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1. PROTOCOL 2032: HLA-haploidentical related marrow grafts for the treatment of primary immunodeficiencies and other nonmalignant disorders using conditioning with low-dose cyclophosphamide, TBI and fludarabine and postgrafting cyclophosphamide.

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2. INTRODUCTION

Hematopoietic cell transplantation (HCT) is often the only curative therapy for patients with non-malignant disorders including immunodeficiencies and other inherited diseases. Unfortunately, less than 30% of children with life-threatening nonmalignant disorders will have a matched family donor[1]. While unrelated registries have expanded donor options, patients who are not of Caucasian ethnicity have difficulty finding donors matched for HLA-A, B, C, DRB1 and DQB1 alleles[2-5]. Therefore, the ability to use related, HLA-haploidentical donors for allogeneic HCT of patients with nonmalignant disorders would provide almost all patients a chance at a potential cure. Several studies have demonstrated HCT using related HLA-haploidentical grafts to be a viable treatment option for patients with immunodeficiency [6-14] and other nonmalignant disorders [15-18]. However, inferior survivals were seen for recipients of HLA-haploidentical grafts compared to recipients of HLA-matched related or unrelated grafts. Several problems were seen including graft failure, delayed immune reconstitution, increased infections, and other significant toxicities including GVHD [10,19]. Therefore, developing safe and effective approaches to HCT from HLA-haploidentical related donors is an important goal in order to increase the number of patients eligible for HCT. A nonmyeloablative approach has several advantages over an ablative approach including decreased regimen related toxicity and recovery of autologous hematopoiesis in the event of graft rejection.

It is important to extend the option of nonmyeloablative HCT for potential therapy of patients with nonmalignant disorders to patients who do not have an HLA-matched donor. Almost all patients would have a related donor identical for one HLA haplotype (haploidentical) and mismatched at HLA-A, -B or -DR of the unshared haplotype. In this protocol, we will use a combination of immunosuppressive agents including cyclophosphamide administered before and after HCT to facilitate engraftment and to remove highly alloreactive T-cell clones presumably involved in GVHD. Feasibility of this approach has been demonstrated in a mouse model, a Phase I clinical trial for patients with hematological malignancies, and preliminary results from a phase II study at the FHCRC and collaborating centers (see below). Encouraged by these early results, a Phase I-II study is warranted to determine the safety and efficacy of such approach in patients with nonmalignant inherited disorders.

3. BACKGROUND

3A. Haploidentical Transplantation for patients with Nonmalignant Disorders.

1) Immunodeficiency disorders

Transplantation with haploidentical donors greatly expanded transplant options for patients with immunodeficiencies and other inherited disorders. The largest experiences reported are for patients with severe combined immunodeficiency disorders (SCID). In the 1980's, several studies demonstrated successful immune reconstitution following transplantation with T-cell depleted haploidentical grafts in patients with SCID [6-8,20,21]. Since that time, several other studies have demonstrated HCT with haploidentical grafts to be a viable treatment option for patients with immunodeficiency [9-14] and other nonmalignant disorders who did not have HLA-matched related or unrelated donors [15,16,16-18].

Fischer et al. compared the outcome of patients with SCID who received either HLA-identical related bone marrow grafts (n=70) versus HLA-nonidentical related T-cell depleted bone marrow grafts (n=100) and found significantly lower overall survivals for patients who received nonidentical versus identical grafts (52% versus 76%, p=0.01) [9]. Pretransplant lung infections were a negative risk factor for survival for recipients of nonidentical grafts. Sustained engraftment was significantly better for those patients who received conditioning prior to HLA-nonidentical marrow grafts versus those patients who did not (86% vs. 50%, p<0.01). Graft-versus-host disease (GVHD) was the primary cause of death in the HLA-nonidentical recipients.

Buckley et al. reported on the outcome of 89 infants with SCID who received T-cell depleted HLA-haploidentical parenteral marrow (n=77) or HLA-identical marrow (n=12) grafts without conditioning or GVHD prophylaxis [10]. GVHD developed in 28 of the 77 infants (36%) given T-cell depleted haploidentical marrow and 6 of the 12 HLA-identical marrow recipients (50%), and was in general mild (<grade II) and not responsible for any deaths. Twenty of the 89 patients (22%) received “booster” transplants due to poor B- or T-cell function or problems with engraftment. Although not clearly detailed, at least 15 of the 20 patients who required booster transplants were patients who received haploidentical grafts. With a median follow up of 5.6 years, 72 of the total 89 patients (81%; 100% HLA-identical, 78% haploidentical) were alive. The most common causes of death were viral infections. Of the 81 patients, infants <3.5 months of age had superior survivals (95%) compared to patients who were 3.5 months or older (76%). T-cell immune recovery was significantly delayed in recipients of T-cell depleted marrow (3-4 months) compared to HLA-identical marrow recipients (2 weeks). Other studies have also found a delay in immune reconstitution in patients with immunodeficiency disorders who received HLA nonidentical T-cell depleted bone marrow transplantation [19].

Filipovich et al. summarized the experience of the International Bone Marrow Transplant Registry and the National Marrow Donor Program for 170 patients with Wiskott-Aldrich Syndrome who received transplantation with HLA-identical sibling donors (n=55), unrelated donors (n=67), and other related relatives (n=48) [12]. The 5-year probabilities of survival by donor type were 87% for HLA-identical sibling recipients, 71% for unrelated donor recipients, and 52% for recipients of “other” related donors. A higher percentage of patients who received grafts from HLA-identical sibling or unrelated donors were cured of their disease (both 75%) versus patients who received grafts from other related donors (57%). The incidence of grade II-IV acute GVHD was 16%, 56%, and 30% for recipients of related, unrelated and “other” related donors, respectively. More recently, Dal-Cortivo et al. reported on the outcome of 40 patients with primary immunodeficiencies (SCID n=32, other n=8) conditioned with busulfan and cyclophosphamide followed by T-cell depleted HLA-haploidentical grafts. Mixed or full donor chimerism (exact numbers not given) was achieved in 24 of the 38 evaluable patients. Grade II-IV GVHD occurred in 11/24 (46%) of patients. Overall survival was 21/40 (53%), with infection being the primary cause of death (8 of 19 deaths) [13]. Klein et al. reported on 19 patients with major histocompatibility complex class II deficiency, who received HLA-identical BMT (n=7) or HLA-haploidentical BMT (n=12). Four of the 8 HLA-identical recipients and 4 of the 12 haploidentical recipients were cured (n=3) or had improvement (n=1) of their disease with mixed or full donor chimerism [14].

Buckley et al. summarized the outcome of patients with immunodeficiencies who received bone marrow transplantation from HLA-identical, unrelated donors, or haploidentical donor grafts [11] (**Table 1**). When comparing overall survivals, patients who received HLA-identical grafts had superior survivals (79%) compared to patients who received unrelated donor grafts (65%) or haploidentical donor grafts (55%).

Table 1: Outcome of patients with Immunodeficiency Disorders who received HLA-identical donor, HLA-unrelated donor or Haploidentical donor grafts			
Type of Immune Defect	HLA-Identical Donor	Unrelated Donor	Haplo Donor
	# survived/ # transplanted	# survived/ # transplanted	# survival/ # transplanted
SCID	105/125	12/17	260/425
Wiskott-Aldrich Syndrome	57/65	20/29	20/58
Combined Immune Deficiency	10/13	0/2	12/35
Omenn Syndrome	9/12	3/6	8/24
Leukocyte Adhesion Defect	6/8	2/2	14/20
MHC Class II antigen deficiency	4/8	2/2	7/20
Chediak-Higashi Syndrome	10/12	5/5	1/5
DiGeorge Syndrome	2/3	0/0	0/3
Chronic Granulomatous Disease	8/12	2/3	0/0
PNP Deficiency	2/5	0/1	2/2
Cartilage-hair Hypoplasia	3/3	0/1	1/3
Hyper-IgM Syndrome	2/3	1/1	1/3
Immunodeficiency with Albinism	3/6	0/0	0/0
X-linked lymphoproliferative disease	3/5	0/1	0/0
Ataxia Telangiectasia	0/1	0/2	0/0
Common variable immunodeficiency	0/4	0/0	0/0
Capping Deficiency	1/1	0/0	3/3
IL-2 Deficiency	0/0	0/0	2/2
Neutrophil Disorders	2/2	0/0	0/0
ZAP70 Deficiency	0/0	0/0	1/1
Chronic Mucocutaneous candidiasis	0/0	0/0	0/1
Neutrophil Actin Dysfunction	1/1	0/0	0/0
All defects	228/289 (79%)	47/72 (65%)	332/605 (55%)

2) Other inherited disorders

Data on patients who were transplanted with haploidentical grafts for inherited disorders other than immunodeficiency disorders is limited. Peters, et al. reported outcome for 54 patients with Hurler syndrome treated with conventional marrow transplantation from HLA-identical related (n=28) or haploidentical donors grafts (n=26). Patients were conditioned with chemotherapy with or without TBI [15]. T-cell depleted grafts were given to 15 recipients of haploidentical marrow, and the remaining patients received T-cell replete grafts. Among the 26 evaluable recipients of HLA-identical related grafts, 15 (58%) achieved full donor chimerism, 7 (27%) achieved mixed donor-host chimerism, and 4 (15%) failed to engraft. Among the 26 recipients of haploidentical grafts, 16 (62%) achieved full donor chimerism, 1 (4%) achieved mixed donor-host chimerism, and 9 (35%) failed to engraft. With a median follow up of 7.3 years for the HLA-identical recipients and 4.6 years for the recipients of haploidentical grafts, the overall actuarial probability of survival at 5 years was 75% for the HLA-identical recipients and 53% for the haploidentical recipients. Grade II-IV acute and extensive chronic GVHD occurred in 29% and 0% of HLA-identical recipients and 46% and 19% of recipients of haploidentical grafts. Death from GVHD occurred in 6 patients (HLA-identical n=1, haploidentical n=5). Enzyme levels were assessed in 28 patients (20 HLA-identical, 8 haploidentical) surviving > 1 year. Among the 10 recipients of homozygous normal donor marrow, levels of leukocyte α -L-iduronidase normalized in 6 and were between 25-100% in 3. Among 18 recipients of heterozygous carrier donor marrow, 15 achieved carrier level of enzyme activity and 3 achieved levels in the lower limit of carrier range. Neurologic outcome was favorably associated with transplantation before age 24 months and achievement of normal enzyme levels. Kapelushnik et al. reported on 1 patient with Hurler's syndrome who received a T-cell depleted 3/6 matched haploidentical PBMC graft from his sister following conditioning with fludarabine (30 mg/m²/day; days -10 to -5), busulfan (4 mg/kg/day; days -7 to -6), ATG (10 mg/kg/day; days -4 to -1) and 750 cGy TBI (day -1) [16]. The patient fully

engrafted, did not have significant GVHD, and had correction of α -L-iduronidase levels at time of last follow up; 30 months after HCT. Although these data are limited, the results are encouraging for the use of haploidentical donors for patients with Hurlers when a related or unrelated donor is unavailable. Extending potential stem cell sources to include haploidentical grafts is particularly important for patients with Hurlers syndrome due to only 30-40% of the patients who need HCT have related donors and the length of time required to find an unrelated donor may be prohibitively long, particularly for patients with rapidly progressive disease.

Osteopetrosis is an autosomal recessive disease resulting from lack of osteoclast resorption of bone, resulting in marrow failure and nerve entrapment, and early death. Normally functioning osteoclasts can be restored by HCT, resulting in amelioration of disease symptoms [2]. Schulz et al. recently reported on the outcome of 7 patients with Osteopetrosis who received T-cell depleted HLA-haploidentical grafts following conditioning with Busulfan, Thiotepa and either cyclophosphamide (n=5) or fludarabine (n=2) [17]. Five of the 7 patients had stable donor engraftment. Of the 2 patients with graft failure, 1 died and the other patient engrafted following transplantation with a second graft from the same donor without conditioning. Three patients did not have any significant complications; however, 4 patients had veno-occlusive disease and respiratory failure. With a median follow up of 47 (range, 25-60) months, 5 patients are alive with stable to improved disease. Although the number of patients treated in this study was small, these results are encouraging; however, transplant related toxicity was high. Therefore, exploring a nonmyeloablative approach for those patients who do not have HLA-matched related or unrelated donors is warranted.

Caillat-Zucman et al. reported on the outcome of 94 children who received HCT from HLA-identical sibling donors (n=31), unrelated donors (n=23) or haploidentical donors (n=40) for primary immunodeficiency disorders (n=64), metabolic diseases (n=12) or nonmalignant hematological diseases (n=18) [18]. Patients primarily received conditioning with busulfan and cyclophosphamide. All recipients of HLA-haploidentical marrow received either T-cell depleted or positive selection of CD34+ cells for GVHD prevention. Of the 90 patients evaluable for engraftment, 83 (92%) engrafted and there was no significant difference in the engraftment rate between the 3 groups. With a median follow up for the living recipients of 63 (range, 41-90) months, superior survivals were seen for recipients of HLA-identical grafts (80.6%) compared to recipients of unrelated grafts (63%) or haploidentical grafts (48%). Infections were the primary cause of death in all patients.

These studies demonstrate myeloablative HCT with HLA-haploidentical grafts to be a viable treatment option for patients with nonmalignant disorders who do not have HLA-matched related or unrelated donors. However, inferior survivals were seen for recipients of HLA-haploidentical grafts. Death from transplant related toxicities primarily infections and GVHD were significant problems. Therefore, strategies aimed at reducing transplant related toxicities without compromising engraftment are needed.

3B. Haploidentical Transplantation Using a Nonmyeloablative Approach

The primary problem of HCT from related, HLA-haploidentical donors is controlling the potent alloreactivity of host T-cells that can lead to rejection of the graft and of donor T-cells that can lead to severe GVHD. Many approaches to this problem are possible. Protocol 2032 will incorporate a combination of immunosuppressive agents in a strategy designed to achieve control of host and donor immune responses with the objective of safe donor engraftment. The platform for this protocol is the approach to nonmyeloablative HCT pioneered by Storb and colleagues at the FHCRC which utilizes an immunosuppressive regimen that includes 2 Gy TBI before transplant (with or without fludarabine) to reduce the risk of graft rejection and post grafting MMF and CSP to control both GVHD and residual HVG reactions [22]. This regimen has been proven to be minimally toxic, well tolerated, and leads to successful engraftment in a high percentage of patients with malignant and nonmalignant hematologic diseases who received HLA-matched related and unrelated grafts [23,24]. However, additional immunosuppression will be needed with this regimen to achieve the objective of safe, nonmyeloablative HCT from HLA-

haploidentical donors [25,26]. Based on the studies of investigators at Johns Hopkins Medical School and the FHCRC (see below), this protocol will provide additional immunosuppression with the alkylating agent cyclophosphamide, before and after transplant, aimed at removing alloreactive host and donor T-cell clones presumably involved in both rejection and GVHD.

3C. Rationale for the Use of Cyclophosphamide

Cyclophosphamide was introduced into the clinical practice of marrow transplantation in 1969/1970, and has remained a major component of many transplantation regimens. Historically, due to its immunosuppressive abilities it was used pre HCT to suppress HVG reactions and improve engraftment [27-29]. The use of cyclophosphamide post HCT was deterred due to concerns that it might be myelotoxic, however, it is now clear that a single low dose of CY does not cause myeloablation [30]. The immunosuppressive rather than myelosuppressive effects of cyclophosphamide are due to its unique pharmacology. Specifically, hematopoietic stem cells express high levels of aldehyde dehydrogenase, an enzyme responsible for cellular resistance to cyclophosphamide. However, B-cells, T-cells and natural killer cells express low levels of this enzyme and are very sensitive to the cytotoxic properties of cyclophosphamide [31]. Therefore, the use of a single dose of cyclophosphamide early after HCT during the activation of alloreactive effector cells, results in cytotoxic activity against both HVG and GVH reactions. Studies have demonstrated that post transplant cyclophosphamide results in destruction of antigen-stimulated, proliferating T-cells (alloactivated) in the peripheral blood [32] and intrathymic clonal deletion of reactive T-cells [32-34], thereby improving engraftment and decreasing GVHD.

3D. Methodology of Using Cyclophosphamide post HCT

1) Pre-clinical data: Results in a Mouse Model of nonmyeloablative HCT using Haploidentical donors

Luznik et al. published his results of transplantation using nonmyeloablative conditioning followed by Haploidentical grafts in the mouse model [35]. In this model, pre-transplant immunosuppression consisted of fludarabine and low-dose total body irradiation (TBI, 200 cGy). High-dose cyclophosphamide was then given on day +3 post-HCT to remove highly alloreactive T-cells of the donor as described by Nomoto's group (for a review, see Mayumi, H., et al) [36]. Successful induction of stable, mixed chimerism was achieved across total MHC disparity in congenic mice (6/6 H-2 mismatch, no minor histocompatibility differences) and in haploidentical strain combinations (3/6 H-2 mismatch, minor histocompatibility differences) with a decreased incidence of GVHD compared to a set of control mice that were not given cyclophosphamide after HCT. Another set of control mice not given a marrow graft recovered autologous hematopoiesis.

2) Results of a Phase 1 Trial Using Cyclophosphamide pre and post HCT.

Based on the data of the effect of cyclophosphamide on prevention of rejection and induction of donor tolerance in the mouse model, a phase I clinical trial was conducted [37]. The major objective of this clinical trial was to determine the minimum dose of pre-transplantation cyclophosphamide required for prevention of allograft rejection. In addition, to reduce the risk of GVHD, all patients received immunosuppression after HCT with high-dose cyclophosphamide plus the combination of mycophenolate mofetil (MMF) and a calcineurin-inhibitor, tacrolimus. Two cohorts of patients existed. The first cohort (n=3) did not receive any pre-transplantation cyclophosphamide. The second cohort (n=10) received 14.5 mg/kg cyclophosphamide on days -6 and -5. All patients received fludarabine (30 mg/m²/day; days -6 to -2) and 200 cGy TBI (day -1) followed by HLA-mismatched (up to 3 HLA antigens) related marrow allografts. In addition, all patients received 50mg/kg cyclophosphamide on day +3. The first 9 patients received MMF (15 mg/kg twice daily; days 4-35) and tacrolimus (initial dose 1 mg daily; days 4-50). Due to 3 of the first 6 patients developing GVHD after day 100, tacrolimus was extended beyond day 90. Median CD34 and CD3 cell doses were 5.3 +/- 1.5 x 10⁶ and 3.2 +/-

0.6×10^7 cells/kg, respectively. Of the patients in cohort 1 (no pre-HCT cyclophosphamide), 1 patient engrafted but died of relapsed disease and the remaining 2 patients did not engraft. One patient had autologous recovery and the other patient died of infection during aplasia. Both patients who rejected had a long history of transfusion-dependence (≥ 1 yr) and, in retrospect, one patient was found to be cross-match positive with her donor prompting a revision of the eligibility criteria to exclude such patients subsequently. Of the 10 patients in cohort #2, sustained donor cell engraftment occurred in 8 patients. Two patients failed to engraft and both had MDS, a long history of transfusion dependence and no prior chemotherapy; known risk factors for graft rejection. Six patients developed acute GVHD (Grade II $n=3$, grade III $n=3$) and 1 patient developed limited chronic GVHD. An additional patient developed grade III GVHD after donor lymphocyte infusion for relapsed AML. With a median follow up for the living recipients of 284 (range 185-423) days, 6 of the 10 patients were alive. Immune reconstitution was evaluated in 6 patients in cohort 2 and demonstrated recovery of CD4 counts to >200 cells/ml by day 40 in 4/6 patients and recovery of NK cells to pretransplantation levels by day 40 in 5/6 patients tested.

