NORTH SHORE –LONG ISLAND JEWISH HEALTH SYSTEM
STEVEN AND ALEXANDRA COHEN CHILDREN'S MEDICAL CENTER OF NY

PROTOCOL

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN PATIENTS WITH HIGH RISK HEMOGLOBINOPATHIES LIKE SICKLE CELL DISEASE AND β-THALESSEMIA-MAJOR USING REDUCED INTENSITY CONDITIONING REGIMEN

PRINCIPAL INVESTIGATOR: Indira Sahdev, MD

Steven and Alexander Cohen Children's Medical Center of NY
North Shore-Long Island Jewish Health System
269-01 76th Avenue
4th Floor Stem Cell Transplant Unit
New Hyde Park, New York 11040
Office Phone #: (718) 470-3611
Fax: (718) 343-2961

TABLE OF CONTENTS

Hypothesis: A preparative regimen will maximize host immunosuppression without myeloablation and will be less toxic, well tolerated and sufficient for engraftment of donor hematopoeitic stem cells (HSC). We plan to test this hypothesis in patients with a non-malignant disease, specifically high risk hemoglobinopathies for which myeloablation is not necessary to eradicate residual disease. Patients will be receiving lower than the conventional doses of chemotherapeutic drugs and more immunosuppressive medications than used in conventional transplants for these disorders. The drugs used will be Campath-1H, Fludarabine and Melphalan to suppress the hyperactive hematopoiesis characteristic of patients with hemoglobinopathies. Cyclosporine/Tacrolimus and Mycophenolate mofetil (MMF) will be administered after hematopoietic stem cell transplantation to enhance engraftment of donor derived HSC and to prevent graft versus host disease (GVHD)

BACKGROUND AND RATIONALE

High-dose chemotherapy with or without radiation followed by allogeneic hematopoietic stem cell transplantation (HSCT) was developed as a curative therapy for a variety of malignant disorders. A standard preparative regimen was designed to deliver myeloablative doses of chemotherapy to impair the host immune system and to eradicate the patient's underlying disease. However, it has become evident that the high-dose therapy does not eradicate the malignancy in many patients. Instead, it is hypothesized that the actual benefit of the transplant is due to an associated immune-mediated graft-versus-host-malignancy effect.

The curative effects of allogeneic bone marrow transplantation may therefore be achieved by directing the therapy at overcoming the immunologic barriers inherent in the transplant procedure (i.e. the host immune system) to initially achieve a stable, mixed hematological chimerism and ultimately a full donor chimerism. The success of engraftment can be measured by mixed chimerism, which is the measurement of the proportion of donor and recipient cells present in the recipient. It is anticipated that, over time, the donor cells may eventually replace the recipient cells; or the donor cells will become tolerant to the allo-antigens from the recipient cells and reconstitute the innate hematopoiesis while maintaining the graft-versus-disease effect (GVD). (21)

Preparative regimens for allogeneic transplantation must address two immunologic barriers to establish successful hematopoietic engraftment: the host-versus-graft effect (HVG) and the graft-versus-host (GVH) effect. High-dose chemotherapy combined with sub lethal doses of radiation therapy has been used to immunosuppress the host sufficiently to prevent rejection of donor cells. Although effective in most patients, the myeloablative regimens are toxic to non-hematopoietic tissue and are associated with a treatment related mortality and morbidity (TRM) approaching 20 to 40%. (66) This excessive TRM limits the potential of this curative treatment in selected patients.

Preparative regimens that are not myeloablative are associated with a greatly decreased incidence of TRM. Non-myeloablative or reduced-intensity immunosuppressive

preparative regimens have achieved a stable, mixed chimerism engraftment and successful allogeneic bone marrow transplants.

With the recognition that the graft-versus-disease (GVD) effect is responsible for many of the observed cures following allogeneic transplantation, preparative regimens have been developed to establish hematopoietic chimerism. Non-myeloablative and reduced-intensity regimens rely on the GVD effect while reducing the myeloablative effect of the transplant. As mentioned earlier, the use of non-myeloablative and reduced-intensity regimens are associated with less toxicity.

Natural History of Sickle Cell Disease

Sickle cell disease (SCD) is a genetic disease with shortened survival and extremely highly prevalence. Over 70,000 people living in United States have sickle cell disease[78]. Disease manifestations vary in severity. Over the last 50 years there have been some advances in health care of children with sickle cell disease. These include newborn screening, early introduction of penicillin, the pneumococcal vaccine and parental education. These and other advances have resulted in improvement in life expectancy of patients with this disease[10]. Yet, this disease continues to be associated with considerable morbidity and mortality particularly as patients reach adult age. The Cooperative Society on Sickle cell Disease demonstrated that mean age of death for patients with this disease in males is 42 years in males and 48 years in females[3]. While this is significantly better than what was expected in 1960, still it constitutes 25 to 30 years loss of life expectancy compared to African-American in general.

Stroke, organ failure, acute chest syndrome and recurrent pain crisis are the major complications that shorten the life expectancy and impair the quality of life[78]. While Hydroxyurea (HU) and chronic transfusion regimens can ameliorate several of the complications of sickle cell disease, allogeneic hematopoietic stem cell transplantation is the only therapy that can cure this disease at the present time. Hematopoietic stem cell transplant successfully establishes donor hematopoiesis and restores cellular, to provide a cure for this non-malignant disorder.

Lack of HLA-matched siblings available for fewer than 15% of otherwise eligible patients is the major obstacle for allogeneic HSCT for high risk SCD[63, 78].

Limitations to SCT in nonmalignant disorders include increased rate of graft rejection due to host immune competence, lack of suitable matched donor, infections, graft versus host disease, and early/late sequelae of conditioning. Strategies to overcome this barrier require successful engraftment of stem cell irrespective of the source with minimal short and long-term transplant related complications.[69]

Goals for stem cell transplant in sickle cell disease are[78]:

1. Decreasing the intensity of transplant related toxicities (mortality/morbidity).
2. Prevention of undesirable GVHD (acute/chronic).
3. Stable donor engraftment.

4. Rapid immune reconstitution.
5. Donor derived immune function.
6. Reversal of established disease related organ damage.
7. Absence of disease progression after HSCT.

Myeloabalative Transplant in Sickle Cell Disease

Transplantation of allogeneic hematopoietic stem cells (bone marrow/peripheral blood/placental blood cell) from HLA-identical healthy siblings after myeloablative therapy has been performed in approximately 150 young patients (<16 yrs of age) with sickle cell disease [3, 44, 45]. This has established normal erythropoiesis, abrogates symptoms and eliminated the need for chronic transfusion and chelation in children with high risk sickle cell disease. Published trials are summarized in Table 1. These studies have shown overall survival rate and effective disease free survival of approximately 90% and less than 10% dying from therapy related complications [55, 48]. Moreover stabilization or reversal of organ damage has been documented after HSCT[11]. However few patients have considered allogeneic HSCT because of paucity of suitable donors, restrictions in eligibility requirements and risk of early death from regimen related side effects. Moreover conventional myeloablative therapy is potentially associated with the risk of late effects including sterility and cancer. A multicenter study from Oakland is in progress with myeloablative busulfan, cytoxan, and rabbit antithymocyte globulin as conditioning in related umbilical cord blood transplants for hemoglobinopathies, and outcomes are compatible to BMT with 89% Overall Survival rate, 84% Disease Free Survival, and low graft rejection rates of 4%. These outcomes show that sickle cell disease patients tolerate SCT and have successful outcomes.

