechanism of action. These mutations were identified in several families, indicating germ line transmission in those kindred's. Thus, *GATA2* joins *RUNX1* and *CEBPA* not only as familial leukemia genes, but also as a cause of a complex congenital immunodeficiency that evolves over decades and combines predisposition to infection and myeloid malignancy.

Table 2. *GATA2* Mutations Identified in MonoMAC Patients

Kindred[*]	**Patient**[*]	**DNA change**[#]	**Codon**[#]	**Null**[&]
1	1.II.1	c.1192 C>T	R398W	
1	1.II.5	c.1192 C>T	R398W	
2	2.II.3	c.1192 C>T	R398W	
3	3.I.1	c.1192 C>T	R398W	
5	5.II.1	c.1061 C>T	T354M	
8	8.I.1	c.243_244delAinsGC	G81fs	+
9	9.III.1	c.1192 C>T	R398W	
10	10.1.1	c.1113 C>G	N371K	
12	12.I.1	c.1083_1094del 12bp	R361delRNAN	
13	13.II.1	c.1-200_871+527del 2033bp	M1del290	+
13	13.I.2	c.1-200_871+527del 2033bp	M1del290	+
15	15.II.1	c.1186 C>T	R396W	
17		c.1061 C>T	T354M	
18		c.1187 G>A	R396Q	
19		c.1061 C>T	T354M	
20		c.778_779ins 10bp	D259fs	+
21		c.1192 C>T	R398W	
22		c. 951_952ins 11bp	N317fs	+

23	c. 751 C>T	P254L
24	c. 1018-1 G>A	D340-381

* - Numbering refers to patient and kindred numbers from Vinh et al. BLOOD 2010.
[#] - Numbering relative to adenine in the ATG start codon of *GATA2* (GenBank NM_001145661.1) and the first methionine (GenBank NP_116027. [&] - Predicted to result in loss of mRNA or protein from mutant allele.

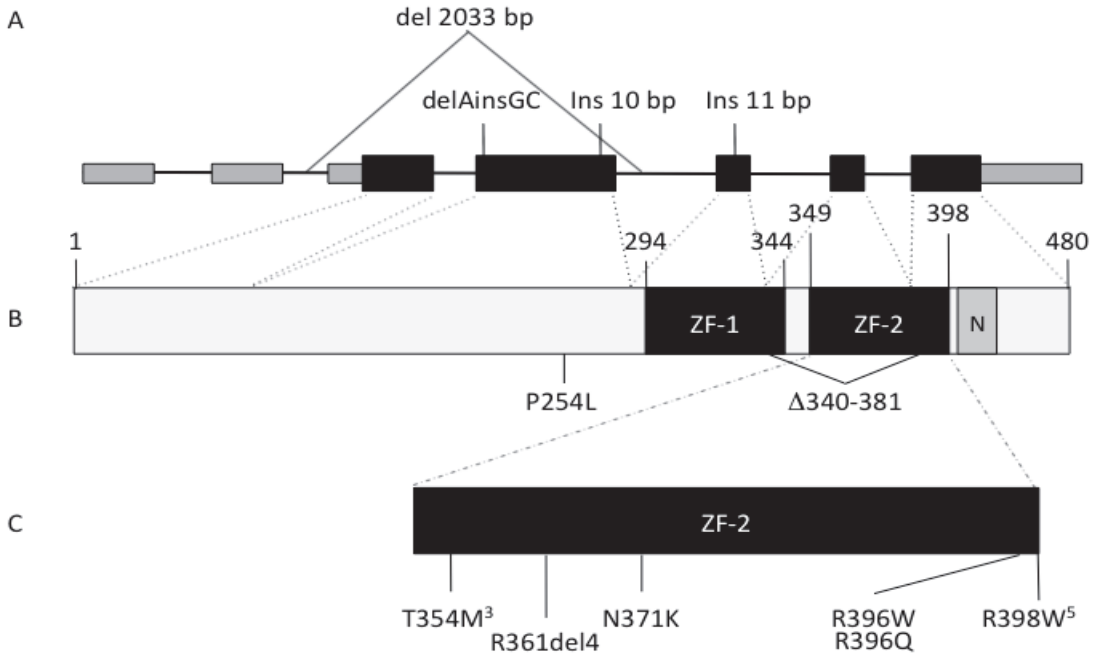


Figure 2. Genomic organization of *GATA2* showing two 5' untranslated and five coding exons. Dark boxes indicate coding regions. Insertion/deletion mutations predicted to result in null alleles are shown above. B. Protein domains of GATA2, showing N and C-terminal zinc fingers (ZF-1, ZF-2) and nuclear localization signal (N). C. Missense and in-frame deletion mutations identified within ZF-2. Superscript numerals indicate number of independent mutations.

1.2.9 HSCT for Myelodysplasia

MDS are a heterogeneous group of clonal hematopoietic disorders characterized by impaired peripheral blood cell production (cytopenias) and most commonly a hypercellular, dysplastic-appearing bone marrow (Nimer 2008). The incidence of MDS increases with increasing age; the median age at diagnosis is in the seventh decade (Greenberg et al. 1997). Survival after diagnosis varies from a few months to several years.

MDS were originally called pre-leukemia, but the term MDS replaced pre-leukemia in the 1970's due to the clinical observation that that many individuals with MDS succumbed to the effects of the cytopenias without transforming to acute myelogenous leukemia (AML) (Nimer

2008). Approximately 50 percent of patients with MDS will develop leukemia. Progressive single or multilineage cytopenias account for the majority of the remaining deaths.

There are a number of different prognostic systems developed to predict outcomes for patients with MDS with the most widely utilized system being the International Prognostic Scoring System (IPSS) (Greenberg et al. 1997). The IPSS uses cytogenetics, bone marrow myeloblast percentage, and peripheral cytopenias to predict leukemia transformation and survival. Patients are grouped into one of four prognostic categories: low-risk, intermediate 1 risk, intermediate 2 risk, and high risk.

In MDS cytogenetic risk groups defined by the IPSS are: good -normal isolated –Y, del (5q), and deletion 20q; poor- complex >3 abnormalities, and/or any chromosome 7 anomalies; intermediate- all other abnormalities (Greenberg et al. 1997). In terms of frequency, deletions of all or part of chromosome 7 represent the second most common cytogenetic abnormality in MDS, next to deletions of 5q. In a recent comprehensive analysis correlating the cytogenetics with the clinical outcome in MDS, monosomy 7 as an isolated abnormality led to a median survival of 14 months (Haase et al. 2007).

Monosomy 7 is a characteristic cytogenetic abnormality in acquired aplastic anemia and congenital neutropenia (Maciejewski et al. 2002). Cells lacking chromosome 7 frequently express a short isoform of the G-CSF receptor, and this short isoform preferentially signals proliferation over differentiation leading to clonal expansion in the setting of neutropenia or cytokine administration (Maciejewski et al. 2002).

Good prognostic factors for HSCT include: age, low IPSS score, short duration of disease (< 3-6 months), blast percentage < 5%, and complete remission before transplant. The long-term disease-free survival with HSCT for MDS from either a matched related or unrelated donor in patients less than 50 years old ranges from 36% for matched related donors to 25% for matched unrelated donors (de Witte et al. 2001).

In patients with mutations in GATA2 allogeneic HSCT represents a potential curative therapy for MDS. Of particular relevance to patients with mutations in GATA2with progression to MDS, many centers are now using a reduced-intensity approach because of age and frequent co-morbidities in patients with MDS. In a Spanish study 20 MDS patients received conditioning with busulfan 10 mg/kg and fludarabine 150 mg/m^2 and peripheral blood stem cells from HLM-matched related donors (Martino et al. 2002). The transplant related mortality at one year was 5%.

In one of the largest studies using reduced-intensity conditioning followed by allogeneic HSCT to treat 148 patients with MDS and MPS, patients received a conditioning regimen consisting of low-dose TBI (200 cGy) alone or with fludarabine 30 mg/m^2/day for 3 days, and post-grafting immunosuppression with CSP and MMF (Laport et al. 2008). Approximately one-half of the patients received matched related donor HSCT, and the other half received matched unrelated donor HSCT. The three-year relapse-free survival and overall survival were 27% for all patients. Recipients of matched unrelated donor grafts did not have higher non-relapse mortality, and

relapse did not constitute the leading cause of death. These results provide support for the use of this regimen in patients with mutations in GATA2 who also have co-morbidities.

1.2.10 Sirolimus and Tacrolimus for GVHD Prophylaxis after HSCT

The optimal series of drugs for GVHD prophylaxis following allogeneic HSCT would include medications that are well tolerated with minimal side effects/toxicities, prevent GVHD, and enable engraftment without an excess rate of disease relapse. Commonly used drugs for the prevention of GVHD after HSCT include: cyclosporine (CSP), methotrexate (MTX), and corticosteroids. For patients undergoing transplantation with either HLA matched or mismatched unrelated donors who are receiving regimens of the above medications the incidence of acute GVHD has been reported being 40-70% (Beatty et al. 1985; Anasetti et al. 1990; Hansen et al. 1998). In addition to incompletely suppressing GVHD, these medications are also associated with a wide variety of undesirable side effects. Due to these problems, there is a continued interest in the development of more efficacious regimens.

In recent studies the immunosuppressive agent sirolimus (Rapamycin) has been shown to prevent GVHD (Antin et al. 2003). Sirolimus inhibits T-cell and dendritic cell function through inhibition of mTOR, which in contrast to the mRNA inhibition associated with calcineurin blockade, operates via a post-transcriptional mechanism to reduce cell signaling and modulates protein translation and phosphorylation (Hackstein et al. 2003). It appears that sirolimus prevents the translation of mRNAs encoding cell cycle regulators and inhibits the ability of lymphocytes to proliferate in response to IL2 (Chakraverty et al. 2002). Sirolimus also has profound effects on dendritic cell function and survival, and this may contribute to prevention of GVHD (Chakraverty et al. 2002). Initial results indicate that sirolimus may be an effective agent for prevention of graft rejection and GVHD when used in conjunction with a second agent.

Recently, sirolimus has been used as a third agent for GVHD prophylaxis after genetically disparate transplantation (Antin et al. 2003). Clinical studies have not evaluated single-agent sirolimus for GVHD prophylaxis, and in fact, murine data indicate that single-agent sirolimus potently abrogates $CD8^+$ T cell mediated GVHD, but not $CD4^+$ T cell mediated GVHD (Blazar, 1998). As such, sirolimus may represent a replacement of calcineurin inhibitor mediated GVHD prophylaxis. In a study of matched sibling allotransplant in sickle cell anemia, sirolimus prevented both graft rejection and GVHD (Hsieh et al. 2009). Sirolimus was also reported to be well tolerated at a dosing schedule designed to maintain a level of 10, with hypertriglyceridemia representing the primary specific drug toxicity.

The use of tacrolimus for the suppression of GVHD has been supported by research in canine and murine models and pilot studies in humans (Markus et al. 1991; Storb et al. 1993; Nash et al. 1995; Fay et al. 1996). Several phase III studies have prospectively compared tacrolimus to CSP with or without MTX in adolescent or adult patients undergoing related or unrelated SCT (Nash et al. 2000; Hiraoka et al. 2001). The incidences of grade II-IV acute GVHD were significantly decreased when patients were treated with tacrolimus. The incidence of acute GVHD for patients receiving tacrolimus versus CSP ranged from 17.5% to 56% and 44.4% to 74%, respectively. However, there was no statistically significant difference in the rate of chronic GVHD between the groups. Data from the first two trials revealed that the relapse rate and OS

were also similar between those receiving tacrolimus and CSA. Both trials found that tacrolimus was well tolerated, but was associated with increased (but transient) nephrotoxicity that resolved over time. Although the third trial supported the previous two with regards to the decreased incidence of acute GVHD and relapse rate, the 2-year OS was worse in the arm receiving tacrolimus than cyclosporine, 50.4% and 40.5%, respectively. When the DFS among patients with no advanced disease was compared, the outcomes were similar.

Although sirolimus and tacrolimus both bind to FKBP12, the two drugs are not competitive and appear to be synergistic (Stepkowski et al. 1997). To take advantage of this synergistic effect, the combination of the two drugs was evaluated in a trial using myeloablative HSCT with matched related or unrelated donors at the Dana Farber Cancer Institute (Antin et al. 2003). In that study the incidence of grade II-IV GVHD was markedly reduced compared to historical controls.

1.2.11 Interim Summary of HSCT for Patients with Mutations in GATA2

Ten patients have been enrolled on this study to date: three patients received a matched related donor hematopoietic stem cell transplant (HSCT), three patients received matched unrelated donor HSCT, and four patients have received umbilical cord transplant. The results to date with HSCT for MonoMAC are summarized below.

Patient # 1: 33 yo man with R81fs mutation in GATA2 on 5 liters of oxygen from extensive pulmonary alveolar proteinosis received his peripheral blood stem cell (PBSC) transplant on 5/12/2009. He tolerated his conditioning regimen (Fludarabine and 200cGY TBI) well. He engrafted within two weeks of transplant and was discharged from the hospital within one month of transplant. He is now 24 months from transplant with a normal hematocrit, white blood cell count, and platelet count. He now has normal levels of monocytes, NK cells, and B-cells. His donor lymphoid chimerism is 100% and his donor myeloid chimerism is 100%. He had a re-admission 6 months post-transplant for chronic GVHD, which occurred when the immunosuppression was tapered. The acute GVHD resolved on corticosteroids, however he now has chronic GVHD. He is now almost three years post-transplant, and off all immunosuppressive agents.

Patient #2: 33 yo man with R398W mutation in GATA2 and myelodysplasia (MDS) received his PBSC from an unrelated donor on 8/12/2009. He tolerated his conditioning regimen (Fludarabine and 200cGY TBI) well with the exception of an episode of emesis. He engrafted within 14 days of HSCT and was discharged. He has had a completely unremarkable course since transplant. He is now nearly two and one-half years after transplant with a normal hematocrit, white blood cell count, and platelet count. He now has normal levels of monocytes, NK cells, and B-cells. His most recent donor lymphoid chimerism was 91% and his donor myeloid chimerism is 100% at 12 months post-transplant. The patient had no acute GVHD and no signs of chronic GVHD at this time. He is off all medications.

Patient #3: 41 yo woman with T354M mutation in GATA2 and MDS with monosomy 6. Following conditioning with Cytoxan 50 mg/kg, Fludarabine 150 mg/m2, and 200 cGy TBI, she underwent a single umbilical cord hematopoietic stem cell transplant on December 29, 2009. The

cord blood unit was a 4/6 match. Chimerism on the bone marrow was 100% at day 28, 60, 100, 6 months, 12 months and two years. Her course was complicated by slow engraftment requiring 100 days. She now is 24 months post-transplant, living at home, and requiring no transfusions. Both lymphoid and myeloid chimerism was 100% 18 months post-transplant. The recipient did not develop acute GVHD and has no signs of chronic GVHD.

Patient #4: 23 yo woman with R396Q mutation in GATA2 and MDS with trisomy 8. Following conditioning with Fludarabine 90 mg/m2, and 200 cGy TBI, she underwent a matched unrelated donor transplant on 8/5/2010. She is now one and one-half years post-transplant and was recently admitted for GVHD requiring immunosuppression.

Patient #5: 15 yo adolescent with D259fs mutation in GATA2 and MDS with monosomy 7. He received a double umbilical cord blood transplant in September of 2010. His course was complicated by autoimmune hemolytic anemia and thrombocytopenia and nephrotic syndrome. He is now one and one-half years post-transplant and has returned to school. He is on no immunosuppressant.

Patient #6: 26 yo man with R361delRNAN in GATA2 along with fibrotic liver injury, and MDS with monosomy 7 who died of sepsis 5 days after double cord blood transplant.

Patient #7: 41 yo woman with mutation in GATA2 and MDS who received conditioning with Fludarabine 90 mg/m2, and 200 cGy TBI followed by a matched related donor transplant on 12/14/2010. She has had an uncomplicated course, however at one-year post-transplant she as found to have recurrence of her cytogenetic abnormality.

Patient #8: 26 yo woman with a 318fs mutation in GATA2 and MDS with primary graft failure after double umbilical cord blood transplant. She received a haplo/cord transplant on April 28, 2011 and engraftment primarily with haploidentical cells. She died 8 months post-second transplant from septic shock from Klebsiella pneumonia.

Patient #9: 32 yo Hispanic man with a T354M mutation in GATA2 and MDS received a matched related donor HSCT. He engrafted on day 14 and has now returned home. He is now more than 100 days post-HSCT.

Patient #10: 39 yo woman with an N371K mutation in GATA2 and MDS who received a matched unrelated donor transplant over 100 days ago. She had engraftment within 14 days and has had no re-admissions. She has returned home.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 INCLUSION CRITERIA - RECIPIENT

a) Patient age of 12-60 years.
b) Mutation in the GATA2 gene performed by the CLIA certified laboratory
c) Clinical history of at least two episodes of life-threatening infection d Available 10/10 HLA-matched related donor, 10/10 or 9/10 matched unrelated donor, 4/6 (or greater) matched umbilical cord blood (UCB) unit(s) with a total dose of greater than or equal to 3.5 $x10^7$ TNC/kg, or a haploidentical donor.
e) Patients may have evidence of MDS with one or more peripheral blood cytopenias and greater than 5% blasts but less than 10% blasts in the bone marrow in the absence of G-CSF**.** Patients previously treated for acute myelogenous leukemia are eligible if they have less than or equal to 5% blasts in the bone marrow in the absence of G-CSF. Subjects 12-17 years of age are required to have MDS with chromosomal abnormalities in addition to mutation in the GATA2 gene for enrollment on this protocol.
f) Left ventricular ejection fraction > 50%, preferably by 2-D echo, or by MUGA, or shortening fraction > 28% by ECHO, obtained within 28 days of enrollment
g) Pulmonary Function Tests: Adult patients: Corrected DLCO diffusion capacity and FEV1 > 10% of expected value obtained within 28 days of enrollment. Pediatric patients: DLCO corrected for hemoglobin and alveolar volume ≥ 20% of predicted.
h) Creatinine: Adult patients: ≤ 2.0 mg/dl and creatinine clearance ≥ 30 ml/min; Pediatric patients: age-adjusted normal serum creatinine (see Table below) OR a creatinine clearance > 60 mL/min/1.73m^2.

ge (years)	aximum Serum Creatinine (mg/dl)
	3
< age < 10	0
< age < 15	2
5	5

d) Serum total bilirubin < 2.5 mg/dl; serum ALT and AST ≤ 5 times upper limit of normal
e) Adequate central venous access potential.
f) Written informed consent/assent obtained from patient/parent or legal guardian.
g) Life expectancy of at least 3 months but less than 24 months.
h) Disease status: Patients are to be referred in remission for evaluation. Should a patient have progressive disease or a donor not be available after enrollment, the patient will be referred back to their primary hematologist-oncologist for treatment. If this course of action is not in the best interest of the patient according to the clinical judgment of the PI/LAI, then the patient may receive standard treatment for the malignant disease under the current study. If under either of these settings, it becomes apparent that the patient will not be able to proceed to transplant, then he/she must come off study. Recipient-Subjects receiving a standard therapy will be told about the therapy, associated risks, benefits alternatives of the proposed therapy, and availability of receiving the same treatment elsewhere, outside of a research protocol.

2.2 EXCLUSION CRITERIA- RECIPIENT

a) HIV infection.
b) Chronic active hepatitis B. Patient may be hepatitis B core antibody positive. For patients with a concomitant positive hepatitis B surface antigen, patients will require a hepatology consultation. The risk-benefit profile of transplant and hepatitis B will be discussed with the patient, and eligibility determined by the PI and the protocol chairperson
c) History of psychiatric disorder which may compromise compliance with transplant protocol, or which does not allow for appropriate informed consent.
d) Active infection that is not responding to antimicrobial therapy.
e) Active CNS involvement by malignancy (patients with known positive CSF cytology or parenchymal lesions visible by CT or MRI).
f) Pregnant or lactating.
g) Sexually active individuals capable of becoming pregnant who are unable or unwilling to use effective form(s) of contraception during time enrolled on study and for 1 year post-transplant. Effective forms of contraception include one or more of the following: intrauterine device (IUD), hormonal (birth control pills, injections, or implants), tubal ligation/hysterectomy, partner's vasectomy, barrier methods, (condom, diaphragm, or cervical cap), or abstinence. The effects on breast-milk are also unknown and may be harmful to the infant; therefore, women should not breast feed during the interval from study entry to one year post-transplant. Males on the protocol must use an effective form of contraception at study entry, and for one year post-transplant. The effects of transplant, the radiation, and the medications used after transplant may be harmful to a fetus.
h) Presence of active malignancy in another organ system other than the hematopoietic.
No available 10/10 HLA-matched related donor,9/10 or 10/10 matched unrelated donor, 4/6 (or greater) matched UCB unit(s) with a total dose of greater than or equal to 3.5 $x10^7$ TNC/kg, or haploidentical donor.
i) Lack of mutation in GATA2 as demonstrated by the CLIA certified laboratory

2.3 INCLUSION CRITERIA- MATCHED RELATED DONOR

a) Related donor matched at HLA-A, B, C, DR, and DQ loci by high resolution typing (9/10 or 10/10 antigen/allele match) are acceptable donors (Maris et al. 2003).
b) Matched related donors for pediatric recipients must be 18 years of age or older. If more than one matched related donor is available, we will select the oldest donor to further decrease the risk of potential disease transmission.
c) Ability to give informed consent
d) Age 18-60 years
e) No history of life-threatening opportunistic infection
f) Adequate venous access for peripheral apheresis, or consent to use a temporary central venous catheter for apheresis.
g) Donors must be HIV negative, hepatitis B surface antigen negative, and hepatitis C antibody negative. This is to prevent the possible transmission of these infections to the recipient.
h) A donor who is lactating must be willing and able to interrupt breast-feeding or substitute formula feeding for her infant during the period of filgrastim administration and for two days following the final dose. Filgrastim may be secreted in human milk, although its bioavailability from this source is not known. Limited clinical data suggest that short-term

administration of filgrastim or sargramostim to neonates is not associated with adverse outcomes (Bernstein et al. 2002).