The level of immunosuppression used in this protocol before and after HCT showed promise in performing nonmyeloablative HCT from related, HLA-haploidentical donors. Transplant-related toxicity was relatively low in an older group of patients with advanced hematologic malignancies who were transplanted with allografts from related, HLA-haploidentical donors containing $>10^7$ CD3⁺ cells/kg. Based on these results a phase II study was developed at the FHCRC to evaluate the efficacy and safety of this regimen in patients with high-risk hematologic malignancies.

3) Nonmyeloablative Hematopoietic Stem Cell Transplantation for Patients with High-Risk Hematologic Malignancies using Related, HLA-Haploidentical Donors: A Phase II Trial of Combined Immunosuppression Before and After Transplantation – Preliminary Results (protocol 1667)

Twenty five patients with high-risk hematologic malignancies (AML ($n=6$), MDS or MDS/AML ($n=5$), CML ($n=2$), ALL ($n=2$), NHL ($n=2$), HD ($n=7$) and MM ($n=1$)) who were ineligible for conventional transplant and did not have an HLA-matched related or unrelated donor were entered onto this phase II clinical study (protocol 1667). (**Table 2**) Patients received Cyclophosphamide (14.5 mg/kg days -6 and -5), Fludarabine (30 mg/m²/day, days -6 to -2), and 200 cGy TBI (day -1) followed by bone marrow grafts on day 0 from HLA-mismatched related donors (8/10 mismatch ($n=4$), 7/10 mismatch ($n=3$), 6/10 mismatch ($n=4$) and 5/10 mismatch ($n=14$)) followed by postgrafting immunosuppression with MMF and Tacrolimus. In addition, all patients received 50mg/kg/dose cyclophosphamide on day +3. The primary endpoints of this study are efficacy (true engraftment rate of 85% with $>50\%$ donor chimerism at day 84) and safety ($<35\%$ grade III-IV GVHD and $<25\%$ day 200 non-relapse mortality). Median age of patients was 44 (range 20-67) years. The median CD34 and CD3 cell doses were $5.3 (2.7-12.8) \times 10^6$ and $4.9 (2.0-7.2) \times 10^7$ cells/kg, respectively. Median neutrophil recoveries occurred at 13 (range, 11-42) days after transplant. Three patients (14%) rejected their grafts at days 12, 13, and 42 after transplant and all had autologous hematopoietic recovery. In addition, all three patients received grafts from 5/10 HLA mismatched related donors (GVHD mismatched vector ($n=1$), bi-directional mismatched vector ($n=2$)). Of the remaining 22 patients, 19 were evaluable for engraftment at day 84. Of the 19 patients, 18 had full donor CD3 and CD33 chimerism defined as donor chimerism $>95\%$. Only \leq grade II acute GVHD was seen (12 patients (67%) grade II, 3 patients (19%) grade I). Five patients (20%) developed extensive chronic GVHD. With a median follow up of the living recipients of 10 (range, 3-26) months, 14 patients have died from relapse ($n=10$), transplant related toxicities (infections, $n=2$), secondary AML ($n=1$), or unknown causes ($n=1$).

Despite the low incidence of graft rejection (3/25 = 12%), protocol 1667 will be amended to increase the pre-transplant cyclophosphamide dose from 14.5 mg/kg (days -6 and -5) to 25 mg/kg (days -6 and -5) with the goal of increasing host immunosuppression and further decreasing the risk of graft rejection.

Table 2: Characteristics and outcome of 25 patients who received HCT with nonmyeloablative conditioning followed by HLA-haploidentical grafts for high-risk hematologic malignancies.

Pt #	Diagnosis	Age	HLA-Mismatch/Donor	Status at HCT	D84 chimerism CD3/CD33	Sustained Engraftment	GVHD		F/U (mos)	Cause of Death
							Acute (Grade)	Ext Chronic		
1	AML	42	5/10 sib	CRp	100/100	+	II	-	†6	Relapse
2	AML	33	5/10 parent	CR2	N/A	-	-	-	†7	Relapse
3	AML	67	7/10 child	CR3	N/A	+	II	-	†3	Relapse
4	AML	35	5/10 parent	CRp	100/100	+	-	-	†4	Relapse
5	AML	51	5/10 child	Ref	100/100	+	-	-	†8	Relapse
6	AML	60	5/10 child	CRt	100/100*	+	-	-	>3	
7	MDS/AML	60	5/10 child	CRp	100/100	+	II	-	†22	Relapse
8	MDS/AML	60	5/10 sib	CR1	N/A	+	-	N/A	†1	Infection
9	MDS/AML	62	8/10 sib	CRp	97/100	+	II	-	†4	Relapse
10	MDS/AML	34	5/10 sib	CR2	N/A	-	N/A	N/A	>7	
11	MDS	37	5/10 parent	CRp	100/100	+	-	+	>3	
12	Ph+ ALL	36	5/10 sib	MRD	N/A	-	N/A	N/A	>16	
13	ALL	51	6/10 child	Ref	100/100	+	II	+	>10	
14	CML	66	8/10 child	AP	92/2	+	II	+	†10	Infection
15	CML	51	6/10 child	Ref	100/100	+	II	-	>13	
16	MM	63	6/10 sib	Ref	100/100	+	II	+	†8	Relapse
17	NHL	59	6/10 sib	Ref	100/100	+	II	-	†5	2° AML
18	NHL	44	7/10 child	CR	100/100	+	II	-	†6	Unknown
19	HD	26	5/10 parent	Ref	N/A	+	-	N/A	†1	Relapse
20	HD	20	7/10 parent	SD	100/100	+	-	-	>26	
21	HD	33	5/10 parent	Ref	100/100	+	-	-	>22	
22	HD	34	8/10 sib	Ref	100/95	+	-	-	†12	Relapse
23	HD	51	8/10 sib	CR	100/100	+	II	+	>11	
24	HD	31	5/10 parent	Relapse	100/100	+	II	-	>4	
25	HD	26	5/10 parent	SD	100/100	+	-	-	>3	

AML = acute myelogenous leukemia, CML = chronic myelogenous leukemia, CR = complete remission, CRp = complete remission (<5% blasts) with pancytopenia or thrombocytopenia, Ext chronic = extensive chronic GVHD, F/U = follow up, HD = Hodgkin disease, MDS = myelodysplastic syndrome, MM = multiple myeloma, MRD = minimal residual disease, N/A = not applicable, NHL = non Hodgkin Lymphoma, Ph+ ALL = Philadelphia chromosome positive acute lymphoblastic leukemia, Ref = refractory, SD = stable disease, > alive, † dead
 * Patient #6 day 56 peripheral blood chimerism was 100% CD3, 100% CD33. The day 84 bone marrow was not sorted but was 100% donor.

Although relapse related mortality in this group of patients with high-risk hematologic malignancies was high (10/14 patients), only 2 patients died from transplant related causes. This is particularly relevant for patients with nonmalignant diseases where death from relapse is not an issue and a low incidence of transplant related mortality is desirable. In addition, although preliminary, > 75% of the patients engrafted and there was a low incidence of grade III-IV (0%) and extensive chronic GVHD (20%). Again, this is particularly appealing for patients with nonmalignant diseases who do not need a graft versus leukemia effect from GVHD. Encouraged by both the phase I and II clinical studies for patients with hematologic malignancies, a Phase I-II study is warranted to determine the safety and efficacy of this approach in patients with nonmalignant disorders. Similar to what will be done for patients with hematologic malignancies; protocol 2032 will use a higher dose of pre-transplant cyclophosphamide with the goal of improving donor engraftment and decreasing graft rejection. Specifically, patients will receive 25 mg/kg (days -6 and -5) instead of 14.5 mg/kg (days -6 and -5).

We have enrolled 5 patients with nonmalignant diseases on protocol 2032 to date, and have observed a high incidence of both acute grades II (n=3) or III (n=1) GVHD and chronic GVHD (n=4). A phase II trial randomized three-arm trial of post-transplant immune suppression following nonmyeloablative conditioning for recipients of unrelated PBSC grafts showed that the addition of sirolimus to a regimen

of tacrolimus and MMF was superior in preventing acute GVHD [Kornblit, B, et al, 2014 Haematologica, In Press]. Specifically, the incidence of grades II-IV acute GVHD were 64% for the arm that included tacrolimus and MMF given similarly to the current 5 patients, compared to 51% for the arm with a lengthened course of MMF, compared to 46% for the arm that included sirolimus in addition to tacrolimus and MMF (hazard ratio 0.59, p=.02). Since relapse is not a concern for patients with nonmalignant diseases, and consequently there is no benefit for GVHD, the protocol was revised to include sirolimus in addition to tacrolimus and MMF for GVHD prophylaxis.

4. OBJECTIVES

It is important to extend the option of nonmyeloablative HCT for potential therapy of patients with nonmalignant disorders to patients who do not have HLA-matched related or unrelated donors. Almost all patients would have a related donor identical for one HLA haplotype (haploidentical) and mismatched at HLA-A, B or DR of the unshared haplotype. The central problem of HCT from related, HLA-haploidentical donors is controlling the potent alloreactivity of host T-cells, which can lead to rejection of the graft and of donor T-cells, which can lead to severe toxicity from GVHD. Many approaches to this problem are possible. This protocol will incorporate a combination of immunosuppressive agents in a strategy designed to achieve control of host and donor immune responses with the objective of safe donor engraftment. Specifically, cyclophosphamide will be administered before and after HCT to facilitate engraftment and to remove highly reactive T-cell clones presumably involved in GVHD.

4A. Primary Objective

1) Safety

Determine safety of nonmyeloablative conditioning and HCT from HLA-haploidentical related donors for patients with nonmalignant inherited disorders who do not have an HLA-matched related or unrelated donor.

4B. Secondary Objectives

1) Efficacy

Determine whether nonmyeloablative conditioning and HCT from an HLA-haploidentical related donor graft can establish mixed chimerism (>5% CD3+ donor T-cell chimerism) in patients with nonmalignant inherited disorders.

- 2) Transplant related mortality at day 100
- 3) Incidence and severity of GVHD
- 4) Immune Reconstitution
- 5) Infections during the first 200 days after HCT

5. PATIENT SELECTION

5A. Patient Inclusions

- 1) Age < 55 years with primary immunodeficiency disorder or other nonmalignant inherited disease (except fanconi anemia) treatable by allogeneic HCT.
- 2) Patients with pre-existing medical conditions or other factors that renders them at high risk for regimen related toxicity or ineligible for conventional myeloablative HCT and who do not have HLA-matched related or unrelated donors.
- 3) Patients with a related donor who is identical for one HLA haplotype.

- 4) Acquired Aplastic anemia: SAA is defined as follows:
- Bone marrow cellularity < 25%, or marrow cellularity < 50% but with < 30% residual hematopoietic cells.
 - Two out of three of the following (in peripheral blood): neutrophils < $0.5 \times 10^9/L$; platelets < $20 \times 10^9/L$; reticulocytes < $20 \times 10^9/L$.

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

5B. Patient Exclusions

- 1) Fanconi Anemia
- 2) **Suitably HLA- matched related or unrelated donors**
- 3) Patients with metabolic storage diseases who have severe CNS involvement of disease, defined as IQ score < 70.
- 4) Patients with the following organ dysfunction:
 - a. **Cardiac:**
 - i) Cardiac ejection fraction < 30% (or, if unable to obtain ejection fraction, shortening fraction < 26%) on MUGA scan or cardiac echo, symptomatic coronary artery disease, other cardiac failure requiring therapy. Patients with a history of, or current cardiac disease should be evaluated with appropriate cardiac studies and/or cardiology consult. Patients with a shortening fraction of <26% must be seen by cardiology for approval.
 - ii) Poorly controlled hypertension despite anti-hypertensive medications.
 - b. **Hepatic:** Patients with clinical or laboratory evidence of liver disease will need to be evaluated for the cause of the liver disease, its clinical severity in terms of liver function and the degree of portal hypertension. Patients will be excluded if they are found to have: fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin > 3mg/dl, or symptomatic biliary disease.
- 5) Positive for human immunodeficiency virus (HIV).
- 6) Females who are pregnant (β -HCG⁺) or breast-feeding.
- 7) Fertile men or women who are unwilling to use contraceptives during HCT and up to 12 months post-treatment.
- 8) Patients with fungal pneumonia with radiological progression after receipt of amphotericin formulation or mold-active azoles for greater than 1 month will not be eligible for this protocol (either regimen A or B).

6. DONOR SELECTION – See Standard Practice Manual

6A. Donor Inclusions

- 1) Related donors who are identical for one HLA haplotype.
- 2) Bone marrow will be the only allowed stem cell source.

6B. Donor Exclusions

- 1) Donor-recipient pairs in which the HLA-mismatch is only in the HVG direction. Patients are homozygous and donor is heterozygous.

- 2) Donors who are not expected to meet the minimum target dose of marrow cells (1×10^8 nucleated cells/kg recipient Ideal Body Weight)
- 3) HIV-positive donors
- 4) A positive anti-donor cytotoxic cross match is absolute donor exclusion.
- 5) Donor < 6 months old and > 75 years old.

7. INFORMED CONSENT

The outpatient attending physician will conduct a conference with the patient and family to discuss this study and alternative treatments available. In a separate conference, the risks of the donation procedure will be outlined to the donor and/or his parent/guardian(s), including the risks of anesthesia, bleeding, and infection for marrow donors. The goals of the study, requirement for data collection, and requirement for release of medical records will be discussed with the patient and/or his/her parent/guardian. All potential risks associated with the use of fludarabine, low dose TBI, Cyclophosphamide, immunosuppressive drugs and allogeneic HCT will be discussed as objectively as possible. Discussion of potential complications should include graft rejection, GVHD, infections, and death. It should be explained that patients offered this protocol have an inherited disorder and are at high risk of early transplant mortality from conventional allogeneic HCT due to associated co-morbidities.

Informed consent from the patient and donor will be obtained using forms approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center. Informed consent will be obtained by the Principal Investigator, Co-Investigator, or Attending Physician who is familiar with the study but not necessarily an investigator. When patients are less than 18 years of age, consent will be obtained from parents or legal guardian(s). All patients enrolled at collaborating centers will engage in institution-specific informed consent conferences after completion of the pre-transplant evaluation. Informed consent from the donor and patient will be obtained using a form approved by the Institutional Review Board for each treatment center. A summary of the conference will be dictated for the medical record detailing what was covered.

8. PROTOCOL REGISTRATION

8A. FHCRC Patients: Eligible patients will be identified by the Clinical Coordinator's Office who will register patients with the Registration office (206-667-4728) between 8:30 am and 4:00 pm, Monday through Friday. After hours, the Registration Office can be reached by paging (206)-995-7437

8B. Collaborating Institutions: Eligible patients will be identified by the principal investigator of the collaborating institution who will register the patient with the FHCRC Registration Office. Registration will include completion of the eligibility checklist and demographic form (**Appendix H of protocol**). This form and a copy of the signed informed consent will be faxed to the Trial Coordinator (206-667-4427). Questions regarding eligibility or protocol information should be directed to the Principal Investigator, Lauri Burroughs (206) 667-2396 or her staff at (206) 667-7385 or (206) 667-4916..

9. PLAN OF TREATMENT

9A. Outline of Treatment Plan (Scheduling is Shown in Table 3)

Days -6, -5

Fludarabine 30 mg/m² IV once per day
Based on estimated creatinine clearance
Cyclophosphamide 25mg/kg IV once per day
Mesna (dosed at 100% cyclophosphamide dose)

Days -4 to -2

Fludarabine 30 mg/m² IV once per day
Based on estimated creatinine clearance

Day -1

- 200 cGy TBI at 6-15 cGy per min
- Patients with acquired aplastic anemia, sickle cell disease, thalassemia, HLH, or other non-malignant disease at high risk for graft rejection (as discussed at the pediatric non-malignant arrival meeting, non-malignant board, patient care conference, or with study PI's) will receive 400 cGy TBI (2 doses of 200 cGy) at 6-15 cGy per min.

Day 0

Infuse marrow allograft

Day +3, +4

Cyclophosphamide 50 mg/kg (First dose must be administered 48-72 hr post-BMT)
Mesna (100% cyclophosphamide dose)

Day +5

Tacrolimus:

0.03 mg/kg/day continuous IV infusion over 22-24 hours and continue until day + 100 with taper through day +180.

Tacrolimus may be converted from IV to oral (twice daily or three times daily dosing per standard practice) when the patient is able to take medications orally and has a therapeutic drug level.

MMF: 15 mg/kg orally every 8 hours and continue until day +30; then BID to day +40 and then if there is no evidence of active GVHD and donor engraftment (CD3) are >95% (or approved by the PI) the MMF may be tapered by roughly 10% per week and discontinued around day +96. MMF can be tapered faster if there is evidence of count suppression following discussion with the PI.

Sirolimus:

≤1.5 m²: 1 mg/m² orally every day until day +180 then taper until approximately day +210.

>1.5 m²: 2 mg orally every day until day +180 then taper until approximately day +210.

G-CSF

(5 µg/kg/day) IV or SC, continue until ANC>500/µl x 3d

Note: Patients with sickle cell disease will not receive G-CSF.

Table 3: Outline of Treatment Plan	
Day	Treatment Plan
-6 to -5	CY, 25 mg/kg/day (with Mesna), Fludarabine, 30 mg/m ² /day (based on estimated creatinine clearance)
-4 to -2	Fludarabine, 30 mg/m ² /day (based on estimated creatinine clearance)
-1	200 cGy TBI at 6-15 cGy/min Or 400 cGy TBI (2 doses of 200 cGy) for patients with Acquired Aplastic Anemia, sickle cell disease, thalassemia, HLH, or other non-malignant diseases at high risk for graft rejection (as discussed at the pediatric non-malignant arrival meeting, non-malignant board, patient care conference, or with study PI's) at 6-15 cGy/min
0	Infuse Marrow allograft
+3, +4	CY, 50 mg/kg/day (with Mesna)
+5	Start G-CSF 5 µg/kg/day and continue until absolute neutrophil count > 500/µl x 3 days. Patients with sickle cell disease will not receive G-CSF. Start MMF (15 mg/kg orally every 8 hours) and continue until day +30; then BID until day +40 and then if there is no evidence of active GVHD and donor engraftment (CD3) are >95% (or approved by the PI) the MMF may be tapered by roughly 10% per week and discontinued around day +96. MMF can be tapered faster if there is evidence of count suppression following discussion with the PI. Start Tacrolimus 0.03 mg/kg/day IV and continue until day +100 with taper through day +180. Tacrolimus may be converted from IV to oral (twice daily or three times daily dosing per standard practice) when the patient is able to take medications orally and has a therapeutic drug level. Start Sirolimus 2 mg (>1.5 m ²) or 1 mg/m ² (≤1.5 m ²) orally per day and continue until day +180 then taper to day +210.

9B. Indwelling central venous catheter

Placement of a double lumen central venous catheter will be required for administration of IV medications and transfusion of blood products.

10. CONDITIONING REGIMEN

Menstruating female patients should be placed on an anti-ovulatory agent prior to initiating the conditioning regimen.

10A. Fludarabine

- 1) Fludarabine dose is based on estimated creatinine clearance:

Adults: creatinine clearance may be estimated by the Cockcroft Formula:

$$C_{Cr} = \frac{(140 - \text{age}) \times \text{IBW (kg)}}{P_{Cr} \times 72} \times 0.85 \text{ (for women)}$$

Pediatrics (Age <17): creatinine clearance may be estimated by the Swartz formula or appropriate calculation for pediatric patients

- 2) Fludarabine is administered by IV infusion over 1 hour on days -6 to -2

If estimated creatinine clearance is less than 60 ml/min, obtain a formal clearance with a 24 hour urine collection, iothalamate or equivalent study.