Table -1 Outcomes following myeloabalative HCT[78]

Author	Number of recipients/ median age in years (range)	Donor source/median follow up (years)	Conditioning	Deaths N (%)	OS N (%)	DFS N (%)	AGVHD N (%)	CGVHD N (%)	Graft rejection N (%)	Comments
Walters[48]	±59/10(3-15)	Sib BM/3.5	Bu/Cy/ATG or alemtuzimab	4(6.7)	55(9.3)	50(84.7)		11(19)	9(15)	Patients with se CNS, renal puln and hepatic dise excluded
Vermylen- 22	50/7.5(0.9-23)	Sib BM/11	Bu/Cy/ ±TLI orATG	2(4)	46(93)	42(85)	20(40)	10(20)	5(10)	Asymptomatic p had better OS a than patients wi disease manifes
Bernaudin2	34/8(2-14) Sib BM/1	Sib BM/1	Bu/Cy/ ±TLI orATG	3(9)	31(91)	30(85)	9(26)	2(6)	1(3) HCT	HCT benefitted stroke patients: deaths were GV induced
Loctelli[58]	11/5(1-20)	Sib UCB/2	Bu/Cy/ ±ATG/ALG Bu/Flu/TT	0	11(100)	10(90.9)	1(9)	(6)	1(9)	7 Patients had d manifestation ot stroke, low rates GVHI

Panepinto[779]	67/10(2-27)	BM,PB, UCB/5	Bu/Cy 63 (94%) other 4(6%)	3(4.4)	64(97)	55(85)	10(4-19) probability(95% CI)	22(13-34) Probability (95%	9(13)	GVHD remaine problem
Adamkiewicz [63]	3/6(3-12)	4/6 URD, UCB/4	Bu/Cy/ATG	0	3	2	3	1	1(33)	URD HCT less successful than sibling transplar

Abbreviations: ATG=antithymocyte globulin; BM= bonemarrow; Bu= busulfan; CI=confidence interval; CNS= Central nervous system; Cy=cytoxan; DFS=disease free survival; Flu=fludarbine; GVHD=graft versus host disease; HCT=hematopoietic stem cell transplant; HU=hydroxyurea; N=number; OS= overall survival; PBSC=peripheral blood stem cell; Sib BM=sibling bone marrow; TBI=total body irradiation; TLI= total lymphoid radiation; TRM=treatment related mortality; TT=thiotepa; UCB=umbilical cord blood; URD= unrelated donor

Natural History of β -Thalassemia- Major

The current conventional treatment of patients with β-Thalassemia-Major consists of lifelong monthly regular blood transfusion, combined with iron chelation therapy from approximately 2 years of age onwards. Iron overload is a frequent cause of morbidity and mortality in these patients with cardiac failure, the major cause of death.

Since, transfusion often causes complications in patients with β -Thalassemia-Major, as per Locatelli et al,[17]patients can be classified accordingly to either of these classes.

PESARO CLASSIFICATION[17]

Pesaro class	inadequate chelation	Hepatomegaly	Hepatic fibrosis
I	No	No	No
II	Any two of the Three risk factors		yes
III	Yes	Yes	Yes

Desferoxamine (iron chelating agent for control of accumulated excess iron) treatment used in these patients is very cumbersome, disruptive and expensive, compliance is a major obstacle especially in adolescence and young adult[17].

HSCT is the only available modality that offers the possibility of cure for patients with Thalassemia. The outcome of HSCT in over 800 of patients with Thalassemia is excellent especially in those who are young and do not have advanced disease[51].

Results with Myeloablative Therapy and HSCT in Patients with β-Thalassemia-Major

A major disadvantage of myeloablative conditioning is transient period of myelo-suppression before bone marrow reconstitution, during which the bone marrow transplant recipient is susceptible to infection. Recently 33 Patients with

thalassemia (19 to class I and 13 class II Pesaro) received umbilical cord blood transplantation from siblings. All UCB were HLA matched, median recipient age was 5 years (range 1-20) years and median follow up was 27 months (range 1-85). All patients survived, 26 out of 33 patients have sustained engraftment[45]. Out of a study involving 68 Thalassemia patients transplanted in 6 Italian BMT centers (age range 2-37 years, median age 15 years) were transplanted from unrelated volunteer donors[69]. Fourteen patients were classified in class risk I, 16 in class risk II, and 38 in class III of Pesaro classification system. All these patients were conditioned with various myeloablative chemotherapeutic agents which included Busulfan/cytoxan, busulfan/thiotepa/cytoxan and busulfan/thiotepa/fludarbine. GVHD prophylaxis consisted of cyclosporine/methotrexate and cyclosporine/ methotrexate/antithymocyte globulin. Overall survival in this cohort of 68 patients was 79.3%, as per Kaplan-Meier estimates of disease free survival with transplant independence was 65.8 %. In a group of 30 Thalassemia patients who were classified to risk classes I and II the probability of Overall Survival and Disease free Survival were 96.7% and 80%, respectively. Thirty-eight patients in class III Overall Survival (OS) was 65.2% and Disease free Survival (DFS) was 54.5%.

Based on the above observations several groups have done trials to investigate new preparative regimen which is less toxic than conventional transplant regimen but is immunosuppressive enough to allow donor engraftment and potentially induce graft effect. Recent report of non-myeloablative regimens which resulted in engraftment of allogeneic stem cell in hematological malignancies raises the possibility that this conditioning regimen might be useful in achieving engraftment in non hematological disorder.

A pilot study was conducted on 5 patients with β-Thalassemia-major who received Busulfan, cyclophosphamide and antithymocyte globulin before cord blood transplantation from unrelated donors (1or 2 of 6 HLA antigens were mismatched). GVHD prophylaxis included cyclosporine and methylprednisolone. All patients are alive with a median follow up of 303 days after transplantation with complete donor chimerism and transfusion independence[67]. Table 2 A and B summarizes the characteristics and results obtained in the study.

Table 2 A: Main clinical and biological characteristics of these patients[67]

Patients

Variable	1	2	3	4	5
Clinical					
AGE (Y)	3.7	2.3	3.6	5.8	11.4
Genotype	IVS II-654 AND P28	Homozygous IVS 654	Homozygous IVS 654	IVS II-654 and codon 43	IVS II-654 and codon 41/42
Disease status	Lucarelli class I	Lucarelli class I	Lucarelli class I	Lucarelli class I	Lucarelli class I
Pretransplantaion serum ferritin (microgram/ml)	515	1583	2461	797	2125
HLA type					
Patient	A2, A24, B46, B48,	A11, --, B4001, B46,	A0203, A2402, B1501,	A0207,	A0201, A2402,

	DRB1, 1312, 1501	DRB1, 0406, 1501	B3802, DRB1 0406, 1602	A2601, B1301, B4601, DRB11202, ---	B1525, B5801, DRB1 0301, 1405
Donor	A2, A24, B46, B48, DRB1 0403, 1501	A1101, B4001, DRB1 0405, 1501	A0203, B3802, DRB1 0403, 1602,	A0206, A1101, B1301, B4601, DRB1 1202,--	A0207, A3303, (A0201, --) B5801, --, (5601,5801) DRB1 0301, 1405 (0301, 1401)
UCB Nucleated cell dose$X10^7$/kg)	8.78	11.83	9.03	4.5	3.25
CD34cell dose ($X10^5$/kg)	2.48	2.43	3.75	2.97	2.31

IVS indicates intervening sequence
Double cord blood transplantation.

Table 2 B: Characteristics of Engraftment, GVHD Grading, Outcome, and chimerism Patients[67]

Variable	1	2	3	4	5
Days until					
ANC>0.5$X10^9$/L	17	12	14	12	12
RBC TRANSFUSION INDEPENDENCE	34	37	27	45	22
PLATELETS>20$X10^9$/L	49	46	43	43	55
GVHD grade	I	II	I	II	III
Outcome	Transfusion independence	Transfusion independence	Transfusion independence	Transfusion independence	Transfusion independence
Days after transplantation	454	344	303	245	152
Transplantation Days of the last chimerism	360	270	270	180	120
Chimerism analysis (%donor cells)	100	100	100	100	100

ANC: indicates absolute neutrophil count; RBC=red blood cell
*Double cord blood transplantation

Non-Myeloablative Conditioning Regimen

Nonmyeloablative HCT represents effective strategy to reduce toxicity of transplantation [56, 57]. There is considerable evidence from preclinical models and from clinical transplantation trials that mixed hematopoietic chimerism is a possible outcome. These observations have supported the development of pilot clinical trials to evaluate nonmyeloablative HCT for hemoglobinopthies. Recent studies using Non-Myeloablative transplant seek to limit side effects using less intensive, better tolerated regimen, successful engraftment of stem cells, irrespective of the source with minimal short and long-term transplant related complications.