2.4 INCLUSION CRITERIA- MATCHED UNRELATED DONOR

a) Unrelated donor matched at 10/10 or 9/10 HLA-A, B, C, DRB1, and DQB1 loci by high resolution typing (Maris et al. 2003).
b) Matched unrelated donors for pediatric recipients must be 18 years of age or older.
c) The evaluation of donors shall be in accordance with existing NMDP Standard Policies and Procedures. General donor inclusion criteria specified in the NMDP Standards (19th Edition, Appendix F).

2.5 INCLUSION CRITERIA- HAPLOIDENTICAL RELATED DONOR

a) A haploidentical donor that shares one haplotype in common with the recipient such that HLA compatibility will be a minimum of 5 out of 10 HLA loci matched. The HLA loci to be tested will be HLA A, B, Cw, DRB1, and DQB1. A minimum number of mismatches is desirable; however if several options are available the selection of a donor will be based on the loci where the mismatch occurs and the relative importance of its potential immunological function. Donor-recipient pairs will initially be typed molecularly to provide a low resolution typing (antigen-level) to aid in the selection of the potential donor. Upon review of the familial inheritance pattern, a qualified HLA staff member will review haplotype inheritance. High resolution (allele-level) typing will be performed. Final selection of a donor will be in consultation with NCI physicians and qualified HLA personnel. Haploidentical related donors for pediatric recipients must be 15 years of age or older. If more than one haploidentical related donor is available, we will evaluate each donor individually according to overall health, ABO matching, CMV, etc. to select the donor
b) Age 15-60 years
c) No history of life-threatening opportunistic infection
d) Adequate venous access for peripheral apheresis, or consent to use a temporary central venous catheter for apheresis.
e) Donors must be HIV negative, hepatitis B surface antigen negative, and hepatitis C antibody negative. This is to prevent the possible transmission of these infections to the recipient.
f) Haploidentical donors will undergo marrow harvest with general anesthesia. Subjects will undergo anesthesia consultation, and meet criteria for eligibility/enrollment. CD34+ fraction will be determined.
g) Subjects will also undergo the Donor Health History Screen to determine donor eligibility using standard DTM criteria in the Dowling Apheresis Clinic by skilled staff in the Blood Services Section for adult patients and age-appropriate questioning when indicated for pediatric subjects.
h) Subjects will undergo follow-up history and physical examination within 1 week of donation.

2.6 EXCLUSION CRITERIA- MATCHED RELATED DONOR

a) Age less than 18 years.
b) HIV infection
c) Chronic active hepatitis B. Donor may be hepatitis core antibody positive.
d) History of psychiatric disorder which in the opinion of the PI may compromise compliance with transplant protocol, or which does not allow for appropriate informed

e) History of hypertension that is not controlled by medication, stroke, or severe heart disease. Individuals with symptomatic angina will be considered to have severe heart disease and will not be eligible to be a donor.
f) Other medical contraindications to stem cell donation (i.e. severe atherosclerosis, autoimmune disease, iritis or episcleritis, deep venous thrombosis, cerebrovascular accident) (Anderlini et al. 1997).
g) History of prior malignancy. However, cancer survivors who have undergone potentially curative therapy may be considered for stem cell donation on a case-by-case basis. The risk/benefit of the transplant and the possibility of transmitting viable tumor cells at the time of transplantation will be discussed with the patient.
h) Donors must not be pregnant. Pregnancy is an absolute contraindication under this protocol. The effects of cytokine administration on a fetus are unknown. Donors of childbearing potential must use an effective method of contraception. Effective forms of contraception include one or more of the following: intrauterine device (IUD), hormonal (birth control pills, injections, or implants), tubal ligation/hysterectomy, partner's vasectomy, barrier methods, (condom, diaphragm, or cervical cap), or abstinence.
i) Thrombocytopenia (platelets less than 150,000 per μl) at baseline evaluation.
j) Donors receiving experimental therapy or investigational agents.
k) Sensitivity to filgrastim or to E. coli-derived recombinant protein products.
l) History of autoimmune disorders, with the exception of thyroid disorders
m) History of documented deep vein thrombosis or pulmonary embolism
n) Mutation in GATA2

2.7 EXCLUSION CRITERIA- MATCHED UNRELATED DONOR

Failure to qualify as an NMDP donor as described in Appendix F.

2.8 EXCLUSION CRITERIA- HAPLOIDENTICAL DONOR

a) Age less than 15 years.
b) HIV infection
c) Chronic active hepatitis B. Donor may be hepatitis core antibody positive.
d) History of psychiatric disorder which in the opinion of the PI may compromise compliance with transplant protocol, or which does not allow for appropriate informed
e) History of hypertension that is not controlled by medication, stroke, or severe heart disease. Individuals with symptomatic angina will be considered to have severe heart disease and will not be eligible to be a donor.
f) Other medical contraindications to stem cell donation (i.e. severe atherosclerosis, autoimmune disease, iritis or episcleritis, deep venous thrombosis, cerebrovascular accident) (Anderlini et al. 1997).
g) History of prior malignancy. However, cancer survivors who have undergone potentially curative therapy may be considered for stem cell donation on a case-by-case basis. The risk/benefit of the transplant and the possibility of transmitting viable tumor cells at the time of transplantation will be discussed with the patient.
h) Donors must not be pregnant. Pregnancy is an absolute contraindication under this protocol. The effects of cytokine administration on a fetus are unknown. Donors of childbearing potential must use an effective method of contraception. Effective forms of contraception include one or more of the following: intrauterine device (IUD), hormonal (birth control

pills, injections, or implants), tubal ligation/hysterectomy, partner's vasectomy, barrier methods, (condom, diaphragm, or cervical cap), or abstinence.

i) Thrombocytopenia (platelets less than 150,000 per μl) at baseline evaluation.
j) Donors receiving experimental therapy or investigational agents.
k) Sensitivity to filgrastim or to E. coli-derived recombinant protein products.
l) History of autoimmune disorders, with the exception of thyroid disorders
m) History of documented deep vein thrombosis or pulmonary embolism
n) Mutation in GATA2

2.9 INCLUSION CRITERIA- UMBILICAL CORD BLOOD UNIT-HLA TYPING AND DOSE

a) At least an HLA UCB 4/6 match (Class I-A, B by low resolution, and Class II-DR by high resolution) to recipient. The following algorithm will be applied to determine if patient will receive single or double umbilical cord graft:
b) For Single UCB SCT:
   - If 6/6 match the unit must have > 3 x 10^7 nucleated cells /kg of recipient body weight.
   - If 5/6 match the unit must have > 4 x 10^7 nucleated cells /kg of recipient body weight.
   - If 4/6 match the unit must have > 5 x 10^7 nucleated cells/kg of recipient body weight. Recipient body weight will be determined as per standard guidelines.
c) If no single UCB with the above characteristics is available, a double UCB will be considered. Units will be selected with the following criteria:
   - Both units will be at least 4/6 match (Class I-A, B by low resolution, Class II-DR by high resolution) to recipient, and should be at least a 4/6 match (Class I-A, B by low resolution, Class II-DR by high resolution) to each other.
   - At least one UCB will have a minimum cell dose of 2.0 X 10^7 TNC/kg of recipient body weight.
   - The minimum combined dose of both units must be at least 3.5 x 10^7 TNC/kg of recipient body weight.
   - The smaller of the two units (UCB2) will have a minimum of 1.5 X 10^7 TNC/kg of recipient body weight.
   - The TNC of non-RBC reduced units will be dose corrected by -25% to allow for cell loss while washing the unit.

2.10 REVIEW OF UCB UNIT HLA TYPING AND SELECTION OF THE UCB UNIT

a) Single UCB SCT: First preference will be given to cell dose, as long as the UCB units are 4/6 matches or greater. Second preference will be given to HLA matching (e.g. if two units have the same cell dose, the unit with a better HLA match will be selected). Priority will be given to Class II matched units.
b) Double UCB SCT: First preference will be given to cell dose, as long as the UCB units are 4/6 matches or greater. Second preference will be given to HLA matching (DR matching will be preferred over Class I matching) (e.g. if two units have the same cell dose, the unit with a better HLA match will be selected). In double UCB SCT, the units should be at least a 4/6 HLA match or greater to the recipient, and at least a 4/6 HLA match to each other. The matching between the UCB units and recipient, and UCB units to each other can be at different loci. Dr. Hickstein or his delegate will review all HLA typing on the patient and potential cord units. High-resolution HLA typing will be reviewed, and, if necessary, an aliquot of the cord unit will be retyped as per standard practice. After review of the typing,

the cord unit(s) will be requested and will be at the DTM Laboratory prior to the start of the conditioning therapy.

2.11 RECIPIENT RESEARCH ELIGIBILITY EVALUATION

The following clinical, laboratory, and radiological assessment must be performed in the patient (recipient) within 28 days before the recipient signs the consent form (unless otherwise specified)

a) Complete medical history and physical examination.
b) Antibody screen for hepatitis A, B, and C; HIV, T.Cruzi (Chagas agent), HTLV-I/II, CMV, adenovirus, EBV, HSV, Toxoplasmosis, and syphilis.
c) PPD test (in recipients considered to be in a high-risk group).
d) The following should be obtained at initial screening and also within 48 hours before starting induction chemotherapy:
   - CBC with differential, PT, and PTT, and ABO typing
   - Acute care panel, hepatic panel, mineral panel, and lipid profile
   - Urine βHCG in women of childbearing potential
e) Urinalysis and 24 hour urine collection (for determination of creatinine clearance).
f) CT scans of head, sinus, chest, abdomen, and pelvis.
g) Cardiac 2-D Echo or MUGA exam.
h) Pulmonary Function Test, including DLCO measurement.
i) Bone marrow aspiration and biopsy; flow cytometry, cytogenetics, and molecular studies (within 60 days of signing consent, unless clinically indicated).
j) All biopsy specimens will be reviewed by the Laboratory of Pathology/CCR/NCI, for confirmation of the histological diagnosis prior to enrollment on study.
k) Lymphocyte phenotyping panel (T, B, NK).
l) Serum troponin.
m) Lipid profile with triglycerides.
n) MRI scan of the head if there is clinical suspicion of CNS disease.
o) Radiation Oncology Consult (within 28 days of initiating conditioning chemotherapy).

2.11.1 Recipient (perform within 90 days before initiation of conditioning chemotherapy)

a) Nutritional assessment (initial consult).
b) Social work consultation.
c) Electrocardiogram.

2.11.2 Recipient (perform within 6 months before initiation of conditioning chemotherapy)

a) Dental consultation to assess need for teeth cleaning or removal.

2.11.3 Recipient pre-transplant evaluation (perform any time before initiation of induction chemotherapy)

a) Typing for HLA-A, -B, -C, and DR.
   PCR test of DNA mini-satellite regions for future determination of chimerism.

2.12 MATCHED RELATED DONOR AND HAPLOIDENTICAL DONOR RESEARCH ELIGIBILITY EVALUATION

2.12.1 Donor Research Evaluation (perform any time before initiation of induction chemotherapy)

a) Typing for HLA A, B, C, DR, DQ.

b) PCR test of DNA mini-satellite regions for future determination of chimerism.

2.12.2 Donor Research Evaluation (perform within 90 days of signing consent)

a) Complete medical history and physical examination.
b) Antibody screen for hepatitis A, B, and C; HIV, HTLV-I/II, CMV, adenovirus, EBV, HSV, Toxoplasmosis, T. cruzi (Chagas agent), and syphilis.
c) CBC with differential, PT, and PTT, and ABO, Rh typing.
d) Acute care panel, hepatic panel, and mineral panel.
e) Urinalysis.
f) Urine β-HCG in women of childbearing potential.
g) Chest radiograph.
h) Electrocardiogram.
i) Screening for Hb S.
j) Lymphocyte phenotyping panel (T, B, NK).

2.13 UNLICENSED CORD BLOOD UNITS

There is a new federal requirement for licensing UCB units. In October, 2009, the FDA released licensure guidance for UCB which outlines how a cord blood bank can apply for licensure of UCB units. If the unit meets the criteria for licensure, then that unit can be distributed as a licensed product. However, starting October 20, 2011, units that do not meet the requirements are considered "unlicensed" and can only be distributed for transplant under an IND.

For those patients whose best cord blood option is an unlicensed unit, the unit will be obtained under an IND which allows access to unlicensed cord blood units. For ease of access, the NMDP has developed a protocol through which most unlicensed CBUs may be obtained. The corresponding NCI protocol number is 12-C-0027. Patients will be consented and signed on to this protocol, unless their CB is obtained from a non-participating cord blood bank. Unlicensed CBs from non-participating cord blood banks will be obtained through a single patient IND if deemed appropriate by the PI, or through the bank's own IND protocol, which will be submitted to the NCI IRB for review.

2.14 PATIENT REGISTRATION

a) Protocol "entry date" is considered to be the day that the informed consent form has been signed by the recipient and donor (matched related donor, when applicable) OR confirmation from the NMDP that the requested donor is available (unrelated donor), meets the inclusion criteria, and has signed the intent to donate form. The treatment start date is considered to be the day the recipient begins his/her conditioning regimen.
b) Authorized staff must register an eligible candidate with Central Registration Office (CRO) no later than 24 hours after the patient (recipient) has signed an informed consent form. The patient may not sign consent before the matched unrelated donor has been identified and cleared by NMDP and the patient has been determined to be eligible, OR the patient may not sign consent before the matched related donor has been identified and has signed consent (when applicable). The patient will not be registered before she/he has signed consent and confirmation from the NMDP that the donor has signed consent. The patient and matched related donor (when applicable) must be registered prior to beginning this study. A

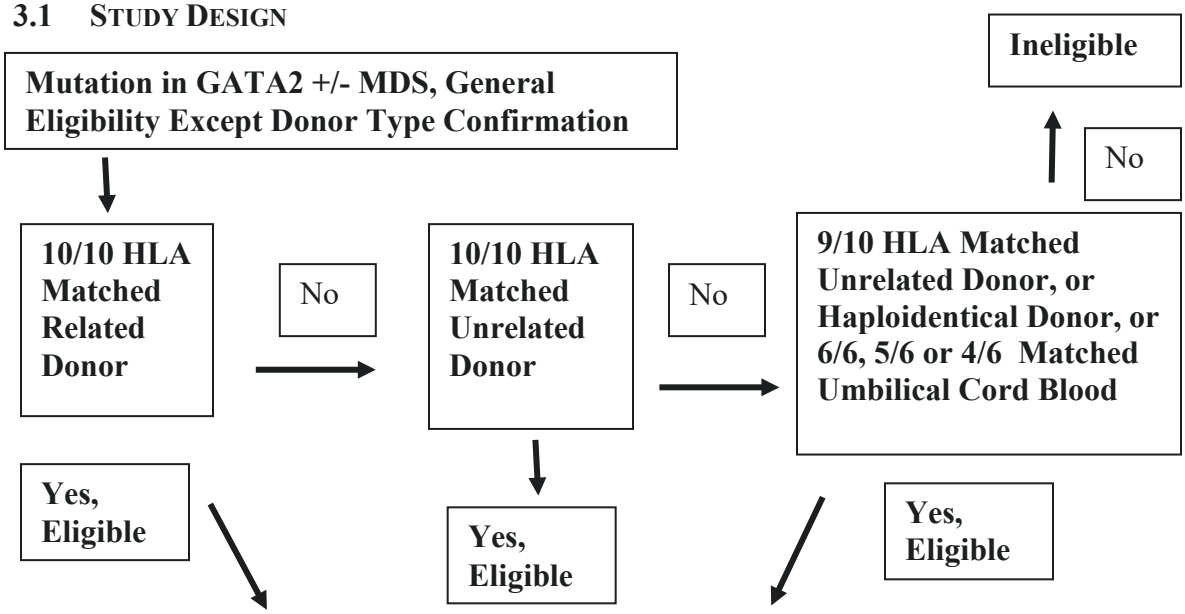
registration Eligibility Checklist is available from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) for NCI patients and must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol. For questions regarding registration authorized staff should call 301-402-1732 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday. Voicemail is available during non-business hours.

2.14.1 Off-Study Procedure:

Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site main page must be completed and faxed to 301-480-0757. The website is: http://home.ccr.cancer.gov/intra/eligibility/welcome.htm)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN



Preparative Regimen for 10/10 MRD and 10/10 URD
1) Fludarabine 30 mg/m^2 per day, IV, Days -4, -3, -2
2) Total Body Irradiation (TBI) 200 cGy, Day -1

Preparative Regimen for 9/10 URD
1) Fludarabine 30 mg/m^2 per day, IV, Days -4, -3, -2
2) Total Body Irradiation (TBI) 300 cGy, Day -1

Preparative Regimen for Haploidentical Donor
1) Fludarabine 30 mg/m^2 per day, IV, Days -6, -5, -4, -3, -2
2) Total Body Irradiation (TBI) 300 cGy, Day -1
3) Cyclophosphamide 14.5 mg/kg IV, Days -6, -5

Preparative Regimen for UCB
1) Fludarabine 40 mg/m^2 per day, IV, Days -6, -5, -4, -3, -2
2) Cyclophosphamide 50 mg/kg on Day -6
3) Total Body Irradiation (TBI) 200 cGy, Day -1
4) Equine ATG 30mg/kg IV on Days -6, -5, -4 (3 doses total)

Allogeneic HSC Transplantation
1) Mobilized PBSC (Day 0); > 5 x 10^6 CD34+ /kg if Matched Related or Unrelated Donor > 3.5 v 10^5 TNC/kg if UCB, and > or equal to 350 x10^6 TNC/kg.2). Sirolimus from Day -2 tapering at Day +180
3) Tacrolimus 0.02 mg/kg continuous IV infusion from Day -3 tapering at Day +180.
4) For Haploidentical Donors Cyclophophamide 50 mg/kg IV on days +3 and +4, and Sirolimus and Tacrolimus from day +5 tapering at day +180.

Figure 3. Protocol schematic.

3.1.1 Donor Stem Cell Mobilization and Collection

a) Matched related and unrelated donors will receive filgrastim as an outpatient for stem cell mobilization at a dose of 10 μg/kg/day by subcutaneous injection. Doses can be rounded to vial sizes (300 mcg and 480 mcg) according to NMDP standards. The dose should not exceed 1200 μg/day, and the volume per injection site shall not exceed 2.0 ml. Filgrastim will be administered upon awakening in the morning. The fifth dose should be given at least one hour prior to apheresis.
b) Apheresis will typically start on the fifth day of filgrastim administration and 15-25 liters will be processed daily based on: (1) donor pre-apheresis circulating CD34 cell count in related donors undergoing apheresis in DTM, or (2) recipient weight for unrelated NMDP donors. (Table 3). The target cell dose will be $\geq 5 \times 10^6$ CD34+ cells/kg-recipient weight.
c) Whole blood apheresis will be performed via a 2-armed approach or a temporary central venous catheter in the femoral position using the Baxter CS3000Plus, Cobe Spectra, or an equivalent instrument (typically 4 to 6 hour procedure).
d) The apheresis procedure will use ACD-A anti-coagulant or heparin at the discretion of the DTM physician.

Table 3 - Blood Volume Processed in Relation to Recipient Weight

Recipient Weight (kg)	Volume Processed (L)	Procedure
≤ 35	12	Single 12 liter apheresis.
36 – 45	15	Single 15 liter apheresis.
46 – 55	18	Single 18 liter apheresis or two 12 L aphereses.
56 – 65	22	Single 22 liter apheresis or two 12 liter aphereses.
> 65	24	Single 24 liter apheresis or two 12 liter aphereses.

e) For related donors, PBSC will be processed, cryopreserved, and stored in liquid nitrogen until the day of transplant upon delivery to the NIH Department of Transfusion Medicine (DTM). Donor cells from unrelated donors will be used without cryopreservation under routine circumstances.
f) The concentration of CD34+ cells in the apheresis product will be determined by flow cytometry, and the number of CD34+ cells in each cryopreserved bag calculated.
g) Matched related donors will be evaluated clinically following apheresis, and they will be seen in the day hospital for follow-up one day following the apheresis. The nurse practitioner or physician assistant covering the Day Hospital will conduct this evaluation.