Creatinine Clearance ml/min	Daily Fludarabine Dose (mg/m ²)
> 60	30
46-60	24
31-45	22.5
21-30	19.5
<20	15

**** The dose of fludarabine is based on M², the calculation is always based on the Actual Body Weight (See appendix L)**

10B. Cyclophosphamide (See Appendix K)

- 1) Cyclophosphamide will be administered at a dose of 25 mg/kg/day as an IV infusion over 1 hour on days -6 and -5 (Total dose = 50mg/kg)
- 2) The dose should be based on the Adjusted Body Weight if the patient's Actual Body Weight is > 100% of the Ideal Body Weight. If the Ideal Body Weight (IBW) > Actual Body Weight, then Actual body weight will be used.
- 3) Please see appendix K for mesna and fluid administration guidelines.

10C. Total body irradiation (See Appendix M for guidelines for TBI administration)

200 cGy TBI will be given on day -1 at a rate of 6-15 cGy per min per TBI administration guidelines (**Appendix M**). Patients with acquired aplastic anemia, sickle cell disease, thalassemia, or other non-malignant disease at high risk for graft rejection (as discussed at the non-malignant board) will receive 400 cGy TBI (2 doses of 200 cGy) on day -1 at a rate of 6-15 cGy per min per TBI administration guidelines (Appendix M)

11. BONE MARROW INFUSION

Donor bone marrow will be harvested with a target yield of 4 x 10⁸ nucleated cells/kg recipient IBW. The minimum acceptable yield should be 1 x 10⁸ nucleated cells/kg recipient IBW. The CD34 and CD3 composition of allografts will be determined by flow cytometry.

See Appendix A for ABO Incompatibility guidelines.

12. POST-TRANSPLANT IMMUNOSUPPRESSION

Immunosuppression to permit engraftment and provide GVHD prophylaxis will be performed with MMF and Tacrolimus. MMF and Tacrolimus treatment are described below and are depicted in Table 3

12A. Cyclophosphamide:

Day +3 and +4: Cyclophosphamide [50mg/kg/day Adjusted Body Weight, unless Ideal Body Weight >Actual Body Weight, then Actual Body Weight] will be given on days +3 and +4 after transplant. (See **Appendix K**) The first dose of Cyclophosphamide (day +3) will be given within 48-72 hr of marrow infusion. Cyclophosphamide will be given as an IV infusion over 1 hr with Mesna and appropriate hydration. (See **Appendix K**) Monitoring of urine output and for hematuria will be performed similarly.

12B. Tacrolimus

Day +5: Starting on day +5, tacrolimus will be given at a dose of 0.03 mg/kg/day continuous infusion over 22-24 hours. Tacrolimus is dosed based on adjusted body weight. If actual weight is less than adjusted body weight; dosing should be based on actual weight. Tacrolimus can be changed to a PO dosing schedule (Q12 or Q8 dosing per standard practice) once oral medications are tolerated and once a therapeutic level (5-10 ng/ml) is achieved. Serum levels of tacrolimus should be measured on day +8 and then twice weekly thereafter and the dose adjusted accordingly to maintain a level of 5-10 ng/ml. Tacrolimus will be tapered after day +100 by 5% per week, provided there is no evidence of GVHD. In addition, in the case of <95% CD3 donor chimerism, continuation of therapeutic tacrolimus beyond day 100 should be determined in consultation with the PI. The combination of sirolimus and tacrolimus can lead to certain toxicities that are increased when the levels of tacrolimus and sirolimus are high. Once a patient has stable donor engraftment, the combined targeted level of sirolimus and tacrolimus should not exceed 16 ng/mL and the tacrolimus level should be <8 ng/mL.

12C. MMF

Day +5: Starting on day +5, MMF will be given orally at a dose of 15 mg/kg based on adjusted body weight every 8 hours. MMF will be switched to BID dosing on day +30 and BID to day +40 and then if there is no evidence of active GVHD and donor engraftment (CD3) are >95% (or approved by the PI) the MMF may be tapered by roughly 10% per week and discontinued around day +96. MMF can be tapered faster if there is evidence of count suppression following discussion with the PI. Myfortic may be used in place of MMF as clinically indicated.

12D. Sirolimus

Day +5: Starting on day +5, Sirolimus will be given orally at a dose of 2 mg (for patients >1.5 m²) or 1 mg/m² (for patients ≤1.5 m²) daily through day +180. Dosing should be adjusted to maintain a target blood level of 3-12 ng/mL. Sirolimus should be tapered at day +180 by 25% per week for 4 weeks and discontinued around day +210, provided there is full donor chimerism. In the case of donor chimerism <95% CD3 or treatment for GVHD, continuation of sirolimus should be determined in consultation with the PI. The combination of sirolimus and tacrolimus can lead to certain toxicities that are increased when the levels of tacrolimus and sirolimus are high. Once a patient has stable donor engraftment, the combined targeted level of sirolimus and tacrolimus should not exceed 16 ng/mL and the tacrolimus level should be <8 ng/mL.

13. GUIDELINES FOR TACROLIMUS DOSE ADJUSTMENT AND MONITORING

13A. If there is nausea and vomiting which prevents oral intake at any time during tacrolimus treatment, the drug should be given intravenously at the appropriate dose that was used to obtain a therapeutic level. (IV: PO ratio = 1: 4).

13B. Whole blood trough levels of tacrolimus (i.e., just prior to the next dose) should be obtained on Day +8 and then twice weekly until the taper is initiated, unless high levels (>20 ng/ml) are detected or toxicity is suspected, in which case more frequent monitoring will be performed as clinically indicated. The dose should be adjusted accordingly to maintain a level of 5-10 ng/ml.

13C. Dose reductions should only be made if Tacrolimus toxicity is present or levels exceed 10ng/ml in the absence of toxicity or as clinically indicated. Dose reductions of Tacrolimus for high levels without toxicity should be conservative, e.g. roughly 25%, to avoid inadequate immunosuppression. If creatinine is greater or equal to 2 times the baseline levels then the tacrolimus dose will be reduced by roughly 25%.

13D. Blood pressure, renal function tests (creatinine, BUN), electrolytes and magnesium need to be followed at least two to three times per week while receiving tacrolimus at full dose and then twice weekly or per attending until tacrolimus is discontinued.

13E. Tacrolimus levels should be performed more frequently when 1) drug is converted from oral to IV or IV to oral, 2) dose adjustments are made due to levels outside the therapeutic range, or 3) voriconazole (see table below) is initiated or withdrawn 4) if toxicity is suspected. Steady state levels will not be achieved for at least 72 hours after any change in dosing, i.e. levels determined earlier may not reflect an accurate steady state concentration.

13F. Patients requiring hemodialysis should have tacrolimus levels maintained in the therapeutic range (5 to 15 ng/ml).

13G.

Decrease Tacrolimus Levels	Increase Tacrolimus Levels
Dilantin	Steroids
Phenobarbital	Fluconazole
Carbamazepine	Ketoconazole
Rifamycin	Itraconazole
Caspofungin	Voriconazole
	Cimetidine
	Macrolide antibiotics
	Calcium channel blockers
	Danazol
	metoclopramide

** When initiating therapy with voriconazole in patients already receiving tacrolimus, it is recommended that the tacrolimus dose be reduced to one-third of the original dose, (a 67% reduction) and followed with frequent monitoring of the tacrolimus blood levels. Increased tacrolimus levels have been associated with nephrotoxicity. When voriconazole is discontinued, tacrolimus levels should be carefully monitored and the dose increased as necessary.

13H. Grapefruit and grapefruit juice affect metabolism of tacrolimus and should be avoided. Oral tacrolimus should be taken consistently with or without food.

14. GUIDELINES FOR MYCOPHENOLATE MOFETIL DOSE ADJUSTMENT AND MONITORING

14A. Initiating MMF therapy: Oral administration of MMF will be at 15 mg/kg orally every 8 hours (45 mg/kg/day) starting the evening of day +5. If there is nausea and vomiting at any time preventing the oral administration of MMF, MMF should be administered intravenously at the appropriate dose. Myfortic may be used in place of MMF as clinically indicated.

14B. Maintaining MMF: Markedly low (<40%) donor T-cell chimerism after HCT may indicate impending graft rejection. MMF should be continued at full dose or, if the MMF taper has been initiated, reinstatement of full dose MMF should occur if clinically feasible. If MMF has been discontinued, MMF should be reinitiated at full dose or as clinically indicated.

15. GUIDELINES FOR MMF DOSE ADJUSTMENT DUE TO DRUG TOXICITY

15A. If in the clinical judgment of the attending physician the observed toxicity is related to MMF administration, a dose adjustment may occur. The discontinuation of MMF at any point should be

discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF)

15B. Gastrointestinal Toxicity. Severe gastrointestinal toxicities such as gastrointestinal hemorrhage have been very rare after nonmyeloablative HCT. In the event of gastrointestinal toxicity that requires medical intervention including medication for control of persistent vomiting or diarrhea that is considered to be due to MMF after day 28, a 20% dose reduction will be made or the drug may be given IV. If severe refractory diarrhea or overt gastrointestinal bleeding occurs, MMF may be temporarily stopped. The MMF should be restarted at 20% reduced dose when the underlying toxicity subsides.

15C. Neutropenia. Based on previous experience in patients after nonmyeloablative HCT, dose adjustments are likely to occur because of hematopoietic adverse effects, in particular neutropenia. A thorough evaluation of neutropenia should occur including peripheral blood chimerism studies, marrow aspiration (if clinically indicated) and review of marrow suppressive medications. If all other potential causes of marrow toxicity are ruled out, dose adjustments will only be made for severe, prolonged neutropenia (ANC <500/ μ l for 5 days or more) that persists after day +21 post-transplant. Dose reductions should be conservative (20%). After day +21, the use of G-CSF will be permitted for severe neutropenia as clinically indicated. MMF may be tapered early following discussion with the protocol PI. The discontinuation of MMF at any point earlier than planned should be discussed with the Study PI and should be documented in the permanent medical record and all Case Report Forms (CRF).

16. GUIDELINES FOR SIROLIMUS DOSE ADJUSTMENT AND MONITORING

- 16A.** Sirolimus should be given at least 4 hours after an oral dose of tacrolimus as concurrent administration leads to elevation of the sirolimus levels. To minimize variability of exposure to sirolimus, the drug should be taken consistently with or without food. Grapefruit juice should not be administered with sirolimus or used for dilution. The dosage should be replaced if the patient vomits within 15 minutes of taking a dose.
- 16B.** Whole blood trough levels of sirolimus (i.e., just prior to the next dose) should be obtained on Day +8 and then twice weekly until day +35, and then weekly until the taper is initiated. More frequent levels may be indicated if high levels (>12 ng/ml) are detected or toxicity is suspected.
- 16C.** Concomittant antifungal therapy: Follow Standard Practice Antifungal Therapy Guidelines for dosing reductions for patients receiving azole antifungal therapy.
- 16D.** Severe neutropenia or thrombocytopenia. The combination of sirolimus and tacrolimus may result in an increased risk of sirolimus toxicity such as anemia, diarrhea, hypokalemia, and/or thrombocytopenia. Dose adjustments should be made for severe neutropenia and thrombocytopenia that persists after day 21 post-transplant, or if the neutropenia is unresponsive to G-CSF. Dose reductions of sirolimus of approximately 50% should occur. If neutropenia persists despite dose reduction, sirolimus should be held until blood counts recover to ANC>1500/ μ L.

16. GROWTH FACTOR SUPPORT

Patients will receive G-CSF at 5 μ g/kg/day IV or SC starting at day +5 and continuing until the ANC >500/ μ L for 3 days. Patients with sickle cell disease will not receive G-CSF.

17. INFECTION PROPHYLAXIS AND THERAPY

17A. Patients will receive prophylaxis for PCP, VZV, and HSV, *S. pneumoniae* and *Candida* as per the standard practice guidelines of the individual institution. Recommendations are listed in **Appendix B of protocol**.

The highlights of the recommendations are:

- 1) Fluconazole until day +75.
- 2) Acyclovir until day +365 (To be continued beyond day +365 until all immunosuppression is discontinued in patients with chronic GVHD).
- 3) Sulfamethoxazole/trimethoprim should be started at day 30 only if the ANC is $>500 \times 3$ consecutive days, and the patient should continue on sulfamethoxazole/trimethoprim until discontinuation of immunosuppressive drugs
- 4) Immunoglobulin (IVIG): see standard practice
 - a. Immunodeficiency disorders: all patients with primary Immunodeficiencies should receive IVIG every month regardless of serum IgG level until they have been off immunosuppression for 3 months.
 - b. Other inherited disorders: see standard practice.

18. EVALUATION OF CHIMERISM

18A. Definitions: For the purposes of this protocol, the following definitions will apply

- 1) **Mixed chimerism:** the detection of donor T-cells (CD3+), as a proportion of the total T-cell population, respectively, of greater than 5% and less than 95% in the peripheral blood.
- 2) **Full donor chimerism:** $> 95\%$ donor CD3+ T-cells.
- 3) **Donor engraftment:** mixed or full donor chimerism will be evidence of donor engraftment
- 4) **Increasing donor chimerism:** a 20% absolute increase in the CD3+ T-cell chimerism compared to the previous months chimerism evaluation.
- 5) **Decreasing donor chimerism:** a 20% absolute decrease in the CD3+ T-cell chimerism compared to the previous months chimerism evaluation.
- 6) **Low donor chimerism:** $<40\%$ CD3+ T-cells after HCT on two consecutive evaluations within a 4-week period. The two evaluations must be at least 14 days apart. Low donor chimerism should always be confirmed with repeat blood T-cell and granulocyte chimerism analysis. A DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) (or VNTR analyses or FISH studies) of sorted peripheral blood CD3+ T-cells will be used to quantitate chimerism. The same assay should be used for a given patient for repeated studies of chimerism. This DNA-based assay (or VNTR and FISH analyses) will also be performed on bone marrow aspirates. Therapeutic decisions (i.e. donor lymphocyte infusion) will be made based on the results of sorted T-cell studies of *peripheral blood*.
- 7) **Rejection:** the inability to detect, or less than 5% donor T-cells (CD3) as a proportion of the total T-cell population, respectively, after nonmyeloablative HCT.
- 8) **Graft failure:** grade IV thrombocytopenia and neutropenia after day 21 that lasts > 2 weeks and is refractory to growth factor support.

18B. Evaluation of Chimerism:

Patients will be evaluated for peripheral blood chimerism on days +28, +56 and +84 post-transplant. Unsorted (whole) bone marrow chimerism will be done on Day +84, and 1 year. In the absence of any further immunotherapy, peripheral blood chimerism studies will then be performed at 6, 12, 18, and 24 months post-transplant, then yearly for 5 years post-transplant or more often if clinically indicated.

18C. Continuation of Immunosuppression:

If a patient has low donor chimerism, immunosuppression should be continued or re-instituted at full dose.

18D. Discontinuation of Immunosuppression:

Immunosuppression should be discontinued as per protocol unless the patient develops GVHD or has falling donor chimerism.

18E. Treatment of Graft Failure/Rejection or Mixed Chimerism with Persistent Disease:

- 1) Patients with *graft failure or rejection* (<5% donor T-cells measured from peripheral blood sample) may be eligible for second marrow transplant, using protocol of institutional preference. At time of second transplant, patients will be scored as graft failure and followed as a separate group for accumulation of descriptive outcome data.
- 2) Patients with mixed chimerism (defined as 5-95% donor T-cells measured from peripheral blood sample) with persistent life threatening disease are eligible for a second nonmyeloablative HCT. Patients with amelioration of disease symptoms in presence of mixed donor chimerism should not be given second HCT.

19. PATIENT AND DONOR CLINICAL AND LABORATORY EVALUATIONS

19A. HLA- typing of Patient and Potential Donors (Pre-transplant Evaluation) – See Standard Practice Manuel

- 1) As broad a range of potential donors as possible should be typed. Included would be parents, siblings, eligible children and spouses.
- 2) Serotyping (HLA-A, B, C) and DNA typing (HLA-A, B, C, DRB1, DQB1) of patient and donor.
- 3) Leukocyte and/or florescence activated cell sorter cross match between the patient and donor.
- 4) Blood samples should be sent to: Clinical Immunogenetics Lab for HLA-typing (green top tube, 10 cc)

19B. Donor

Donors will be evaluated according to Standard Practice Guidelines and should include:

- 1) Complete history and physical examination prior to and the day after bone marrow harvest to include the following:
 - a. *Medical problems, including pulmonary and upper airway disease, cardiovascular disease, diabetes, arthritis, or abnormalities of the spine.*
 - b. *Last menstrual period*
 - c. *Previous exposure to anesthetics, and family history of anesthesia-associated complications*
 - d. *Blood transfusions*
 - e. *Medications*
 - f. *Vaccinations*
 - g. *Allergies*
 - h. *Height and weight*
- 2) **Lab tests:**
 - a. *CBC with differential including platelet counts on day –2 and day 0.*
 - b. *Serum sodium, potassium, Chloride, CO2, Glucose, Bun, Creatinine, Calcium, Magnesium, Phosphorus, Uric acid, LDH, alkaline phosphatase, Total bilirubin, AST, ALT and albumin*

- c. *Hepatitis screen, CMV, syphilis, HIV and HTLV I serologies (tests must be completed within 30 days of transplantation)*
 - d. *ABO Rh blood typing. If the donor has antibodies against red cell antigens of the recipient, the titers will be determined. Cytotoxic cross match between patient and donor (HLA Laboratory) will be performed.*
 - e. *Female donors of menstruation age: serum HCG*
 - f. *Sickle cell analysis for any patients of African-American ethnicity*
- 3) A heparinized blood sample (green top tube, 10 cc) as a donor reference for subsequent determination of donor chimerism should be sent to the Cytogenetics Lab if the donor-recipient pair is sex-mismatched or to the Clinical Immunogenetics Lab if sex-matched. Label "Protocol 2032"

19C. Patient

Refer to FHCRC/SCCA Standard Practice Manual for Pre-Transplant Evaluation Guidelines for Allogeneic Transplant as clinically indicated (results of tests and/or procedures conducted as per standard of care for pretransplant workups may be used for eligibility determination if conducted within an appropriate window prior to screening)

1) Pre-transplant Baseline Evaluation

- a. **History:** *A complete history with full details of the patient's prior treatment and response including the following:*
 - i) *Hematologic findings at diagnosis (including biochemical markers, cytogenetic and molecular markers, disease process, and immunologic criteria)*
 - ii) *Transfusion history (including type of donor, i.e. random or family donor)*
 - iii) *Current medical problems*
 - iv) *Current medications*
 - v) *Female patients who have gone through puberty – pregnancy history, menstrual history, and date of last sexual intercourse*
- b. **Physical Exam:** *Careful physical examination*
- c. **Laboratory evaluation:**
 - i) *CBC with differential*
 - ii) *BUN/Creatinine*
 - iii) *Total bilirubin, fractionated bilirubin, alkaline phosphatase, AST, ALT, GGT*
 - iv) *ABO/Rh typing, direct coombs*
 - v) *Quantitative serum immunoglobulins (tough level if on IVIG if possible)*
 - vi) *Pre-transplant viral testing*

Pre Transplant Viral Testing	All 2032 Patients	Patients with Primary Immune Deficiency Disorders	Patients with Antibody Deficiencies or on IVIG	If Clinically Indicated
Hepatitis Screen	X			
CMV Serology	X			
VZV Serology	X			
HSV Serology	X			
Toxoplasma Serology	X			
Anti HIV Serology	X			
Hepatitis B PCR			X	
Hepatitis C PCR			X	
HIV PCR or p24 antigen testing			X	
CMV PCR	X*			
EBV PCR		X*		X*
Adenovirus PCR		X*		X*
VZV PCR				X
HSV PCR				X
Toxoplasma PCR				X

* Approximately 1 month prior to conditioning

viii) *As a pre-transplant reference for subsequent determination of donor chimerism 10 cc of heparinized peripheral blood from the patient and the donor will be drawn and sent to:*

- A)** Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. *Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).*
- B)** Clinical chimerism studies post-transplant should only be sent to the relevant laboratory that performed the chimerism analysis pre-transplant.

d. See staging tables for further evaluation

e. Radiological evaluation: The following studies should be obtained prior to the start of the transplant

- i)** CXR-PA and lateral view (Other x-rays as clinically indicated) within approximately 1 month of HCT as clinically indicated. If a CT scan of the chest has been done a CXR is not needed.
- ii)** CT scan of the chest, abdomen and pelvis within approximately 30 days of HCT if clinically indicated
- iii)** EKG within approximately 3 months of HCT if clinically indicated
- iv)** Echo or MUGA for calculation of cardiac ejection fraction within approximately 3 months of HCT, if clinically indicated. If unable to obtain ejection fraction, then please obtain shortening fraction calculation.

f. Bone marrow evaluation:

If clinically indicated, a bone marrow aspirate should be obtained within approximately 4 months of start of transplant conditioning for morphology, flow cytometry, and cytogenetics. We recommend that all patients with bone marrow failure disorders, aplastic anemia, and hemophagocytic lymphohistiocytosis have a bone marrow aspirate and biopsy within approximately 1 month of start of transplant conditioning for morphology, flow cytometry, cytogenetics, and MDS FISH panel (bone marrow failure and aplastic anemia) in order to assess disease status prior to HCT.