It is a challenge to establish a full or partial donor chimerism in a patient who is stable and immunocompetent with a hematological disorder. A series of 7 patients with SCD and β-Thalassemia-major underwent HLA-identical sibling HCT after nonmyeloabaltive conditioning. The regimen consisted of fludarbine for 5 days followed by radiation which produced minimal toxicity and resulted in transient donor engraftment in 6 out of 7 patients with hemoglobinopathies. The duration of transient mixed chimerism ranged from 97 to 441 days after transplantation in 4 out of 6 patients, respectively and persisted until

immunosuppressive drugs were discontinued after transplantation. Donor engraftment after nonmyeloablative HCT is more difficult to achieve among immunocompetent pediatric patients with hemoglobinopathies than among patients with hematological malignancies, perhaps in part because recipients may have been sensitized to minor histocompatibility antigens of their donors by preceding blood transfusions, Published data is summarized in the Table 3

Table 3: Review of literature of non-myeloablative transplant[57] with SCD and β-Thalasemia-major

							Donor			Graft Composi-tion		
Patient NO	Age (y)/sex	Disease	Major complications before transplantation	RBC TXN		Stem cell source	Host donor ABO	Hb type	SEX	TNC per kilogram ($X10^8$)	$Cd34^+$ per kilogram ($x10^6$)	CD3+ per kilo-gram ($X10^7$)
1	7/M	Hb SD	Infarct on MRI neurocognitive changes	2.5Y	FluX5,200cGY,FK-506/ MMF	Unprocessed BM	Compatible	AS	F	5.5	11.3	3.9
2	9/M	Hb SS	Right middle and anterior cerebral artery strokes while on transfusion for TIA	5 Y	FluX5,200cGY,FK506/ MMF	Unprocessed BM	Compatible	AA	M	5.1	9.6	5.3
3	20/F	Hb SS	Frequent VOE, ataxia and dysarthria from idiopathic cerebritis	1Y	FluX5,200 cGY,FK506 /MMF	Plasma depleted BM	A^-/O^+	AS	F	1.21	1.11	5.3
4	11/F	β^0 Thal	Cholelithiasis	11Y	FluX5, 200Cgy,CsA/ MMF,ATG	PBSC	Compatible	AA	M	17	9.7	31
5	3/M	Hb SS	ACSX1,splenic sequestration, spleenectomy	16Y	FluX5, 200Cgy,CsA/ MMF, ATG	Unprocessed BM	Compatible	AS	F	5.8	8.3	3
6	5/F	Hb SS	Hospitalization for VOE X 2	<5Y	FluX3, 200 cGY, CsA/MMF	Unprocessed BM	Compatible	AA	M	4.6	NA	NA
7	12/M	Hb SS	Frequent VOE,ACSX1,aplastic crisis, seizures, ischemic changes on MRI, NIMRA, NI TCD	6Y	FluX3, 200cGY, CsA/MMF	RBC-depleted BM	O^+/A^+	AS	F	1.5	4.8	9.2

β^0 indicates β Thalassemia major; MRI, magnetic resonance imaging; TIA, transient ischemic attack; VOE, painful vas-occlusive episode; ACS, acute chest syndrome; NI, normal; MRA, magnetic resonance arteriography; TCD, Transcranial Doppler; RBC, red blood cell transfusion; Flu, fludarabine; FK-506, tacrolimus; MMF, mycophenolate mofetil; CSa, Cyclosporine; BM, bone marrow; TNC, total nucleated cells; N/A, not available; txn, transfusion exposures/duration; Y, years; U, units.

*Patients 1, 2, and 3 were treated at John Hopkins, Patients 4 and 5 at Stanford, Patient 6 at Oakland, and patient 7 at the Fred Hutchison Cancer Research Center.

+Fludarabine dose: 30mg/m^2/dose; ATG dose, 10mg/kg/dX4 days.

± Patient 3 also received hydroxyurea for 1 year.

A pilot study was conducted by Krishnamurti et al, for evaluating the safety of nonmyeloabaltive therapy in patients with hemoglobinopathies. Patients in this study had allogeneic transplantation from HLA identical sibling donor with hemoglobinopathy. The conditioning regimen consisted of busulfan/fludarabine/antithymocyte globulin and total lymphoid radiation of 500 Centi-grey as single fraction shielding liver, lungs, heart and gonads. GVHD prophylaxis consisted of cyclosporine and mycophenolate mofetil.[39, 40] Outcomes of this trial on first 3 patients is summarized in the Table 4

Table 4

Diagnosis and follow up	Organ toxicity	Duration of ANC<500 DAYS	GVHD acute/chronic	ENGRAFTMENT % DONOR (BONE MARROW)		
				D+30	D+100	D+360
1.Scd D+800	None	7	None/ None	89%	100%	100%
2. α Thal D+500	None	5	None/ None	60%	17%	05%
3. Scd D+450	None	8	Grade II skin/none	75%	81%	81%

Reduced Intensity Conditioning.

An important reason for developing new transplant strategies with reduced-intensity conditioning is to off set some of the early and late toxicities associated with the conditioning regimen. Though targeted to reduce regimen related toxicity, a reduced intensity conditioning could tip of the balance in favor of graft rejection in immunocompetent host.[7]

Studies have targeted at developing ideal and safe regimen that is less intense well tolerated with limited side effects.

Review of literature for Reduced Intensity Conditioning[78] Table 5: Outcomes following reduced intensity HCT

Author	Number of recipients/age in years (range)	Donor s source follow up (years)	Conditioning	Deaths N	OS/DFS N	aGVHD	C GVHD	Graft rejection N	Comments
Van Besien[34]	2/40, 56	Sib BM	Flu/Mel/ATG	2	0	2	—	–	High mortality in older patients
Schleuning[52]	1/22	Sib BM/I	Flu/CY	–	1/1	0	Yes	–	Reduced intensity conditioning well tolerated
Iannone[57]	6/8(3-20)	Sib BM/PBSC	Flu/TBI±ATG_	0	6/0	0		6	All children eventually rejected the graft
Horan[68]	4/25(9-30)	Sib BM/I	Flu/ATG/200Cgy TBI	1	3/1	0	–	2	Increased graft rejection in alloimmunized patients
Jacobsohn[65]	3/14(4-22)	Sib (2) and URD PBSC	Bu//Flu/ATG	1	2/0	1		2	Increased graft rejection in sickle cell disease recipients

Mazur[75]	**1/8**	**4/6URD UCB/2**	**HU/Rituximab/alemtuzu /mab/thiotepa/TBI 6cGY**	**--**	**1/1**	**0**	**_**	**0**	**Second transplant successful after increased immunosuppression with preparative regimen**
Krishnam urti[38]	**1/8**	**Sib BM/1**	**Bu/Flu/ATG/TLI 500cGY**	**--**	**1/1**	**0**	**_**	**0**	**Patient with stroke-tolerated conditioning well**
Shenoy[78]	**6/11(2-17)**	**3Sib BM 3 urd/0.8**	**Alemtuzumab/Flu/Mel 140**	**--**	**6/6**	**0**	**1**	**0**	**Tolerated preparative regimen; patient with stroke; stable mixed chimerism; low rate of GVHD**

Abbreviations: ATG=antithymocyte globulin; BM=bone marrow; Cy=cytoxan; DFS=disease free survival; Flu=fludarabine; GVHD=graft versus host disease; HCT=hematopoietic stem cell transplant; HU=hydroxyurea; N=number; OS= overall survival; PBSC=peripheral blood stem cell; Sib BM=sibling bone marrow; TBI=total body irradiation; TLI= total lymphoid radiation; TRM=treatment related mortality; TT=thiotepa; UCB=umbilical cord blood; URD= unrelated donor

A new reduced intensity conditioning regimen aimed at significantly suppressing the recipient's immune system to facilitate engraftment and minimize toxicity in nonmalignant disorder transplant was developed by Shenoy et all.16 recipients with nonmalignant disorder received the SCT using the best available stem source. With median follow-up of 281 days (78-907) 12/16 patients are stable, or cured. Results of the study are summaries in Table 6.[78]

Review of literature for reduced intensity conditioning stem cell transplant for non-malignant cell disorder

Table 6: Characteristics of stem cell transplant[78]