3.1.2 Cord Blood Collection:

The cords will be obtained from qualified cord blood banks through the NMDP. The cords will be scheduled for shipping in order to arrive 1 week prior to the patient beginning the transplant conditioning regimen. They will remain frozen until ready for use. If the cord has an attached segment, the segment will be sent to the HLA lab where DNA will be extracted

from the segment for HLA confirmatory typing. The DNA will be shared with the Chimerism Lab who will use it to develop the STR profile for later use. If there is more than one segment, the second segment will be used to check viability of the cord. This will be done using 7-AAD which is a flow cytometric marker which separates viable from apoptosed cells. Note: The viability of the segment does not necessarily correlate with the viability of the cord unit. The number and type of available cord blood specimens vary by bank, collection date and typing history. Viable cell aliquots and spot cards may also be used for HLA testing and STR analysis.

Once they are thawed, the cords will be diluted 1:1 with a dextran/HSA mix and transferred to a larger bag. From that bag, CBC, nucleated cell count, colony forming units, flow cytometry, and sterility will be sent. This volume should total about 1.5 mL. After the CBC is done, the concentration of cells will be known and the number of cells can be calculated. If the unit did not have a detached segment, HLA testing and the STR profile will be done from this sample. The viability will be repeated on the diluted cord unit.

3.1.3 Haploidentical Donor Bone Marrow Harvest

a) Haploidentical donors will undergo marrow harvest with general anesthesia. Subjects will undergo anesthesia consultation, and meet criteria for eligibility/enrollment. CD34+ fraction will be determined. Subjects will also undergo the Donor Health History Screen to determine donor eligibility using standard DTM criteria in the Dowling Apheresis Clinic by skilled staff in the Blood Services Section for adult patients and age-appropriate questioning when indicated for pediatric subjects. Subjects will undergo follow-up history and physical examination within 1 week of donation. The target dose is > or equal to 3.5×10^8 total nucleated cells per kilogram. Of this product, 5 million total donor nucleated cells will be reserved for biologic studies and go to the clinical core of ETIB (Bldg 10, Room 12C-216, 301 402 3627. Cell selection will not be performed on the HSC grafts.

3.1.4 Transplant Procedure (Day 0)

a) On day 0, the patient will receive fresh or cryopreserved peripheral blood stem cells from an HLA-matched related or unrelated donor, umbilical cord blood units, or fresh or cryopreserved haploidentical donor bone marrow. The cryopreserved PBSC product will be thawed and immediately administered intravenously. The target dose of the PBSC is $\geq 5 \times 10^6$ $CD34^+$ cells per kg. If the collection exceeds 5×10^6 $CD34^+$ cells per kg, the PI will decide if greater than 5×10^6 $CD34^+$ cells per kg will be infused. The target dose for haploidentical bone marrow is $> 3.5 \times 10^8$ nucleated cells/kg recipient body weight.
b) Approximately 5×10^6 donor total nucleated PBSC will be reserved for Dr. Fran Hakim (Bldg. 10, Room 12C-216, Tel 301-402-3627).

3.1.5 Determination of Engraftment

a) Neutrophil Recovery: Designated by the first of 3 consecutive days with an ANC above $500/mm^3$.
b) Platelet Recovery: Designated by the first of 7 days where the platelet count remains above $20{,}000/mm^3$ without transfusion support.
c) Sustained Donor Engraftment: Neutrophil recovery associated with complete donor chimerism at day 100. Any patient who dies before day 28 will not be evaluated for engraftment.
d) Primary Graft Failure: Failure to achieve sustained donor engraftment

e) Secondary Graft Failure: Documented sustained donor engraftment as defined above followed by: 1) severe neutropenia (ANC < 500/mm^3) and bone marrow biopsy revealing a cellularity of less than 25% or 2) absence of donor cells in the marrow or blood as demonstrated by a chimerism assay without subsequent improvement occurring either spontaneously or after growth factor treatment. Improvement is defined as ANC > 500/mm^3 consistently. Severe neutropenia with marrow cellularity > 25% is not secondary graft failure.
f) Aplasia: Less than 5% marrow cellularity as measured on bone marrow biopsy.

3.1.6 Complication of Mixed Chimerism or Persistent/Progressive Disease

a) Donor Lymphocyte Infusion: Patients with persistent or progressive MDS post-HSCT, or mixed chimerism that does not improve after tapering or discontinuing immune suppression may be eligible to receive donor lymphocytes (donor lymphocyte infusion, or "DLI") or G-CSF mobilized DLI if they can be obtained from the donor. Additional lymphocytes will be collected by apheresis from the patient's stem cell donor, either in steady state (i.e. no donor therapy) or after filgrastim. DLI or mobilized DLI may be administered alone or after chemotherapy.
b) DLI may be sequentially administered for mixed chimerism or progression of the MDS at doses 1 -10 x 10^6 $CD3^+$ T cells per kg. Recipients with mixed chimerism or progression of MDS will receive DLI consisting of unmanipulated donor T cells.
c) Alternatively, in cases where additional donor stem cells are desired, the donor product may be administered without lymphocyte purification.
d) Recipients with mixed chimerism at day +30 will undergo repeat chimerism assessment at day +60 and every 30 days thereafter as deemed necessary. If there is persistent mixed donor-recipient chimerism (< 95% donor chimerism on whole blood, lymphoid, or myeloid subsets), recipients without Grade 2 or higher GVHD, patients will be eligible to receive DLI. DLI may be repeated every 4 weeks with dose escalation as indicated above, until donor chimerism > 95% is achieved or until GVHD develops.
e) Recipients with progression of MDS and without Grade 2 or higher GVHD will be eligible to receive DLI every 4 weeks with dose escalation as indicated above, until donor chimerism > 95% is achieved or until GVHD develops.
f) In addition to DLI, patients with persistent or progressive disease may be offered therapy on other NCI protocols. Alternatively, persistent or progressive disease may be treated with any approved therapy thought to be in the best interest of the patient including chemotherapy, radiation therapy, or monoclonal antibody therapy.

3.2 DRUG ADMINISTRATION

3.2.1 Transplant Conditioning Regimen for 10/10 Matched Related Donor or a 10/10 Matched Unrelated Donor Transplant

a) Central line access will be required prior to the start of the preparative regimen, and a triple lumen Hickman catheter is recommended, however clinical determination for the most appropriate line type and placement will be made on a case-by-case basis.
b) Conditioning Regimen with 200 cGy TBI and Fludarabine for 10/10 matched related and 10/10 matched unrelated donor transplant. If anesthesia is required in pediatric patients, an additional consent will be obtained for TBI anesthesia.

Agent	Dose	Days
Fludarabine	30 mg/m^2 per day	Transplant Days –4, -3, -2

	IV infusion over 30 min, daily for 3 days	
Total body Irradiation	200 cGy	Transplant Day -1

c) The dose of Fludarabine will be dose adjusted for renal dysfunction. For Creatinine Clearance of 30-70 mL/min/1.73m2, there will be a 20% dose reduction. If the Creatinine Clearance is less than 30mL/min/1.73m2, Fludarabine will not be administered.

3.2.2 Transplant Conditioning Regimen for 9/10 Matched Unrelated Donor Transplant

a) Central line access will be required prior to the start of the preparative regimen, and a triple lumen Hickman catheter is recommended, however clinical determination for the most appropriate line type and placement will be made on a case-by-case basis.
b) Conditioning Regimen with 300 cGy TBI and Fludarabine for 9/10 matched matched unrelated donor transplant. If anesthesia is required in pediatric patients, an additional consent will be obtained for TBI anesthesia.
c) The dose of Fludarabine will be dose adjusted for renal dysfunction. For Creatinine Clearance of 30-70 mL/min/1.73m2, there will be a 20% dose reduction. If the Creatinine Clearance is less than 30mL/min/1.73m2, Fludarabine will not be administered.

Agent	**Dose**	**Days**
Fludarabine	30 mg/m^2 per day IV infusion over 30 min,daily for 3 days	Transplant Days –4, -3, -2
Total body Irradiation	300 cGy	Transplant Day -1

3.2.3 Transplant Conditioning Regimen for Haploidentical Related Donor Transplant

a) Central line access will be required prior to the start of the preparative regimen, and a triple lumen Hickman catheter is recommended, however clinical determination for the most appropriate line type and placement will be made on a case-by-case basis.
b) Conditioning Regimen with 200 cGy TBI and Fludarabine and Cyclophosphamide for Haploidentical Related Donor.
c) The dose of Fludarabine will be dose adjusted for renal dysfunction. For Creatinine Clearance of 30-70 mL/min/1.73m2, there will be a 20% dose reduction.

Agent	**Dose**	**Days**
Fludarabine	30 mg/m^2 per day IV infusion over 30 min,daily for 3 days	Transplant Days -6, -5. –4, -3, -2
Cyclophosphamide	14.5 mg/kg	Transplant days -6, -5
Total body Irradiation	200 cGy	Transplant Day -1

3.2.4 Transplant Conditioning Regimen for Umbilical Cord Blood

a) A triple lumen Hickman catheter will be placed by an interventional radiologist or surgeon at least 7 days before the start of the preparative regimen.
b) Conditioning with 200 cGy TBI, Fludarabine, and Cyclophosphamide for umbilical cord blood transplant will occur as follows:

Day - -6 Fludarabine 40 mg/m^2 IV, Cyclophosphamide 50 mg/kg IV, Equine ATG 30mg/kg IV
Day- -5 Fludarabine 40 mg/m^2 IV, Equine ATG 30 mg/kg IV
Day- -4 Fludarabine 40 mg/m^2 IV, Equine ATG 30 mg/kg IV
Day- -3 Fludarabine 40 mg/m^2 IV
Day- -2 Fludarabine 40 mg/m^2 IV
Day- -1 TBI 200 cGy
Day 0 Cord blood transplant

If anesthesia is required in pediatric patients, an additional consent will be obtained for TBI anesthesia.

Agent	Dose	Days
Fludarabine	40 mg/m^2 per day IV infusion over 30 min, daily for 5 days	Transplant Days -6, -5, -4, -3, -2
Cyclophosphamide	50 mg/kg	Transplant Day -6
Total body Irradiation	200 cGy	Transplant Day -1
Equine ATG	30 mg/kg IV daily for 3 days	Transplant Days -6, -5, -4

c) Equine ATG Instructions

1. Equine ATG will be administered at a dose of 30 mg/kg/day for 3 consecutive days (days -6, -5, -4) for a total dose of 90 mg/kg.
2. Equine ATG will be infused IV over 4 hours. Infusion times may be extended up to 24 hours to improve tolerance if necessary.
3. An epinephrine Auto-Inj 0.3 mg (EpiPen) should be kept at bedside on days of equine ATG administration
4. Because of risk of allergic reaction to ATGAM, at least 24 hours before the first infusion of ATGAM, patients will be tested with an intradermal injection of 0.1 ml of a 1:1000 dilution (5 µg horse ATG) of ATGAM in sodium chloride injection and a contralateral sodium chloride injection control. The patient should be observed every 15 to 20 min over the first hour of intradermal injection. A local reaction of 10 mm or more with a wheal or erythema or both should be considered a positive test. If there is a positive skin test, the patient will undergo ATG desensitization per standard of care guidelines or receive a 24-hour infusion of ATG.
5. Subjects will receive pre-medication prior to Equine ATG infusion consisting of:
   - Oral acetaminophen 650 mg
   - Oral diphenhydramine 25-50 mg IV

   The dosages of these drugs may be adjusted for weight based dosing if necessary following pediatric dosing recommendations.

   Oral prednisone at 1mg/kg/day will begin on day -5 prior to the first dose of h-ATG for serum sickness prophylaxis, and will be continued at this dose for 10 days. Thereafter, prednisone will be slowly tapered over the next 2 weeks until it is discontinued on day

+19. Likewise, patients where it is clinically indicated to use a shorter course or taper schedule will be dosed individually as clinically indicated. Those patients who develop serum sickness may require a longer tapering schedule and will be dosed individually as clinically indicated. Infusion reactions will be treated symptomatically (e.g. antiemetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators, and meperedine. In cases of moderate to severe reactions, hydrocortisone will be given and the infusion will be discontinued and restarted at a slower rate once the symptoms have subsided. If a patient has a persistent severe infusion reaction that does not respond to measures to ameliorate the sings/symptoms associated with the infusion, the infusion will be discontinued and further study participation will continue as planned with the exception that no further ATG will be administered.

d) The dose of Fludarabine will be dose adjusted for renal dysfunction. For Creatinine Clearance of 30-70 mL/min/1.73m^2, there will be a 20% dose reduction. If the Creatinine Clearance is less than 30mL/min/1.73m^2, Fludarabine will not be administered.
e) Cyclophosphamide will be dosed on actual body weight. Hydration will be carried out according to the NIH supportive care guidelines for the prevention of hemorrhagic cystitis as outlined below.

Background:
Hemorrhagic cystitis (HC) is a well documented complication of cyclophosphamide therapy. The mechanism of cyclophosphamide-induced HC is secondary to bladder wall damage from contact with the acrolein metabolite of cyclophosphamide. The risk of cyclophosphamide-induced HC is greater with high dose cyclophosphamide regimens such as those employed in hematopoetic stem cell transplant (HSCT) preparative regimens. Routine prevention strategies including intravenous hydration, forced diuresis, and mesna are imperative to reduce the risk of HC. This guideline is intended to provide a consistent approach to HC prevention in patients receiving high-dose cyclophosphamide in chemotherapy preparative regimens prior to HSCT at the NIH.

Definitions:
High-dose cyclophosphamide: There is no absolute cut off for defining a dose of cyclophosphamide that would necessitate an aggressive strategy for HC prevention. Cyclophosphamide doses employed in HSCT preparative regimens at the NIH that equal or exceed 50 to 60 mg/kg/dose OR 1200 mg/m2/dose should always be accompanied by the HC prevention strategies outlined below.

- Intravenous hydration should be initiated at a minimum of 12 hours prior to the first dose of cyclophosphamide
- The rate of intravenous infusion for hydration is 0.9% Sodium Chloride Inj with 10 mEq / liter of potassium chloride infused at a rate of 90 mL/m2/hour.
- Continue hydration until 24 hours after last cyclophosphamide infusion.
- Furosemide 20mg intravenously will be given once daily - *Adult Patients Only* - (preferably in the AM hours) on a scheduled basis on the days of cyclophosphamide administration to maintain diuresis. Pediatric patients may receive furosemide as clinically indicated at a dose of 1mg/kg up to a maximum of 20 mg. (Furosemide doses may be adjusted based on patient weight, diuretic response or other clinical factors;
    - Monitor intake and output closely (2 hour intervals). Accurate documentation of intake and output is essential to make good clinical decisions regarding diuresis.

- If fluid intake exceeds urine output by 500 ml (10 ml/kg for pediatric patients < 50 kg) over an 8 hour period, an additional 20 mg of furosemide (1mg/kg for pediatric patients up to a maximum of 20 mg) will be administered intravenously. Notify PI/LAI when PRN doses of furosemide are administered.
- Additional doses of furosemide maybe administered as needed for weight gain due to fluid retention. In general, furosemide doses should be separated by at least a four hour observation interval.
- Notify PI/LAI if patient's input is greater than output by 1 liter (20 ml/kg for pediatric patients < 50 kg) in an 8 hour period during cyclophosphamide hydration.
- Stop IV hydration (24 hours after last cyclophosphamide dose).

- **MESNA**

  For detoxification of acrolein, the urotoxic metabolite of cyclophosphamide, Mesna will be given in a dose equal to that of cyclophosphamide. **Total MESNA dose should = the cyclophosphamide dose.** Mesna will be administered as a continuous intravenous infusion over 24 hours on each day that cyclophosphamide is administered. The initial bag of mesna should be started concurrently with the start of cyclophosphamide.

  Example: For preparative regimens that dose cyclophosphamide at 60 mg/kg/day, the MESNA will also be given at a dose of 60 mg/kg administered as a continuous intravenous infusion over 24 hours.

  The MESNA infusion should continue for a minimum of 22-24 hours after the last dose of cyclophosphamide. The entire MESNA dose prescribed should be administered each day of cyclophosphamide therapy

3.2.5 GVHD Prophylaxis with Sirolimus

a) Patients will receive GVHD prophylaxis with sirolimus and tacrolimus.
b) For recipients of matched related and unrelated donors, and umbilical cord blood, Sirolimus will be administered continuously from day –2 by mouth as Rapamune tablets at an initial "loading dose" of 12 mg, p.o. in adults > 18 years of age. In pediatric patients >40 kg the loading dose will be 6 mg. In pediatric patients <40kg, the loading dose will be 3mg/m2.
c) For recipients of haploidentical donors, Sirolimus will be administered continuously from day +5 by mouth as Rapamune tablets at an initial "loading dose" of 12 mg, p.o. in adults > 18 years of age. In pediatric patients >40 kg the loading dose will be 6 mg. In pediatric patients <40kg, the loading dose will be 3mg/m2.
d) Sirolimus will be administered each day in the morning.
e) From days -1 in matched related and unrelated donor transplants, and umbilical cord blood transplants, and continuing post-transplant, Sirolimus dosing will be Rapamune tablets, 4 mg, p.o., each day in adult patients. In pediatric patients > 40 kg the initial dose will be 2 mg every 24 hours. For patients < 40 kg, the initial dose will be 1 mg/m^2. This may be delayed or held during the peri-engraftment phase for severe oral mucositis or delayed platelet engraftment. Sirolimus dose should be adjusted to maintain a target therapeutic concentration of 10 ng/L with a range of 5 to 15 ng/L.
f) In haploidentical blood recipients Sirolimus dosing will be Rapamune tablets, 4 mg, p.o., each day in adult patients. In pediatric patients > 40 kg the initial dose will be 2 mg every 24 hours. For patients < 40 kg, the initial dose will be 1 mg/m^2. This may be delayed or held during the peri-engraftment phase for severe oral mucositis or delayed

platelet engraftment. Sirolimus dose should be adjusted to maintain a target therapeutic concentration of 10 ng/L with a range of 5 to 15 ng/L

g) Sirolimus levels will be drawn on a Monday, Thursday. For sirolimus levels, 2.5 cc of blood will be collected into EDTA tubes and analyzed at the NIH Clinical Center.
h) Because sirolimus has a long half-life (approximately 60 hours), sirolimus levels should be monitored once per week beyond day 60 until levels are undetectable. This information will be informative relative to the duration of time post-transplant that individual subjects have exposure to biologically significant levels of sirolimus.
i) The total dose of sirolimus will continue with tapering beginning at day +180. (+/- two days) unless clinically indicated per PI discretion.
j) The initial Sirolimus dosing maybe adjusted if clinically indicated for known drug interactions that cannot be avoided.

3.2.6 GVHD Prophylaxis with Tacrolimus

a) Tacrolimus will be used as the second agent in the immunosuppressive regimen, and in recipients of matched related and unrelated donors and umbilical cord blood, Tacrolimus will be initiated on day -3 before transplant as a continuous intravenous infusion at 0.02 mg/kg/day for a target level of 5 to 10 ng/ml
b) For recipients of haploidentical donors, Tacrolimus will be administered continuously from day +5.
c) When the patient is reliably taking oral medications, tacrolimus will be converted to an equivalent oral dose using a 1:3 conversion from the IV form. The total daily dose will be divided into two equal doses, one dose given approximately every 12 hours.
d) The total dose of tacrolimus will continue with tapering starting at day +180, unless clinically indicated per PI.
e) The initial Tacrolimus dosing may be adjusted if clinically indicated for known drug interactions that cannot be avoided.

3.2.7 GVHD Prophylaxis with Cyclophosphamide for Haploidentical Recipients

a) Cyclophosphamide will be used as the third agent in the immunosuppressive regimen for haploidentical recipients and will be given on days +3 and +4 after transplant as an infusion over 90 min.
b) Cyclophosphamide will be accompanied by Mesna 80% of cyclophosphamide dose in 4 divided doses on day +3, the day of high dose cyclophosphamide.
c) Tacrolimus and Sirolimus will be started on day +5 for haploidentical recipients.

3.2.8 Post Transplant Treatment with Filgrastim (G-CSF)

Filgrastim will be administered at a dose of 5 micrograms/kg/day IV starting on day 0 for matched related, unrelated, and cord blood recipients, and on day +4 in haploidentical recipients, and continued until the ANC is greater than 1000 for 3 days or greater than 5000 for 1 day.

3.3 TREATMENT MODIFICATIONS

3.3.1 Donor Hematopoietic Stem Cell Harvest Modifications

a) The dose of filgrastim for matched related donor stem cell mobilization will not be changed from a dose of 10 μg/kg/day by subcutaneous injection.
b) There will be no additional dose of filgrastim if a sixth day of apheresis is performed.

c) For apheresis an additional 12 L apheresis may be performed on day 6 if the target dose of 5 x 10^6 CD34_+ cells/kg is not reached on the day 5 collection.
d) If greater than 5 x 10^6 CD34+ cells per kg are harvested after apheresis, then no further mobilization or apheresis will be performed, and the patient will be eligible to receive the stem cell transplant with that dose of CD34+ cells.
e) In the event that < 3 x 10^6 CD34+ cells per kg are harvested, the donor will be given two weeks of rest, and then will be re-treated with filgrastim 8 µg/kg subcutaneously BID for five days followed by repeat blood stem cell harvesting.
f) If the donor stem cells are cryopreserved, the apheresis product will be cryopreserved in Plasmalyte A, Pentastarch, human serum albumin, DMSO, and preservative free heparin (10U/ml). In the event that unrelated donor cells are to be collected and cryopreserved prior to transplant, permission will be obtained from the NMDP.
g) If an ABO incompatibility exists between the donor and patient, the donor erythrocytes will be removed from the graft in the cell-processing laboratory, according to standard DTM operating procedures.
h) Bone marrow may also be obtained from the donor if conditioning has already begun and the PBSC collection is not to be carried out or results in a suboptimal collection.