2) Post HCT Evaluation:

Note: Day + 28, 56, 84, etc. evaluations to be performed. This is a recommended evaluation schedule. The clinical team can decide to not do these studies if clinically indicated.

Once patients are discharged they should be evaluated at least twice weekly in the out patient department until day 28 and then weekly. Patients with GVHD or graft rejection should have more frequent follow up. Please refer to instructions below and in the Standard Practice Manual for guidelines regarding evaluation of patients following low-dose regimens.

a. History and physical exam:

- i) Once outpatient: clinic evaluation twice weekly until day 28, then weekly. More frequent evaluations are indicated for patients with signs of GVHD or graft failure.
- ii) Weekly weights

b. Laboratory Evaluation:

- i) **CBC daily from day 0 until ANC > 500/uL for 3 days after nadir reached.** Thereafter, a CBC should be checked three times a week until day +28. After day +28, a CBC should be checked twice weekly until 2 months post-transplant and later if clinically indicated. Daily platelet counts if the platelet count is <20,000/ μ L.
- ii) **Electrolyte panel and serum BUN/creatinine** should be checked 3 times a week until day 28 and then twice weekly while on full dose Tacrolimus or per attending until Tacrolimus is discontinued.
- iii) **Calcium, magnesium, phosphate** should be checked twice weekly until day 28 and then weekly.
- iv) **Serum albumin** should be checked weekly.
- v) **Hepatic function** including AST, ALT, alkaline phosphatase, and total and direct bilirubin should be checked two times a week until day +28 and then every week.
- vi) **Patients on steroids** should have surveillance blood cultures checked two times a week until steroids have been tapered to less than 0.5 mg/kg/day
- vii) **Iron studies:** Ferritin level, binding capacity including serum iron, total iron binding capacity, direct and % transferrin saturation checked on Day + 84, and 365 and yearly x 5 years if clinically indicated.
- viii) **Quantitative Immunoglobulins per standard practice.**
- ix) **Infection Monitoring:** Infection monitoring per institutional standard practice, although the following is recommended
 - A) CMV surveillance: Per institutional standard practice

B) Adenovirus surveillance:

All Patients
Adenovirus PCR weekly until day 100. Monitoring beyond day 100 should continue if the patient's absolute lymphocyte count < 300 cells/microliter or as clinically indicated.

C) EBV lymphoproliferative syndrome surveillance:

	All Patients
If stable copies by PCR	Weekly until day 100
If rising copies by PCR	Twice weekly until day 100

- x) Serum triglyceride levels (fasting) should be drawn every two weeks through day 56, then monthly while on sirolimus, or more often if clinically indicated.
- xi) Cholesterol level (fasting) should be drawn around day 84 while on sirolimus, or more often if clinically indicated.
- xii) Haptoglobin every other week until Day +56, then as clinically indicated. Evaluation of schistocytes weekly with CBC through Day + 56.
- xiii) *Disease Specific analysis, per staging table*

d. See staging tables for further evaluation

e. Radiology evaluation: Two view chest x-ray (PA and lateral) as clinically indicated

3) Additional evaluation at day 80-100:

Please refer to instructions below and Standard Practice Manual Guidelines or institutional guidelines.

- a. A patient with an uncomplicated HCT would be discharged after the day +80-100 workup and screening for chronic GVHD are completed and analyzed. Since the patient may be discharged prior to starting Tacrolimus and MMF taper, instructions should be provided for preventing and detecting GVHD as per standard practice of collaborating institution.

GVHD evaluation guidelines are as follows:

- i) History and physical exam
- ii) CBC, serum IgG, total bilirubin, alkaline phosphatase, ALT and AST
- iii) GVHD evaluation (histopathology)
 - A)** Oral mucosal biopsy – 3 mm incisional biopsy (if deemed necessary by oral medicine)
 - B)** Skin biopsy - 3 mm punch biopsy from affected area or posterior iliac crest
- iv) Schirmer's tear test if clinically indicated: if the Schirmer's test is abnormal (<15 mm OS or OD) include ophthalmologic evaluation
- v) Pulmonary function test

- vi) Oral medicine evaluation
- vii) Dietitian assessment
- viii) Gynecological assessment (adult female)
- ix) Staging workup, as indicated by staging table and text

See **section 20.G** for diagnosis and treatment guidelines of acute and chronic GVHD

19D. Additional evaluation guidelines following 100 Days post HCT

- 1) **First month** – weekly evaluations by referring physician, including physical examination and weights (children <17 years – record height and weight every 3 months)
- 2) **Weekly** - CBC, BUN, creatinine, ALT, AST, Alkaline phosphatase, Total bilirubin and direct bilirubin. If stable, monthly evaluations as above for 2 years, then annually
- 3) Per institutional standard practice, immunization antibody titers should be obtained at approximately 1 year (if patient on IVIG, do not obtain): including Hepatitis B, Tetanus, Pertussis, Pneumococcal, Haemophilus Influenzae Type b, and polio.

STAGING TABLE

ALL DISEASES

	Pre	Day +28	Day +56	Day +84	Day +180	Day +365	18 months	24 months	Years 3-5
BMA w/ pathology, flow cytometry, cytogenetics, FISH ¹ and chimerism ⁷ (recommended)	X ¹	X ⁸		X ⁸		X ⁸		X ¹	X ¹
PB chimerism ⁹	X ²	X ³	X ³	X ^{3,4}	X ^{3,4}	X ^{3,4}	X ^{3,4}	X ^{3,4}	X ⁵
Quantitative Immunoglobulins (Serum Troughs) ⁶	X	X	X	X		X			

¹ If clinically indicated

² For storage

³ CD3, CD33, CD56

⁴ CD19 recommended for PID patients or as clinically indicated

⁵ CD3 and CD33. In addition, we recommend that CD56 chimerism be sent if mixed chimerism was present at the previous timepoint or as clinically indicated. We recommend that CD19 chimerism also be sent if the patient has a primary immunodeficiency disorder and mixed chimerism was present at the previous time point or as clinically indicated.

⁶ Per standard practice.

⁷ Bone marrow aspirate (unsorted) to Clinical Chimerism Lab for chimerism studies on around day +84 and 1 year after HCT. Send to: Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).

⁸ Recommend for patients with acquired Aplastic anemia: Bone marrow aspiration and biopsy on Days +28, 84 and 365 or as clinically indicated: send for pathology, flow cytometry, cytogenetics, and for chimerism analysis.

⁹ For FHCRC patients: blood to clinical flow lab for sorting, then to the chimerism lab for quantifying of peripheral blood. Send to Clinical Immunogenetics Lab (206- 667-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).

DISEASE SPECIFIC STAGING

Primary Immunodeficiency Diseases

The following tests are to be done for all primary immunodeficiency diseases

	Pre	Day +28	Day +56	Day +84	Day +180	Day +365	Yearly x5
Consultation with Pediatric Immunologist	X ⁵			X		X	X ⁷
Infectious Disease Referral	x ^{1,5}						
Lymphocyte (phenotype) subsets ^{2,3}	X ⁵			X		X	X ⁶
Lymphocyte Function Analysis ^{2,4}	X ⁵			X		X	X ⁶
Quantitative Immunoglobulins (serum troughs)	X	X	X	X		X	X ⁶

¹ All patients with primary immunodeficiency disorders should be referred to infectious disease for consultation prior to transplantation if they have not been seen by infectious disease. If infectious disease team determines a consult is not needed, an ID consult does not need to occur.

² We recommend for patients with primary immunodeficiency disorders (or as clinically indicated) that patients undergo an evaluation of lymphocyte phenotype and function if it has not already been done: Of note, patients with chronic granulomatous disease and leukocyte adhesion deficiency do not need lymphocyte phenotype or function testing. In addition, patients with HLH do not need lymphocyte function testing:

³ Lymphocyte phenotype (subsets): For FHCRC patients, send 2-3 ml in EDTA to Seattle Children’s Cell Marker Lab (206) 987-2560 for flow cytometry to determine number of CD3, CD4, CD8, CD19/20, and CD16/56 cells. For patients <20 kg where blood volume is a concern, please call the Cell Marker Lab for recommendations regarding the total blood volume needed to complete this study. Sample should be obtained around day +84. Repeat around 12 months and then annually x 5 years post HCT if clinically indicated.

⁴ Lymphocyte function: For FHCRC patients send 10 ml in heparin to Seattle Children’s Cell Marker Lab 206-987-2560 for proliferation to mitogens (PHA & CD3 only) testing. For patients <20 kg where blood volume is a concern, please call the Cell Marker Lab for recommendations regarding the total blood volume needed to complete this study. For outside centers send to appropriate lab. Samples should be obtained around day+84. Repeat around 12 months and then annually x 5 years if clinically indicated.

⁵ If not already completed pre conditioning

⁶ As clinically indicated

⁷ As directed by immunology

Disease Specific Tests: The following tests are to only be done if the patient has the specific primary immunodeficiency disease listed below.

	Pre	Day +28	Day +56	Day +84	Day +180	Day +365	Yearly x5
Leukocyte Adhesion defect Analysis of CD18 expression by flow cytometry ³	X ¹			X		X	X ²
Wiskott-Aldrich Syndrome Gene Sequencing ⁴	X ¹						
Determination of WASP expression by flow cytometry ⁵	X ¹			X		X	X ²
IPEX Determination of FOXP3 expression by flow cytometry ⁶	X ¹			X		X	X ²
Chronic Granulomatous Disease Neutrophil Oxidative Burst ⁷	X ¹			X		X	X ²
Genetic analysis of cytosolic components of NADPH oxidase system ⁸	X ¹						
CD40LD Determination of CD40 Ligand expression by flow cytometry ⁶	X ¹			X		X	X ²
SCID Maternal T Cell Engraftment ⁹	X						

¹ If not already completed pre conditioning

² If most recent result abnormal

³ Send 5 ml blood in heparin (green top) to Seattle Children’s Cell Marker Lab

⁴ Send 10mL blood in EDTA tube to Immunology Diagnostic Laboratory Seattle Children’s Research Institute 1900 9th Avenue Seattle 98101, attn. IDL lab (206-987-7442)

⁵ Send 5-10 ml blood (green top) to Immunology Diagnostics Laboratory Seattle Children’s Research Institute 1900 9th Avenue Seattle 98101, attn. IDL lab (206-987-7442).

⁶ Send 5-10 ml blood in heparin (green top) to Immunology Diagnostics Laboratory Seattle Children’s Research Institute 1900 9th Avenue Seattle 98101, attn. IDL lab (206-987-7442).

⁷ Send 1-2 ml blood in heparin (green top) to Seattle Children’s Cell Marker Lab or appropriate outside lab

⁸ Send 5 ml blood in EDTA (purple top) to Genedx (www.genedx.com)

⁹ SCIDs patients only: 5 cc blood in Na Heparin – sort (UWMC Hematopathology), then FISH vs. VNTR.

- A. **For male patients**, send 5 ml blood in heparin to flow cytometry lab for sorting T cells and then to Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants).
- B. **For female patients**, send 5 ml blood in heparin from patient to flow cytometry lab for sorting T cells for then to Clinical Immunogenetics Lab (206-288-7700; SCCA G7-200) for a DNA-based assay that compares the profile of amplified fragment length polymorphisms (ampFLP) of the patient and donor. Outside institutions may use VNTR analysis (sex- matched transplants) or sex chromosome FISH-analysis (sex-mismatched transplants). In addition, from mother send 5 ml blood in EDTA (purple top) to Clinical Immunogenetics Laboratory.

Other Disease Specific Tests:

	Pre	Day +28	Day +56	Day +84	Day +365	Yearly x5
Sphingolipidoses						
Consultation with Metabolic Disorders specialist	X ³			X	X	X
Nerve Conduction studies	X ³				X	
Consultation with Neurologist	X ³			X	X	X
MRI of Head	X ³	X	X	X	X	X
Mucopolysaccharidoses						
Disease specific enzyme level ¹	X ³	X	X	X	X	X
Consultation with Pediatric Specialist in Metabolic Disorders	X ³			X	X	X
Marrow, skin, liver, and/or gut biopsy as indicated according to disease for determination of storage material	X ³			X	X	
MRI of Head if indicated	X ³			X	X	
LP for storage determination if indicated	X ³			X	X	
Osteopetrosis						
Bone biopsy for pathology ²	X ³			X	X	X
Head MRI	X ³			X	X	X
Acquired Aplastic Anemia						
Flow cytometry of peripheral blood to assess for absence of PIG1-associated surface proteins characteristic of paroxysmal nocturnal hemoglobinuria.	X ³					
Peripheral blood for mitomycin C and DEB chromosome breakage analyses to assess for Fanconi anemia.	X ³					
Hemophagocytosis Lymphohistiocytosis (HLH)						
Lymphocyte phenotype (subsets)	X ³			X	X	X ⁴
Soluble Interleukin-2 Receptor/Interleukin 2	X ³			X	X	
Sickle Cell Disease						
Percent Hemoglobin S	X ⁵			X		
Dyskeratosis Congenita						
Telomere length analysis	X ³					

	Pre	Day +28	Day +56	Day +84	Day +365	Yearly x5
Other Diseases						
Send disease specific markers after consultation with a specialist in the disorder.	X ³					

¹ For FHCRC patients send to Seattle Children’s laboratory.

² For FHCRC patients send to Seattle Children’s pathology.

³ Disease specific testing does not need to be done pre HCT if it has already been performed pre start of conditioning.

⁴ If abnormal at previous interval or if clinically indicated.

⁵ On arrival and then within 1 week prior to start of conditioning.

20. DRUGS, IRRADIATION, COMPLICATIONS INCLUDING GVHD AND TOXICITIES:

20A. Cyclophosphamide

1) Description:

Cyclophosphamide is an alkylating agent which prevents cell division primarily by cross-linking DNA strands. Cyclophosphamide is cell cycle non-specific. Cyclophosphamide is not stem cell toxic.

2) Storage and Administration:

Cyclophosphamide for injection is commercially available in 2000 mg vials, which are reconstituted with 100 ml sterile water for injection. The concentration of the reconstituted product is 20 mg/ml. The calculated dose will be diluted further in 250-500 ml of Dextrose 5% in water. Each dose will be infused over 1-2 hr (depending on the total volume).

3) Side Effects and Toxicity:

Clinical toxicities of cyclophosphamide include alopecia, nausea and vomiting, headache and dizziness, hemorrhagic cystitis, cardiotoxicity, immunosuppression, myelosuppression, pulmonary fibrosis, increased hepatic enzymes and syndrome of inappropriate anti-diuretic hormone (SIADH).

20B. Mesna

1) Description:

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazaphosphorine (cyclophosphamide and iphosphamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazaphosphorine, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazaphosphorine.

2) Storage and Administration:

Mesna is commercially available in 200 mg, 400 mg and 1000 mg vials containing a 100mg/ml solution. Each dose of mesna will be diluted further in 50 ml of normal saline to be infused over 15 min. Mesna dose will be based on the cyclophosphamide dose being given. The total daily dose of mesna is equal to 100% of the total daily dose of cyclophosphamide.

3) Side Effects and Toxicity:

At the doses used for uroprotection mesna is virtually non-toxic. However, adverse effects, which may be attributable to mesna, include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

20C. Fludarabine (side effects):

1) Description:

Fludarabine's active metabolite 2-fluoro-ara-A is an antimetabolite that inhibits DNA primase, DNA polymerase alpha and Ribonucleotide nuclease.

2) Dosage and Administration: Fludarabine monophosphate is commercially available as a 50 mg/vial, which is reconstituted with 2 ml of sterile water, resulting in a 25mg/ml solution. The desired dose is further diluted to concentrations of 0.04-1 mg/ml in normal saline or 5% dextrose (50-100ml) for injection and will be administered by IV infusion over 30 minutes or longer. Fludarabine will be administered by IV infusion over 1 hr in a dose of 30 mg/m²/day on days -6 to -2.

3) Side Effects and Toxicities: The dose of fludarabine used in this protocol is nonmyeloablative; however, it can cause severe immunosuppression particularly in the CD4+ T cell compartment. Immunosuppression increases the risk of infection, which can be life threatening. In addition, clinical toxicities of fludarabine monophosphate include: myelosuppression, primarily lymphopenia and granulocytopenia, alopecia, rash, dermatitis, nausea, vomiting, anorexia, stomatitis, diarrhea, somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness, hepatocellular toxicity with elevation in serum transaminases, neurotoxicity and interstitial pneumonitis. These effects are reversible when the drug is discontinued. Immunosuppression observed with the use of fludarabine increases the risk of infection which can be life-threatening.

20D. TBI (side effects)

TBI given at high doses in conventional transplants may cause nausea, vomiting, diarrhea, temporary hair loss, and painful swelling of the salivary glands for a few days. TBI may destroy normal bone marrow cells in addition to the cancer cells. The dose of TBI (200 cGy or 400 cGy for patients with Acquired Aplastic Anemia, sickle cell disease, thalassemia, or other non-malignant disease at high risk for graft rejection (as discussed at the non-malignant board or the patient care conference)) used in this protocol is about one-sixth (200 cGy) to one-third (400 cGy) of that used in conventional transplant protocols, and severe acute side effects have so far not been observed. TBI has been associated with causing sterility and there is a risk of major genetic damage to any children conceived after transplantation. There is a risk that a small percentage of patients may develop a secondary cancer resulting from this treatment.

20E. Tacrolimus

See **sections 12 and 13. Pages 16-18** for information about administration and dosage adjustments. (See Standard Practice Manual)

1) Introduction

Tacrolimus, also known as FK-506, is a macrolide immunosuppressive agent. Tacrolimus inhibits lymphocytes by forming a complex with FKBP-12, calcium, and calmodulin, leading to the decrease in the phosphatase activity of calcineurin. Calcineurin mediates the first intracellular signal required for T-cell activation after antigen recognition by the T-cell receptor. This drug is used with corticosteroids for prophylaxis of organ rejection in patients receiving allogeneic liver transplants and for prophylaxis of GVHD in the setting of HCT. It is also used for

immunosuppression after kidney, cardiac, pancreas, pancreatic islet cell and small bowel transplantation. This drug is well-absorbed orally. It is metabolized in the liver by unknown mechanisms, but demethylation and hydroxylation have been proposed based on *in vitro* studies. The metabolized products are excreted in the urine.

2) Administration

a. Oral

- i) *Tacrolimus capsules – 0.5 mg, 1 mg or 5 mg capsules . (There is currently no oral solution available.)*
- ii) *For better absorption, it is recommended that Tacrolimus capsules be taken on an empty stomach.*
- iii) *Tacrolimus should not be taken with grapefruit juice as it may increase blood levels.*
- iv) *If patient vomits within one hour of oral administration, repeat dose.*
- v) *If vomiting persists, switch to IV administration.*

b. Intravenous

- i) *Sterile solution of 5 mg/ml ampules in polyoxyethylated castor oil (Cremophor FCL).*
- ii) *Diluted in D₅W in glass or other non-PVC container.*
- iii) *Final dilution volume: 50-250 ml dependent upon patient size and Tacrolimus dose.*
- vi) *Infusion time: Standard – 22-24 hours (continuous infusion).*

c. Conversion from IV to PO dosing of tacrolimus.