PT NO	Diagnosis (Age in years)	Stem Cell source/ HLA match	TNC 10^8/ kg	CD34 10^6/kg	Myeloid engraftment (days)ANC> $50x10^9$/L	Platelet engraft-ment (days) ANC> $50x10^9$/L	BM chimerism (% Donor)	PB chimerism (%donor)		GVHD acute/ chronic	Follow-up days
								Lymphoid	Myeloid		
1	Hurler syndrome (1.5)	Unrelated BM 6/6	3.2	2.3	12	40	>95	>95	>95	0/0	907
2	Langerhans' cell histiocytosis-(MULTI ORGAN –LCH) (19)	Sibling BM 6/6	3.3	1.2	13	63	>95	86	>95	GrI/0	467
3	Hb E/β Thalassemia (100)	Sibling BM6/6	2.2	4.9	17	103[b]	>95	53	>95	0/0	447
4	Evan syndrome (12)	Unrelated UCB 4/6	0.09	0.06	17	46	>95	>95	>95	0/0	102[c]
5	Severe Aplastic Anemia (SAA) (12)	Sibling PB 6/6	5.0	3.7	10	13	>95	>95	>95	GrI/0	399
6	X-Adrenoleuko-dystrophy (X-ALD) (3.5)	Sibling PB 6/6	4.5	2.5	12	16	>95	>95	>95	0/0	319
7	Sickle cell Anemia (SCA)(2)	Sibling BM6/6	3.3	4.0	13	20	>95	>95	>95	0/0	293
8	Hemophogocytic lymphohistiocy-tosis-HLH (3)	Sibling BM 6/6	3.7	6.2	12	18	>95	>95	>95	0/0	262
9	Severe Aplastic	Unrelated	0.2	0.2	36	1-	>95	>95	>95	0/-	49[c]

	Anemia (SAA)(12)	UCB 5/6									
10	Severe Aplastic Anemia (SAA)(12)	Unrelated BM 5/6	7.8	3.6	16	26	>95	>95	>95	GrI/0	242
11	Hemophogocytic lymphohistiocy-tosis-HLH (18)	Sibling PB 6/6	10-.3	3.4	-	-	-	-	-	-	14[c]
12	Severe Aplastic Anemia (SAA)(40)	Sibling PB 6/6	3.4	2.8	-	-	-	-	-	-	6[c]
13	Congenital dyserythropoietic anemia Type I (CDA)(2)	Unrelated UCB 4/6	0.9	0.6	16	27	94	>95	86	0/-	88
14	XLAAD (7)	Unrelated BM 6/6	12.6	7.9	12	30	94	>95	>95	0/-	92
15	Hurler (1)	Sibling PB 6/6	8.3	13.0	12	12	>95	>95	93	0/-	78
16	Severe Aplastic Anemia (SAA)(12)	Sibling BM 5/6	4.2	5.1	15	22	>95	>95	>95	GR2/0	269

[a]Agvhd was restricted to skin in all recipients and responded to brief topical immunosuppression.
[b]following partial splenectomy in recipient with Hb E/β Thalassemia.
[c] Expired
[d]XLAAD=X-linked autoimmunity- allergic disregulation syndrome; melphlan dose 70mg/m^2 de-escalation study.

In an effort to achieve stable engraftment with any suitable donor stem cell source and to minimize toxicity we have developed a new reduced intensity conditioning regimen for high risk hemoglobinopathies with the main aim of significantly suppressing the recipient's immune system and facilitate engraftment.

STUDY DESIGN

This is a descriptive pilot study. The plan is to enroll a total of 29 patients. There are no control subjects. Partial completers and drop outs will not be included in this study. This is a reduced intensity conditioning regimen consisting of Campath-1H, Fludarabine, Melphalan, in combination with the immunosuppressive medications Cyclosporine or Tacrolimus and Mycophenolate-Mofetil. This is aimed at suppressing the transplant recipient's immune system to facilitate engraftment and to minimize toxicity in subjects. The subjects enrolled will have a diagnosis of high risk hemoglobinopathies and will receive the best available human stem cell source for transplantation as part of standard of care. Patients will be admitted to Cohen Children's Medical Center of New York, Bone Marrow Transplant Unit to receive the reduced intensity conditioning regimen and immunosuppressive drugs.

Primary Objectives

To evaluate the response of reduced intensity regimen consisting of Campath-1H, Fludarabine and Melphalan in Sickle Cell and β-Thalassemia Major patients receiving Human Leukocyte Antigen (HLA) matched/mismatched, related or unrelated hematopoietic stem cell transplantation (Bone marrow/Peripheral blood/Cord blood) in combination with the immunosuppressants Cyclosporine or

Tacrolimus and Mycophenolate Mofetil for treatment of high risk hemoglobinopathies (Sickle cell disease and β Thalassemia-major).

Secondary Objectives

To determine the incidence of:

- Sustained remission of disease at 2 years.
- Engraftment of donor cells, including the development of chimerism at 30 days, 100 days, 6 months and 1 year.
- Determine the % of donor chimerism at day ≥ 30, 6 months, 1 year and successful transplantation
- Treatment-related morbidity and mortality
- Acute and chronic graft-versus-host disease
- To determine that administration of Cyclosporine/Tacrolimus and MMF for preventing GVHD and to enhance engraftment of donor derived HSCT.
- To determine the effect of HSCT on cerebral perfusion and diffusion.
- To determine the rate of T cell immune reconstitution in children with sickle cell disease following myeloablative compared to non-myeloablative stem cell transplantation using immunophenotype assays and measurement of T cell specific donor engraftment.

PATIENT ELIGIBILITY CRITERIA

All patients must meet the inclusion and none of the exclusion criteria for standard of care Bone Marrow Transplant as defined below.

Patient Inclusion Criteria for Sickle Cell Disease

- Patients at least one year of age to less than or equal to 21 years of age with (Sickle Cell Disease-SS or Sickle Cell-S-β-Thalassemia and with one or more of the following disease complications:
- Development of stroke on chronic transfusion protocol.
- Allosensitization on chronic transfusion therapy
- Impaired neuropsychological function and abnormal MRI scan
- Abnormal Transcranial Doppler studies
- Acute chest syndrome (2 to 3 episodes of acute chest syndrome in last 3 to 4 years).
- Ferritin level ≤ 1500 mg/ml
- Recurrent painful priapism; 3-4 episodes/year requiring intervention.
- Recurrent vaso-occlussive crisis of at least 3 to 4 episodes/year.
- Osteonecrosis of multiple bones with documented destructive changes.
- Signed informed consent
- Patients physically and psychologically capable of undergoing transplantation and a period of strict isolation.
- Ferritin ≤ 1500
- Liver Iron Concentration < 6mg/g

Patient Inclusion Criteria for β Thalassemia major

Patients less than or equal to 21 years of age with B- Thalassemia major on routine monthly transfusion protocol or with one or more of the following complications;

1. Hepatomegaly.
2. Liver biopsy revealing evidence of portal fibrosis as
   A) Mild
   B) Moderate
3. Ferritin level≤ 1500ng/ml
4. Liver Iron Concentration (LIC) < 6mg/g

Exclusion Criteria for Both Sickle Cell and β Thalassemia Major Patients

1. HIV positive result confirmed by Western Blot.
2. Pregnancy (Pregnancy testing for females of child-bearing age will be performed and those with a positive serum β-Human Chorionic Gonadotropin will be excluded) and lactating females.
3. Creatinine greater than two times the upper limit of normal for the laboratory,
4. Pulmonary disease with FVC, FEV1 or DLCO parameters < 50% predicted (corrected for hemoglobin) or stage 3 or 4 sickle lung disease.
5. Cardiac insufficiency or coronary artery disease requiring treatment
6. Active infection requiring systemic antibiotic therapy with antibacterial, antifungal or antiviral agents
7. Lansky performance score <70%- (Appendix B)
8. Acute hepatitis/biopsy evidence of cirrhosis.
9. Pulmonary Hypertension

RECRUITMENT PROCEDURES

Subjects will be recruited from the Hematology-Oncology Department at **Cohen Children's Medical Center, (CCMC)** and also may be referred from other institutions as mentioned below. The investigators who participate in the clinical care of these patients in **Cohen Children's Medical Center, (CCMC)** will identify potential subjects who may be eligible for the study and approach the subject, parent or legal guardian. This communication will be documented in the patient's medical record. Subjects and parents/legal guardians of subjects who meet the inclusion criteria will be approached by the study investigators during routine visits conducted as per standard of care.