3.3.2 Transplant Treatment Modification

a) If donor apheresis on days 5, 6, and 7 yields a total of ≥ 3 x 10^6 $CD34^+$ cells per kg, this level of $CD34^+$ cell dose will be allowed.
b) If PBSC cell transplant results in DMSO-related toxicities (chills, muscle aches), diphenhydramine 25 mg IV and meperidine 50 mg IV may be administered one time.

3.3.3 Post-transplant Rejection

This protocol uses a nonmyeloablative-conditioning regimen, thus autologous recovery is anticipated in patients who fail to engraft. Patients who have less than 1% donor neutrophils in the peripheral blood on two occasions more than one month apart and occurring more than one month after transplant will be considered to have graft rejection.

3.3.4 Dose Modification for Sirolimus

a) The total duration of sirolimus will be determined by the presence or absence of GVHD and the level of donor/recipient chimerism, however the schedule is to continue sirolimus until day +180 (+/- 2 days) and then taper as long as the severity of GVHD is less than grade II, and the patient is not requiring systemic steroids. Sirolimus will subsequently be reduced by one-third on day +180 (+/- 2 days as long as the severity of GVHD is less than grade II, the patient is not receiving systemic steroids, and the donor chimerism is 100 percent. If the donor chimerism is less than 100%, sirolimus will be continued without taper for up to 2 years at the discretion of the PI.
b) Because prolonged dosing of sirolimus is associated with increased cholesterol and triglycerides, a lipid panel that includes cholesterol and triglycerides should be measured every 2 weeks during sirolimus therapy. If elevated cholesterol and triglyceride levels are documented on two consecutive tests, therapy with an HMG-CoA reductase inhibitor ("statin" therapy) will be initiated. Since the patients will be on Fluconazole, it is recommended to start Pravastatin 40 mg/day. In the event that this 40 mg daily dose is not sufficient (elevation of cholesterol or triglycerides at a level 50% above baseline values), then consideration may be given to increase the dose of the Pravastatin therapy to 80 mg.

3.3.5 Dose Modification for Tacrolimus

a) Subsequent doses of tacrolimus will be adjusted according to levels monitored at least bi-weekly and/or upon symptoms or alterations in renal function. The target level will be 5 to 10 ng/ml
b) When the patient is reliably taking oral medications, tacrolimus will be converted to an equivalent oral dose using a 1:3 conversion from the IV form. The total daily dose will be divided into two equal doses, one dose given approximately every 12 hours.
c) The total dose of tacrolimus will continue until day +180 and then begin tapering until day + 365 (+/- two days), per PI discretion as long as the severity of GVHD is less than grade II, and the patient is not requiring systemic steroids.

3.4 PHARMACOKINETIC STUDIES (NOT APPLICABLE)

3.5 PROTOCOL EVALUATION

3.5.1 Recipient pre-transplant evaluation (perform within 48 hours of the initiation of pre-transplant conditioning)

a) CBC with differential
b) PT, and PTT
c) Acute Care Panel
d) Hepatic Panel
e) Mineral Panel
f) Uric Acid, Serum
g) Protein, Total, Serum
h) Lactate Dehydrogenase
i) Creatine Kinase
c) Urine βHCG in women of childbearing potential

3.5.2 Evaluation During Transplantation (see Appendix B)

The following studies will be obtained in the recipient during hospitalization for transplantation:

a) CBC twice daily; differential count daily
b) Acute Care Panel BID
c) Hepatic Panel
d) Mineral Panel
e) Uric Acid, Serum
f) Protein, Total, Serum
g) Creatine Kinase
h) LDH daily
i) Lipid panel weekly
j) Type and screen twice a week
k) Sirolimus levels two times a week
l) Tacrolimus levels will be evaluate twice weekly
m) Weekly CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
n) Donor: Recipient Chimerism Studies: Determination of donor vs. host chimerism will be performed by VNTR-PCR of microsatellites. A CLIA-certified lab will perform these assays. Initial baseline determination on donor and recipient DNA will be done at the time of study entry. Post-transplant chimerism will be determined at the discrete time points (+/- 2 days) starting with day 30 post-transplant to evaluate T-lymphoid, myeloid,

and total blood chimerism. Blood chimerism will also be evaluated at day +100, 6 months, 12 months, and 24 months post-transplant (+/- 3 days), and as clinically indicated (i.e., mixed chimerism). Chimerism may be measured at other time-points if clinically indicated (e.g., to determine effect of manipulating immune suppression in order to increase donor chimerism). Send 5 cc blood in yellow top ACD tube to Jody Keary, Clinical Center Pathology.

o) A bone marrow aspirate will be collected at days 30 and 100 post-transplant (+/- 3 days) to help elucidate the contribution of donor hematopoietic progenitor cells to the graft, and to determine whether there are differences between the levels of donor chimerism in the peripheral blood compared to the bone marrow. DNA chimerism will be performed by CLIA certified laboratories. If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed on days 30, 100, 6 months and 12 months post-transplant. A portion of the sample will be sent to Dr. Fran Hakim, Bldg 10, Room 12C-216.

p) A lymphocyte phenotyping panel (T, B, NK) will be performed on days +30, +60, +100, 6 months, 12 months, and 24 months post-transplant. All times are +/- 3 days. A CBC with absolute monocyte count will be performed at these same time points.

q) In order to determine the rate and contribution of each UCB unit or units to engraftment after UCB transplant, unsorted chimerism studies using quantitative polymerase chain reaction (PCR) for polymorphic variable number tandem repeat (VNTR) or short tandem repeat (STR) microsatellite regions from DNA from peripheral blood will be evaluated. A CLIA-certified lab will perform these assays. Initial baseline determination on donor and recipient DNA will be at time of study entry. Send 5 cc of blood in purple top tube to Dr. Roger Kurlander, Clinical Center pathology.

r) The patient will also be evaluated at least twice weekly during the first 100days after transplantation for the development of acute GVHD. Weekly CMV, Adenovirus, EBV, HHV6 PCR, and Toxoplasmosis will be performed.

3.5.3 Post-Transplant Evaluation (also see Appendix B)

After completion of therapy the patient will be followed for potential complications related to allogeneic SCT. The patient will be seen in follow-up to evaluate disease status and late problems related to allogeneic HSCT at days +30, +60, and +100; and at 6, 12, and 24 months post-transplant. At these times patients will have the following tests performed to determine clinical response (+/- 3days):

a) CBC with differential count
b) Acute Care Panel
c) Hepatic Panel
d) Mineral Panel
e) Uric Acid, Serum
f) Protein, Total, Serum
g) Lactate Dehydrogenase
h) Creatine Kinase
i) Lipid panel (weekly)
j) Type and screen
k) Sirolimus level
l) Tacrolimus level

m) CMV, Adenovirus, EBV, HHV6, and toxoplasmosis weekly
n) Donor: Recipient Chimerism Studies: A CLIA-certified lab will perform these assays. Initial baseline determination on donor and recipient DNA will be done at the time of study entry. Post-transplant chimerism will be determined at the discrete time points (+/- 3 days) starting with day 30 post-transplant to evaluate T-lymphoid, myeloid, and total blood chimerism. Blood chimerism will also be evaluated at day +100, 6 months, 12 months, and 24 months post-transplant, and as clinically indicated (i.e., mixed chimerism). Chimerism may be measured at other time-points if clinically indicated (e.g., to determine effect of manipulating immune suppression in order to increase donor chimerism).
o) In order to determine the rate and contribution of each UCB unit or units to engraftment after UCB transplant, unsorted chimerism studies using quantitative polymerase chain reaction (PCR) for polymorphic variable number tandem repeat (VNTR) or short tandem repeat (STR) microsatellite regions from DNA from peripheral blood will be evaluated on days +30, +100, 6, 12, and 24 months post-transplant. A CLIA-certified lab will perform these assays. Initial baseline determination on donor and recipient DNA will be at time of study entry.
p) A bone marrow aspirate will be collected at day 100 post-transplant (+/- 3 days) to help elucidate the contribution of donor hematopoietic progenitor cells to the graft, and to determine whether there are differences between the levels of donor chimerism in the peripheral blood compared to the bone marrow. DNA chimerism will be performed by CLIA certified laboratories. A sample will also be delivered to Dr. Fran Hakim, Bldg 10, Room 12C-216.
q) If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed on days +30, +100, 6 months, 12 months, and 24 months post-transplant (+/- 2 days). If the patient has normal cytogenetics at day +30 cytogenetic will not be repeated until day 100.
r) A lymphocyte phenotyping panel (T, B, NK) will be performed on days +30, +100, 6 months, 12 months, and 24 months post-transplant. All times are +/- 3 days. A CBC with absolute monocyte count will be performed at these same time points.
s) The patient will also be evaluated at least twice weekly during the first 100 days after transplantation for the development of acute GVHD.

3.6 CONCURRENT THERAPIES

Please see section 4.1 for concurrent therapy with anti-bacterial, anti-fungal, and anti-viral agents during transplant.

3.7 SURGICAL GUIDELINES (NOT APPLICABLE)

3.8 RADIATION GUIDELINES

a) Patients receiving 10/10 matched related and 10/10 matched unrelated donor hematopoietic stem cells, and haploidentical donor bone marrow cells, will receive 200 cGy total body irradiation on day -1. Patients with 9/10 matched unrelated donors will receive 300 cGy TBI on day -1. Following consultation with the Radiation Oncology Branch, patients will be measured to determine the maximal lateral separation at the level of the hip with the patient lying supine. The lateral head and neck separation will also be measured to determine the appropriate thickness of compensators for this treatment. On day -1 patients will receive a total body dose of either 200 or 300 cGy delivered to the lateral midplane at a source distance

of 6 meters to isocenter. The treatment will be delivered with opposed lateral treatment fields with head and neck compensation to minimize dose heterogeneity. Modifications to the radiation treatment palm, with the exception of the total dose, may be made to the treatment technique at the discretion of the treating radiation oncologist based on the patient's thickness or other technical factors. Approximately one hour prior to radiation, patients should be given one 8mg tablet of Zofan (Ondansetron) orally with water. Pediatric patients who require anesthesia will be requested to sign an additional consent for anesthesia.

3.9 OFF TREATMENT CRITERIA- NOT APPLICABLE

3.10 OFF STUDY CRITERIA

The donor or recipient will be removed from protocol for any of the following reasons:

a) Inadequate stem cell collection from the selected donor. If this occurs, another eligible donor may be enrolled and may undergo filgrastim mobilization and PBSC collection as outlined in the donor selection criteria. If no other eligible donor is available, or if there is insufficient time to arrange donor cell collection, then the recipient will be removed from the trial and referred back to their physician.
b) Unacceptable toxicity (> grade 3) from the initiation of G-CSF until cells are harvested, for the donor.
c) The donor or recipient refuses to continue therapy.
d) In addition, the patient may at any time be removed from protocol at the principal investigator's discretion, if the PI deems the patient to be at unacceptable risk to remain on study. Reasons for this action may include (but are not limited to): declining organ function/performance status before transplantation; inadequate family/caregiver support; noncompliance.
e) Lost to follow-up
f) Death
g) Off-Study Procedure: Authorized staff must notify Central Registration Office (CRO) when a patient is taken off-study. An off-study form from the web site (http://intranet.cancer.gov/ccr/welcome.htm) main page must be completed and faxed to 301-480-0757.

*Note: Donors will be considered "Off Study" 1 day post collection of cells or until resolution of a pending adverse event.

3.11 POST STUDY EVALUATION (FOLLOW-UP)

a) Patients will be followed post-therapy at least weekly in the outpatient setting until at least day +100 (post transplant) to evaluate disease status and problems related to allogeneic HSCT
b) Clinic visits will be scheduled at day's +30, +60, and +100; and at 6, 9, 12, 18, and 24 months post-transplant at which time the following tests will be performed to determine clinical response:
    - CBC with differential count
    - Acute Care Panel
    - Hepatic Panel
    - Mineral Panel
    - Uric Acid, Serum
    - Protein, Total, Serum

- Lactate Dehydrogenase
- Creatine Kinase
- CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
- Donor: Recipient Chimerism Studies:
- Lymphocyte phenotype panel (T, B, NK). A CBC with absolute monocyte count will be performed at these same time points.

c) A bone marrow aspirate will be collected at day 100 post-transplant (+/- 3 days) to help elucidate the contribution of donor hematopoietic progenitor cells to the graft, and to determine whether there are differences between the levels of donor chimerism in the peripheral blood compared to the bone marrow.

d) If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed on days +30, +100, and 6, 9, 12, 18, and 24 months post-transplant (+/- 3days). If the patient has normal cytogenetics at day +30 cytogenetics will not be repeated until day 100.

e) The patient will also be evaluated at least twice weekly during the first 100 days after transplantation for the development of acute GVHD.

3.12 IMMUNOLOGIC STUDIES

a) Immune reconstitution of donor leukocyte subsets will be assessed on days +30, +100, 6 months, 12 months, and 24 months post-transplant (+/- 2 days). Peripheral blood (five green (CPT) tubes will be collected) will be sent to the Dr. Fran Hakim, ETIB, Bldg 10, Room 12C-216. After lysis of RBC, flow sorting of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Jodi Keary.

3.13 RESTRICTIONS FOR BLOOD DRAWN FOR RESEARCH SAMPLES:

Research blood sample aliquot size will be minimized for patients < 18 years of age and the total amount restricted. The amount of blood drawn from adult (those 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, in an 8 week period.

The amount of blood drawn for research purposes from pediatric patient subjects (those under 18 years of age) will not exceed 5 mL/kg in a single day, and no more than 9.5 mL/kg over an 8 week period.

In the event that blood draws are limited due to patient size, research studies will be performed in order of priority. Small volume apheresis collections will *not* be included in this calculation since such is performed isovolumetrically with minimal red cell loss.

4 SUPPORTIVE CARE

4.1 INFECTION PROPHYLAXIS

(For a full description of infection prophylaxis please refer to the NIH BMT Consortium Supportive Care Guidelines at http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml). Infection prophylaxis for pediatric patients should follow the NIH BMT Consortium Supportive Care Guidelines found at http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml

4.2 MANAGEMENT OF ENGRAFTMENT SYNDROME

Engraftment syndrome may occur at the time of neutrophil recovery. Its clinical manifestations include noninfectious fever, rash, and vascular leak causing non-cardiogenic pulmonary edema, weight gain, and renal insufficiency (Spitzer 2001). Diagnostic criteria and a treatment schema for engraftment syndrome are included in Appendix E.

4.3 TREATMENT OF GRAFT-VERSUS-HOST DISEASE

a) In patients in whom GVHD is suspected, standard clinical criteria and biopsy findings (when clinically indicated) will be used to establish the diagnosis. Acute GVHD will be assessed according to 1994 Consensus Conference on Acute GVHD Grading criteria (Przepiorka et al. 1995). Chronic GVHD will be assessed according to 2005 Chronic GVHD Consensus Project (Filipovich et al. 2005). See Appendix D for details concerning the assessment of acute and chronic GVHD.
b) Patients with clinical Stage 1 or 2 (Grade I) GVHD of the skin without any other organ involvement will be treated with a topical corticosteroid cream.
c) In general, patients with ≥ Grade II acute GVHD will be treated with high-dose, systemic corticosteroids.
d) Patients who fail to respond to corticosteroids will be considered for second-line immunosuppressive therapy. These agents include Tacrolimus as a continuous intravenous infusion at 0.02 mg/kg/day; Mycophenolic acid 1 Gm po BID, Infliximab (Remicade) 10 mg/kg/dose IV every week for a maximum of 4 doses, Daclizumab 1mg/kg IV on Days 1, 4, 8, 15, and 22, or other experimental acute GVHD protocols, if they are available.

4.4 MENSES SUPPRESSION AND CONTRACEPTION

a) For a full description of menses suppression and contraception please refer to the NIH BMT Consortium Supportive Care Guidelines at:
b) http://intranet.cc.nih.gov/bmt/clinicalcare/guidelines.shtml
c) Pre-menopausal women who have not undergone hysterectomy will be placed on a monophasic oral contraceptive to provide both menses suppression and contraception. This therapy will begin upon enrollment and continue until platelet recovery after transplantation (> 50,000/µl without transfusion). Treatment should be started prior to the onset of thrombocytopenia usually when withdrawal bleeding begins. Once thrombocytopenia occurs therapy is less likely to be effective. As general recommendations: Prescribe Lo-Ovral (300µg norgestrol and 30 µg ethinyl estradiol) 1 tablet daily. Instruct patient not to take placebo tablets. If the patient is on another oral contraceptive, she should be switched to Lo-Ovral. If Lo-Ovral is started when the patient is bleeding and bleeding persists for more than 2-3 days, increase the dose to 1 tablet twice daily. If bleeding persists for more than an additional 2-3 days (total 5 to 6 days after starting hormones), consult the NIH Gynecology Consult Service.

d) Female transplant recipients will be advised to use an effective form of contraception for at least 1 year after transplantation, and to have their male partners use condoms.
e) Male transplant recipients will be advised to use contraception, preferably condoms, for 1 year after transplantation.

4.5 BLOOD PRODUCT SUPPORT

a) Patient's blood counts will be monitored daily during the hospitalization. Patients will receive packed red blood cells and platelets as needed to maintain Hb > 8.0 gm/dl, and platelets > 10,000/mm^3 (or higher, if clinically indicated).
b) All blood products, with the exception of the stem cell product and DLI, will be irradiated.
c) Leukocyte filters will be utilized for all blood and platelet transfusions to decrease sensitization to transfused leukocytes and decrease the risk of CMV infection. The patient, who is seronegative for CMV and whose donor is seronegative, should receive CMV-negative blood products whenever possible.
d) Transfusion of irradiated blood and cellular blood products will begin at a minimum of two weeks prior to hematopoietic cell collection. Transfusion of irradiated blood and cellular blood products will continue to at least one year after transplantation. Patients receiving immunosuppressive medication will continue to have all blood and cellular blood products irradiated until discontinuation of immunosuppressive treatment.
e) Patients sensitized to HLA or platelet specific antigens should receive HLA matched apheresis platelet collections.

4.6 NUTRITIONAL SUPPORT

If mucositis or GVHD prevent adequate PO intake, parenteral hyperalimentation will be instituted and discontinued with input from the Pharmacy and Nutrition Departments. Oral intake will resume when clinically appropriate under the supervision of the dietary service of the Clinical Center.

4.7 ANTI-EMETIC USAGE

Anti-emetic usage will follow recommendations from the Pharmacy.

4.8 INTRAVENOUS IMMUNE GLOBULIN (IVIG)

a) Will follow Guidelines for Infection Management in Allogeneic HSCT Recipients by the NIH Blood and Marrow Consortium standard practices.
b) IgG level will be measured only in patients who have had ≥ 2 respiratory infections that required antibiotic therapy in the six months prior to transplant.
c) IgG values less than 400 mg/dl, 500 mg/kg IVIG will be administered intravenously.

4.9 HEPATIC FUNCTION SUPPORT

All patients will receive ursodeoxycholic acid (Ursodiol) for the prevention of hepatic complications after allogeneic stem cell transplantation. Ursodiol will start day -4 prior to the transplant and will continue until day +100 post-transplant. Patients weighing less than 90 kg will receive 300mg orally twice daily (600mg total dose each day). Those patients weighing more than 90 kg will receive 300 mg orally each morning and 600 mg orally each evening (900 mg total dose each day).

4.10 CENTRAL NERVOUS SYSTEM PROPHYLAXIS

Pediatric patients < 18 years of age may receive standard intrathecal (IT) chemotherapy on a monthly basis on a case by case basis after conferring with the PI and the treating physician, to prevent meningeal dissemination in patients with select hematologic malignancies.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

a) Data will be prospectively collected and entered in real time into the Cancer Center Clinical Data System database (NCI C3D database) (information at http://ccrtrials.nci.nih.gov). It is expected that clinical data be entered into C3D no later than after 10 business days of the occurrence. The NCI PI and research nurse will have access to these data via web access.