Patients should be converted to an oral dose at 4 times the IV dose to be given in divided (Q12 hour) doses. For children aged < 6 years old who have sub-therapeutic levels, the dose interval may need to be reduced to every 8 hours.

3) Side effects are generally reversible and may include:

- a) **Renal** – Rise in serum creatinine, hemolytic uremic syndrome.
- b) **Neurological** – Peripheral: paresthesia, tremor. Central: seizures, headache, insomnia, dizziness, depression, confusion, hallucinations, psychosis, myoclonus, neuropathy, agitation.
- c) **Gastrointestinal** – Nausea, vomiting, anorexia, constipation, diarrhea.
- d) **Cardiovascular** – Hypertension, myocardial hypertrophy.
- e) **Endocrine** – Hyperglycemia, hyper/hypokalemia, hypophosphatemia, hypomagnesemia.
- f) **Integument** – Itching, rash.
- g) **Hematologic** – Leukocytosis, thrombocytopenia, leukopenia, anemia, PTLD, thrombotic microangiopathy.
- h) **Liver** – Abnormal liver function tests.
- i) **Ocular** – Blurred vision, photophobia.
- j) **Respiratory** – Pleural effusion, atelectasis, cough, dyspnea.
- k) **Musculoskeletal** – Arthralgia.

20F. Mycophenolate Mofetil (MMF) – side effects:

See **section 14** for information about administration and dosage adjustments.

1) Description

MMF is the morpholinylethylester of mycophenolic acid (MPA) and reversibly inhibits inosine monophosphate dehydrogenase, particularly the type II isoform that is more prominent in activated lymphocytes. As a result of the inhibition of de novo purine synthesis, proliferation of B and T lymphocytes is blocked and antibody production is inhibited.

2) Storage and Administration

MMF is available in an oral and an intravenous formulation. The oral formulation is supplied in 250mg hard gelatin capsules and can be stored at room temperature. MMF for IV administration is supplied as a lyophilized powder in a glass vial containing the equivalent of 500mg.

3) Side Effects and Toxicity

- a) Precautions: MMF has been studied extensively among patients after nonmyeloablative HCT. Previous clinical studies in patients after allografting suggest that the principal adverse reactions associated with the administration of MMF include nausea, vomiting, neutropenia, diarrhea, and on one occasion bloody diarrhea. In the setting of marrow transplantation, several etiologic factors may contribute to alterations in gastrointestinal and hematologic parameters. MMF has an increased incidence of digestive system adverse events, including GI tract ulceration, and hemorrhage (3% of patients receiving MMF). GI tract perforations have rarely been observed. Most patients in these studies were also on other drugs known to be associated with these complications. Up to 2% of patients receiving MMF for prevention of rejection developed severe neutropenia (ANC <500). The development of neutropenia may be related to MMF itself, concomitant medications, viral infections or some combination of these causes. MMF dose adjustments will be made if clinically indicated if in the opinion of the attending physician, no other cause is thought to be responsible for the abnormality. These adjustments should be discussed with the principal investigator and documented in the medical records and the clinical reporting form (CRF). Dose adjustments are described in Sections 14 & 15.

20G. Sirolimus

- a) Formulation, Storage, and Administration: Sirolimus is supplied as an oral solution (Rapamune Oral Solution) 1 mg/mL or as 1 mg tablets. Rapamune Oral Solution pouches should be stored protected from light and refrigerated at 2-8°C. If necessary, the patient may store the pouches at room temperatures up to 25°C for a short period of time (no longer than 30 days). The tablets should be stored at 20-25°C and be protected from light. Refer to section 12D for dosing instructions. To minimize variability of exposure to sirolimus, this drug should be taken consistently with or without food. Grapefruit juices should not be administered with sirolimus or used for dilution. If patients are receiving Rapamune Oral Solution, the dose should be mixed well with 60 mL of water or orange juice and taken immediately. It is recommended that the container be refilled with a minimum of 120 mL of water or orange juice, mixed well, and this rinse solution should be swallowed.
- b) Adverse Reactions: The incidence of adverse reactions was determined in two randomized double-blind multicenter controlled trials in which 499 renal transplant recipients received Rapamune oral solution 2 mg/day and 477 received 5 mg/day. Specific adverse reactions associated with the administration of Rapamune oral solution included hypocholesterolemia, hyperlipidemia, hypertension, and rash. At the higher dose of 5 mg, these adverse effects included anemia, arthralgia, diarrhea, hypokalemia, and thrombocytopenia. Additional toxicities from studies in stem cell transplantation include: hemolytic uremic syndrome, seizures, and neutropenia.
- c) Clinically Significant Inducers or Inhibitors of the Cytochrome P450 Enzyme System: Sirolimus levels may increase in patients taking fluconazole, itraconazole, cyclosporine,

methylprednisolone, or tacrolimus. Other medications that may affect metabolism of sirolimus are listed below:

Agents <u>likely to increase</u> sirolimus levels	Agents which may <u>increase</u> sirolimus levels	Agents <u>likely to decrease</u> sirolimus levels	Agents which may <u>decrease</u> sirolimus levels
Diltiazem Nicaripine Verapamil Erythromycin Ketoconazole Voriconazole Clarithromycin	Cimetidine	Carbamazepine Phenobarbital Phenytoin Rifampin	Primidone Valproic acid Rifabutin

d) Management of Toxicities:

Severe neutropenia or thrombocytopenia: Refer to section 16D.

Hyperlipidemia: Sirolimus is known to cause elevations in serum cholesterol and triglyceride levels. Serum triglyceride levels should be drawn every two weeks through day 56, then monthly while on sirolimus, or more often if clinically indicated. Cholesterol levels will be drawn at the Discharge evaluation (around day 80). In 10-20% of patients, the triglyceride level exceeds 1000 mg/dL. To avoid complications due to pancreatitis, patients should be treated with gemfibrozil or atorvastatin for triglyceride levels >800 mg/dL.

20H. GVHD

The major toxicity of T-replete HCT from related, HLA-haploidentical donors is GVHD. In nonmyeloablative HCT from related, HLA-haploidentical donors the incidence of GVHD ≥Grade II was >90% (50% Grades III and IV) in studies described by **Sykes and co-workers [38,39]** using a different conditioning and immunosuppressive regimen. Overall mortality was 75%. However, preliminary results of the Phase II clinical trial (protocol 1667) discussed above in Section E. #3 (pages 8-9) show that only ≤ acute Grade II GVHD was observed in 12/25 patients and none of the patients have died as a result of GVHD or its therapy.

1) **Diagnosis:** Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspicious for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (**Appendices C and D**).

2) **Recommended Treatment:**

a. **Patients Developing Grade II-IV Acute GVHD and Tacrolimus has started to taper:**

i) *If acute GVHD develops during Tacrolimus taper, reinstate tacrolimus at the last dose prior to the initiation of the taper. If off tacrolimus, reinstate Tacrolimus at 0.06 mg/kg PO q12 hours. If there is concern of GI absorption, use IV route (0.03 mg/kg/d continuous IV infusion over 22-24 hours, pediatric patients or 0.015 mg/kg every 12 hours, adult patients).*

ii) *Prednisone (2 mg/kg/day) should be added if severe GVHD occurs and/or no GVHD response occurs by 72 hours after initiation of Tacrolimus. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6 week taper. A faster prednisone taper may be initiated if clinically indicated.*

b) **Patients developing acute GVHD ≥ grade II on full dose Tacrolimus and full dose MMF:**

- i.) *Prednisone (2 mg/kg/day) or intravenous equivalent is to be added. Patients who respond to steroids after 10 to 14 days of treatment, should begin a 6 week taper. A faster prednisone taper may be initiated if clinically indicated.*
 - ii.) *When steroids are tapered to 0.5 mg/kg PO QD then an MMF taper may be initiated. In the absence of GVHD flare, the MMF and prednisone tapers should continue until completion. If nausea and/or vomiting prevent the oral administration of MMF, MMF should be administered intravenously at 15 mg/kg q 8hrs.*
 - iii) *Patients are eligible for institutional trials of GVHD therapy.*
- c) **Patients with Chronic Extensive GVHD:**
- i) **Tacrolimus dosing:**
Adults: 0.01-0.02 mg/kg PO q12hrs
Pediatric doses for patients < 17 years of age: 0.05-0.08 mg/kg PO q12hrs.
 - ii) **Prednisone (1mg/kg/day qd.)**
Taper of prednisone as per recommendations of the Long Term Follow Up (LTFU) at FHCRC and collaborating institutions standard practice. Patients can also be treated on other protocols eligible at that time. Patients off immunosuppression who develop concurrent manifestations of GVHD that satisfy criteria for \geq grade II acute GVHD (e.g. erythematous rash, diarrhea, hyperbilirubinemia) **and** are pathognomonic of clinical extensive chronic GVHD (e.g. lichenoid oral changes, ocular sicca, scleroderma, bronchiolitis obliterans, contractures), should receive prolonged immunosuppressive therapy similar to that for chronic extensive GVHD.
 - iii) **Antibiotic prophylaxis with daily Bactrim and anti-viral prophylaxis with acyclovir.**

20I. Myelosuppression:

- 1) Grade IV myelosuppression will be defined as a decrease in ANC to $\leq 500/\mu\text{L}$ and/or platelet count to $\leq 20,000/\mu\text{L}$.
- 2) Myelosuppression may occur in this patient population for a number of reasons such as direct toxic effect of drugs (MMF, ganciclovir etc.), rejection, and relapse.
- 3) Patients with myelosuppression may be managed as follows:
 - a) **Suspected MMF toxicity:** refer to section 15, Guidelines for MMF dose adjustment above for management recommendations.
 - b) **Suspected ganciclovir toxicity:** consider changing to foscarnet.

Thrombocytopenic patients will receive platelet transfusion as per standard care.

21. PROTOCOL DEVELOPMENT AND SPECIAL CONSIDERATIONS

PROJECTED TARGET ACCRUAL ETHNIC AND GENDER DISTRIBUTION CHART

All ethnic groups and both genders will be enrolled according to the patient distribution in the participating institutions, as outlined in the following Chart. Skewing of gender may occur if a preponderance of X-linked syndromes are enrolled.

TARGETED / PLANNED ENROLLMENT: Number of Subjects 20			
Ethnic Category	Sex / Gender		
	Females	Males	Total
Hispanic or Latino	0	1	1
Not Hispanic or Latino	7	12	19
Ethnic Category Total of All Subjects*	7	13	20
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	1	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	1	1
White	7	11	18
Racial Categories: Total of All Subjects*	7	13	20

22. DATA AND SAFETY MONITORING PLAN AND ADVERSE EVENT REPORTING

22A. Monitoring the progress of trials and the safety of participants

The principal investigators (PI) monitor multi-institutional clinical trials, with oversight by a Data Safety and Monitoring Board (DSMB), the Data and Safety Monitoring Committee (DSMC) and the Institutional Review Board (IRB). The PI reviews outcome data for each individual patient on an ongoing basis.

Serious adverse events are reported to the trial coordinator, the study nurse or directly to the PI. The trial coordinators at collaborating centers or the local PIs will fax an official report of a serious adverse event to the coordinating center (FHCRC) within ten days. The serious adverse event report is reviewed by PI. If the serious adverse event meets the FHCRC criteria for reporting then an official signed report is submitted to the FHCRC Institutional Review Office (IRO). All deaths, regardless of the cause, are reported to the IRB. Multi-center protocols have a DSMB responsible for monitoring patient safety on this clinical trial. The DSMB meets twice a year and all outcome data is reviewed including all adverse events reported to the coordinating center (FHCRC) along with those officially reported to the FHCRC IRO. The DSMB confirms that the trial has not met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the FHCRC IRB as well as the trial coordinators/local PIs of this protocol. The DSMB will discontinue the review of outcomes when this protocol is closed to accrual. Furthermore, the FHCRC also has a DSMC that reviews the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual IRB review and approval is also required.

With respect to safety, patients are monitored for the development of GVHD, myelosuppression, infections and rejection. All patients, regardless of diagnosis, will be considered in the safety analysis. GVHD events will be closely monitored and severity of GVHD graded. Formal stopping rules for GVHD grade IV and day-100 mortality are provided.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the FHCRC Trial Coordinator. At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. This documentation is extracted by the study nurse within 140 days +/- after HCT via chart review and collection of copies of source documents and entered into a hard copy or electronic Case Report Form (CRF). Thus, multiple

health care providers provide independent observations and participate in monitoring this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the primary health care provider (fellow or physician assistant), floor or outpatient nurse and the PI or other attending clinician involved with the patient averting possible conflict of interest having the PI as the attending clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene on an ad hoc basis as necessary to review the primary data and render a decision.

All centers have an IRB that reviews the protocol and that the local PIs contact when an adverse event on the protocol occurs. Most of the centers have internal auditing mechanisms that assure accurate assessment of clinical outcomes. Clinical outcome data are summarized and transmitted from collaborating centers as case report forms (CRFs). CRFs from external sites are verified and signed by the local investigators at that site. When possible, primary source documents regarding patient outcomes are collected with patients' names removed and replaced by Unique Patient Numbers (UPNs). The CRFs are generated from the collaborating centers at defined time points (100 days, 6 months, and yearly).

22B. Plans for assuring compliance with requirements regarding the reporting of adverse events

The adverse event reporting in these multi-institution clinical trials will follow the FHCRC Guidelines for serious adverse event (SAE) reporting (**see Appendix E of the protocol**). These guidelines detail the expedited reporting requirements and definitions of particular events. All SAEs that meet expedited reporting are reported to the IRO within 10 days of the investigator, trial coordinator, or research nurse upon learning of the event. The IRO must receive a completed SAE report form, signed by the PI, within 10 calendar days. For patients being cared for at the FHCRC, health care providers communicate with the PI, trial coordinator or research nurses as events occur triggering subsequent reporting. For patients not being cared for at FHCRC the outside facilities communicate with the PI, trial coordinator, or research nurse for these reporting purposes. All serious adverse events are reported to the IRB at the time of annual renewal and at the biannual mixed chimerism meeting. The PI for a study is responsible for this reporting and the IRO assures adverse event reporting on an annual basis. The PI in the annual application for grant continuation will summarize reports of toxicities. Furthermore, stopping rules and interim analysis provides an additional safeguard for adverse event analysis and reporting in this protocol. All collaborating PIs have fulfilled all NIH requirements for training in human subjects protection.

22C. Plans for assuring that any action resulting in a temporary or permanent suspension of an NCI-funded clinical trial is reported to the NCI grant program director responsible for the grant

This clinical research trial uses commercial agents and there is no associated Investigational New Drug (IND) or Investigational Device Exemption (IDE). Any temporary or permanent suspension, as determined by the PI, IRB, or DSMC, of this clinical research trial will be reported to the NCI grant program director by the PI.

22D. Plans for assuring data accuracy and protocol compliance

This protocol has a DSMB that is responsible for reviewing protocol data and safety endpoints. The DSMB meets on a twice yearly basis, reviews a report of appropriate endpoint data, and compiles a report that is submitted to the Institutional Review Board, DSMC Chairman, and Protocol Office.

At the FHCRC, health care providers and rotating attending physicians assess patients and record their observations in the medical record. This documentation is extracted by one of the study nurses at 100 days after HCT via chart review and entered into an electronic Case Report Form (CRF). The study nurses also continue to follow patients after day 100, review source documentation, and complete CRFs

at 6 month and then yearly intervals per protocol. The CRFs are printed directly from the database, and the PI reviews the CRFs and the primary source documents for data accuracy. When the CRFs are verified, they are signed by the PI. Thus, multiple health care providers provide independent observations and participate in assessments on this trial. The study has dedicated nurses who at a minimum follow patients to confirm eligibility; reporting of adverse events; reporting of events, which are part of the safety-monitoring plan, and protocol adherence. The PI, data coordinators, and research nurses are responsible for review and maintenance of all patient records to ensure data integrity and protocol adherence.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the FHCRC Trial Coordinator. Clinical outcome data are summarized and transmitted from collaborating centers as CRFs. CRFs from external sites are verified and signed by the local investigators at that site. The data are incorporated into a central database by the data manager. Collaborating sites send signed consents, eligibility forms, and CRFs with source documents demonstrating eligibility, treatment, and serious adverse events (if applicable) to the study staff. These are reviewed for eligibility, adherence to the protocol, accuracy, and completeness by the study staff. Queries are sent to the collaborating investigators if CRFs are inaccurate or incomplete.

The study is monitored under the FHCRC Monitoring Plan. The FHCRC Data and Safety Monitoring Plan details the full scope and extent of monitoring and provides for immediate action in the event of the discovery of major deviations.

23. RECORDS

Clinical records will be maintained as confidentially as possible by all collaborating institutions. Data will be collected from the collaborating institutions and will be maintained by Clinical Statistics. The investigator will ensure that data collected conform to all established guidelines for coding, collection, keyentry and verification. Each patient is assigned a unique patient number to assure patient confidentiality. Any publication or presentation will refer to patients by this number and not by name. The licensed medical records department, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents. Patient research files are kept in a locked room. They are maintained by the FHCRC data collection staff, which is supervised by an A.R.T. Access is restricted to personnel authorized by the Division of Clinical Research.

24. STATISTICAL CONSIDERATIONS AND TERMINATION OF THE STUDY

We plan to enroll 20 patients on this study. The primary endpoint of this study is to determine safety. We will consider this study a success if we do not reach the stopping rules. All patients, regardless of diagnosis, will be considered in the safety analysis. The study will be suspended and referred to the DSMB for consideration of results and appropriate modification or termination of the study if there is sufficient evidence that the probability of transplant related mortality (death not due to the underlying disease) by day 100 exceeds 25% or that the probability of grades III-IV acute GVHD exceeds 25%. This will be deemed to occur if the lower level of a one-sided 80% confidence interval for either proportion exceeds 25% and will be evaluated after 5 patients have been followed for at least 100 days. Operationally, the study will be suspended if: 3 out of the first 5, 5 out of the first 10, 6 out of the first 15, or 8 of the first 20 patients die before day 100 or develop grade III or IV acute GVHD before diagnosis of chronic GVHD. (See **appendix C for grading of acute GVHD**). In addition, this study will be suspended and referred to the DSMB for consideration of results and appropriate modification or termination of the study if there is sufficient evidence that the probability of graft rejection/graft failure by day 84 exceeds 20%. (See Section 18 for definitions of graft rejection and graft failure) This will be considered true if the lower bound of a one-sided 80% confidence interval exceeds 20%. Operationally, the study will be suspended if: 3 out of the first 5, 4 out of the first 10, 5 out of the first 15, or 6 out of the first 20 patients have graft rejection or fail to engraft.

Therefore, **stopping rules** will be imposed for:

- Day 84 Graft rejection/graft failure > 20% (3/5, 4/10, 5/15, 6/20).
- Day 100 TRM >25% (3/5, 5/10, 6/15, 8/20)
- Grade III/IV acute GVHD preceding diagnosis of chronic GVHD >25% (3/5, 5/10, 6/15, 8/20)

In addition, at the conclusion of the study, all unexpected toxicities will be summarized and reported. The following adverse outcomes will be monitored carefully throughout the study:

1. Assessment of Adverse events - Adverse events will be assessed using an adapted version of the Common Toxicity Criteria (**Appendix J**).
2. Infections. Every effort will be made to obtain tissue documentation of infection as consistent with appropriated medical management. For details on grading infections see **Appendix J**. Death due to infection will be scored as TRM, including for patients with primary immunodeficiency disorders.