Patients may also be referred by other hematologists and their treatment staff about participating in the research study. The clinician or staff will note such communication in the patient's medical record. If the patient agrees to be referred to the researcher, the following language is suggested: *"I discussed the possibility of referring the patient to Dr. Sahdev[team] for [describe research study]. The patient agreed to the referral, and to sharing information about his/her condition with the researcher."*

A clinician, who is not the researcher, and that clinician's treatment staff, may give the patient the researcher's name and contact information. The patient may then choose to contact the researcher directly.

A clinician, who is not the researcher, and that clinician's treatment staff, may discuss a patient's eligibility with the research personnel as long as all information about the patient has been de-identified. If the research personnel think the de-identified patient would be eligible for the study, the treatment personnel could then obtain the patient's permission to give the research personnel the patient's name or give the patient the researcher's contact information.

Informed consent for participation and authorization for the use and disclosure of the subject's Protected Health Information (PHI) will be obtained prior to the implementation of any research procedures.

The PI or study investigators will discuss the study with subjects and parents/legal guardian of minor subjects. The investigator will review and explain the study and elements of informed consent, including the purpose of the research, its procedures, risks, benefits, alternatives, costs, etc. An opportunity to ask questions and have them answered prior to signing the consent form will be provided. A copy of the consent form can be taken home so that those interested may have the opportunity to further discuss it with family and friends. Only those physician investigators listed in the study personnel list and approved by the IRB as being able to obtain consent will obtain informed consent. If the subject, or parents/legal guardian agree to participation, the consent form will be signed before participation begins. Subjects may withdraw or be withdrawn from the

study at any time, but not after receiving the preparative regimen chemotherapy, because doing so may decrease blood counts and put the subject at risk for infection/sepsis/bleeding, which in turn, may result in death.

Assent of minor subjects will be obtained as appropriate. The investigators will follow the IRB's guidance of an age range of 7-9 years and up, based on maturity. Minor subjects, who turn 18 during the study, will be re-consented as adults upon reaching the age of maturity.

A signed copy of the executed consent form will be provided to all participants. Informed consent will be documented in the medical record; a copy of the consent document will be placed in the subject's medical record and research record.

Standard of care procedures such as the Hematopoietic Stem cell transplantation, supportive medications and care, follow-up visits, laboratory testing, etc. and associated risks will be discussed with subjects as part of standard of care procedures. Subjects will be provided separate consent for all standard of care procedures.

METHODS

As part of research study subjects will receive the reduced intensity conditioning regimen and immunosuppressive medications as described below. In addition, we will also collect the results of clinical data obtained as part of standard care in order to assess overall clinical status or health status of the subjects. We will also assess the engraftment status by measuring the percentage of donor chimerism, treatment related mortality and morbidity, acute and chronic graft versus host disease.

The research component of this study is the reduced intensity conditioning regimen. The stem cell transplant procedure and all drugs used as preparative regimens would be administered regardless of participation in the research. The administration of reduced dosages of these preparative regimens is the only investigational aspect of the study. There is no drug wash out period. All subjects will undergo investigational reduced intensity conditioning regimen followed by standard allogeneic hematopoietic stem cell transplantation.

1. **Reduced Intensity Conditioning Regimen**
    1. Fludarabine + Melphalan (chemotherapy agents that suppress bone marrow)
    2. Campath-1H (immunosuppressant)

2. **GVHD Prophylaxis**:
   Cyclosporine/Tacrolimus + Mycophenolate-Mofetil

3 **Standard Allogeneic Hematopoietic Stem Cell Transplantation**

4 **Data Collection**
- Results of routine labs, tests, procedures
- Follow-up visits

The Reduced Intensity Conditioning Regimen: This is a combination of treatments which prepares the patient's body to accept the donor's stem cells by partially destroying the patient's bone marrow and suppressing the immune system. It involves three chemotherapy medications, which are explained in greater detail below. Two of the three chemotherapy medications are Fludarabine and Melphalan, and these are given to suppress the bone marrow. Campath-1H (Alemtuzumab) is the third drug, which will be given to the patient to suppress the immune system. The dose adjustment of the medication is based on patient age and body surface area. Cyclosporine/Tacrolimus and MMF will be administered after hematopoietic stem cell transplant to suppress the immune system, enhance engraftment of donor derived (HSCT) and to prevent GVHD.

All drugs are Food and Drug Administration (FDA) approved. Reduced dosage of these medications in this pediatric population is also approved, but the use of these drugs in combination is experimental.

Conditioning Chemotherapeutic Agents:
- Campath-1H is a humanized monoclonal antibody, which targets the CD52 antigen on the surface of normal human lymphocytes as well as malignant lymphocytes.
- Campath-1H (Alemtuzumab) 3 mg intravenous as a test dose on day -20 followed by 15mg/dose for **>10 yrs** of age infused **once** daily x 3 days on days –19, –18 and -17.
- Campath-1H (Alemtuzumab) 10mg/dose for **<10 yrs** of age **once** daily x 3 days on days –19, –18 and –17
- Fludarabine- Fludarabine is the 2-fluro, 5-phosphate derivative of vidarbaine (FLU) at a dose of 35 mg/m2 intravenous over one hour on each of four consecutive days: days -7, -6, -5, and -4.
- Melphalan- **Alkylating agent** (Mel-) 70mg/m^2 intravenous once daily for 2 consecutive days –3, day –2.

This is a reduced intensity preparative regimen that can maximize host immmunosuppression without myeloablation, may minimize toxicity and will be sufficient for engraftment of donor hematopoietic stem cells.

SCHEMA

<u>If <10 years of age</u>	
CAMPATH-1H: 3mg intravenous as a test dose on	**DAY- 20**
CAMPATH-1H: 10mg/dose for <10 yrs of age	**DAY: -19**
CAMPATH-1H: 10mg/dose	**DAY: -18**
CAMPATH-1H: 10mg/dose	**DAY: -17**
<u>If > 10 years of age</u>	
CAMPATH-1H: 3mg intravenous as a test dose on	**DAY- 20**
CAMPATH-1H: 15mg/dose for >10 yrs of age	**DAY: -19**
CAMPATH-1H: 15mg/dose	**DAY: -18**
CAMPATH-1H: 15mg/dose	**DAY: -17**
<u>All Subjects</u>	
FLUDARABINE: 35 mg/m2	**DAY: -7**
FLUDARABINE: 35 mg/m2	**DAY: -6**
FLUDARABINE: 35 mg/m2	**DAY: -5**
FLUDARABINE: 35 mg/m2	**DAY: -4**
<u>All Subjects</u>	
Melphalan: 70mg/m^2 q 24 hours	**DAY: -3**
Melphalan: 70mg/m^2 q 24 hours	**DAY: -2**
DAY OF REST	**DAY: -1**
STEM CELL INFUSION: **(Via the central catheter)**	**DAY: 0**

<u>Graft Versus Host Disease (GVHD) Prophylaxis</u>: These medications are used as a part of standard of care in transplant patients for GVHD prophylaxis but the use of these drugs in combination is investigational:

TACROLIMUS and MYCOPHENOLATE MOFETIL for PBSC, BM and CORD Blood Transplant

OR

CYCLOSPORINE and MYCOPHENOLATE MOFETIL for PBSC, BM and CORD Blood Transplant

Acute GVHD will be based on standard grading (see Appendix C). Prednisone will be initiated for ≥ Grade II GVHD at 2 mg/kg/day by mouth. Intravenous solumedrol can be substituted at equivalent doses should oral formulation not be tolerated

Cyclosporine A (CSA): dosage is based on actual body weight. CSA is to start on day –1 at a dose of 1.5 mg/kg intravenously every 12 hours and given over 2 hours. The dose of CSA is adjusted to maintain a level between 150-300 ng/mL. CSA levels should be monitored 2 times per week. Intravenous CSA will continue until patient tolerates oral liquid or pill without vomiting, as determined by the treating physician. At this time, patients will start oral CSA at a dose of 6 mg/kg/dose twice a day to maintain a blood level between 150-300 ng/mL. CSA should be taken with food if possible. If at day +60, ≥50% donor chimerism or <50% host is demonstrated, then CSA will continue until day +365. If at or around day +180, donor chimerism remains around 100%, CSA will be tapered. If at any time after day +60 there is no evidence of mixed chimerism or if donor chimerism is<10% and no GVHD, then CSA will be withdrawn.