5.1.1 Adverse Events during the Conditioning Therapy phase:

5.1.1.1 Grade 1 and 2 adverse events will not be recorded, with the exception of All Grade 2 events that are not in the consent form, but are possibly, probably or definitely related to the research;

5.1.1.2 All expected and unexpected Grade 3 and 4 adverse events possibly, probably or definitely related to the research will be recorded with the exception of:

a) Blood/Marrow abnormalities (will include grade 3&4 hemolysis)
b) Coagulation abnormalities
c) Metabolic abnormalities (will include grade 3&4 LFTs and creatinine)

5.1.1.3 All grade 5 adverse events will be recorded regardless of attribution.

5.1.2 Adverse Events during the Transplant phase (Day 0 to Day 100):

5.1.2.1 Grade 1 adverse events will not be recorded.

5.1.2.2 Grade 2 adverse events that will be recorded:

a) those potentially, probably and definitely related to GVHD.
b) infection including fever in the absence of neutropenia.
c) Those unexpected potentially, probably, and definitely related to the research.
d) Any that are deemed clinically significant by the PI

5.1.2.3 All expected and unexpected Grade 3 and 4 adverse events possibly, probably or definitely related to the research will be recorded with the exception of:

a) Blood/Marrow abnormalities (will include grade 3 &4 hemolysis)
b) Coagulation abnormalities with the exception of TTP/HUS which will be recorded
c) Metabolic abnormalities (will include grade 3 &4 LFTs and creatinine)

5.1.2.4 All grade 5 adverse events will be recorded regardless of attribution.

5.1.3 Adverse Events during the Follow-up phase (beyond Day 100):

5.1.3.1 Grade 1 adverse events will not be recorded.

5.1.3.2 Grade 2 adverse events that will be recorded:

a) those potentially, probably and definitely related to GVHD.
b) infection including fever in the absence of neutropenia.

5.1.3.3 All expected and unexpected Grade 3 and 4 adverse events possibly, probably or definitely related to the research will be recorded with the exception of:

a) Electrolyte abnormalities (will include grade 3&4 LFTs and creatinine)

5.1.3.4 All grade 5 adverse events will be recorded regardless of attribution.

a) After obtaining Informed Consent, a file will be created in the database with standardized forms. The NCI C3D will maintain data for described endpoints in the protocol. The medical record will maintain complete records on each patient including any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records. The NCI C3D will serve as the primary source from which all research analyses will be performed.

b) Data collection will include the eligibility criteria checklist, patient history, , specialty forms for pathology, radiology, weekly toxicity monitoring, monitoring of GVHD, engraftment data, chimerism data, immune reconstitution and relapse data and an off-study summary sheet, including a final assessment by the treating physician. After patients are seen in clinic on day +30, +100 and 6, 9, 12, 18, and 24 months post-transplant, the NCI C3D will be updated in real-time. Additional data that is obtained during the transplant process may be utilized in the analysis of this protocol (e.g. chimerism testing performed as clinically indicated on dates other than those specifically required under the guidelines of the protocol). Please see appendix H for detailed data entry requirements for C3D.
c) Data will also be sent to the International Bone Marrow Transplant Registry (IBMTR).
d) Unrelated donor recipients will be offered to participate in a cross-institute data and repository sample collection protocol though NIAID which fulfills the NMDP requirements for data reporting as described in the Clinical Center's Transplant Center Agreement with NMDP.
e) Protocol Deviations: Any protocol deviations should be directly reported to the PI or LAI. These deviations will be reported to the NCI IRB.

5.2 RESPONSE CRITERIA

a) Patients will also be assessed for complete clinical and hematological response at day +30, +60, and +100, and 6, 12, and 24 months post-transplant.

   In the unlikely events that bone marrow is used as the source of stem cells for HSCT, the data from these individuals will be analyzed separately for the primary and secondary end-points, however this data will be included in the stopping point criteria.

   - Parameters to be monitored:
     - Total nucleated cell dose, CD34+ cell dose, CD3+ cell dose
     - Degrees of reconstitution of monocytes, B-lymphocytes, NK cells, T-lymphocytes, by lymphocyte phenotype on peripheral blood leukocytes for CD3+, CD20+, CD56+, and CD14+ cells
     - Absolute neutrophil and absolute monocyte count
     - Neutrophil recovery (days to neutrophil count of 5×10^9/l
     - Platelet recovery (days to platelet count of 50×10^9/l, days to transfusion independence.
     - Red cell recovery (days to RBC transfusion independence)
     - Incidence and severity of acute GVHD
     - Incidence and severity of chronic GVHD
     - Incidence of graft rejection
     - Non-hematological effects attributable to the preparative regimen
     - Transplant related mortality
     - Incidence and type of infectious episodes
     - Disease-free and overall survival

b) Complete hematological response (CR) for GATA2 Mutation Syndrome consists of normal levels of T-lymphocytes, B-lymphocytes, NK cells, monocytes, and neutrophils at 100 days post-transplant. CRIS order to be entered for TBNK cells by flow cytometry.

c) A partial hematological response (PR) for GATA2 mutation syndrome consists of normalization at 100 days post-transplant of one or more of the leukocyte subsets which were severely decreased prior to transplant, i.e., B-lymphocytes, NK cells, or monocytes.
d) No response for GATA2 mutation syndrome consists of lack of normalization any of the 3 leukocyte subsets that were severely decreased prior to transplant
e) Complete response (CR) for MDS consists of normalization of the peripheral blood counts (ANC > 1,500/µl and platelets > 100,000/µl) without circulating blasts, bone marrow cellularity > 20% with normal maturation and fewer than 5% blasts in bone marrow 100 days after transplant (Schetelig et al. 2003). The absence of specific molecular or cytogenetic markers of disease that were previously present further defines molecular or cytogenetic remission, respectively.
f) Partial response (PR) for MDS is defined as improvement in markers of leukemia or by having all criteria for complete remission satisfied, except that the bone marrow may contain > 5% but < 25% blasts, or < 5% blasts are present.
g) No response for MDS is defined as bone marrow and peripheral blood morphological features consistent with relapse or progression, including rising blast count and re-emergence of specific molecular or cytogenetic markers.
h) Samples will be collected and analyzed at day's +30, +60, and +100, and at 6 and 12 months post-transplant for clinical and hematological response.

5.3 TOXICITY CRITERIA:

Adverse Events on this study will be reported using the NCI Common Terminology Criteria for Adverse Events version 3.0 (CTCAE v3.0). A copy of the CTCAE v3.0 can be downloaded http://ctep.info.nih.gov/CTC3/default.html.

All appropriate treatment areas should have access to a copy of the CTCAE version 3.0.

5.4 STATISTICAL CONSIDERATIONS

a) The primary objectives of this feasibility study are:
   1) In a pilot fashion, to determine whether reduced-intensity allogeneic HSCT results in engraftment and restores normal hematopoiesis by day +100 in patients with GATA2 mutation syndrome
   2) To determine the safety of this HSCT regimen in patients with GATA2 mutation syndrome, including transplant related toxicity, the incidence of acute and chronic graft-versus-host disease, immune reconstitution, overall survival, and disease-free survival
b) Up to 15 patients in total will be prospectively recruited over a 2-year period for this HSCT protocol. We currently have six patients with mutations in GATA2 who are candidates for transplant. This study utilizes a matched related donor if one is present, however if no matched related donor is identified, but a 10/10 matched unrelated donor is available, then that donor will be utilized. If neither a 10/10 matched related donor nor a 10/10 matched unrelated donor is available, then either a 9/10 matched unrelated donor, a haploidentical donor or UCB units will be used if they are available and meet the dose and matching requirements. The choice between a 9/10 matched unrelated donor, an UCB donor, or a haploidentical donor will depend upon the nature of the HLA mismatch, the clinical condition of the patient, and the urgency for transplant among other factors. This decision whether to use a 9/10 matched unrelated donor, an UCB donor, or a haploidentical donor will be made by the PI or LAI.

c) It is estimated that approximately one-quarter of the patients with GATA2 deficiency will have a 10/10 matched related donor, and that approximately one-quarter of the patients will have a 10/10 matched unrelated donor. It is anticipated that the remaining patients will have 9/10 matched unrelated donors, umbilical cord blood donors, or haploidentical donors. Thus, it is anticipated that no more than 5 patients from each donor category will be enrolled. To date we have enrolled 3 matched related donor recipients, three matched unrelated donor recipients, and 4 umbilical cord blood recipients

d) Our goal is to learn if the approach is likely to be of beneficial and have an acceptable level of toxicity. The definition of benefit is whether the patient engrafts the donor HSC and normal hematopoiesis is reconstituted. For purposes of sample size calculations, we will aim for 50% with benefit and consider this procedure to be of less benefit if fewer than half (50%) may truly benefit. As an illustration, we will aim for 50% with benefit and consider the results to be less promising if consistent with only 20% having benefit. If there are 15 evaluable patients, considered without regard to type of donor, the probability of having 6 or more with a successful outcome is 6.1% if the true probability of benefit were 20% and the probability is 84.9% if the true probability of benefit were 50%. Thus, observation of benefit in 6 or more of 15 patients provides evidence that this method is likely to be useful for these patients. At the conclusion of the trial, 90% and 95% confidence intervals will be formed around the observed benefit results. Given the rarity of this condition, this is intended to be a guideline, but findings of lesser benefit may be considered useful for further consideration if the toxicity profile is not excessively negative.

e) Stopping Rules: Although the patients with this disease are debilitated from the immunodeficiency disease and the frequency of infections, we anticipate that most will survive the transplant itself. The following stopping rules will be implemented in order to provide patient safety:
   1) Separately within each donor category, if two patients fail to engraft, or fail to survive the first 100 days following HSCT, then we will stop enrollment, reevaluate the protocol, and present this data to the IRB.
   2) If while accrual is proceeding, if 1/3 or greater of the patients have a grade 3 or higher GVHD, this will also pause accrual to the trial, including IRB review, prior to re-starting accrual or amending the trial appropriately.

5.5 DATA AND SAFETY MONITORING PLAN

In accordance with NCI IRB policy, this pilot study will require independent monitors or review by a Safety Monitoring Committee (SMC).

The PI, LAI, and Scientific Chairperson will provide continuous, close monitoring, with prompt reporting of serious adverse events to the IRB. On a weekly basis, the PI, LAI, Protocol Research Nurse, and the NCI Transplant Coordinator will review clinical and laboratory data from patient. A complete summary of patient outcomes will be provided every six months to the IRB in the form of a continuing review application. The research nurse will verify enrollment data entered into the C3D for each subject within 4 weeks of signing consent. Ongoing data entered into C3D will be verified by the research nurse monthly, and at the time of continuing review. Documentation of data verification will be tracked in C3D.

6 HUMAN SUBJECTS PROTECTIONS

6.1 RATIONALE FOR SUBJECT SELECTION

Subjects for this pilot and feasibility study will be males and females of all races and ethnic groups with mutations in GATA2 and a life-expectancy of greater than 3 months but less than 24 months. The GATA2 mutation syndrome is characterized by: 1) the onset of disease beyond infancy with least two episodes of a life-threatening opportunistic infection including MAC, 2) a unique T+, B-, NK-, monocyte- phenotype on flow cytometry, 3) propensity to progress to MDS/AML, and 4) mutations in the GATA2 gene. This patient group was chosen because allogeneic hematopoietic stem cell transplant represents a potentially effective treatment for both GATA2 mutations and MDS. There are no treatment options for patients with this disease that provide any significant survival advantage. The approximate survival of these patients with conventional treatment is approximately five years. Because myeloablative preparative regimens are associated with a high degree of morbidity and mortality in patients with co-morbidities, such individuals are most likely to benefit from the reduced-intensity transplant approach used in this study. Individuals with HIV disease will not be candidates for this protocol due to the high rate of post-transplant complications in this group. Individuals who are pregnant or lactating will not be candidates for this protocol due to the risk to the fetus or newborn.

Information about this protocol will be posted on www.clinicaltrials.gov, NIH Clinical Center studies, and the NCI Patient recruitment websites. The protocol will also be listed in the physician's data query (PDQ).

6.1.1 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

6.1.2 Participation of Children

Children below the age of 12 will not be included on this protocol. Donors less than 15 years of age will not be included on this protocol since the protocol involves greater than minimal risk without prospect of direct benefit to individual patients for this population.
Physicians, nurses, and multidisciplinary support teams of the POB, NCI, and CC will provide patient care. The staff of the POB has expertise in the management of children with complex oncologic disorders and complications of therapy. Full pediatric support and subspecialty services are available at the NIH CC.

6.2 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS- PATIENTS

6.2.1 Related to the Transplant in General

The patients may obtain direct benefit from the transplant. However, the radiation and immunosuppression utilized in this study carry the risk of direct toxicity. In addition, immune suppression is associated with an increased risk of opportunistic infections. However, it is anticipated that these risks will be lower than the risks associated with traditional allogeneic transplantation utilizing total body irradiation in which the mortality may be as high as 40%. Although our data as well as that of others suggest a significant reduction in transplant related mortality with reduced-intensity conditioning, the procedure nevertheless carries significant risk. It is therefore only appropriate to carry out this experimental procedure in the context of debilitating or life-threatening conditions and with full informed consent from the patient. The use of 200 cGy low-dose TBI may increase both the degree of myelosuppression and immunosuppression without significantly altering the side effect profile. The specific hazards of

this study using a reduced-intensity conditioning regimen are graft rejection, graft-versus-host disease, and disease relapse. Of these complications, GVHD remains the major cause of morbidity and mortality. Therefore, the patient will receive sirolimus (Rapamycin) and tacrolimus for GVHD prophylaxis. It is not known whether study recipients will be protected against GVHD by this regimen. The major discomforts of sirolimus and/or tacrolimus are: nausea, anorexia, diarrhea, fever and malaise, and intolerance of the period of confinement (see section 8.2 and 8.3 for full toxicity profile). Side effects of those drugs novel to reduced-intensity transplantation are described in detail in Section 8.

6.2.2 Risks related to matched related donor transplant

Engraftment is more predictable using a 10/10 matched donor transplant than with a 9/10-matched unrelated donor transplant. Treatment with both total body irradiation and fludarabine is designed to enhance donor engraftment to greater than 90%. Graft-versus-host disease remains a formidable risk with approximately 50% of patients developing GVHD, and 25% of patients having GVHD requiring additional treatment. The risk of GVHD is least with a matched related donor compared to the other two donor sources.

6.2.3 Risks related to matched unrelated donor transplant

Engraftment is also predictable using a 10/10 matched unrelated donor transplant with approximately 90% or more of patients achieving engraftment. Treatment with both total body irradiation and fludarabine, and the increase to 300 cGy TBI for 9/10 matched unrelated donors, is designed to enhance donor engraftment. Graft-versus-host disease remains a formidable risk with approximately 60% of patients developing some GVHD, and 30% of patients having GVHD requiring additional treatment. The risk of GVHD is greater with a matched unrelated donor than with a matched related donor.

6.2.4 Risks Related to Haploidentical Transplant

Haploidentical donor transplant with this conditioning regimen results in 90% or more of patients achieving engraftment. The risk of GVHD using a haploidentical donor and this conditioning regimen is approximately 50%, however only 10% have grade 3 to 4 GVHD.

6.2.5 Risks related to UCB transplant

Engraftment is lowest in this group compared to the other two groups. This results in an increased risk of infection and bleeding. However, the risk of GVHD is lower in this group compared to matched unrelated donor transplant.

6.2.6 Related to Total Body Irradiation

The side effects of radiation have been well described. The most common include nausea and mucositis. There also exists a risk of hypothyroidism, cataracts, interstitial pneumonitis, nephropathy, and an unspecified long term risk of developing secondary malignancies (Buchali et al. 2000) (Deeg et al. 1983). Importantly, the majority of the non-neoplastic effects were subclinical and/or reversible (Thomas et al. 2001). Studies attempting to evaluate the risk induced by radiation alone suggest that there is a higher rate of solid tumors after radiation-based regimens. Curtis et al. reported on 19,229 patients and found a cumulative incidence rate of 2.2% at 10 years, and 6.7% at 15 years, with higher doses of TBI associated with a higher risk of solid cancers (Curtis et al. 1997). In a study in 1999, no relationship could be found to radiation but the highest risk factor was felt to be the presence of chronic graft versus host disease, and long-term treatment with cyclosporine (Kolb et al. 1999). Pediatric patients who require sedation

in order to undergo TBI will be asked to sign a separate consent outlining the risk of the selected anesthesia.

6.2.7 Related to Cyclophosphamide (Cytoxan)
Commercially available. See package insert for further information.

Toxicity:

	Common	Occasional	Rare
Immediate: Within 1-2 days of receiving drug	Anorexia (L), nausea (L), vomiting(L)	Metallic taste (L), Inappropriate ADH[1]	Transient blurred vision[1] cardiac toxicity with arrhythmias[1] myocardial necrosis [2](L)
Prompt: Within 2-3 weeks, prior to the next course	Myelosuppression (L), alopecia (L)	Hemorrhagic cystitis (L)	
Delayed: Any time later during therapy, excluding the above conditions	Immunosuppression, gonadal dysfunction /sterility (L)		Pulmonary fibrosis[3](L)
Late: Any time after completion of treatment			Secondary malignancy, bladder fibrosis
Unknown Frequency and Timing: **Fetal and teratogenic toxicities and toxicities in breast-fed children			

[1] Less common with lower doses.
[2] Only with very high doses.
[3] Risk increased with chest radiation.
(L) Toxicity may also occur later.

**Fetal toxicities and teratogenic effects of cyclophosphamide (alone or in combination with other antineoplastic agents) have been noted in humans. Toxicities include: chromosome abnormalities, multiple anomalies, pancytopenia, and low birth weight.
**Cyclophosphamide is excreted into breast milk. Neutropenia has been reported in breast-fed infants. Cyclophosphamide is considered to be contraindicated during breast feeding because of the reported cases of neutropenia and because of the potential adverse effects relating to immune suppression, growth, and carcinogenesis.

6.3 RISKS IN RELATION TO BENEFIT

6.3.1 Recipients:
Patients on this study may be directly benefited by this treatment protocol. Considerable clinical data has demonstrated long-term disease-free survival for patients with immunodeficiency diseases as well as high-risk hematological malignancies following allogeneic HSCT. Thus, the patient on this study may be directly benefited by this treatment. In particular, reduced-intensity

allogeneic stem cell transplantation may offer the patient a curative therapy for his disease while reducing the risks of a myeloablative transplant. This carefully defined patient cohort is a reasonable one in which to explore strategies to improve the results of allogeneic HSCT from unrelated donors, which historically has achieved prolonged remission and survival for some patients, but has been associated with significant morbidity and high transplant-related mortality. It is hypothesized that the transplant approach used in this study will result in the successful transfer of allogeneic stem cells with reduced morbidity and mortality from the transplant preparative regimen, and with reduced graft-versus-host disease. Clinically the approach is ethically acceptable because we are treating patients with at least one, and in some cases two, lethal hematological diseases that are both incurable with conventional treatments. The protocol aims to decrease the risk of transplant related toxicity, thus making more patients candidates for this potentially curative therapy. Therefore the research involves more than a minor increase over minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102). The potential risks to the transplant recipient have been carefully considered and are felt to be significantly less than would otherwise occur with myeloablative allogeneic HSCT. The risks graft rejection, acute and chronic GVHD, infection, and relapse will be present, as discussed previously.

6.3.2 Donors:

Healthy 10/10 matched related donors, 10/10 matched unrelated donors,9/10 matched unrelated donors, and haploidentical donors will be co-enrolled onto this study. The collection aspect of this protocol is not investigational. There is potential benefit for donors, as they may derive psychological benefit from participating in a clinical trial designed to improve the health of the recipient of the prior transplant donation. Other potential benefits include the diagnosis of previously unknown illnesses (such as viral hepatitis) at the time of donor screening.

6.4 CONSENT AND ASSENT PROCESSES AND DOCUMENTS

a) Informed consent will be obtained from all patients, and/or the patient's parents or legal guardian (if he/she is < 18 years of age), and matched related donors entered on this trial. The procedures and treatments involved in this protocol, with their attendant risks and discomforts, potential benefits, and potential alternative therapies will be carefully explained to the recipient and/or parent/guardian. If applicable, the parent or guardian will sign the designated line on the informed consent attesting to the fact that the child has given assent.
b) The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. Because many patients must travel to the NIH from long distances at substantial expense, requiring both parents to be present for the consent process could be a financial hardship for many families. When necessary, the protocol will be reviewed by telephone with the other parent and their approval will be verified. When guardianship status of the child is uncertain, a social worker will be asked to investigate and, if necessary, seek documentation of custody status. The PI or an associate investigator on the trial will obtain consent. Where deemed appropriate by the clinician and the child's parents or guardian, the child will also be included in all discussions about the trial and verbal assent will be obtained. The parent or guardian will sign the designated line on the informed consent attesting to the fact that the child has given assent.
c) Similarly, the procedures and treatments involved in this protocol, with their attendant risks and discomforts, will be carefully explained to the matched unrelated donors at the respective donor center as required by NMDP. The original signed informed consent goes to Medical Records; a copy will be kept for the research record. The Central Registration Office (CRO),

Data Management Section will also retain a copy of the informed consent document. A copy of the signed informed consent document will also be given to the recipient (and matched related donor, when applicable). b) The Central Registration Office (CRO) will ascertain the date of IRB approval before registering the first patient.

d) The Principal Investigator and/or an authorized designee will consent the patient and matched related donor (when applicable) and will be available to answer all patient questions.
e) If any new information becomes available relating to risks, adverse events, or toxicities, while patients are participating in this protocol, this information will be provided orally and/or in writing to all enrolled and prospective patient participants. Documentation will be provided to the IRB and if necessary the informed consent amended to reflect relevant information.