As a secondary endpoint we will also evaluate the proportion of patients who achieve >5% donor T-cell chimerism by day 84. From this study we will obtain a preliminary estimate of incidence of successful T-cell chimerism for use in designing future studies. Although not definitive, for the endpoint of mixed chimerism given the small number of patients, we shall consider this study a success if the observed rate of successful establishment of mixed chimerism (>5% donor T-cell chimerism) is 80% or greater (16/20). If the true rate is 90%, then the probability that 16 or more patients among 20 will develop mixed chimerism is approximately 93%. If the true rate is 70%, 60%, or 50% then this probability is 38%, 17%, or 5%, respectively.

- In June 2016 the Data Safety Monitoring Board met to discuss the possibility of the study having met stopping rules based on the fact that 5 patients out of the first 10 patients developed grade III or IV acute GVHD. Per the protocol the study will be suspended if 3 out of first 5, 5 out of the first 10, 6 out of the first 15, or 8 out of the first 20 develop grade III or IV acute GVHD.

The DSMB met and made the following recommendation: The DSMB members agreed that current protocol findings met criteria for convening the DSMB. The DSMB members noted that, while there is an apparent relatively high proportion of patients with Grade 3-4 GVHD, there has been a very low rate of non-relapse mortality with only 1 death. The DSMB members agreed to continue accrual on the protocol with the requirement that subsequent patients be enrolled no sooner than 100 days after the previous patient's transplant day 0. If an additional patient develops Grade 3-4 acute GVHD, then the DSMB should be reconvened and will likely result in study closure.

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APPENDICES – TABLE OF CONTENTS

APPENDIX A	ABO INCOMPATIBILITY
APPENDIX B	INFECTIOUS DISEASE GUIDELINES
APPENDIX C	ACUTE GRAFT-VERSUS-HOST DISEASE GRADING
APPENDIX D	CHRONIC GRAFT-VERSUS-HOST DISEASE GRADING
APPENDIX E	STUDY COORDINATOR’S MANUAL INCLUDING PROCEDURE FOR REPORTING ADVERSE EVENTS
APPENDIX F	ADVERSE EVENT REPORT
APPENDIX G	NOTICE OF DEATH FORM
APPENDIX H	PATIENT DEMOGRAPHICS AND REGISTRATION FORM
APPENDIX I	OUTSIDE CENTER CORE CASE REPORT FORM
APPENDIX J	ADAPTED COMMON TOXICITY CRITERIA
APPENDIX K	CYCLOPHOSPHAMIDE ADMINISTRATION GUIDELINES
APPENDIX L	WEIGHT CALCULATIONS FOR DRUG DOSING
APPENDIX M	TOTAL BODY IRRADIATION GUIDELINES
APPENDIX N	COORDINATING CENTER FUNCTIONS
APPENDIX O	HEMATOPOIETIC CELL TRANSPLANT-COMORBIDITY INDEX (HCT-CI)

APPENDIX A

ABO INCOMPATIBILITY

Red Blood Cell - Incompatibility (Major):

Occasional patients may have antibodies directed against red blood cell antigens found on the donor's cells. These are generally ABO or Rh antigens, although incompatibility with other red cell antigens identified by donor-recipient crossmatch may occur. Although the volume of red blood cells (RBC) in most PBMC products will only be 2-5% of the product volume before infusion, the small quantity may cause a hemolytic transfusion reaction. According to the FHCRC policy it is generally acceptable to infuse a volume of about 10ml RBCs per product. If the recipient shows an anti-donor titer of $\geq 1:32$ or the RBC volume is greater than 10ml (or > 20 ml in two products combined) the PBMC components should be RBC depleted by Starch Sedimentation (flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse red cell depleted PBMC products within 2 hours of depletion.

Expected Results: Red blood cell depleted PBMC products will contain < 10 ml of red blood cells and $\geq 90\%$ nucleated cell recovery.

Red Blood Cell - Incompatibility (Minor):

Occasional donors may have antibodies directed against red blood cell antigens (ABO, Rh, or other antigen system) found on the recipient's cells. The risk of hemolysis of recipient red cells immediately after transplant is not of very much clinical import. Due to the high number of lymphocytes in the PBMC inoculum, recipients may be at much greater risk for a delayed type of hemolysis that can be severe. PBMC products contain < 200 ml of plasma according to FHCRC policy and no deleterious effects have been observed so far. However, if donors show an anti-recipient titer $\geq 1:256$, the PBMC component should be plasma depleted (see flowsheet below). *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

Timing: Every attempt should be made to infuse plasma-depleted PBMC within 2 hours of depletion.

Expected Results: The plasma depletion should not affect the nucleated cell recovery.

Red Blood Cell – Bidirectional Incompatibility:

Patients undergoing transplants for bidirectional RBC incompatibility should be managed according to both algorithms shown below. Most red cell depletion techniques also deplete plasma from the PBMC component with no additional cell loss. *Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding management of a specific patient.*

Post transplant blood component support will be according to Standard Practice Guidelines.

ABO INCOMPATIBILITY

MAJOR ABO INCOMPATIBLE

Recipient anti-Donor titer	\geq 1:32	<20ml RBC total	\Rightarrow	Infuse without modification
		>20ml RBC total	\Rightarrow	RBC depletion of component
	\leq 1:16		\Rightarrow	Infuse without modification

MINOR ABO INCOMPATIBLE

Donor anti-Recipient titer	\geq 1:256	Plasma depletion of component
	\leq 1:128	Infuse without modification

APPENDIX B

INFECTIOUS DISEASE GUIDELINES

Herpes Simplex and Varicella Zoster Virus Prevention and TreatmentPg



HSV VZV Prevention
and Treatment v6Jur

CMV Prevention: Surveillance and Preemptive Therapy 8/16/17



cmvprevention
v16Aug17.pdf

CMV Disease: Diagnosis and Treatment 10/19/16



cmvdiseasetreatme
nt v19Oct16.pdf

Antifungal Therapy Guidelines 3/16/16



antifungal_therapy
v16Mar16.pdf

Pneumonia / Pneumocystis Carinii Prophylaxis 10/19/16



pneumocystisjirove
ci v19Oct16.pdf

Antibiotic Prophylaxis for Encapsulated Bacteria in Allogeneic Patients with Chronic GvHD Requiring Immunosuppressive Therapy 10/19/16



antibioticprophylax
is v19Oct16.pdf

Vaccinations 6/21/18



ltfu_guidelines.
vaccinations v12Sep

Foscarnet 8/16/17



foscarn
v16Aug17.pdf

APPENDIX C

ACUTE GRAFT-VERSUS-HOST-DISEASE STAGING AND GRADING TABLES

Clinical Stage of Acute GVHD According to Organ System

Stage	Skin	Liver	Intestine ⁽¹⁾
1	Maculopapular rash <25% of body surface	Bilirubin 2-3 mg/dl	>500-1000 mL diarrhea per day or (nausea, anorexia or vomiting with biopsy (EGD) confirmation of upper GI GVHD
2	Maculopapular rash 25-50% of body surface	Bilirubin 3.1-6 mg/dl	>1000 -1500 mL diarrhea per day
3	Maculopapular rash >50% body surface area or Generalized erythroderma	Bilirubin 6.1-15 mg/dl	>1500 mL diarrhea per day
4	Generalized erythroderma with bullous formation and desquamation	Bilirubin >15 mg/dl	Severe abdominal pain with or without ileus

Overall Clinical Grading of Severity of Acute GVHD

Grade	Skin	Liver	GI
I	1-2	0	0
II ^A	3 and/or	1 and/or	1
III ^{A,B}	4 and/or	2-4 and/or	2-4
IV ^{A,C}	4 and/or	2-4 and/or	2-4

- A. Grade II-IV GVHD with only single organ involvement should be biopsy confirmed.
- B. Non-fatal GVHD
- C. Fatal GVHD

⁽¹⁾ For pediatric patients the following diarrhea volumes will apply:

Stage 1 - > 10 ml/kg/day

Stage 2 - > 20 ml/kg/day

Stage 3 - > 30 ml/kg/day

**APPENDIX D
EVALUATION OF CHRONIC GRAFT-VERSUS-HOST DISEASE^a**

In all cases, concomitant processes (i.e. infections or drug reactions) must be ruled out. Karnofsky or Lansky Clinical Performance scores, 60%, > 15% weight loss, and recurrent infections are usually signs of clinical extensive chronic GVHD. Abnormalities that could indicate chronic GVHD are categorized by organ systems as listed below.

Skin	Erythema, dryness, pruritus, pigmentary changes (i.e. hyperpigmentation, vitiligo), mottling, papulosquamous plaques, nodules, exfoliation, macular-papular or urticarial rash, scleroderma, morphea (one or several circumscribed, indurated and shiny lesions)
Nail	Ridging, onychodystrophy, onycholysis
Hair	Premature graying, (scalp hair, eyelashes, eyebrows), thinning scalp hair, alopecia, decreased body hair
Mouth	Dryness, burning, gingivitis, mucositis, striae, atrophy, erythema, lichenoid changes, ulcers, labial atrophy or pigmentary changes, tooth decay, tightness around the mouth
Eyes	Dryness, burning, blurring, gritty eyes, photophobia, pain
Vagina/vulva	Dryness, dyspareunia, stricture or stenosis, erythema, atrophy or lichenoid changes not included
Liver	Elevated liver function tests not due to other causes (alkaline phosphatase \geq 3x upper limit of normal, AST or ALT \geq 4x upper limit of normal or total serum bilirubin \geq 2.5; in the absence of chronic GVHD involving other organs, liver biopsy is required to confirm diagnosis)
Lung	Bronchiolitis obliterans (see diagnostic indicators), cough, wheezing, dyspnea on exertion, history of recurrent bronchitis or sinusitis
GI	Anorexia, nausea, vomiting, weight loss, dysphasia, odynophagia, malabsorption
Fasciitis	Stiffness and tightness with restriction of movement, occasionally with swelling pain, cramping, erythema and induration, most commonly affecting forearms, wrists and hands, ankles, legs, and feet, inability to extend wrists without flexing the fingers or the elbows, contractures
Serositis	Chest pain or cardiopulmonary compromise due to pericarditis or pleuritis
Muscle	Proximal muscle weakness, cramping
Skeletal	Arthralgia of large proximal girdle joints and sometimes smaller joints

Appendix D (continued)

Laboratory testing and diagnostic indicators of chronic GVHD^a

Eye	Schirmer's test with a mean value \leq 5mm at 5 minutes, or symptomatic with values of 6-10mm or keratitis detected by slit lamp examination
Liver	Elevated liver function tests not due to other causes (see definition of clinical limited and extensive chronic GVHD)
Lung	New obstructive lung defect defined as $FEV_1 < 80\%$ of predicted with either an $FEF_{25-75} < 65\%$ of predicted or $RV > 120\%$ of predicted, or a decrease of FEV_1/FVC by $> 12\%$ within a period of less than 1 year. A diagnosis of bronchiolitis obliterans requires negative microbiological tests from bronchoalveolar lavage and evidence of air trapping by high resolution end-expiratory and end-inspiratory CAT scans of the chest. A thoracoscopic lung biopsy may be necessary in order to confirm the diagnosis of bronchiolitis obliterans in patients who have obstructive lung disease without air trapping when chronic GVHD involving other organs is absent
Esophagus	Esophageal web formation, stricture or dysmotility demonstrated by barium swallow, endoscopy or manometry
Muscle	Elevated CPK or aldolase, EMG findings consistent with myositis
Blood	Thrombocytopenia (usually 20,000-100,000/ μ l), eosinophilia, hypogammaglobulinemia, hypergammaglobulinemia, and autoantibodies occur in some cases

^a From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

Appendix D (continued)

GRADING OF CHRONIC GRAFT-VERSUS-HOST DISEASE

A. Clinical limited chronic GVHD

1. Oral abnormalities consistent with chronic GVHD, a positive skin or lip biopsy, and no other manifestations of chronic GVHD
2. Mild liver test abnormalities (alkaline phosphatase $\leq 2x$ upper limit of normal, AST or ALT $\leq 3x$ upper limit of normal and total bilirubin ≤ 1.6) with positive skin or lip biopsy, and no other manifestation of chronic GVHD
3. Less than six papulosquamous plaques or limited skin rash or dyspigmentation ($< 20\%$ of the body surface), positive skin biopsy, and no other manifestations of chronic GVHD
4. Ocular sicca (Schirmer's test ≤ 5 mm), positive skin or lip biopsy, and no other manifestations of chronic GVHD
5. Vaginal or vulvar abnormalities with positive biopsy, and no other manifestations of chronic GVHD

B. Clinical extensive chronic GVHD

1. Involvement of two or more organs with symptoms or signs of chronic GVHD, with biopsy documentation of chronic GVHD in any organ
2. $> 15\%$ base line body weight loss not due to other causes, with biopsy documentation of chronic GVHD in any organ
3. Skin involvement more extensive than defined for limited chronic GVHD, confirmed by biopsy
4. Scleroderma or morphea
5. Onycholysis or onychodystrophy thought to represent chronic GVHD, with documentation of chronic GVHD in any organ
6. Decreased range of motion in wrist or ankle extension due to fasciitis caused by chronic GVHD
7. Contractures thought to represent chronic GVHD
8. Bronchiolitis obliterans
9. Positive liver biopsy; abnormal liver function tests not due to other causes with alkaline phosphatase $> 2x$ upper limit of normal, AST or ALT $> 3x$ upper limit of normal, or total bilirubin > 1.6 , and documentation of chronic GVHD in any organ
10. Pericarditis or pleuritis not due to other causes
11. Positive upper or lower GI biopsy

^a From Standard Practice Guidelines for "Chronic Graft-versus-Host Disease Classification at the time of presentation" developed by Long Term Follow-Up at the FHCRC

APPENDIX E

Study Coordinator's Manual

I. Introduction

The mixed chimerism protocols have been opened to multiple sites to increase the referral base and accrual. Because of this expansion of collaborators, the data collection procedures are being revised. The procedure manual was created to assure consistency of data reporting across the centers and to assure compliance with regulations. General expectations of collaborators are that they will comply with appropriate regulatory requirements, specified protocol requirements, and provide outcome data.

The manual translates working procedures for study coordination. Its goal is to describe the procedures with sufficient clarity to ensure that all study centers will use the same procedures and follow-up schedules for participant data management and reporting. Changes to the manual and relevant forms will be made as soon as practical and will become effective on receipt of the revised procedures at the study centers, unless otherwise noticed.

II. Institutional Review Board Review of Protocols and Modifications

All research protocols proposed for use that involves human subjects must be reviewed and approved by the Institutional Review Board (IRB) prior to implementation. New protocols will undergo review at the FHCRC IRB and then will be distributed to sites that wish to participate for their IRB's review. For Centers that have a Federal Wide Assurance (FWA), formal collaboration includes submission of a form 310 and a copy of the IRB approved protocol and consent forms to the FHCRC. For sites without a FWA, an FWA form needs to be filed. Once the paperwork is submitted to the Office for Human Research Protection, the approval process can take up to a couple of months, and must be completed before collaboration on a protocol can begin.

In addition, all amendments and/or revisions to on-going, approved activities must be submitted for review and approved prior to implementation at an institution. No revisions may be implemented at outside institutions without the prior approval of the FHCRC Principal Investigator. The FHCRC and the local site's IRB must review all protocol activities at least once annually. This must be done within 365 days of the last review regardless of the policies of the institution. A copy of annual renewal approvals must be received for collaboration to continue for the next year.

III. Registrations

Collaborating Institutions: The principal investigator of the collaborating institution who will register the patient with the FHCRC will identify eligible patients. Registration will include completion of the eligibility checklist/demographic form (Appendix H) and receipt of signed consent. Fax these documents to 206-667-4427 prior to treatment initiation. Patients should be registered prior to treatment initiation for valid registration

APPENDIX E (continued)

IV. Reporting Adverse Events

The following guidelines are the minimum serious adverse event (SAE) reporting guidelines for Category 1 and 2 studies conducted at the Fred Hutchinson Cancer Research Center.

Expedited Reporting Requirements

All unexpected **and** serious adverse events which may be due to study treatment or intervention **must** be reported to the FHCRC Institutional Review Office as soon as possible but within at least 10 calendar days of the investigator learning of the event.

Definitions

Adverse Event - Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, medical treatment or procedure and which does not necessarily have to have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, medical treatment or procedure whether or not considered related to the medicinal product.

Life-threatening Adverse Event – Any adverse event that places the patient or subject, in view of the investigator, at immediate risk of death from the reaction. Study toxicities are graded using the adapted NCI Common Toxicity Criteria (where appropriate use the criteria for transplant patients.) All Grade 4 (life-threatening) toxicities occurring between first protocol intervention and day 200 that meet expedited reporting requirements must be reported as soon as possible but within at least 10 calendar days of the investigator learning of the event.

Unexpected Adverse Event – An adverse event, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational product or package insert/summary of product characteristics for an approved product). If applicable product information is not available, such as for studies that do not involve pharmaceutical products or devices, an unexpected adverse event is an adverse event that was not described in the study protocol or informed consent.

Serious Adverse Event (SAE) – Any adverse event occurring that results in any of the following outcomes:

- Death – first protocol intervention until day 200, regardless of cause,
- a life-threatening adverse event (see above)
- a persistent or significant disability/incapacity,
- a congenital anomaly
- requires intervention to prevent permanent impairment or damage.

APPENDIX E (continued)

Hospitalization, in general, will not be considered a serious adverse event as approximately half of evaluable MRD patients AND the majority of evaluable URD patients receiving nonmyeloablative transplants were hospitalized. Hospitalization will be considered a serious adverse event if it fulfills the criteria for a serious and unexpected adverse event as described above.

To ensure no confusion or misunderstanding exist of the differences between the terms “serious” and “severe,” which are not synonymous the following note of clarification is provided:

The term “severe” is often used to describe the intensity (severity) or a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious,” which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory obligations.

Attribution - The FHCRC designation for the determination of whether an adverse event is related to a medical product, treatment or procedure will be as follows:

- Related – includes adverse events that are definitely, probably, or possibly related to the medical treatment or procedure.
- Not Related – includes adverse events are doubtfully related or clearly not related to the medical treatment or procedure.

The FHCRC Serious Adverse Event (SAE) Report Form should be completed for all adverse events that meet the expedited reporting requirements. All available information should be submitted but it is acceptable to fax an incomplete report form at the initial report. A completed report should be faxed as soon as possible but must be received within 10 calendar days.

It is the responsibility of the FHCRC Principal Investigator to notify the sponsor, NIH, FDA or other agencies of serious adverse events as required in the protocol.

Serious adverse events that do not meet the requirement for expedited reporting (not related to study treatment or expected) will be reported to the IRB as part of the annual renewal of the protocol.

FHCRC is acting as the Coordinating Center for this multi-institutional study, and it is the responsibility of the FHCRC Principal Investigator (or designee) to complete the FHCRC Serious Adverse Event Report for all serious adverse events that meet the expedited reporting requirements that are received from the participating sites.

Procedure for Reporting Serious and Unexpected Adverse Events from Participating Sites

Regulations defining the responsibilities for reporting serious and unexpected adverse reactions are defined above. Serious and unexpected adverse events must be reported to the FHCRC Investigator within 10 days of learning of the event. This includes patient deaths, regardless of cause (serious, unexpected, and related/possibly related), occurring from the first protocol intervention and day 200 post-transplant procedure. The Fred Hutch trial coordinator contact information is telephone (206) 667-7385 or (206) 667-4916 and must be followed by fax at **(206) 667-4427** of a detailed written report (See **Appendix F of protocol**) within 10 working days. The report must include the date and time of onset, severity and duration of the event, the relationship to the study, the treatment given and eventual outcome. Follow-up information to a SAE report must be submitted as soon as the relevant information is available.