Tacrolimus (prograf): Macrolide lactone immunosuppressant. Dosage is based on actual body weight. Tacrolimus is to start on day -1 at a dose of 0.03mg/kg intravenously as a continuous 24 hours infusion. The dose of Tacrolimus is adjusted to maintain a level between 12-15 ng/mL. Tacrolimus levels should be monitored 2 times per week for the first month and once a week thereafter while on it. Intravenous tacrolimus will continue until patient tolerates oral liquid or pill without vomiting as determined by the treating physician. At this time, patients will start oral tacrolimus at a dose of 0.06 mg/kg/dose twice a day to maintain a blood level between 12-15 ng/mL. Initial doses of tacrolimus should be reduced if total bilirubin is elevated. If at day +60, >50% donor chimerism or <50% host is demonstrated, then tacrolimus will continue until day +365. If at day +60, >50% donor chimerism or <50% host is demonstrated, then tacrolimus will continue until day +365. If at or around day +180, donor chimerism remains around 100%, tacrolimus will be tapered. If at any time after day +60 there is no evidence of mixed chimerism or if donor chimerism is<10% and no GVHD, then tacrolimus will be withdrawn.

Mycophenolate Mofetil (MMF): Immunosuppressive agent, inosine monophosphate dehydrogenase inhibitor. Dosage is based on actual body weight. MMF is to start on day -1 at a dose of 600mg/m2 or 15 mg/kg/dose administered intravenously every 12 hours and given over 2 hours. The dose is same for both oral and intravenous formulation. MMF can be administered on an empty stomach or with food if possible. No dosage adjustment is recommended in patients with hepatic impairment. If at day +60, >50% donor chimerism or <50% host is demonstrated then MMF will continue until day +365. If at day +60, >50% donor chimerism or <50% host is demonstrated, then MMF will continue until day +365. If at any time after day +60 there is no evidence of mixed chimerism or if donor chimerism is<10% and no GVHD, then MMF will be withdrawn.

COLLECTION OF CLINICAL DATA AND INFORMATION FROM MEDICAL CHART (TO BE COLLECTED FROM ALL PARTICIPANTS)

Information to be collected from the medical chart includes: relevant information regarding name, age, sex, demographic data, medical history, past history, family and social history, history of blood transfusions, any complications, iron overload, physical exam findings, screening laboratory results, any previous imaging study results if available, previous history of hospitalizations, current medications, and surgical information.

The results of lab results performed as part of standard care will be collected from subjects' medical records for purposes of the research. The following tests and procedures are performed as part of standard care:

1. Complete history and physical exam.
2. Complete blood count (CBC), comprehensive metabolic panel (CMP), blood type, serum ferritin. (should be <1500ng/ml)
3. Peripheral blood for chimerism studies and hemoglobin electrophoresis.
4. Serological testing which includes hepatitis panel, Epstein Barr Virus (EBV), HIV(Human Immunodeficiency Virus) and HTLV(Human T-cell Lymphotrophic Virus) I and II (blood tests for AIDS virus), cytomegalovirus (CMV), syphilis (testing for sexually transmitted disease).
5. Transcranial Doppler (TCD), MRI and MRA of the brain to evaluate if there is disease related ischemia.
6. Chest and sinus x-rays, electrocardiogram (ECG), cardiac echocardiogram (ECHO) and MUGA (Multiple Gated Acquisition Scan) obtained for baseline determination of heart function.
7. Bone marrow samples and CT (computed tomography) scans and/or MRI (magnetic resonance imaging) studies may be necessary, depending on the patient's clinical condition to assess subject's current clinical disease status.
8. MRI of the heart T2*, liver R2* and liver biopsy (if applicable) to assess for hemochromatosis.
9. To keep pre-transplant hemoglobin S% <35 %, If pre-transplant hemoglobin is more that 35% patients will undergo exchange transfusion (removal of sickle cell load in blood) in order to satisfy transplant criteria.
10. 24 hour urine collection (to evaluate kidney function) and pulmonary function tests (PFT) to evaluate lung function.
11. MRI of the hip, knee or spine for evidence of osteonecrosis.
12. Blood tests for pregnancy in women of childbearing age will be done. If the test is positive, subject will not be able to receive standard of care BMT and will be ineligible to participate in the research study.
13. Sperm Banking will be encouraged as applicable.

FOLLOW UP PROCEDURES

No additional tests or procedures will be performed. Study visits will be conducted during routine follow-up visits. Subjects will continue to have physical examinations and blood tests as a part of standard of care. The results of these tests and procedures will be collected for purposes of the research. Standard tests, procedures and follow up are described in Appendix-D. Immunosuppressive medications will be continued for at least one year as per standard of care.

MEASUREMENT OF EFFECT

To assess the response to treatment, the following end points will be used (which are part of standard of care for transplant). We will collect the results of the following from the medical chart:

1. Engraftment Parameters

- Neutrophil engraftment will be monitored as the number of days to an absolute granulocyte count (neutrophil and band forms) of >500/uL x 3 consecutive days.
- Platelet engraftment will be monitored as the number of days to a platelet count of >50,000/uL independent of platelet transfusion for at least 7 days.
- The total number of red blood cell and platelet transfusions will be monitored.

2. Hemoglobin electrophoresis

Peripheral blood will be monitored for success of engraftment by monitoring for sickle cell load/ adult to fetal hemoglobin ratio in thalassemia major by electrophoresis at approximately 30, 60, 100 days, 6 months, 1 year, 15 months, 18 months and at 2 years post stem cell transplant.

3. Chimerism

Chimerism will be defined as the presence of both donor and recipient DNA. Chimerism will be evaluated using polymerase chain reaction (PCR)-based analysis of polymorphic, microsatellite DNA regions for various number of tandem repeats (VNTR) in sex-matched cases; and fluorescent *in situ* hybridization (FISH) studies will be used to detect the X and Y chromosome in sex-mismatched cases.

4. Management of Mixed Chimerism/Graft Failure.

Chimerism will be measured at the time of engraftment, and at approximately 30, 60, and 100 days post transplant and then monthly until 100% chimerism has been established or as clinically warranted.

Graft failure will be defined as less than 10% donor cells on or after day +60 of transplant.
Graft rejection will be defined as chimerism of at least 30% that is lost at any point post-transplant.

RISKS/DISCOMFORTS

Since this is a reduced intensity conditioning regimen, there is risk of

1. Graft failure

2. Graft rejection
3. Low chimerism

Patients with SCD/ β Thalassemia major undergoing transplantation are heavily transfused. This leads to sensitization of HLA antigens and therefore will increase the risk of graft rejection. The incidence of graft rejection after myeloablative chemotherapy with allogeneic HCST is (5-10%). It is likely that incidence of graft rejection with reduced intensity conditioning regimen is increased as compared to patients getting myeloabaltive transplantation thus leading to low chimerism. However, a rapid taper of immunosuppressive therapy appears to increase the likelihood of graft rejection in patients after myeloablative transplant. By prolonging the immunosuppressive therapy with gradual tapering in this study, there is a good chance of reducing the graft rejection, thereby decreasing the sensitization of HLA antigens which plays a role in graft rejection.

Side Effects of Reduced Intensity Conditioning Regimen:

Melphalan:
The risks associated with the administration of Melphalan include loss of appetite, nausea,
vomiting, mouth sores, hair loss, if drug leaks from vein there are chances of extravasation to the skin(local irritation to the skin) and also may decrease in the number of red and white blood cells and platelets made in the bone marrow. Additional side effects include but are not limited to:

1. Low blood pressure
2. Excessive perspiration
3. Allergic reaction
4. Weakness
5. Weight loss
6. Damage/scarring of lung tissue
7. Inability to conceive (have children) (sperm banking prior to initiating chemotherapy is encouraged)
8. On rare instances there is a possibility of a cancer or leukemia which may result from this treatment.