6.4.1 Telephone re-consent procedure

Reconsent on this study may be obtained via telephone according to the following procedure: the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent. The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

7 DATA REPORTING

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted above in Section **5.1**.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per section **7.2**.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study

- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH definition)

Any change, divergence, or departure from the IRB approved research protocol..

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subject.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and the characteristics of the subject population being studied; **AND**
- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB REPORTING

7.2.1 NCI-IRB Expedited Reporting of Unanticipated Problems and Deaths

The protocol PI will report to the NCI-IRB:

- All deaths, expect deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:

    - All Grade 2 unexpected events that are possibly, probably or definitely related to the research;
    - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
    - All Grade 5 events regardless of attribution;
    - All Serious Events regardless of attribution.

    NOTE: Grade 1 events are not required to be reported.

All Grade 4 and 5 unexpected serious adverse events that are related to commercially available agents will be reported directly to the FDA via Medwatch (http://www.fda.gov/medwatch).

8 PHARMACEUTICAL INFORMATION

8.1 FLUDARABINE (FLUDARA, BERLEX LABORATORIES)

a) Supply - Fludarabine monophosphate will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH. Fludara is stored at room temperature.
b) Preparation - FLUDARA IV should be prepared for parenteral use by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7–8.5. Fludarabine will be diluted in 100 to 125ml of either 5% dextrose in water or 0.9% sodium chloride, and infused IV over 30 minutes.
c) Storage and Stability - Reconstituted FLUDARA IV should be stored in the refrigerator between 36 and 46 degrees F. Because reconstituted FLUDARA IV contains no antimicrobial preservative, care must be taken to assure the sterility of the prepared solution; for this reason, reconstituted FLUDARA IV should be used or discarded within 8 hours.
d) Administration - Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.
e) Toxicities – Fludarabine toxicities include myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, auto-immune hemolytic anemia, auto-immune thrombocytopenia, paresthesias, peripheral neuropathy, renal, and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only rarely been demonstrated at the 25-30 mg/m^2/day dosage of fludarabine. Very rarely described complications include transfusion-associated graft-versus-host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome following fludarabine administration has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed post-fludarabine, especially in heavily pre-treated individuals, and in individuals receiving fludarabine combined with other agent.
f) Dose reduction for renal impairment- For Creatinine Clearance of 30-70 mL/min/1.73m2 there will be a 20% dose reduction. If the Creatinine Clearance is less than 30mL/min/1.73m2, Fludarabine will not be administered.

8.2 SIROLIMUS (RAPAMUNE®)

a) General: Sirolimus is an immunosuppressant that is FDA approved for use in combination with cyclosporine and corticosteroids for prophylaxis of organ rejection after kidney transplant. Sirolimus is administered SIROLIMUS (Rapamycin) (Rapamune®, Wyeth-Ayerst Laboratories)
b) Supply - For patient administration, oral tablets will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.
c) Storage and Stability - Oral tablets should be stored 20-25 C° (68-77 F°). Oral solution should be refrigerated (2-8 C° or 36-46 F°).
d) Administration: Because fatty food can increase the absorption of sirolimus, the tablets

should be administered in the setting of a steady diet that is relatively constant with respect to fat content.

e) Toxicities: 1) Sirolimus induces immune suppression, which has been associated with opportunistic infection and an increased rate of malignancy, particularly skin cancer. 2) Some individuals may develop hypersensitivity to sirolimus. 3) May cause an increase in cholesterol and triglycerides, which may be associated with pancreatitis. 4) With long-term administration, may result in impaired renal function. 4) Co-administration of voriconazole is strongly discouraged on this protocol, as the combination of voriconazole and sirolimus results in an approximate 10-fold increase in sirolimus blood levels.

f) Drug Interactions: May increase the in vivo drug levels of simvastatin.

8.3 TACROLIMUS (FK506, PROGRAF)

a) Supply: Tacrolimus will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (0.5 mg, 1mg, and 5mg), and as a parenteral concentrate for injection (5 mg/ml, 1 ml ampules).

b) Preparation: For parenteral doses, tacrolimus injection concentrate (5 mg/ml) should be diluted to a final concentration of 0.004 to 0.02 mg/ml in dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of tacrolimus will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing.

c) Storage and Stability: Capsules and ampules of parenteral concentrate bear expiration dates and are stored at room temperature. Tacrolimus concentrate for injection that has been diluted to a final concentration of 0.004 to 0.02 mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for sorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.

d) Administration: Tacrolimus may be given intravenously over 24 hours or orally.

e) Toxicities: Acute tacrolimus nephrotoxicity is usually manifested by a moderate decline in renal excretory function, which is readily reversible by a decrease in drug dosage. Although some degree of transient renal dysfunction may occur in patients with therapeutic levels of tacrolimus, significant renal toxicity is associated with elevated trough or steady state levels. In addition to an increase in BUN and creatinine, hyperkalemic hyperchloremic acidosis, low fractional excretion of sodium and the onset of hypertension with hypomagnesemia are seen with tacrolimus nephrotoxicity. Insulin dependent post transplant diabetes mellitus was reported in 20% kidney transplant patients, which was reversible in 50% of the patients at 2 years post transplant. Hypertension occurs in up to 60% of patients. Hypomagnesaemia can be associated with neurologic symptoms, including seizures, cerebellar ataxia and depression. Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has been reported. The drug carries a warning for increased susceptibility to infection and the possible development of lymphoma may result from resulting immunosuppression.

8.4 FILGRASTIM (G-CSF; NEUPOGEN ®)

(Amgen), Filgrastim Classification: glycoprotein

a) Supply – Commercially available as filgrastim injection in a concentration of 300μg/ml in 1ml (300μg) and 1.6ml (480μg) vials from the Clinical Center Pharmacy Department.

b) Preparation – For subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water to a concentration greater than 5µg/ml just prior to administration; storage of the diluted filgrastim is not recommended. Dilution of filgrastim to a final concentration of less than 5µg/ml is not recommended at any time. Do not dilute with saline at any time; product may precipitate. Filgrastim diluted to concentrations between 5 and 15µg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes. The dose may be "rounded down" to within 10% of patient's calculated dose to use the drug cost-effectively.
c) Storage and Stability – Filgrastim for injection should be stored in the refrigerator at 2^{o} to 8^{o}C (36^{o} to 46^{o}F). Avoid shaking.
d) Administration – Subcutaneous injection is preferred. If clinically indicated, filgrastim may be administered as an intravenous infusion over 4 or 24 hours.
e) Toxicities – Medullary bone or skeletal pain is the most commonly reported toxicity. In addition, reversible elevations in uric acid, lactate dehydrogenase, and alkaline phosphatase are common laboratory abnormalities. Four cases of splenic rupture have been reported in healthy donors when given filgrastim or other myeloid growth factors for peripheral blood stem cell mobilization; 1 of these cases resulted in fatality. Five additional cases of splenic rupture have been reported in cancer patients undergoing chemotherapy or peripheral blood stem cell mobilization; splenic rupture may have contributed to deaths in 2 of these cases. One additional death due to splenic rupture after filgrastim therapy was reported to the manufacturer without additional information. According to the manufacturer, the reporting rate for splenic rupture with filgrastim is less than 1 in 486,000.

8.5 DIPHENHYDRAMINE

a) Supply – Commercially available. Diphenhydramine HCl injection is available in an injectable solution at a 50mg/ml concentration in single dose ampules, syringes and vials as well as multi-dose vials from multiple manufacturers.
b) Preparation – Diphenhydramine HCl may be given by direct intravenous injection without additional dilution. Alternatively the prescribed dose may be diluted in a small volume (e.g. 25-50ml) of 5% dextrose in water (D5W) or 0.9% sodium chloride (NS) and infused over 10-15 minutes.
c) Storage and Stability – Store commercially available injectable product at controlled room temperature.
d) Administration – Diphenhydramine HCl injection may be administered by direct IV injection (IV push) at a rate generally not exceeding 25mg/min. Alternatively, diphenhydramine HCl injection may be diluted and given over 10-15 minutes (see Preparation).
e) Toxicities – Sedation, sleepiness, dizziness, disturbed coordination, epigastric distress, and thickening of bronchial secretions. Diphenhydramine can provide additive effects with alcohol or other CNS depressants. Diphenhydramine can cause anticholinergic side effects (e.g. dry mouth, fixed or dilated pupils, flushing, urinary retention). Diphenhydramine

should be used with caution in patients with a history of bronchial asthma, increased intraocular pressure, hyperthyroidism, cardiovascular disease or hypertension.

8.6 ACETAMINOPHEN

a) Supply: Commercially available as 325 mg or 500 mg tablets for oral administration from multiple manufacturers.
b) Storage: Store at controlled room temperature.
c) Administration: Oral. For analgesia and antipyretic, the usual dose is 650 to 1000 milligrams every 4 to 6 hours, to a maximum of 4 grams/day.
d) Toxicities: No toxicities are anticipated to result from single doses of acetaminophen administered as premedication for rituximab infusions.

8.7 ACYCLOVIR

a) Supply: Commercially available.
b) Product description: Acyclovir is available in 200mg capsules, 400mg tablets, and 800mg tablets. It is also available in an oral suspension at a concentration of 200mg/5ml. Parenteral acyclovir sodium is available in vials containing 500mg or 1gm powder for injection of acyclovir as the sodium salt. Acyclovir sodium for injection is also available as a 50mg/ml solution for injection in 1gm/20ml vials.
c) Preparation: For parenteral administration, acyclovir sodium powder for injection should be reconstituted with sterile water for injection to a concentration of 50mg/ml (10ml of diluent for 500mg vial and 20ml of diluent for 1gm vial). The calculated dose should be further diluted in 0.9% sodium chloride or 5% dextrose in water to a concentration of < 7mg/ml prior to administration.
d) Storage and Stability: Oral capsules, tablets and oral solution should be stored at 15^{o} to 25^{o}C (59^{o} to 77^{o}F). Capsules and tablets should be protected from moisture. Acyclovir powder for injection and solution for injection should be stored at 15^{o} to 25^{o}C (59^{o} to 77^{o}F). The powder for injection once reconstituted should be used within 12 hours. After final dilution for administration, the dose may be stored at room temperature; it should be used within 24 hours.
e) Route of administration: Oral and parenteral. Parenteral doses should be administered by slow intravenous infusion over one hour at concentrations of 7mg/ml or less.
f) Toxicities: Oral administration: nausea and/or vomiting, diarrhea, headache, malaise, skin rash, asthenia; Parenteral administration: inflammation or phlebitis at the injection site, transient elevations of serum creatinine or BUN, nausea and/or vomiting, itching, rash or hives, elevation of transaminases, and encephalopathic changes. Renal toxicity can occur and is secondary to precipitation of acyclovir crystals in the renal tubules. The risk of toxicity can be minimized by avoiding the administration of high concentrations and rapid infusions.

8.8 TRIMETHOPRIM/SULFAMETHOXAZOLE (TMP/SMX, COTRIMOXAZOLE, BACTRIM, SEPTRA)

a) Supply: Commercially available as a single strength tablet containing trimethoprim 80mg and sulfamethoxazole 400mg and a double strength (DS) tablet containing trimethoprim 160mg and sulfamethoxaole 800mg. It is also available in an oral suspension at a concentration of 40mg of trimethoprim and 200mg sulfamethoxazole per 5ml. Parenteral

TMP/SMX is available in a solution for injection at a concentration of 80mg of trimethoprim and 400mg of sulfamethoxazole per 5ml.

b) Preparation: For parenteral administration, the commercial solution for injection must be diluted prior to administration. It is recommended that each 5ml of the solution for injection be diluted with 100-125 ml or, if fluid restriction is required, in 75ml of dextrose 5% in water. 0.9% NaCl may be substituted as a diluent but the resulting solutions have reduced stability. Consult with pharmacy for questions regarding diluent, volume, and expiration.
c) Storage and Stability: Oral tablets and oral suspension should be stored at 15^{o} to 30^{o}C (59^{o} to 86^{o}F) in a dry place and protected from light. TMP/SMX for injection should be stored at room temperature between 15^{o} to 30^{o}C (59^{o} to 86^{o}F) and should not be refrigerated. Stability of intravenous doses after final dilution is dependent on concentration and diluent. Consult with pharmacy for questions regarding stability and expiration dating.
d) Administration – Oral and parenteral. Parenteral doses should be administered by an intravenous infusion over 60 to 90 minutes.
e) Toxicities: 1) the most common adverse effects from TMP/SMX are gastrointestinal disturbances (nausea, vomiting, anorexia) and allergic skin reactions (such as rash and urticaria). 2) Fatalities associated with the administration of sulfonamides, although rare, have occurred due to severe reactions, including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia and other blood dyscrasias. 3) For TMP/SMX injection, local reaction, pain and slight irritation upon IV administration are infrequent. Thrombophlebitis has rarely been observed.

8.9 URSODEOXYCHOLIC ACID (URSODIOL, ACTIGALL®)

a) Supply – Commercially available as 300 mg capsules.
b) Pharmacology - About 90% of a therapeutic dose of Ursodiol is absorbed in the small bowel after oral administration. After absorption, Ursodiol enters the portal vein and undergoes efficient extraction from portal blood by the liver where it is conjugated with either glycine or taurine and is then secreted into the hepatic bile ducts. Only small quantities of Ursodiol appear in the systemic circulation and very small amounts are excreted into urine. The sites of the drug's therapeutic actions are in the liver, bile, and gut lumen.
c) Storage and Stability – Oral capsules should be stored at 15°C-30°C (59°F-86°F).
d) Administration – Oral.
e) Toxicities – Nausea, vomiting, dyspepsia, metallic taste, abdominal pain, biliary pain, cholecystitis, constipation, stomatitis, flatulence, diarrhea, pruritus, rash, dry skin, urticaria, headache, fatigue, anxiety, depression, sleep disorders. Less common side effects include sweating, thinning of hair, back pain, arthralgia, myalgia, rhinitis, cough.
f) Drug Interactions - Bile acid sequestering agents such as cholestyramine and colestipol may interfere with the action of Ursodiol by reducing its absorption. Aluminum-based antacids have been shown to adsorb bile acids in vitro and may be expected to interfere with Ursodiol in the same manner as the bile acid sequestering agents. Estrogens, oral contraceptives, and clofibrate increase hepatic cholesterol secretion, and encourage cholesterol gallstone formation and hence may counteract the effectiveness of Ursodiol.

8.10 CEFTAZIDIME (FORTAZ ®)

a) Supply: Supplied frozen, sterile, iso-osmotic non-pyrogenic solution in plastic containers.

b) Preparation: For IV infusion, reconstitute the 1- or 2 Gm infusion pack with 100 mL sterile water. Consult with pharmacy for questions regarding diluent, volume, and expiration.
c) Storage and Stability: Commercially available in dry state stored between 15 and 25 degrees C protected from light. Also supplied frozen in 50-mL single dose plastic containers as a premixed solution of ceftazidime sodium and should not be stored above -20˚C
d) Administration: The usual dosage is 2 grams administered intravenously every 8 to 12 hours. Parenteral doses should be administered by an intravenous infusion over 20 to 30 minutes.
e) Toxicities: 1) the most common adverse effects from ceftazidime are local reactions and allergic and gastrointestinal reactions. These reactions include: 1) phlebitis and inflammation at the site of injection, 2) pruritis, rash, and fever, 3) nausea, vomiting , and abdominal pain, 4) headache, dizziness, and paresthesias,

8.11 LEVOFLOXACIN (LEVAQUIN ®)

a) Supply: Levofloxacin for injection is supplied in single-use vials with each vial containing a concentrated solution with the equivalent of 500 mg of levofloxacin in 20 mL vials and 750 mg in 30 mL vials. Levofloxacin is also supplied as a premix in 5% dextrose with each bag containing a dilute solution with the equivalent of 250, 500, or 750 mg respectively of levofloxacin.
b) Preparation: For IV infusion, the single-use vial must be diluted to 5 mg/mL with D5W or 0.9% Sodium Chloride prior to administration. For the levofloxacin in premixed, ready to use solution in D5W, no further preparation is necessary.
c) Storage and Stability: Levofloxacin injection in Single-Use vials should be stored at controlled room temperature and protected for light. Levofloxacin injection Premix should be stored at or below 25 ˚C. Avoid excessive heat and protect from light.
d) Administration: Levofloxacin injection is 250 or 500 mg administered by slow infusion over 60 minutes every 24 hours or 750 mg administered by slow infusion over 90 minutes every 24 hours.
e) Toxicities: 1) Moderate to severe photoxicity reactions have been observed in patients exposed to direct sunlight while receiving levofloxacin, 2) predispose to seizure, 3) disturbances in blood glucose, 4) peripheral neuropathies.

8.12 CYCLOPHOSPHAMIDE (CTX, CYTOXAN)

a) Supply – Cyclophosphamide will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources and is supplied as a lyophilized powder in various vial sizes.
b) Preparation - will be reconstituted with sterile water for injection to yield a final concentration of 20 mg/ml as described in the package insert.
c) Storage and Stability - The vials are stored at room temperature. Following
d) reconstitutions as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8° C.
e) Route of Administration - The cyclophosphamide used in this regimen will be mixed in 100 ml 0.9% sodium chloride, Inj., and given at a dose of 50 mg/kg as an IVPB over 2 hours on day -6. .e) Toxicities:
    1) Nausea and vomiting - variable; symptomatically improved with standard antiemetics and/or benzodiazepines [e.g., lorazepam].
    2) Water retention – cyclophosphamide may rarely provoke the syndrome of inappropriate antidiuretic hormone secretion and resultant hyponatremia, usually manifested 12-48 hrs

after IV administration, necessitating frequent accurate assessment [q 1-2 hrs] of intake, urine output and urine specific gravity. This effect can be counteracted by furosemide. Fluid restriction is not feasible during administration of high dose cyclophosphamide.

3) Cardiomyopathy - cyclophosphamide may cause severe, sometimes lethal, hemorrhagic myocardial necrosis or congestive cardiomyopathy. Patients may present with congestive cardiomyopathy as late as 2 weeks after the last dose of cyclophosphamide. The clinical syndrome has been observed in patients receiving the dose of cyclophosphamide used in this protocol. In an attempt to minimize this complication, patients with significant cardiac dysfunction are excluded from this protocol [see patient eligibility]. Congestive failure is managed according to standard medical therapeutics.
4) Hemorrhagic cystitis – this is a serious, potentially life-threatening complication related to injury of the bladder epithelium by cyclophosphamide metabolites.
5) Although sub-clinical hematuria is not uncommon at this dose level, clinically significant hematuria or serious hemorrhage can usually be avoided by maintaining a high urine volume and frequent voidings and the administration of Mesna. Diuresis is maintained for 24 hrs after completion of last dose by parenteral infusions of normal saline with potassium chloride. Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to ensure this diuresis. Continuous bladder irrigation may be used for control of significant hematuria.
6) Sterility
7) Less common but serious complications include pulmonary fibrosis and secondary malignancies. Less common but reversible toxicities include alopecia and skin rash.

8.13 EQUINE ANTI-THYMOCYTE GLOBULIN (ATGAM)

a) Supply- Purified, concentrated sterile gamma globulin, primarily monomeric IgG, from hyperimmune serum of horses immunized with human thymus lymphocytes. Each ml of ATGAM contains 50 mg of horse gamma globulin stabilized in 0.3 molar glycine to a PH of approximately 6.8.
b) Preparation- ATGAM should be reconstituted at a concentration not to exceed 4 mg/ml on 0.9% sodium chloride.
c) Storage and Stability- ATGAM is stored as 5 ml ampules in a refrifgerator at 2˚ to 8˚ C. DO NOT FREEZE.
d) Administration- Dose of 30 mg/kg IV every day on days -6, -5, and -4, for a total dose of 90 mg/kg. Diluted ATGAM should be allowed to reach room temperature before infusion. The drug should be infused IV through an in-line filter with a pore size of 0.2 to 1.0 micron. ATGAM should be infused over 4 hours.
e) Toxicities- Because of risk of allergic reaction to ATGAM, at least 24 hours before the first infusion of ATGAM, the manufacturer recommends that patients be tested with an intradermal injection of 0.1 ml of a 1:1000 dilution (5 µg horse ATG) of ATGAM in sodium chloride injection and a contralateral sodium chloride injection control. The patient should be observed every 15 to 20 min over the first hour of intradermal injection. A local reaction of 10 mm or more with a wheal or erythema or both should be considered a positive test. Additional toxicities include: leucopenia, thrombocytopenia, fever, chills, serum sickness like symptoms.