APPENDIX E (continued)

Obligation of Investigators

All adverse events using the modified NCI Common Toxicity Criteria (**Appendix J** of the protocol) occurring between first protocol intervention and day 100 during the study, whether or not attributed to the study, that are observed by the Investigator or reported by the patient will be recorded on the Case Report Form (**Appendix I** of the protocol). Attributes will include a description, date of onset, maximum severity, and assessment of relationship to the study agent or other suspect agent(s).

Adverse events will be graded accordingly: 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life threatening or debilitating, and 5 = fatal. All Grade 4 (life-threatening) or Grade 5 (fatal) events on the NCI scale meet expedited reporting requirements.

Association or relatedness to the study agent will be graded as follows: 1 = unrelated, 2 = unlikely, 3 = possibly, 4 = probably, and 5 = definitely related.

V. Case Report Forms

Case report forms must be completed for all patients registered onto the protocols and submitted to the FHCRC data coordinating center. The first case report form (days 0-100) is due on day 120. A Staging Form must accompany the form with the patient staging at diagnosis, transplant and day 100. Staging forms should also be completed with each Follow Up Form completed on day 180, 1 year, 1.5 years, 2 years, 3 years, and yearly thereafter. A DLI Form must be completed and submitted for every infusion given.

VI. Protocol Monitoring

As the coordinating center, FHCRC will monitor accrual at the outside institutions. The guidelines below are intended to guide the reviewers in their assessment of items that significantly alter the clinical effectiveness of the treatment or the evaluation of its toxicity.

A. Registration/Randomization

1. Patient was registered prior treatment
2. Information given at registration represents actual data in medical records (stage, diagnosis, cell type, etc.)

B. Informed Consent/IRB Approval Dates

1. The consent was signed prior to registration
2. The consent is in language was approved by the institution's IRB. IRB approval and reapproval are documented including appropriate use of full-board review and proper review of appropriate amendments or revisions
3. Consent was dated and has written witness signature. IRB approval was obtained prior to the patient signing the consent form and start of treatment.

C. Patient Eligibility

1. Eligibility criteria and exclusion criteria were met
2. Treatment/Intervention Administration
3. Doses were modified according to protocol
4. Accurate documentation of drug administration

D. Study Tests/Evaluation

1. Protocol specified laboratory tests or diagnostic studies are available
2. Appropriate record of protocol intervention is documented.

APPENDIX E (continued)

E. Study Events/Adverse Drug Experience

1. Serious Adverse Events reported according to protocol specifications

F. Follow-Up

1. Disease status assessed according to the required protocol guidelines documenting response to treatment.
2. Accurate determination of cancer progression

APPENDIX F
Fred Hutchinson Cancer Research Center
SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ FHCRC/SCCA Other

Gender: Male Female Age: _____

FHCRC Principal Investigator: _____

Phone Number: _____ Mailstop: _____

Date of Report: _____

Initial Report Follow-Up Report # _____ Other

Date study staff became aware of event: _____

Date Serious Adverse Event Started: _____

Date Ended: _____ Or Ongoing (if ongoing – must submit follow up report)

Adverse Event: _____

Describe the Serious Adverse Event including a summary of all relevant clinical information.
(Or attach a MedWatch Form or other SAE reporting form if one has been completed.) Use Page 2, if necessary:

Outcomes Attributed to adverse event: (Check all that apply)

- | | |
|---|---|
| <input type="checkbox"/> Death / / | <input type="checkbox"/> Disability |
| <input type="checkbox"/> Life-Threatening | <input type="checkbox"/> Congenital Anomaly |
| <input type="checkbox"/> Hospitalization (initial or prolonged) | <input type="checkbox"/> Required intervention to prevent permanent impairment/damage |

Specify Agent(s) and/or Procedure(s) involved in this protocol:

#1 _____ #2 _____

Pharmaceutical treatment/procedure	product/medical	Pharmaceutical treatment/procedure	product/medical
---	------------------------	---	------------------------

- | | |
|---|---|
| <input type="checkbox"/> Not Related (Unrelated, Unlikely) | <input type="checkbox"/> Not Related (Unrelated, Unlikely) |
| <input type="checkbox"/> Related (Possible, Probable, Definite) | <input type="checkbox"/> Related (Possible, Probable, Definite) |

- | | |
|--|---|
| <input type="checkbox"/> Follow-up Report Required | <input type="checkbox"/> Final Report (PI must sign final report) |
|--|---|

Report Completed by: _____ Date: _____

The PI has determined that the consent form must be revised: Yes No

Does this study involve the deliberate transfer of recombinant DNA or DNA or RNA derived from recombinant DNA, into human subjects (human gene transfer)? yes no If yes and the activity involves the SCCA outpatient clinic, a copy of this Protocol Modification Form and any supporting documents to be reviewed and approved, will be forwarded to the FHCRC's Institutional Biosafety Committee (IBC) by the Protocol Office (Mailstop: LM-230).

Signature of Principal Investigator _____ Date: _____

APPENDIX F (continued)
Fred Hutchinson Cancer Research Center
Clinical Research Division
Institutional Review Office
SERIOUS ADVERSE EVENT REPORT (SAE) Form IRO-08

FHCRC IR File Number: _____ FHCRC Protocol Number: _____

FHCRC Unique Patient # _____ Date of Report: _____

Describe the Serious Adverse Event including a summary of all relevant clinical information.

APPENDIX G

NOTICE OF DEATH

Patient ID: _____ Date of Death: _____

Place of Event: _____

Apparent cause of death (Please be specific. Attach hospital summary or death summary when possible):

Form completed by: _____ Date: _____

APPENDIX H

Protocol 2032 Patient Demographics and Eligibility Form

Please Fax this completed form to (206)-667-4427 for patient registration.
 Questions regarding eligibility should go to Lauri Burroughs, M.D., 206-667-2396.

UPN: _____	
Patient Name: _____ (Last)	_____ (First) _____ (MI)
Date of Birth: ____/____/____ (Mo) (Day) (Year)	Age: _____
Diagnosis: _____	Planned Day 0: ____/____/____ (Mo) (Day) (Year)
<p>Ethnicity (choose one): Instruct the patient to <u>select one</u> of the following.</p> <p><input type="checkbox"/> Hispanic (A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. Term "Spanish Origin" can also be used in addition to "Hispanic" or "Latino".)</p> <p><input type="checkbox"/> Not Hispanic or Latino</p> <p><input type="checkbox"/> Declined to Report</p> <p><input type="checkbox"/> Research Subject does not know ethnicity</p>	
<p>Race (check all that apply): Instruct the patient to <u>select one or more</u> of the following.</p> <p><input type="checkbox"/> American Indian/Alaska Native (A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliations or community attachment).</p> <p><input type="checkbox"/> Asian (A person having origins in any of the original peoples of the Far East, Southeast, Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand and Vietnam).</p> <p><input type="checkbox"/> Black or African American (A person having origins in any of the black racial groups of Africa).</p> <p><input type="checkbox"/> Native Hawaiian/Pacific Islander (A person having origins in any of the original peoples of Hawaii, Guam, Samoa or other Pacific Islands).</p> <p><input type="checkbox"/> White (A person having origins in any of the original peoples of Europe, the Middle East or North Africa).</p> <p><input type="checkbox"/> Research subject does not know race</p> <p><input type="checkbox"/> Declined to report</p>	

TBI DOSE LEVEL

- 200 cGy
- 400 cGy: 2 doses of 200 cGy for patients with acquired aplastic anemia, sickle cell disease, thalassemia, HLH, or other non-malignant disease at high risk for graft rejection (as discussed at the non-malignant board or the patient care conference).

Signature of **Local** Principal Investigator: _____ Date: _____
 (or Designee)
 Transplant Center: _____

Signature of **FHCRC** Principal Investigator: _____ Date: _____
 (or Designee)

Protocol 2032 Eligibility

I) Inclusion Criteria:

1) Yes No Patient signed IRB approved consent form. Date: _____
IRB File Number: _____

2) Yes No The consent was approved by your institution's IRB on Date: _____

The following criteria questions must be marked "Yes" or "NA" for the patient to enter on 2032.

3) Yes No Age < 55 years with a primary immunodeficiency or other nonmalignant inherited disease (except fanconi anemia) treatable by allogeneic HCT.

4) Yes No Patients with pre-existing medical conditions or other factors that render them at high risk for regimen related toxicity or ineligible for a myeloablative conventional HCT. Pre-existing condition precluding conventional myeloablative transplant: _____

5) Yes No Patient does not have an HLA-matched related or unrelated donor.

6) Yes No NA Patient with acquired aplastic anemia.

- Bone marrow cellularity < 25%, or marrow cellularity < 50% but with < 30% residual hematopoietic cells.
- Two out of three of the following (in peripheral blood): neutrophils < 0.5 x 10⁹/L; platelets < 20 x 10⁹/L; reticulocytes < 20 x 10⁹/L.

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

7) Yes No Patient with a related donor who is identical for one HLA haplotype.

Patient					
A: _____	A: _____	C: _____	C: _____	B: _____	B: _____
DRB1: _____	DRB1: _____	DQB1: _____	DQB1: _____		
Donor					
A: _____	A: _____	C: _____	C: _____	B: _____	B: _____
DRB1: _____	DRB1: _____	DQB1: _____	DQB1: _____		

II) Exclusion criteria:

Each of the following questions must be marked "No" Or "NA" for the patient to enroll on 2032.

8) Yes No Patients with fanconi anemia

9) Yes No Patients with metabolic storage diseases who have severe CNS involvement of disease, defined as IQ score < 70.

10) Yes No Patients who are positive for human immunodeficiency virus (HIV).

11) Yes No NA Females who are pregnant (β-HCG⁺) or breast-feeding.

12) Yes No NA Fertile men or women who are unwilling to use contraceptives during HCT and up to 12 months post-treatment.

13) Yes No Patients with fungal pneumonia with radiological progression after receipt of amphotericin formulation or mold-active azoles for greater than 1 month will not be eligible for this protocol (either regimen A or B).

14) Yes No Organ dysfunction. Please check yes if patient meets any of the following:

a. Yes No Cardiac ejection fraction < 30% (or, if unable to obtain ejection fraction, shortening fraction <26%) on MUGA scan or cardiac echo, symptomatic coronary artery disease, other cardiac failure requiring therapy. Patients with a history of, or current cardiac disease should be evaluated with appropriate cardiac studies and/or cardiology consult. Patients with a shortening fraction <26% must be seen by cardiology for approval.

Date: ____ / ____ / ____

Ejection Fraction Value: _____ % or

Shortening Fraction Value: _____ %

b. Yes No Poorly controlled hypertension despite anti-hypertensive medications.

c. Yes No Patients with clinical or laboratory evidence of liver disease will need to be evaluated for the cause of the liver disease, its clinical severity in terms of liver function and the degree of portal hypertension. Patients will be excluded if they are found to have: fulminant liver failure, cirrhosis of the liver with evidence of portal hypertension, bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, uncorrectable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal liver abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin > 3mg/dl, or symptomatic biliary disease.

Date: ____ / ____ / ____

ALT: _____ U/L

AST: _____ U/L

Total Bilirubin: _____ mg/dL Alk.phos: _____ U/L

Signature of person completing form: _____ Date: _____

FHCRC Patients:

Signature of Principal Investigator: _____ Date: _____
(or Designee)

OR

Outside Center Patients:

Signature of **Local** Principal Investigator: _____ Date: _____
(or Designee)

Signature of **FHCRC** Principal Investigator: _____ Date: _____
(or Designee)

APPENDIX I
Outside Center Core Case Report Forms
For Peripheral Blood or Bone Marrow Transplantations



OSC CRF Version
5.0 (2018) - PB BM.p

APPENDIX J

Adapted from
COMMON TOXICITY CRITERIA (CTC)

Grade		
Adverse Event	3	4
ALLERGY/IMMUNOLOGY		
Allergic reaction/hypersensitivity (including drug fever)	Symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angioedema	Anaphylaxis
Vasculitis	Requiring steroids	Ischemic changes or requiring amputation
Allergy/Immunology – Other (Specify, _____)	Severe	Life-threatening or disabling
BLOOD/BONE MARROW		
Hemolysis (e.g., immune hemolytic anemia, drug-related hemolysis, other)	Requiring transfusion and/or medical intervention (e.g., steroids)	Catastrophic consequences of hemolysis (e.g., renal failure, hypotension, bronchospasm, emergency splenectomy)
For BMT studies, if specified in the protocol.	>4 u pRBC in 24 hours	Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin
For pediatric BMT studies, if specified in the protocol.	>30mL/kg in 24 hours	Hemorrhage or hemolysis associated with life-threatening anemia; medical intervention required to improve hemoglobin
CARDIOVASCULAR (ARRHYTHMIA)		
Cardiovascular/Arrhythmia -Other (Specify, _____)	Symptomatic, and requiring treatment of underlying cause	Life-threatening (e.g., arrhythmia associated with CHF, hypotension, syncope, shock)
CARDIOVASCULAR (GENERAL)		
Acute vascular leak syndrome	Respiratory compromise or requiring fluids	Life-threatening; requiring pressor support and/or ventilatory/support
Cardiac-ischemia/infarction	Angina without evidence of infarction	Acute myocardial infarction

APPENDIX J (continued)

APPENDIX J (continued)

Grade		
Adverse Event	3	4
CARDIOVASCULAR (GENERAL) cont'd.		
Cardiac left ventricular function	CHF responsive to treatment	Severe or refractory CHF or requiring intubation
Cardiac troponin I (cTnI)	Levels consistent with unstable angina as defined by the manufacturer	Levels consistent with myocardial infarction as defined by the manufacturer
Cardiac troponin T (cTnT)	≥ 0.1 - <0.2 ng/mL	≥ 0.2 ng/mL
Hypotension	Requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	Shock (associated with acidemia and impairing vital organ function due to tissue hypoperfusion)
Myocarditis	CHF responsive to treatment	Severe or refractory CHF
Pericardial effusion/pericarditis	With physiologic consequences	Tamponade (drainage or pericardial window required)
Syncope (fainting) is graded in the NEUROLOGY category.	-	-
Thrombosis/embolism	Deep vein thrombosis, requiring anticoagulant therapy	Embolic event including pulmonary embolism
Vein/artery operative injury is graded as Operative injury of vein/artery in the CARDIOVASCULAR (GENEARL) category.		
Cardiovascular/General – Other (Specify, _____)	Severe	Life-threatening or disabling

APPENDIX J (continued)

Grade		
Adverse Event	3	4
COAGULATION		
<p>DIC (disseminated intravascular coagulation)</p> <p>Also consider Platelets. Note: Must have increased fibrin split products or D-dimer in order to grade as DIC.</p>	<p>Laboratory findings present with <u>no</u> bleeding</p>	<p>Laboratory findings <u>and</u> bleeding</p>
<p>Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura/TTA or hemolytic uremic syndrome/HUS)</p> <p>Also consider Hemoglobin, Platelets, Creatinine. Note: Must have microangiopathic changes on blood smear (e.g., schistocytes, helmet cells, red cell fragments).</p>	<p>Laboratory findings present without clinical consequences</p> <p>Evidence of RBC destruction with creatinine (>3 x ULN) not requiring dialysis</p>	<p>Laboratory findings and clinical consequences, (e.g., CNS hemorrhage/bleeding or thrombosis/embolism or renal failure) requiring therapeutic intervention</p> <p>Evidence of RBC destruction with renal failure requiring dialysis and/or encephalopathy</p>
<p>Coagulation - Other (Specify, _____)</p>	<p>Severe</p>	<p>Life-threatening or disabling</p>
CONSTITUTIONAL SYMPTOMS		
<p>Weight gain associated with Veno-Occlusive Disease (VOD) for BMT studies, if specified in the protocol.</p> <p>Also consider Ascites Edema, Pleural effusion (non-malignant).</p>	<p>>10% or as ascites</p>	<p>>10% or fluid retention resulting in pulmonary failure</p>

APPENDIX J (continued)

Grade		
Adverse Event	3	4
DERMATOLOGY/SKIN		
Erythema multiforme (e.g., Stevens-Johnson syndrome, toxic epidermal necrolysis)	Severe or requiring IV fluids (e.g., generalized rash or painful stomatitis)	Life-threatening (e.g., exfoliative or ulcerating dermatitis or requiring enteral or parenteral nutritional support)
Rash/desquamation associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	Symptomatic generalized erythroderma or symptomatic macular, papular or vesicular eruption, with bullous formation, or desquamation covering $\geq 50\%$ of body surface area	Generalized exfoliative dermatitis or ulcerative dermatitis or bullous formation
GASTROINTESTINAL		
Ascites(none-malignant)	Symptomatic, requiring therapeutic paracentesis	Life-threatening physiologic consequences
Colitis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Melena/GI bleeding, Rectal bleeding/hematochezia, Hypotension.	Abdominal pain, fever, change in bowel habits with ileus or peritoneal signs, and radiographic or biopsy documentation	Perforation or requiring surgery or toxic megacolon
Diarrhea associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol. <i>For pediatric BMT studies, if specified in the protocol.</i> Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Pain, Dehydration, Hypotension.	>1500mL of diarrhea/day >15mL/kg of diarrhea/day	Severe abdominal pain with or without ileus

APPENDIX J (continued)

Grade		
Adverse Event	3	4
GASTROINTESTINAL cont'd.		
Duodenal ulcer (requires radiographic or endoscopic documentation)	Uncontrolled by outpatient medical management; requiring hospitalization	Perforation or bleeding, requiring emergency surgery
Gastric ulcer (requires radiographic or endoscopic documentation) Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Bleeding without perforation, uncontrolled by outpatient medical management; requiring hospitalization or surgery	Perforation or bleeding, requiring emergency surgery
Gastritis Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia.	Uncontrolled by out-patient medical management; requiring hospitalization or surgery	Life-threatening bleeding, requiring emergency surgery
Pancreatitis Also consider Hypotension. Note: Amylase is graded in the METABOLIC/LABORATORY category.	Abdominal pain with pancreatic enzyme elevation	Complicated by shock (acute circulatory failure)

APPENDIX J (continued)

Grade		
Adverse Event	3	4
GASTROINTESTINAL cont'd.		
<p>Mucositis</p> <p>Note: Radiation-related mucositis is graded as Mucositis due to radiation.</p>	<p>Painless erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support</p>	<p>Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia</p>
<p>Typhlitis (inflammation of the cecum)</p> <p>Also consider Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia, Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia, Hypotension, Febrile neutropenia.</p>	<p>Abdominal pain, diarrhea, fever, and radiographic or biopsy documentation</p>	<p>Perforation, bleeding or necrosis or other life-threatening complication requiring surgical intervention (e.g., colostomy)</p>

APPENDIX J (continued)

Grade		
Adverse Event	3	4
HEMORRHAGE		
<p>Notes: Transfusion in this section refers to pRBC infusion.</p> <p>For <u>any</u> bleeding with grade 3 or 4 platelets (<50,000), <u>always</u> grade Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia. Also consider Platelets, Transfusion: pRBCs, and Transfusion: platelets in addition to grading severity by grading the site or type of bleeding.</p> <p>If the site or type of Hemorrhage/bleeding is listed, also use the grading that incorporates the site of bleeding: NS Hemorrhage/bleeding, Hematuria, Hematemesis, Hemoptysis, Hemorrhage/bleeding with surgery, Melena/lower GI bleeding, Petechiae/purpura (Hemorrhage/bleeding into skin), Rectal bleeding/hematochezia, Vaginal bleeding.</p>		
<p>Hemorrhage/bleeding with grade 3 or 4 thrombocytopenia Also consider Platelets, Hemoglobin, Transfusion: platelets, Transfusion: pRBCs, site or type of bleeding. If the site is not listed, grade as Hemorrhage – Other (Specify site, _____).</p> <p>Note: This adverse event must be graded for any bleeding with grade 3 or 4 thrombocytopenia.</p>	<p>Requiring transfusion</p>	<p>Catastrophic bleeding, requiring major non-elective intervention</p>
<p>Hemorrhage/bleeding without grade 3 or 4 thrombocytopenia Also consider Platelets, Hemoglobin, Transfusion: platelets, Transfusion: pRBCs, Hemorrhage – Other (Specify site, _____).</p> <p>Note: Bleeding in the absence of grade 3 or 4 thrombocytopenia is graded here only if the specific site or type of bleeding is not listed elsewhere in the HEMORRHAGE category. Also grade as Other in the HEMORRHAGE category.</p>	<p>Requiring transfusion</p>	<p>Catastrophic bleeding requiring major non-elective intervention</p>