Fludarabine
Fludarabine alone may cause central nervous system disturbances, including confusion and/or seizures. This could lead to coma that could be irreversible. Additional side effects include but are not limited to:

1. Alopecia (temporary hair loss)
2. Skin rash
3. Nausea and vomiting
4. Anorexia (inability to eat)
5. Diarrhea or constipation
6. Abdominal cramps. It may also cause a serious disturbance of the normal function of the kidneys and /or liver
7. Increase liver enzymes

8. Fatigue
9. Hypotension (low blood pressure) and chest pain
10. Muscle weakness and fever

Campath-1H (Alemtuzumab): Infusion reactions are common

1. Hypotension
2. Rash
3. Nausea
4. Fever and rigors
5. Leukopenia, neutropenia and thrombocytopenia
6. Increased risk for opportunistic infections
7. Skeletal pain and asthenia
8. Peripheral edema
9. Back or chest pain.
10. Malaise
11. Head ache
12. Dysthesias.
13. Dizziness
14. Sometimes hypertension
15. Sinus or supraventricular tachycardia.
16. Sterility.

Risk of Immunosuppression: Immunosuppression is generally given to prevent the body from rejecting stem cell transplant and treating graft-versus-host disease. Many side effects of immune suppression are due in part to the weakened immune system of transplant recipients. Because of the high amount of redundancy in immune cells, immunosuppressants, which aim to decrease immunologic rejection of the transplant, inadvertently handicap the ability of the immune system as a whole. The immune system therefore has a decreased capacity to protect the individual against opportunistic infectious agents (bacteria, viruses, fungi). So infection is a frequent side effect of immunosuppression in transplant recipients. Fortunately, the infections can usually be controlled by the appropriate antibiotic, antiviral or anti fungal drugs.

Side Effects of GVHD Prophylactic Medication: These medications are used as a part of standard of care in transplant patients for GVHD prophylaxis but combination of drugs is a part of the study:

Cyclosporine

Cyclosporine: The immediate and most common side effect of this drug may include nausea or vomiting when given orally. Other possible common side effects include but not limited to:

1. High blood pressure (hypertension),
2. Shaking of the hands (tremor),
3. Increased hair growth,
4. Effect on kidney function,
5. Rarely seizures

These effects are generally reversible upon decreasing the dose of the drug. An occasional patient has had a seizure but it is unclear whether cyclosporine, other drugs, or a combination of drugs was responsible. Some patients given intravenous cyclosporine for the treatment of GVHD have experienced a painful sensation in the hands or feet or both. The pain subsided with the improvement of the GVHD or when the cyclosporine was switched from the intravenous to the oral form. Few patients may experience a change of liver or kidney function, in which case, the dose of cyclosporine may need to be reduced or possibly even withheld. This effect on kidneys seems to increase when other drugs which might cause kidney problems are given at the same time, especially certain antibiotics. Occasionally the kidney damage is severe enough to require the use of an artificial kidney machine (hemodialysis). The risk may be higher if renal function is markedly impaired prior to the transplant. During treatment, cyclosporine blood levels will be monitored periodically to determine if there are increased risks of side effects that warrant adjusting the dose.

Tacrolimus:
The most common known immediate side effects are

1. Tremors
2. Parathesias.
3. Insomnias
4. Expressive dysphasia
5. Seizures
6. Renal insufficiency
7. Hypertension
8. Impaired glucose tolerance and hyperglycemia
9. Hyperurecemia
10. EBV-associated lymphoproliferative disease in organ transplant patient
11. And on short IV infusion of tacrolimus majority had
    a) Head ache
    b) Nausea and vomiting
    c) Hyperesthesia.

Mycophenolate Mofetil: The principal adverse reactions associated with administration are:

1. Diarrhea
2. Vomiting
3. Leukopenia
4. Sepsis
5. Higher frequency of certain type of infections
6. Hypersensitivity reactions

Risk of Medical Information:

There is a risk of breach of confidentiality when the required medical information for this study is collected from the medical chart. This is explained in detail in confidentiality section.

BENEFITS OF TAKING PART IN THE STUDY

The primary possible benefit of participating in this study is that using reduced intensity chemotherapy as a preparative regimen may cause less injury to vital organs. This may be more tolerable and may cure the disease without causing some of the side effects associated with higher doses of chemotherapy which is used in conventional standard transplants. The results of this research will help us in providing data for a more comprehensive study for treating SCD and β Thalassemia major in the future. If this study shows sustained mixed donor chimerism, the paradigm that only severely affected patients with SCD or β Thalassemia major be offered HSCT may shift thus allowing more low risk patients the alternative of cure without long term toxicity and morbidity.

BENEFIT OF THIS STUDY TO SOCIETY

The benefits of the reduced dose conditioning followed by hematopoietic stem cell transplantation to society may be that of new treatment options for patients with hemoglobinopathies in the future. We hope that this study will lead to alternative treatment strategy like different combination of reduced intensity conditioning regimen and different combination of immunosuppressants. A large group of patients may have access to this care being proposed as compared to myeloablative transplants in which only few people are eligible for transplant because of paucity of suitable donors, restrictions in eligibility requirements and risk of early death from transplant related toxicity due to myeloablative preparative regimen.

ALTERNATIVE THERAPY

The alternative therapy for subjects who do not wish to participate in this study, but still interested in getting transplant for their specific disease can receive high dosage standard myeloablative chemotherapy. They will be informed of any new findings that might affect their willingness to continue participating in the study.

EVALUATION OF TOXICITY

i. The study reduced dosing conditioning, and overall transplant-related toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0 as part of standard of care. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be downloaded from the CTEP home page (http://ctep.cancer.gov)

ii. GVHD will be graded according to standardized criteria (refer to Appendix C).

iii. Survival/death will be recorded by the day of death and cause of death.

EVALUATION OF DISEASE RESPONSE

1. Patients will be evaluated for disease free survival by looking at the engraftment of donor cells by evaluating the ratio of donor to recipient chimerism.
2. Complete response to reduced intensity conditioning with combination of chemotherapy and immunosuppressive medication is defined as disappearance of all evidence of disease (>95% of donor pattern), Mixed (50-95% of donor pattern), Partial response is defined as a 50% but >30% of donor pattern and absent response (<10% donor pattern).
3. Donor derived immune function.
4. Rapid immunoreconstitution.
5. Reversal of established disease related organ damage.

DATA SAFETY MONITORING BOARD AND STOPPING RULES

Adverse events will be graded as described above according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) v3.0. In order to assure the safety of patients on study, all adverse events will be reported in real time by the transplant team to the DSMB which is composed of Dr. Suchitra Acharya, Dr. Arlene Redner, Dr. Sharon Singh are the other members in Data Safety Monitoring Board who are the experts in the field of hematology but are not a part of the study. The board will meet once every year. They will review all the data that we have collected and the results that that we have retrieved from the medical records of the enrolled subjects. They will also look into toxicity grading, GVHD grading and also adverse events when the subject is enrolled on the study. A report of adverse events will be provided to the IRB yearly or after the enrollment of 3 subjects and the completion of 100 days post bone marrow transplant. Stopping rules for safety will include monitoring of day 100 mortality and graft failure. Enrollment will be temporarily suspended after groups of 3 patients are enrolled and all are 100 days post transplant to determine if stopping rules which includes a true rate of day 100 mortality exceeding 10% or a rate of graft failure more than 25% have been met for toxicity or graft failure.

CONFIDENTIALITY

If patient agrees to participate in this study, they authorize us to collect and share his/her health information. Protected Health Information (PHI) will be collected and used in the study. No PHI will be disclosed outside of the study team or health system. Only study personnel will have access to subject information.

A copy of the signed Consent Form will be placed in the subject's inpatient hospital medical record. A signed copy of the Consent Form will be given to the subjects.