9 APPENDICES

9.1 APPENDIX A: PREPARATIVE REGIMEN

Administration of Fludarabine and Total Body Irradiation (TBI) for 10/10 Matched Related and Unrelated Donor Transplantation

Agent	Dose	Days
Fludarabine	30 mg/m^2 per day IV infusion over 30 minutes, daily for 3 days	Transplant Days –4, -3, -2
TBI	200 cGy	Transplant Day -1

Administration of Fludarabine and Total Body Irradiation (TBI) for 9/10 Matched Unrelated Donor Transplantation

Agent	Dose	Days
Fludarabine	30 mg/m^2 per day IV infusion over 30 minutes, daily for 3 days	Transplant Days –4, -3, -2
TBI	300 cGy	Transplant Day -1

Administration of Fludarabine, Cyclophosphamide, Equine ATG and TBI for Umbilical Cord Blood Transplant

Agent	Dose	Days
Fludarabine	40 mg/m^2 per day (25 mg/m^2 per day in patients < 50 kg) IV infusion over 30 min, daily for 5 days	Transplant Days -6, -5, -4, -3, -2
Equine ATG	30 mg/kg per day	Transplants Days -6, -5, -4
Cyclophosphamide	50 mg/kg	Transplant Day -6
Total body Irradiation	200 cGy	Transplant Day -1

Administration of Fludarabine, Cyclophosphamide, and TBI for Haploidentical Transplant

Agent	Dose	Days
Fludarabine	30 mg/m^2 per day (25 mg/m^2 per day in patients < 50 kg) IV infusion over 30 min, daily for 5 days	Transplant Days -6, -5, -4, -3, -2
Cyclophosphamide	14.5 mg/kg per day	Transplant Day -6,-5
Total body Irradiation	200 cGy	Transplant Day -1

Total Body Irradiation

Patients receive irradiation given from a linear accelerator. Protocols specify the irradiation exposure and schedule of administration. TBI will be given at a dose of 200 cGy on day -1.

Fludarabine

Fludarabine is given as an IV infusion over 30 min. Dosing is based on actual weight. Cyclophosphamide dosing is based upon actual body weight.

9.2 APPENDIX B: PROTOCOL EVALUATION CHECKLIST

1. Recipient pre-transplant evaluation (perform 48 hours prior to the initiation of pre-transplant conditioning)
   a) CBC with differential
   b) PT, PTT
   c) ABO Typing
   d) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
   e) Urine ß-HCG if female of childbearing potential
2. During hospitalization for transplant
   a) CBC BID with differential in am
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
   c) Lipid panel weekly
   d) Type and screen twice a week
   e) Sirolimus levels twice a week
   f) Tacrolimus levels twice a week
   g) Weekly CMV PCR Adenovirus, EBV, HHV6 PCR, toxoplasmosis

3. Post-transplant evaluation
   a) CBC with diff twice weekly or weekly at the discretion of the PI.
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum weekly or weekly at the discretion of the PI
   c) Lipid panel weekly or weekly at the discretion of the PI
   d) Type and screen twice weekly or weekly at the discretion of the PI
   e) Sirolimus levels twice weekly or weekly at the discretion of the PI
   f) Tacrolimus levels twice weekly or weekly at the discretion of the PI
   g) Weekly CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis or weekly at the discretion of the PI

4. Day + 14
   a) CBC with differential,
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
5. Day + 30
   a) CBC with differential,
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
   c) Peripheral Blood Lymphocyte Phenotyping (T, B, NK). Send 5 cc blood in purple top tube to Clinical Center pathology.
   d) CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
   e) Donor: Recipient Chimerism Studies to evaluate T-lymphoid, myeloid, and total blood chimerism. Send yellow top ACD tube to Clinical Center pathology, attn; Jody Keary.

f) Leukocyte subset donor chimerism. Blood (Five green CPT tubes) will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216. After lysis of RBC, flow sorting of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Dr. Roger Kurlander.
g) If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed (5mLs will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216).

6. Day + 60
   a) CBC with differential
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum

7. Day + 100
   a) CBC with differential
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
   c) Peripheral Blood Lymphocyte Phenotyping (T, B, NK). Send 5 cc blood in purple top tube to Clinical Center pathology.
   d) CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
   e) Donor: Recipient Chimerism Studies to evaluate T-lymphoid, myeloid, and total blood chimerism. Send yellow top ACD tube to Clinical Center pathology, attn; Jody Keary. Leukocyte subset donor chimerism. Blood (five green CPT tubes) will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216. After lysis of RBC, flow sorting of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Dr. Roger Kurlander.
   f) A bone marrow aspirate with cytogenetics will be performed on all patients (5mLs will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216).

8. 6 Months Post-Transplant
   a) CBC with differential
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
   c) Peripheral Blood Lymphocyte Phenotyping (T, B, NK). Send 5 cc blood in purple top tube to Clinical Center pathology.
   d) CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
   e) Donor: Recipient Chimerism Studies to evaluate T-lymphoid, myeloid, and total blood chimerism. Send yellow top ACD tube to Clinical Center pathology, attn; Jody Keary. Leukocyte subset donor chimerism. Blood (Five green CPT tubes) will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216. After lysis of RBC, flow sorting

of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Dr. Roger Kurlander.

f) If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed (5mLs will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216).

9. 12 Months Post –Transplant
   a) CBC with differential
   b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
   c) Peripheral Blood Lymphocyte Phenotyping (T, B, NK). Send 5 cc blood in purple top tube to Clinical Center pathology.
   d) CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
   e) Donor: Recipient Chimerism Studies to evaluate T-lymphoid, myeloid, and total blood chimerism. Send yellow top ACD tube to Clinical Center pathology, attn; Jody Keary. Leukocyte subset donor chimerism. Blood (Five green CPT tubes) will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216. After lysis of RBC, flow sorting of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Dr. Roger Kurlander.If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed (5mLs will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216).

10. 18 Months Post –Transplant
    a) CBC with differential
    b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum
    f) Peripheral Blood Lymphocyte Phenotyping (T, B, NK). Send 5 cc blood in purple top tube to Clinical Center pathology.
    c)CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis
    d0 Donor: Recipient Chimerism Studies to evaluate T-lymphoid, myeloid, and total blood chimerism. Send yellow top ACD tube to Clinical Center pathology, attn; Jody Keary. Leukocyte subset donor chimerism. Blood (Five green CPT tubes) will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216.. After lysis of RBC, flow sorting of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Dr. Roger Kurlander.

e) If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed (5mLs will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216).

11. 24 Month Post-Transplant

a) CBC with differential

b) Acute care panel, Hepatic panel, Mineral panel, Protein-total serum, Creatinine kinase, Lactate Dehydrogenase, Uric acid-serum

c) Peripheral Blood Lymphocyte Phenotyping (T, B, NK). Send 5 cc blood in purple top tube to Clinical Center pathology.

d) CMV, Adenovirus, EBV, HHV6 PCR, Toxoplasmosis

e) Donor: Recipient Chimerism Studies to evaluate T-lymphoid, myeloid, and total blood chimerism. Send yellow top ACD tube to Clinical Center pathology, attn; Jody Keary. Leukocyte subset donor chimerism. Blood (Five green CPT tubes) will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216. After lysis of RBC, flow sorting of T-lymphocytes (CD3+), B-lymphocytes (CD20+), NK cells (CD56+), and monocytes (CD14+) will be carried out. If 10,000 to 100,000 cells can be obtained from the individual cell population, then chimerism will be carried out on the individual cell populations. Sorted cell populations will be analyzed for donor chimerism by the Clinical Center pathology, attn: Dr. Roger Kurlander.

f) If MDS is present pre-transplant, then a bone marrow aspirate with cytogenetics will be performed (5mLs will be sent to Dr. Fran Hakim, ETIB, Bldg 10, Room 12-C-216).

9.3 APPENDIX C: IMMUNIZATIONS POST-TRANSPLANT

Immunizations: All recipients will receive immunizations after transplant, according to the following table (http://intranet.cc.nih.gov/bmt/_pdf/table_II.pdf):

9.4 APPENDIX D: GRADING AND MANAGEMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE

Clinical Staging of Acute GVHD

Stage	Skin	Liver	Gut
+	Rash < 25% BSA	Total bilirubin 2-3 mg/dl	Diarrhea 500-1000ml/d
++	Rash 25-50% BSA	Total bilirubin 3-6 mg/dl	Diarrhea 1000-1500ml/d
+++	Generalized erythoderma	Total bilirubin 6-15 mg/dl	Diarrhea >1500ml/d
++++	Desquamation and bullae	Total bilirubin > 15 mg/dl	Pain +/- ileus

BSA = body surface area; use "rule of nines" or burn chart to determine extent of rash.

Clinical Grading of Acute GVHD

	Stage			
Grade	**Skin**	**Liver**	**Gut**	**PS**
0 (none)	0	0	0	0
I	+ to ++	0	0	0
II	+ to +++	+	+	+
III	++ to +++	++ to +++	++ to +++	++
IV	++ to ++++	++ to ++++	++ to ++++	+++

Treatment of Acute GVHD

This schema is intended to serve as a guideline and to promote consistency in our clinical practice; it may be modified for individual patients as clinical circumstances warrant.

Grade 0-I GVHD:

1) Topical corticosteroids (usually 0.1% triamcinolone; 1% hydrocortisone to face) applied to rash BID.

Grade II-IV GVHD:

1) Methylprednisolone (MP) 1 mg/kg per dose IV, BID for 4 consecutive days.
2) If no response after 4 days, continue until response (7-day maximum trial); the dose may be doubled (4 mg/kg/day).
3) If response within 7 days, taper as follows:
   a) 0.75 mg/kg per dose IV BID for 2 days.
   b) 0.5 mg/kg per dose IV BID for 2 days.
   c) 0.375 mg/kg per dose IV BID for 2 days.
   d) If clinically appropriate, change MP to oral prednisone to equivalent of IV dose) daily for 2 days. MP may be converted to prednisone later in the taper at the investigators' discretion.
   e) After this, steroids will be reduced by 10% of starting oral dose each week until a dose of 10 mg/day is reached. Subsequent reductions will be made at the investigators' discretion.

f) If GVHD worsens during taper, steroids should be increased to previous dose.
g) During steroid taper, maintain cyclosporine at therapeutic levels (Section 3.5.1).

4) If no response is observed within 7 days of MP treatment:
   a) Increase Methylprednisolone to 10 mg/kg per dose IV, BID for 2 days.
   b) If there is no improvement, consideration will be given to using second-line immunosuppressive therapy, e.g., tacrolimus, mycophenolic acid, monoclonal antibodies, or studies of investigational agents for acute GVHD, if they are available.
5) Antifungal prophylaxis with agents effective against mould will be started when it is anticipated that the patient will be receiving steroids at $\geq$ 1 mg/kg/day of methylprednisolone (or equivalent) for $\geq$ 2 weeks. Voriconazole, caspofungin, liposomal amphotericin B (Ambisome), posaconazole or amphotericin B lipid complex (Abelcet) are valid alternatives. During prophylaxis with any of the above agents, fluconazole should be discontinued. In patients with therapeutic cyclosporine levels at the initiation of voriconazole therapy, the cyclosporine or tacrolimus dose should be decreased by approximately 50%. In patients with therapeutic sirolimus levels at the initiation of voriconazole therapy, the sirolimus dose should be decreased by approximately 90%.
6) Determination of GVHD treatment response should be made within 96 hours of starting the treatment. The following are criteria to determine definitions of response to GVHD treatment:
   a) Complete response: Complete resolution of all clinical signs and symptoms of acute GVHD.
   b) Partial Response: 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Maintenance of adequate performance status (Karnofsky Score $\geq$ 70%).
   c) Non-responder: < 50% reduction in skin rash, stool volume or frequency, and/or total bilirubin. Failure to maintain adequate performance status (Karnofsky Score $\leq$ 70%).

   d) Progressive disease: Further progression of signs and symptoms of acute GVHD, and/or decline in performance status after the initiation of therapy.

Treatment

This schema is intended to serve as a guideline and to promote consistency in our clinical practice; it may be modified for individual patients as clinical circumstances warrant.

9.5 APPENDIX E: MANAGEMENT OF ENGRAFTMENT SYNDROME

Clinical Definition of Engraftment Syndrome

A constellation of clinical symptoms and signs has been observed during neutrophil recovery following HSCT, most commonly termed "engraftment syndrome" (ES), but interchangeably termed "capillary leak syndrome" and "autoaggression syndrome". Engraftment syndromes have been observed after both autologous and allogeneic HSCT; in the latter setting, the clinical sequelae of neutrophil recovery were historically attributed to an early, sometimes "hyperacute" graft-versus-host reaction. Our current understanding of ES holds the overproduction of pro-inflammatory cytokines at the time of neutrophil recovery to be the initiating event. The study of ES has been somewhat hindered by the lack of uniform definition for this clinical entity; therefore, the following criteria have been propose (Spitzer 2001):

Major criteria:	• Fever > 38.3° with no identifiable infectious etiology • Erythrodermatous rash involving more than 25% of body surface area and not attributable to a medication • Noncardiogenic pulmonary edema, manifested by diffuse pulmonary infiltrates and hypoxia
Minor criteria	• Hepatic dysfunction with either total bilirubin > 2 mg/dl or transaminases levels > two times normal • Renal insufficiency (serum creatinine > two times baseline) • Weight gain > 2.5% of baseline body weight • Transient encephalopathy unexplainable by other causes

A diagnosis of ES is established by the presence of all three major criteria, or two major criteria and one or more minor criteria; the clinical signs and symptoms should appear within 96 hours of neutrophil recovery, according to the above proposed definition.

- Neutrophil recovery after transplantation may result in "engraftment syndrome" (which may include fever, rash, edema, hypoxia)
- The following criteria will be utilized to diagnose engraftment syndrome (must have 3 major criteria or 2 major + 1 minor criteria):
- To promote consistency of our clinical practice, the following will be the recommended therapy for engraftment syndrome. However, this schedule may be modified (taper either accelerated or delayed) for individual patients as clinical circumstances warrant.

Treatment of Engraftment Syndrome

The mainstay of therapy for ES is high-dose corticosteroids, based upon the literature on diffuse alveolar hemorrhage in the setting of bone marrow transplantation – a complication that many investigators now believe to be part of the spectrum of ES (Metcalf et al. 1994). Our group has adopted the following treatment schema for patients diagnosed with ES, with satisfactory results:

Day 1: Methylprednisolone 250 mg IV Q6 hours x 4 doses
Day 2: Methylprednisolone 250 mg IV Q8 hours x 3 doses
Day 3: Methylprednisolone 250 mg IV Q12 hours x 2 doses
Day 4: Methylprednisolone 125 mg IV Q12 hours x 2 doses

Day 5:	Methylprednisolone 60 mg IV Q12 hours x 2 doses
Day 6:	Methylprednisolone 30 mg IV Q12 hours x 2 doses
Days 7-8:	Prednisone 60 mg PO QD x 2 days
Days 9-10:	Prednisone 50 mg PO QD x 2 days
Days 11-12:	Prednisone 40 mg PO QD x 2 days
Days 13-14:	Prednisone 30 mg PO QD x 2 days
Days 15-16:	Prednisone 20 mg PO QD x 2 days
Days 17-18:	Prednisone 10 mg PO QD x 2 days
Day 19:	Discontinue prednisone

In the event that symptoms or clinical signs of ES recur during the steroid taper, patients should be retreated with methylprednisolone at a minimum dose of 60 mg IV QD. *This schema is intended to serve as a guideline and to promote consistency in our clinical practice; it may be modified for individual patients as clinical circumstances warrant.* ***In particular, steroid dosing for pediatric patients (< 18) will be determined on a case-by-case basis.***

9.6 APPENDIX F- NMDP CRITERIA FOR MATCHED UNRELATED DONOR

a) HLA-testing performed by high resolution typing, and donors matched at HLA (matched 10/10) -A, B, C, and DRB1 and DQ loci or 9/10 loci are acceptable donors.
b) An NMDP designated physician, in accordance with the most recent NMDP Standards and FDA Guidance, will perform unrelated donor eligibility determination. In general, the following inclusion criteria apply:
    a. Age 18 – 60
    b. Ability to give informed consent
c) Determination of Medical Suitability
    a. Medical suitability will be determined as specified in the 19th Edition NMDP Standards, section 9.3000, and according to the NMDP standard operating procedure A00220. The NMDP adheres to the strictest interpretation of the FDA Guidance for Industry "Eligibility Determination for Donors of Human Cells, Tissue, and Cellular and Tissue-Based Products (HCT/P's)", February 2007.
    b. Per NMDP Standards, a physical exam is performed on all potential marrow and PBSC donors. The assessment includes, but is not limited to:
        i. Complete medical history and physical exam with special note of:
            1. Deferral of voluntary blood donation, prior experience or problems with anesthetic agents, or other possible contraindications to donation for the prospective donor
            2. Vital signs, including height and weight
            3. List of medications
            4. Venous access for potential apheresis donors
            5. If information provided in the health history screening questionnaire or other relevant medical records indicates a potential risk for transmission of a communicable disease, the donor may be deferred or a physical examination including specific assessment for signs or symptoms of a communicable infection will be performed.
        ii. Examinations including but not limited to:
            1. Chest X-Ray
            2. Urinalysis
            3. Electrocardiogram
            4. Labs
                a. Hemoglobin and Hematocrit
                b. CBC with differential
                c. Platelet Count
                d. ABO and RH
                e. Screening for Hemoglobin S
                f. Electrolytes
                g. Alkaline Phosphatase
                h. BUN and Creatinine
                i. LDH
                j. Bilirubin

k. ALT,SBPT
l. Glucose
m. Serum Total Protein plus albumin or serum protein electrophoresis
n. Serum Beta-HCG Pregnancy

c. Infectious Disease Testing
   i. Donor Infectious Disease Markers are tested at the time of donor's confirmatory typing, within 30 days of collection, and again on the day of collection according to FDA regulations. Interpretation of infectious disease markers is performed by the donor center physician according to NMDP Standard A00223 version 2.0 "IDM Eligibility and Labeling Guide"
   ii. The following markers are tested
      1. Hepatitis B surface antigen (HBsAg)
      2. Hepatitis B core antibody (Anti HBc)
      3. Hepatitis C antibody (Anti-HCV)
      4. Anti-HTLV I/II
      5. Antibody to T.cruzi
      6. NAT testing for HIV-1/HCV
      7. Anti-HIV 1 and Anti-HIV 2
      8. Syphilis serologic screening test (STS)
      9. Anti-CMV (IgG or total)
      10. WNV-NAT testing (only required on day of collection during optional testing period)
   iii. Any abnormal findings that may increase the risk of transmitting a communicable disease or that could increase the risk of transplant for the recipient are disclosed to the transplant center. The patient's physician will be notified, and a determination will be made whether or not to proceed with the collection. In the event the donation process continues a Letter of Urgent Medical Need will be generated.

d) Documentation of donor clearance and consent will be provided to NIH through the search-coordinating unit at the NMDP.

9.7 APPENDIX G: EXPERIMENTAL TRANSPLANTATION AND IMMUNOLOGY BRANCH PRECLINICAL SERVICE POLICY FOR SAMPLE HANDLING

Storage/Tracking in the Preclinical Development and Clinical Monitoring Facility (PDCMF)

- Normal patient blood and tissue samples, collected for the purpose of research under IRB approved protocols of the Experimental Transplantation and Immunology Branch (ETIB) may be archived by the ETIB Preclinical Development and Clinical Monitoring Facility (PDCMF). All data associated with archived clinical research samples is entered into the ETIB PDCMF's Microsoft Excel databases on frozen cells and plasma. These databases are stored on the NCI group drive in the ETIB 'PRECLINSERVICE' folder. Access to this folder is limited to PDCMF staff and ETIB clinical staff, requiring individual login and password. All staff in the PDCMF laboratory receives annually updated NIH/CIT training and maintains standards of computer security.
- The data recorded for each sample includes the patient ID, trial name/protocol number, date drawn, treatment cycle/post-transplant time point, cell source (e. g. peripheral blood, lymphapheresis, mobilized peripheral blood stem cells, marrow, urine, skin or oral biopsy as well as box and freezer location. Patient demographics that correlate treatment outcomes and therapies with the samples can be obtained only through the NCI/ETIB clinical records. As of January 2007, all newly received samples receive a unique bar code number, which is included in the sample record in the PDCMF database. Only this bar code is recorded on the sample vial and the vials will not be traceable back to patients without authorized access to the PDCMF database. All non-coded samples previously archived will be stripped of identifiers prior to distribution for any use other than as a primary objective of the protocol under which they were collected.
- Samples are stored in locked freezers. All samples will be labeled solely with a bar code (which includes the date, and serially determined individual sample identifier). The key will be available to a restricted number of ETIB investigators and associate investigators on the protocol. Coded samples will be stored frozen at -20°, -80° or liquid nitrogen vapor phase to -180 C according to the stability requirements in a single location under the restricted control of the PDCMF of ETIB.
- Access to samples from a protocol for research purposes will be by permission of the Principal Investigator of that protocol in order to be used (1) for research purposes associated with protocol objectives for which the samples were collected, or (2) for a new research activity following submission and IRB approval of a new protocol and consent, or (3) for use only as unlinked or coded samples under the OHSRP Exemption Form guidelines stipulating that the activity is exempt from IRB review. Unused samples must be returned to the PDCMF laboratory.
- Samples and associated data will be stored permanently unless the patient withdraws consent. If researchers have samples remaining once they have completed all studies associated with the protocol, they must be returned to the PDCMF laboratory.
- These freezers are located onsite at the Preclinical Service laboratory (12C216) (-85° freezer) or in ETIB common equipment space (CRC/3-3273).

Protocol Completion/Sample Destruction

- Once research objectives for the protocol are achieved, researchers can request access to remaining samples, providing they have both approval of the Principal Investigator of the original protocol under which the samples or data were collected and either an IRB approved protocol and patient consent or the OHSRP Exemption Form stipulating that the activity is exempt from IRB review.
- The PDCMF staff will report to the Principal Investigators any destroyed samples, if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container), lost in transit between facilities or misplaced by a researcher.
- The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

9.8 Appendix H: Data Collection Elements Required By Protocol

All of the following elements will be recorded in the C3D database.