APPENDIX J (continued)

Grade		
Adverse Event	3	4
HEMORRHAGE cont'd		
CNS hemorrhage/bleeding	Bleeding noted on CT or other scan with no clinical consequences	Hemorrhagic stroke or hemorrhagic vascular event (CVA) with neurologic signs and symptoms
Hemoptysis	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Melena/GI bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Rectal bleeding/hematochezia	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Vaginal bleeding	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
Hemorrhage – Other (Specify site, _____)	Requiring transfusion	Catastrophic bleeding, requiring major non-elective intervention
HEPATIC		
Bilirubin	>3.0 – 10.0 x ULN	>10.0 x ULN
Bilirubin associated with graft versus host disease (GVHD) for BMT studies, if specified in the protocol.	>6 - <15 mg/100mL	>15 mg/100mL
INFECTION/FEBRILE NEUTROPENIA		
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection)	Present	Life-threatening sepsis (e.g., septic shock)
Infection/Febrile Neutropenia – Other (Specify, _____)	Severe	Life-threatening or disabling

APPENDIX J (continued)

Grade		
Adverse Event	3	4
NEUROLOGY		
Aphasia, receptive and/or expressive, is graded under Speech impairment in the NEUROLOGY category.		
CNS cerebrovascular ischemia	Transient ischemic event or attack (TIA)	Permanent event (e.g., cerebral vascular accident)
Leukoencephalopathy associated radiological findings	Severe increase in SAS; severe ventriculomegaly; near total white matter T2 hyperintensities or diffuse low attenuation (CT); focal white matter necrosis (cystic)	Severe increase in SAS; severe ventriculomegaly; diffuse low attenuation with calcification (CT); diffuse white matter necrosis (MRI)
Seizure(s)	Seizure(s) in which consciousness is altered	Seizures of any type which are prolonged, repetitive, or difficult to control (e.g., status epilepticus, intractable epilepsy)
PULMONARY		
Adult Respiratory Distress Syndrome (ARDS)	-	Present
Apnea	Present	Requiring intubation
Carbon monoxide diffusion capacity (DLCO)	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
FEV1	>25 - <50% of pretreatment or normal value	<25% of pretreatment or normal value
Hypoxia	Decreased O2 saturation at rest, requiring supplemental oxygen	Decreased O2 saturation, requiring pressure support (CPAP) or assisted ventilation

APPENDIX J (continued)

Grade		
Adverse Event	3	4
RENAL/GENITOURINARY		
Creatinine <i>Note: Adjust to age-appropriate levels for pediatric patients.</i>	>3.0- 6.0 x ULN	>6.0 x ULN
Renal failure	Requiring dialysis, but reversible	Requiring dialysis and irreversible
SECONDARY MALIGNANCY		
Secondary Malignancy – Other (Specify type, _____) excludes metastasis from initial primary	-	present

Appendix K
Protocol 2032.00
Cyclophosphamide Guidelines

Introduction

Cyclophosphamide is frequently used as a cytotoxic agent. Cyclophosphamide is converted to its active form in vivo by hepatic enzymes. After a single dose, most of the active metabolites are degraded by tissue enzymes. After high doses (> 40 mg/kg), the alkylating activity in the plasma is minimal by 24 hours. Several of the metabolites appear to have toxic actions. One of the metabolic products, acrolein (CH₂=CH-CHO), is known to be toxic to the bladder urothelium and can cause hemorrhagic cystitis when cyclophosphamide is administered at high doses. In the bladder, the free thiol groups of a drug called mesna (sodium-2-mercaptoethanesulfonate) react with the double bond in acrolein producing an inactive compound, which is eliminated in the urine. Urinary excretion of mesna is almost complete within 2-4 hours after IV administration.

Dosing of cyclophosphamide

Cyclophosphamide doses are specified in the protocol. Adjusted body weight should be used for calculating initial doses if patient's actual weight is > 100% of ideal body weight. If the Ideal Body Weight > Actual Body Weight, then the Actual Body Weight should be used. Doses \geq 5000mg must be infused IV over 2 hours. Lower doses may be administered over one hour.

Note: cyclophosphamide is administered to adults as inpatients or outpatients but to children only as inpatients.

Side Effects of Cyclophosphamide

1. Fluid Retention

Cyclophosphamide can cause an antidiuretic effect with development of inappropriate ADH secretion.

2. Cardiomyopathy

Within 0-10 days after high-dose CY (>100 mg/kg), a clinical syndrome of severe CHF may develop characterized by cardiomegaly, pericardial effusions, diffuse voltage decrease on ECG and decreased LVEF. Mortality is >50%. There are nine retrospective studies of post-transplant cardiomyopathy published between 1976 - 2001. In the four studies of >100 patients, incidence was 0.4 – 4%. The relationship between pre-transplant LVEF or pre-existing cardiac abnormalities and post-transplant cardiomyopathy is equivocal but the number of patients studied has been small. At present, cardiac status is assessed on an individual basis.

References: Appelbaum, et al. Lancet (1976); Goldberg, et al. Blood 68: 1114 (1986); Cazin, et al. Cancer 57: 2061 (1986); Bearman, et al. BMT 5: 173 (1990); Braverman, et al. J. Clin. Oncol. 9: 1215 (1991); Hertenstein, et al. J. Clin. Oncol. 12: 998 (1994); Lee, et al. BMT 18: 573 (1996); Fujimaki, et al. BMT 27: 307 (2001); Murdych and Weisdorf BMT 28: 283 (2001).

(Side Effects of Cyclophosphamide- Continued)

3. Hemorrhagic Cystitis

Cyclophosphamide can cause hemorrhagic cystitis. To prevent the development of hemorrhagic cystitis, patients are kept well hydrated, and mesna therapy or bladder irrigation is used.

The recommended prophylaxis is mesna therapy unless (i) contraindicated clinically, e.g., bladder outlet obstruction as in BPH or (ii) otherwise specified per protocol or Standard Treatment Plan.

a. Bladder Protection

- **Mesna**

The dose of mesna is based on the cyclophosphamide dose being given. Use of mesna should be considered for all patients receiving >2 g/m² of cyclophosphamide. The total daily dose of mesna is equal to 100 % of the total daily dose of cyclophosphamide. Mesna is administered in 4 divided doses IV at 30 minutes prior to start of cyclophosphamide infusion and then at 3, 6 and 8 hours after the start of the cyclophosphamide infusion.

- **Bladder Irrigation**

- Adults > 17 years of age: Insert a #16 French of larger three-way Foley catheter and administer urological saline at a rate of 500 mL/hr. Bladder irrigation should begin 1 hour prior to the start of the first cyclophosphamide infusion and may be discontinued 24 hours after the last dose of cyclophosphamide providing heme testing of urine is negative.
- Children > 12 years of age: Assess individually to determine whether or not it is possible to place a #16 French three-way catheter. If possible, administer urological saline at a rate of 500 mL/hr. Bladder irrigation should begin 1 hour prior to start of the first cyclophosphamide infusion and may be discontinued 24 hours after the last dose of cyclophosphamide providing heme testing of urine is negative.
- Children 1.5-12 years of age: Unless there is an anatomic abnormality, insert a simple Foley catheter to straight drainage as follows:
 - 11-12 years #14 French with balloon
 - 8-10 years #12 French with balloon
 - 1.5-7 years #10 French with balloon

**(Side Effects of Cyclophosphamide-
3. Hemorrhagic Cystitis- Continued)**

b. Fluid Administration

MESNA

Adult Patients

Outpatient (patients who are not at risk for tumor lysis syndrome)

- Solution: NS with 20 mEq KCl/L

- Rate of infusion: 3 ml/kg/hr
- Duration of infusion: Begin at least 2 hours prior to start of cyclophosphamide infusion and continue until at least 8 hours post each dose of cyclophosphamide

Inpatient

- Solution:
 - Without sodium bicarbonate: D5 ½ NS with 20 mEq KCl/L
 - With sodium bicarbonate: D5 ¼ NS with NaHCO₃ 50 mEq/L plus 20 mEq KCl/L (recommended ONLY for patients with high tumor burden such as >20,000 cells/mm³ or mass lesions i.e., at risk for tumor lysis syndrome)
 - Sodium bicarbonate is added when clinically indicated to promote alkalinization of urine to prevent crystallization of uric acid. However, alkalinization of urine is known to increase formation of acrolein.
- Rate of infusion: 2 x maintenance (~3000 mL/m²/24 hours)
- Duration of infusion: Begin fluid administration 4 hours prior to the first dose of cyclophosphamide and continue until 24 hours after the last dose of cyclophosphamide is given.

(Side Effects of Cyclophosphamide-

3. Hemorrhagic Cystitis

b. Fluid Administration

MESNA- Continued

Pediatric patients

Cyclophosphamide is ONLY administered in the hospital

- Solution:
 - Without sodium bicarbonate: D5 ¼ NS with KCl 20 mEq/L
 - With sodium bicarbonate: D5 ¼ NS with NaHCO₃ 40 mEq/L plus KCl 20 mEq/L (recommended ONLY for patients with high tumor burden such as >20,000 cells/mm³ or mass lesions or patients on allopurinol, i.e., at risk for tumor lysis syndrome) .
 - Sodium bicarbonate is added when clinically indicated to promote alkalinization of urine to prevent crystallization of uric acid. However, alkalinization of urine is known to increase formation of acrolein.
- Rate of Infusion:
 - Patients ≥ 20 kg: 2 x maintenance rate
 - Patients < 20 kg: 1.5 x maintenance rate
- Duration of Infusion: Begin fluid administration 4 hours prior to the first dose of cyclophosphamide and continue until 24 hours after the last dose of cyclophosphamide is given.

BLADDER IRRIGATION

- Adult patients (see above, same as for inpatient fluid administration for Mesna)
- Pediatric patients (see above, same as for Mesna)

c. Monitoring

MONITOR	CHILDREN	ADULTS
Weight	Twice Daily	Twice Daily
I&O's	Every 2 hours	Every 4 hours (patients without foley catheter or bladder irrigation must void every 2 hours until at least 8 hours after each dose of cyclophosphamide)
Posturals	N/A	BID
Urine pH	(only patients receiving NaHCO ₃) Keep pH > 7	(only patients receiving NaHCO ₃) Keep pH > 7
Urine for red cells	before cyclophosphamide and around 24 hours after each infusion of cyclophosphamide	before cyclophosphamide and around 24 hours after each infusion of cyclophosphamide

(Side Effects of Cyclophosphamide-

3. Hemorrhagic Cystitis

c. Monitoring- Continued

- **Urine for red cells:**

Test urine by dipstick PRIOR to administration of first dose of cyclophosphamide and once around 24 hours after infusion of each dose of cyclophosphamide. If positive, send urine to lab for complete urinalysis including microscopic evaluation.

- **Urine output:**

- **Adults:** For patients >60 kg, urine output must be at least 3 mL/kg over 2 hours before administering a dose of cyclophosphamide. Urine output must be maintained at ≥ 600 mLs for the first 4 hours post-cyclophosphamide. If urine output < 600 ml over 4 hours, consider either (i) a NS fluid bolus based on current weight, I&O and postural vital signs or (ii) administration of furosemide (10 mg/m² IV)

NOTE: furosemide should not be administered until after the last daily dose of mesna

- **Adults** For patients <60 kg, urine output must be at least 3 mL/kg over 2 hours before administering a dose of Cyclophosphamide. Urine output must be maintained at 2-3 ml/kg/hour for the first 4 hours post-cyclophosphamide. If urine output is less, consider either (i) a NS fluid bolus based on current weight, I&O and postural vital signs or (ii) administration of furosemide (10 mg/m² IV)

NOTE: furosemide should not be administered until after the last daily dose of mesna

- **Children < 40 kg:** Urine output should be maintained at 2-3 mL/kg/hr until 24 hours after the last dose of cyclophosphamide is administered.

If urine output in children (≤ 40kg) is <2-3 ml/kg/hr, give NS challenge, 10 ml/kg over 20 minutes. If inadequate urinary response in 2 hours, give furosemide 0.5 mg/kg IV

NOTE: furosemide should not be administered until after the last daily dose of mesna

Patients without foley catheter or bladder irrigation must void every 2 hours until at least 8 hours after each dose of cyclophosphamide.

- **Weight:** Both inpatients and outpatients should be weighed before each dose of cyclophosphamide. Then, outpatients should be weighed after completion of mesna therapy; inpatients should be weighed twice daily. If the patient's weight is > 2 kg above the pre-treatment weight, consideration should be given to the administration of a diuretic. **NOTE: furosemide should not be administered until after the last daily dose of mesna.**

(Side Effects of Cyclophosphamide-Continued)

4. Nausea/Vomiting/Anorexia

(See Anti-emetics Institutional Standard Practice Guidelines)

5. Other Toxicities

- Myelosuppression
- Gonadal abnormalities
- Alopecia
- Rare pulmonary toxicity

Approved by Medical Executive Committee 6/11/91 (AEP.SPM) cyclophosphamide.doc

Revised by Standard Practice Committee 6/29/94

Approved by Medical Executive Committee 6/28/94

Revised by the Standard Practice Committee July 29, Sept 16, 1998

Abbreviations removed May 1, 1999

Discussed and revision concept accepted in Clinical Advisory Committee August 2002

Revised in Standard Practice Meeting without finalization April 24, 2002, seen in committee November 20, 2002, then with all final information supplied by committee members, January 28, 2003, **February 18, 2003**

Appendix L



weight_for_drug_dosage
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Appendix M
Radiotherapy Treatment Guidelines per Standard Practice



TBI_Adult_Non_Myel
oablative



TBI_Pediatric_NON_
Myeloablative

**APPENDIX N
COORDINATING CENTER FUNCTIONS**

Outside Center – PI Communication in Hematologic Malignancies

I. Study Management, data analysis, and Data and Safety Monitoring

a. Study Management:

- i. Each local PI is responsible for selection, training and oversight of local study coordinators
- ii. The Coordinating Center registers subjects on the study and assigns study IDs
- iii. One copy of the research data is retained by the site. Another data set (identified only by study IDs) is transmitted to the Coordinating Center to create the master data file. All data are kept in locked areas and password protected databases accessible only to study staff
- iv. The quality of data is monitored in an ongoing fashion with the study team and corrective action plans instituted as necessary

b. Data Analysis:

- i. Study staff review data for completeness as it is submitted by the sites
- ii. The study statistician is responsible for data cleaning and the conduct of analyses as outlined in the protocol and grant

c. Data Safety and Monitoring:

- i. The trial coordinators at collaborating centers or the local PIs will fax an official report of an SAE (as defined by the protocol) to the Coordinating Center within ten days
- ii. The SAE report is reviewed by the Overall PI. If the SAE meets the FHCRC criteria for reporting then an official signed report is submitted to the IRB
- iii. An independent DSMB will meet at six-month intervals and all outcome data is reviewed including all adverse events and SAEs reported to the Coordinating Center along with those officially reported to the IRB
- iv. A report from the DSMB is submitted to the IRB as well as the trial coordinators/local PIs participating in the protocol

II. Protocol and informed consent document management

- a. A master protocol is maintained by the Coordinating Center and distributed to the sites for customization and local IRB review
- b. All protocol and consent modifications initiated by the Coordinating Center are sent to the Collaborating Sites following approval by the Coordinating Center IRB, for review and approval by the local IRB
- c. Changes required by local IRBs are reviewed by the Coordinating Center and approved prior to implementation at local sites

III. Assurance of local IRB OHRP-approved assurance

- a. Each site provides their OHRP assurance number and evidence of IRB certification
- b. Study staff monitor maintenance of institutional assurance and IRB certification

IV. Assurance of local IRB approvals

- a. The Coordinating Center maintains copies of the most current collaborating site Consent Forms and IRB approval documentation
- b. No site may enroll subjects until the Coordinating Center has received confirmation of local IRB approval
- c. Each site is responsible for preparation and submission of their continuing reviews. Any changes to the protocol or consent form will be communicated to the Coordinating Center
- d. Sites are required to have active IRB approvals to participate in any study related activities

- V. Any substantive modification by the Collaborating Institution related to risks or alternative procedures is appropriately justified**
 - a. The Coordinating Center reviews any modifications to consent forms to ensure that site consents do not delete or change the basic or additional elements or alternatives required in the sample consent form

- VI. Informed consent is obtained from each subject in compliance with HHS regulations**
 - a. Subjects must provide written informed consent prior to study participation
 - b. The Coordinating Center verifies eligibility and signed consent prior to assigning a study ID number

APPENDIX O
The Hematopoietic Cell Transplant-Comorbidity Index (HCT-CI) 9/7/10
Assign scores appropriately if the patient has any of these comorbidities

<i>Patient</i> _____ (<i>name</i>), <i>UPN</i> _____		<i>Date</i> _____	
Comorbidities	Definitions	HCT-CI scores	Actual Lab Values/Comments
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, and ventricular arrhythmias requiring treatment <i>in the patient's past history</i>	1	
Cardiac	Coronary artery disease†, congestive heart failure, myocardial infarction <i>in patient's past history</i> or EF of $\leq 50\%$ <i>at time of HCT</i>	1	
Inflammatory bowel disease	Crohn's disease or ulcerative colitis requiring treatment <i>in the patient's past history</i>	1	
Diabetes	Requiring treatment with insulin or oral hypoglycemic, but not diet alone, <i>at time of HCT</i>	1	
Cerebro-vascular disease	Transient ischemic attack or cerebro-vascular accident <i>in patient's past history</i>	1	
Psychiatric disturbance	Depression/anxiety requiring psychiatric consult or treatment <i>at time of HCT</i>	1	
Hepatic – mild	Chronic hepatitis, Bilirubin $>ULN- 1.5 X ULN$, or AST/ALT $>ULN-2.5XULN$ <i>at time of HCT</i>	1	
Obesity	Patients with a BMI of >35 for adults or with BMI-for-age percentile of ≥ 95 th percentile for children <i>at time of HCT</i>	1	
Infection	Documented infection or fever of unknown etiology requiring anti-microbial treatment <i>before, during and after</i> the start of conditioning regimen	1	
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica <i>in patient's past history</i>	2	
Peptic ulcer	Requiring treatment <i>in patient's past history</i>	2	
Renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation <i>at time of HCT</i>	2	
Moderate pulmonary	DLco and/or FEV ₁ $>65\%-80\%$ or Dyspnea on slight activity <i>at time of HCT</i>	2	
Prior solid tumor	Treated at any time point <i>in the patient's past history</i> , excluding non-melanoma skin cancer	3	
Heart valve disease	<i>At time of HCT</i> excluding mitral valve prolapse	3	
Severe pulmonary	DLco and/or FEV ₁ $\leq 65\%$ or Dyspnea at rest or requiring oxygen <i>at time of HCT</i>	3	
Moderate/severe hepatic	Liver cirrhosis, Bilirubin $>1.5 X ULN$, or AST/ALT $>2.5XULN$ <i>at time of HCT</i>	3	
Please provide (KPS): Karnofsky Performance Score = _____%		Total Score =	Signature of Provider:

†One or more vessel-coronary artery stenosis, requiring medical treatment, stent, or bypass graft. EF indicates ejection fraction; ULN, upper limit of normal; SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV₁, forced expiratory volume in one second; AST, aspartate aminotransferase; ALT, alanine aminotransferase.