A. Patient Enrollment

Recipient

- Date of birth, age, gender, race, ethnicity
- Performance Status
- Date of original diagnosis
- Date of Informed Consent signature, consent version and date of registration
- Baseline History/Physical
- Baseline Symptoms

Donor

- Date of birth, age, gender, race, ethnicity
- Height
- Weight
- Baseline History/Physical (Y/N)

B. Study Drug administration and response for each course of therapy given

- Dates study drugs given
- Dose level, actual dose, schedule and route given
- Response assessment for each restaging performed

C. Laboratory and Diagnostic Test Data

- All Clinical laboratory and diagnostic test results done at screening(recipient and donor) and until day 100 post transplant with the following exceptions:
- Screening labs that are not automatically loaded for example (HIV, anti hep B and C).
- Diagnostic tests which are not specified in the protocol, and if the results are not needed to document the start or end of an adverse event that requires reporting (all CTs should be recorded).
- All clinical laboratory and diagnostic tests done after day 100 that support a possible, probable or definite diagnosis of GVHD, infection or secondary malignancy and those done to document a change in grade and the end of these adverse events.
- All tests done to document resolution of adverse events that occurred in the first 100 days post transplant
- HLA data (patient and donor).
- Blood, bone marrow, and tumor chimerism data

D. Adverse Events Recorded per section 5.1

E. Concomitant Measures

- GVHD prophylaxis and treatment (start and stop dates)
- All immunosuppressive agents

F. Treatment of Persistent/Progressive Disease with Standard Therapy

- Chemotherapy
- Immunotherapy
- Radiation therapy
- Donor Lymphocyte Infusion

G. Response

Restaging studies performed at protocol specified time points and as clinically indicated.

H. Off study

- Date and reason for off study
- Date and cause of death
- Autopsy findings

10 REFERENCES

Anasetti, C., P. G. Beatty, et al. (1990). "Effect of HLA incompatibility on graft-versus-host disease, relapse, and survival after marrow transplantation for patients with leukemia or lymphoma." Hum Immunol **29**(2): 79-91.

Anderlini, P., M. Korbling, et al. (1997). "Allogeneic blood stem cell transplantation: considerations for donors." Blood **90**(3): 903-8.

Antin, J. H., H. T. Kim, et al. (2003). "Sirolimus, tacrolimus, and low-dose methotrexate for graft-versus-host disease prophylaxis in mismatched related donor or unrelated donor transplantation." Blood **102**(5): 1601-5.

Aoudjhane, M., M. Labopin, et al. (2005). "Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT)." Leukemia **19**(12): 2304-12.

Appelbaum, F. R. (2007). "Hematopoietic-cell transplantation at 50." N Engl J Med **357**(15): 1472-5.

Ash, R. C., J. T. Casper, et al. (1990). "Successful allogeneic transplantation of T-cell-depleted bone marrow from closely HLA-matched unrelated donors." N Engl J Med **322**(8): 485-94.

Bach, F. H., R. J. Albertini, et al. (1968). "Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome." Lancet **2**(7583): 1364-6.

Barker, J. N., T. P. Krepski, et al. (2002). "Searching for unrelated donor hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow." Biol Blood Marrow Transplant **8**(5): 257-60.

Bearman, S. I., F. R. Appelbaum, et al. (1988). "Regimen-related toxicity in patients undergoing bone marrow transplantation." J Clin Oncol **6**(10): 1562-8.

Beatty, P. G., R. A. Clift, et al. (1985). "Marrow transplantation from related donors other than HLA-identical siblings." N Engl J Med **313**(13): 765-71.

Bernstein, H. M., D. A. Calhoun, et al. (2002). "Use of myeloid colony-stimulating factors in neonates with septicemia." Curr Opin Pediatr **14**(1): 91-4.

Bray, R. A., C. K. Hurley, et al. (2008). "National marrow donor program HLA matching guidelines for unrelated adult donor hematopoietic cell transplants." Biol Blood Marrow Transplant **14**(9 Suppl): 45-53.

Brunstein, C. G., J. N. Barker, et al. (2007). "Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease." Blood **110**(8): 3064-70.

Buchali, A., P. Feyer, et al. (2000). "Immediate toxicity during fractionated total body irradiation as conditioning for bone marrow transplantation." Radiother Oncol **54**(2): 157-62.

Buckley, R. H. (2003). "Treatment options for genetically determined immunodeficiency." Lancet **361**(9357): 541-2.

Buckley, R. H., S. E. Schiff, et al. (1999). "Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency." N Engl J Med **340**(7): 508-16.

Burroughs, L. M., R. Storb, et al. (2007). "Intensive postgrafting immune suppression combined with nonmyeloablative conditioning for transplantation of HLA-identical hematopoietic cell grafts: results of a pilot study for treatment of primary immunodeficiency disorders." Bone Marrow Transplant **40**(7): 633-42.

Carvallo, C., N. Geller, et al. (2004). "Prior chemotherapy and allograft CD34+ dose impact donor engraftment following nonmyeloablative allogeneic stem cell transplantation in patients with solid tumors." Blood **103**(4): 1560-3.

Chakraverty, R., K. Peggs, et al. (2002). "Limiting transplantation-related mortality following unrelated donor stem cell transplantation by using a nonmyeloablative conditioning regimen." Blood **99**(3): 1071-8.

Champlin, R. E. (1990). "T-cell depletion for bone marrow transplantation: effects on graft rejection, graft-versus-host disease, graft-versus-leukemia, and survival." Cancer Treat Res **50**: 99-111.

Childs, R., A. Chernoff, et al. (2000). "Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation." N Engl J Med **343**(11): 750-8.

Childs, R., E. Clave, et al. (1999). "Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses." Blood **94**(9): 3234-41.

Copelan, E. A. (2006). "Hematopoietic stem-cell transplantation." N Engl J Med **354**(17): 1813-26.

Curtis, R. E., P. A. Rowlings, et al. (1997). "Solid cancers after bone marrow transplantation." N Engl J Med **336**(13): 897-904.

de Witte, T., S. Suciu, et al. (2001). "Intensive chemotherapy followed by allogeneic or autologous stem cell transplantation for patients with myelodysplastic syndromes (MDSs) and acute myeloid leukemia following MDS." Blood **98**(8): 2326-31.

Deeg, H. J., R. Prentice, et al. (1983). "Increased incidence of malignant tumors in dogs after total body irradiation and marrow transplantation." Int J Radiat Oncol Biol Phys **9**(10): 1505-11.

Dufour, C., R. Rondelli, et al. (2001). "Stem cell transplantation from HLA-matched related donor for Fanconi's anaemia: a retrospective review of the multicentric Italian experience on behalf of AIEOP-GITMO." Br J Haematol **112**(3): 796-805.

Engel, M. E., D. D. Hickstein, et al. (2006). "Matched unrelated bone marrow transplantation with reduced-intensity conditioning for leukocyte adhesion deficiency." Bone Marrow Transplant **37**(7): 717-8.

Fay, J. W., J. R. Wingard, et al. (1996). "FK506 (Tacrolimus) monotherapy for prevention of graft-versus-host disease after histocompatible sibling allogenic bone marrow transplantation." Blood **87**(8): 3514-9.

Filipovich, A. H., R. S. Shapiro, et al. (1992). "Unrelated donor bone marrow transplantation for correction of lethal congenital immunodeficiencies." Blood **80**(1): 270-6.

Filipovich, A. H., D. Weisdorf, et al. (2005). "National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report." Biol Blood Marrow Transplant **11**(12): 945-56.

Flomenberg, N., L. A. Baxter-Lowe, et al. (2004). "Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-

C mismatching is associated with a strong adverse effect on transplantation outcome." Blood **104**(7): 1923-30.

Gatti, R. A., H. J. Meuwissen, et al. (1968). "Immunological reconstitution of sex-linked lymphopenic immunological deficiency." Lancet **2**(7583): 1366-9.

Giralt, S., E. Estey, et al. (1997). "Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy." Blood **89**(12): 4531-6.

Giralt, S., P. F. Thall, et al. (2001). "Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation." Blood **97**(3): 631-7.

Gluckman, E. (2000). "Current status of umbilical cord blood hematopoietic stem cell transplantation." Exp Hematol **28**(11): 1197-205.

Gluckman, E., V. Rocha, et al. (1997). "Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group." N Engl J Med **337**(6): 373-81.

Greenberg, P., C. Cox, et al. (1997). "International scoring system for evaluating prognosis in myelodysplastic syndromes." Blood **89**(6): 2079-88.

Haase, D., U. Germing, et al. (2007). "New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients." Blood **110**(13): 4385-95.

Hackstein, H., T. Taner, et al. (2003). "Rapamycin inhibits IL-4--induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo." Blood **101**(11): 4457-63.

Hansen, J. A., T. A. Gooley, et al. (1998). "Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia." N Engl J Med **338**(14): 962-8.

Hegenbart, U., D. Niederwieser, et al. (2006). "Treatment for acute myelogenous leukemia by low-dose, total-body, irradiation-based conditioning and hematopoietic cell transplantation from related and unrelated donors." J Clin Oncol **24**(3): 444-53.

Hiraoka, A., Y. Ohashi, et al. (2001). "Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation." Bone Marrow Transplant **28**(2): 181-5.

Honig, M., M. H. Albert, et al. (2007). "Patients with adenosine deaminase deficiency surviving after hematopoietic stem cell transplantation are at high risk of CNS complications." Blood **109**(8): 3595-602.

Horwitz, M. E., A. J. Barrett, et al. (2001). "Treatment of chronic granulomatous disease with nonmyeloablative conditioning and a T-cell-depleted hematopoietic allograft." N Engl J Med **344**(12): 881-8.

Hsieh, M. M., E. M. Kang, et al. (2009). "Allogeneic hematopoietic stem-cell transplantation for sickle cell disease." N Engl J Med **361**(24): 2309-17.

Hsu, A. P., E. P. Sampaio, et al. "Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome." Blood.

Huhn, R. D., J. F. Tisdale, et al. (1999). "Retroviral marking and transplantation of rhesus hematopoietic cells by nonmyeloablative conditioning." Hum Gene Ther **10**(11): 1783-90.

Kang, E. M., Y. Hanazano, et al. (2001). "Persistent low-level engraftment of rhesus peripheral blood progenitor cells transduced with the fanconi anemia C gene after conditioning with low-dose irradiation." Mol Ther **3**(6): 911-9.

Kapoor, N., D. Kirkpatrick, et al. (1981). "Reconstitution of normal megakaryocytopoiesis and immunologic functions in Wiskott-Aldrich syndrome by marrow transplantation following myeloablation and immunosuppression with busulfan and cyclophosphamide." Blood **57**(4): 692-6.

Kato, T., I. Tsuge, et al. (1999). "Successful bone marrow transplantation in a child with X-linked hyper-IgM syndrome." Bone Marrow Transplant **23**(10): 1081-3.

Kernan, N. A., G. Bartsch, et al. (1993). "Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program." N Engl J Med **328**(9): 593-602.

Khouri, I. F., M. Keating, et al. (1998). "Transplant-lite: induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies." J Clin Oncol **16**(8): 2817-24.

Khouri, I. F., R. M. Saliba, et al. (2001). "Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality." Blood **98**(13): 3595-9.

Kolb, H. J., G. Socie, et al. (1999). "Malignant neoplasms in long-term survivors of bone marrow transplantation. Late Effects Working Party of the European Cooperative Group for Blood and Marrow Transplantation and the European Late Effect Project Group." Ann Intern Med **131**(10): 738-44.

Kroger, N., H. G. Sayer, et al. (2002). "Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality." Blood **100**(12): 3919-24.

Kurtzberg, J., M. Laughlin, et al. (1996). "Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients." N Engl J Med **335**(3): 157-66.

Laport, G. G., B. M. Sandmaier, et al. (2008). "Reduced-intensity conditioning followed by allogeneic hematopoietic cell transplantation for adult patients with myelodysplastic syndrome and myeloproliferative disorders." Biol Blood Marrow Transplant **14**(2): 246-55.

Laughlin, M. J., J. Barker, et al. (2001). "Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors." N Engl J Med **344**(24): 1815-22.

Laughlin, M. J., M. Eapen, et al. (2004). "Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia." N Engl J Med **351**(22): 2265-75.

Le Deist, F., S. Blanche, et al. (1989). "Successful HLA nonidentical bone marrow transplantation in three patients with the leukocyte adhesion deficiency." Blood **74**(1): 512-6.

Lee, S. J., J. Klein, et al. (2007). "High-resolution donor-recipient HLA matching contributes to the success of unrelated donor marrow transplantation." Blood **110**(13): 4576-83.

Longhurst, H. J., D. Taussig, et al. (2002). "Non-myeloablative bone marrow transplantation in an adult with Wiskott-Aldrich syndrome." Br J Haematol **116**(2): 497-9.

Luznik, L., L. W. Engstrom, et al. (2002). "Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation." Biol Blood Marrow Transplant **8**(3): 131-8.

Luznik, L., P. V. O'Donnell, et al. (2008). "HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide." Biol Blood Marrow Transplant **14**(6): 641-50.

Maciejewski, J. P., A. Risitano, et al. (2002). "Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia." Blood **99**(9): 3129-35.

Mackinnon, S., L. Barnett, et al. (1994). "Minimal residual disease is more common in patients who have mixed T-cell chimerism after bone marrow transplantation for chronic myelogenous leukemia." Blood **83**(11): 3409-16.

Maris, M. B., D. Niederwieser, et al. (2003). "HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies." Blood **102**(6): 2021-30.

Markus, P. M., X. Cai, et al. (1991). "Prevention of graft-versus-host disease following allogeneic bone marrow transplantation in rats using FK506." Transplantation **52**(4): 590-4.

Martin, P. J., J. A. Hansen, et al. (1988). "Graft failure in patients receiving T cell-depleted HLA-identical allogeneic marrow transplants." Bone Marrow Transplant **3**(5): 445-56.

Martino, R., M. D. Caballero, et al. (2002). "Evidence for a graft-versus-leukemia effect after allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning in acute myelogenous leukemia and myelodysplastic syndromes." Blood **100**(6): 2243-5.

McSweeney, P. A., D. Niederwieser, et al. (2001). "Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects." Blood **97**(11): 3390-400.

Metcalf, J. P., S. I. Rennard, et al. (1994). "Corticosteroids as adjunctive therapy for diffuse alveolar hemorrhage associated with bone marrow transplantation. University of Nebraska Medical Center Bone Marrow Transplant Group." Am J Med **96**(4): 327-34.

Mielcarek, M., B. E. Storer, et al. (2007). "Outcomes among patients with recurrent high-risk hematologic malignancies after allogeneic hematopoietic cell transplantation." Biol Blood Marrow Transplant **13**(10): 1160-8.

Munchel, A., C. Kesserwan, et al. "Nonmyeloablative, HLA-haploidentical bone marrow transplantation with high dose, post-transplantation cyclophosphamide." Pediatr Rep **3 Suppl 2**: e15.

Myburgh, J. A., J. A. Smit, et al. (1980). "Transplantation tolerance in primates following total lymphoid irradiation and allogeneic bone marrow injection. II. Renal allografts." Transplantation **29**(5): 405-8.

Myburgh, J. A., J. A. Smit, et al. (1984). "Total lymphoid irradiation in kidney and liver transplantation in the baboon: prolonged graft survival and alterations in T cell subsets with low cumulative dose regimens." J Immunol **132**(2): 1019-25.

Nash, R. A., J. H. Antin, et al. (2000). "Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors." Blood **96**(6): 2062-8.

Nash, R. A., R. Etzioni, et al. (1995). "Tacrolimus (FK506) alone or in combination with methotrexate or methylprednisolone for the prevention of acute graft-versus-host disease after marrow transplantation from HLA-matched siblings: a single-center study." Blood **85**(12): 3746-53.

Niederwieser, D., M. Maris, et al. (2003). "Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or

mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases." Blood **101**(4): 1620-9.

Nimer, S. D. (2008). "Myelodysplastic syndromes." Blood **111**(10): 4841-51.

O'Donnell, P. V., L. Luznik, et al. (2002). "Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide." Biol Blood Marrow Transplant **8**(7): 377-86.

Orsini, E., E. P. Alyea, et al. (2000). "Conversion to full donor chimerism following donor lymphocyte infusion is associated with disease response in patients with multiple myeloma." Biol Blood Marrow Transplant **6**(4): 375-86.

Petersdorf, E. W. (2006). "Immunogenomics of unrelated hematopoietic cell transplantation." Curr Opin Immunol **18**(5): 559-64.

Petrus, M. J., J. F. Williams, et al. (2000). "An immunoablative regimen of fludarabine and cyclophosphamide prevents fully MHC-mismatched murine marrow graft rejection independent of GVHD." Biol Blood Marrow Transplant **6**(2A): 182-9.

Przepiorka, D., D. Weisdorf, et al. (1995). "1994 Consensus Conference on Acute GVHD Grading." Bone Marrow Transplant **15**(6): 825-8.

Rao, K., P. J. Amrolia, et al. (2005). "Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen." Blood **105**(2): 879-85.

Rocha, V., M. Labopin, et al. (2004). "Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia." N Engl J Med **351**(22): 2276-85.

Rocha, V., J. E. Wagner, Jr., et al. (2000). "Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources." N Engl J Med **342**(25): 1846-54.

Rubinstein, P., C. Carrier, et al. (1998). "Outcomes among 562 recipients of placental-blood transplants from unrelated donors." N Engl J Med **339**(22): 1565-77.

Sanz, G. F., S. Saavedra, et al. (2001). "Standardized, unrelated donor cord blood transplantation in adults with hematologic malignancies." Blood **98**(8): 2332-8.

Sauer, M., C. Bettoni, et al. (2005). "Complete substitution of cyclophosphamide by fludarabine and ATG in a busulfan-based preparative regimen for children and adolescents with beta-thalassemia." Bone Marrow Transplant **36**(5): 383-7.

Schetelig, J., C. Thiede, et al. (2003). "Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the Cooperative German Transplant Study Group." J Clin Oncol **21**(14): 2747-53.

Shulman, L. N., N. J. Tarbell, et al. (1998). "Low-dose total body irradiation and G-CSF without hematopoietic stem cell support in the treatment of relapsed or refractory acute myelogenous leukemia (AML), or AML in second or subsequent remission." Int J Radiat Oncol Biol Phys **42**(5): 1113-7.

Slavin, S., A. Nagler, et al. (1998). "Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases." Blood **91**(3): 756-63.

Spitzer, T. R. (2001). "Engraftment syndrome following hematopoietic stem cell transplantation." Bone Marrow Transplant **27**(9): 893-8.

Spitzer, T. R., S. McAfee, et al. (2000). "Intentional induction of mixed chimerism and achievement of antitumor responses after nonmyeloablative conditioning therapy and HLA-matched donor bone marrow transplantation for refractory hematologic malignancies." Biol Blood Marrow Transplant **6**(3A): 309-20.

Stepkowski, S. M., L. Tian, et al. (1997). "Synergistic mechanisms by which sirolimus and cyclosporin inhibit rat heart and kidney allograft rejection." Clin Exp Immunol **108**(1): 63-8.

Storb, R., R. F. Raff, et al. (1993). "FK-506 and methotrexate prevent graft-versus-host disease in dogs given 9.2 Gy total body irradiation and marrow grafts from unrelated dog leukocyte antigen-nonidentical donors." Transplantation **56**(4): 800-7.

Storb, R., C. Yu, et al. (1999). "Stable mixed hematopoietic chimerism in dog leukocyte antigen-identical littermate dogs given lymph node irradiation before and pharmacologic immunosuppression after marrow transplantation." Blood **94**(3): 1131-6.

Thomas, E. D., H. L. Lochte, Jr., et al. (1957). "Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy." N Engl J Med **257**(11): 491-6.

Thomas, O., M. Mahe, et al. (2001). "Long-term complications of total body irradiation in adults." Int J Radiat Oncol Biol Phys **49**(1): 125-31.

Truitt, R. L. and A. A. Atasoylu (1991). "Impact of pretransplant conditioning and donor T cells on chimerism, graft-versus-host disease, graft-versus-leukemia reactivity, and tolerance after bone marrow transplantation." Blood **77**(11): 2515-23.

Veys, P., K. Rao, et al. (2005). "Stem cell transplantation for congenital immunodeficiencies using reduced-intensity conditioning." Bone Marrow Transplant **35 Suppl 1**: S45-7.

Vinh, D. C., S. Y. Patel, et al. (2010). "Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia." Blood **115**(8): 1519-29.

Wagner, J. E., J. N. Barker, et al. (2002). "Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival." Blood **100**(5): 1611-8.

Wagner, J. E. and J. Kurtzberg (1997). "Cord blood stem cells." Curr Opin Hematol **4**(6): 413-8.

Wong, R., S. A. Giralt, et al. (2003). "Reduced-intensity conditioning for unrelated donor hematopoietic stem cell transplantation as treatment for myeloid malignancies in patients older than 55 years." Blood **102**(8): 3052-9.