Storing Documents in Hard Copy

The Original Consent Form and all study documents (study file) will be kept in a master file in a locked cabinet in a secure location in the PI's private office. Original Consent Forms will be kept for a minimum of ten years from the completion of the study. Study subjects initials will be used to identify progress notes and study source documents. The initials will be used instead of names.

Storing Documents Electronically
Any documents that contain PHI (e.g., consent forms, link between the ID to subjects' identifiers) will be stored in a password protected computer document/database that will be stored on the NSLIJ network server, separately from any de-identified research data files. Only IRB approved personnel will be the only individuals with access to any research documents containing PHI. De-identified data files will be stored on a password protected computer network.

Storing Documents on Portable Electronic Devices
No PHI or research data will be stored on any Portable Electronic Devices (e.g., laptops, tablets, flash drives, etc.)

Emailing Data
Any research data that will be emailed will be de-identified and encrypted. PHI will not be emailed to any commercial email addresses (e.g., gmail, yahoo, hotmail, etc.)

If the results of the study are published or used in any presentation, all information will be de-identified. It will be impossible to recognize individuals.

COMPENSATION FOR RESEARCH RELATED INJURY
If patient is hurt from being in the study, he/she will receive medical care and treatment as needed from the North Shore-Long Island Jewish Health System. However, patient will be responsible for the costs of such medical treatment, directly or through your medical insurance and/or other forms of medical coverage.

COSTS
Chemotherapy is a part of standard of care for transplant patients. However this study involves reduced intensity conditioning chemotherapy which is again chemotherapy but in reduced dosage and the investigators feel that it would be appropriate to charge this to the subject's insurance company as this study may cause less toxicity, less days of neutropenia leading to less days of hospitalization. Subjects may have unforeseen added costs from being in this study. Prior authorization will be obtained from insurance company before a subject enrolls on this study. If subjects have no insurance then they cannot participate in this study. However, if the subject wants to participate in the study without insurance approval then they will be responsible for the cost of the study

STATISTICAL ANALYSIS
On a formal consultation with the bio-statistical department it was determined that for each outcome descriptive statistics will be calculated.

Specific Aims
1. To describe the response of sickle cell patients and β-Thalassemia-Major patients undergoing the reduced intensity conditioning prior to receiving human leukocyte antigen (HLA) matched/mismatched, related or unrelated hematopoietic stem cell transplantation
2. To describe secondary responses to treatment

3. To describe adverse events

Endpoint Variables
All outcomes will be evaluated at 30 days, 60 days, 100 days, 6 months, 1 year, 15 months, 18 months, 2 years and 3 years unless otherwise noted.

Aim 1:
For sickle cell patients, a patient will be considered a responder if the sickle cell load is < 25%
For β-Thalassemia-Major patients, a patient will be considered a responder if all of the following conditions are met:

- Hemoglobin level ≥9 and ≤10gm/dl
- Ferritin level<_1000ng/ml (decrease in iron load as age appropriate).
- Hepatomegaly<2 cm (as age appropriate)

For both diseases, any patient who cannot be classified as a responder will be considered a non-responder.

Aim 2:
All responses will be described for the two diseases combined, (sickle cell disease and β-Thalassemia-Major), except where noted.

Categorical outcomes:

- Sustained remission of disease at 3 years
- Engraftment of donor cells
- Development of chimerism defined as 75% donor chimerism *(a range around 70-80%)*
- Treatment-related morbidity
- Treatment-related mortality
- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Cerebral perfusion and diffusion

Continuous outcomes:

- Percent of donor chimerism
- The rate of T cell immune reconstitution.

Aim 3:
Adverse events to be described include:

- Risks of transplant:
- Low blood counts *(i.e. WBC <500 as appropriate)*
- Bleeding and failure of the donor stem cell to grow
- Infections
- Transfusion reactions
- Loss of fertility

- Transplant related morbidity and mortality
- Graft failure
- Graft rejection
- All other adverse events

STATISTICAL METHODS

For all aims, and for each outcome, descriptive statistics will be calculated:

- For categorical outcomes, the percent of patients with that outcome and the associated 95% exact binomial confidence interval will be calculated.
- For continuous outcomes means and standard deviations will be calculated.

Study Endpoints

In this study, the endpoint is sustained remission and development of stable mixed chimerism. Stable mixed chimerism is as 75% donor engraftment on day 100. The goals are to determine the probability of success and the probability of surviving 100 days.

Sample Size Justification

This is a pilot study, and the proposed sample size of 10 subjects is based on feasibility and availability of resources. The purpose of the present study is to gather information required to design a larger, more comprehensive study, including data required for a formal power analysis. Although patients with either sickle cell disease or β-Thalassemia-Major are eligible for this study, it is possible that out of the 10 patients enrolled, all or most will have sickle cell disease or all or most will have or β-Thalassemia-Major. However, the purpose of this pilot is to evaluate this regimen in high risk hemoglobinopathies, rather than in a specific disease.

REFERENCES

1. Lucarelli G, Galimberti M, Polchi P, et al: Bone Marrow Transplantation in patients with thalessemia. N Eng Journ Med. 1990:322:417-420.
2. Bernaudin F, Souillet G, Vannier J, Plouvier E, Lemerle S, Michael G, et al: Treatment of sickle cell anemia with bone marrow allograft: French experience (15 cases0 Nouv Rev Fr Hematology 1993:35:319-325.
3. Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Stein berg MH, KlugPP, Mortality in sickle cell disease. Life Expectancy and risk factors of early death.NEng Journ Med. 1994:330:1639-1644
4. Oliveri NF, Nathan DG, et al: survival in medically treated patient's with homozygous Thalassemia. N Eng Journ Med 1994:331: 574-578.
5. Kalyinyak KA, Morris C, Ball WS, et al: Bone Marrow Transplantation in a young child with sickle cell anemia. Am J Hematology 1995: 48: 256-261.
6. Wagner JE, Rosenthal J, Sweetan R, et al, successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors; Analysis of engraftment and acute graft versus host disease. Blood; 1996:88:795-802.
7. Walters MC, Patience M, Leisenring W, et al: Bone Marrow Transplantation for sickle cell disease. N Eng J Med 1996:335:369-376.
8. Abboud MR, Jackson SM, et al: bone marrow transplantation for sickle cell anemia, Ame J Ped Hemat Oncol: 16:86-89:1994.

9. Brittenham GM, Griffith PM, et all: efficacy of deferoxamine in preventing complications of iron overload in patients with Thalassemia .N Eng J Med: 331; 567-573:1994.
10. Serjeant GR. Natural history and determinants of clinical severity of sickle cell disease. Curr Opin Hemat; 2; 103-108:1995.
11. Ferster A, Coraazza F, et al: transplant sickle cell disease patients with autologous bone marrow recovery after graft failure develop increased level of foetal hemoglobin which corrects disease severity. Br J Hemat; 90:804-808:1995.
12. Lucarelli G, Galimberti M, Clift M, et al: Bone Marrow Transplantation in patients with Thalassemia. Results in class 3 patients. Blood: 87:2082-2088; 1996.
13. Walters MC, Patience M, Leisenring W, et al; Barriers to Bone Marrow Transplantation. Biology of blood and Bone Marrow transplantation: 2: 100-104(1996).
14. Vermylen C, Cornu G. Bone marrow transplantation for sickle cell anemia. Curr Opin Hemat; 3; 163-166:1996.
15.Walters MC, Patience M, Leisenring W, et al; Collaborative multicenter investigation of marrow transplantation in sickle cell disease: current results and future directions. Biology of blood and Bone Marrow transplantation: 3: 310-315(1997).
16. Werner E, Yanovich S, Rubinstein p, ET AL: Unrelated placental cord blood transplantation in-patient with sickle cell anemia and acute non-lymphocytic leukemia. Blood: 90: 1997.
17. Lucarelli G, GiardiniC, Angelucci E, et al: Bone marrow transplantation in thalessemia.
In Blood stem cell transplantation. J.N.Winter, ED 305-315. Kluwer academic Boston; 1997.
18. Hoffbrand AV, Davis B, et al; long term trial of deferiprone I 51 transfusion dependent iron overload patients. Blood: 91:295-300:1998.
19.Peterdorf E W, Gooley TA, Anasetti C, et al optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. Blood: 92; 3515-3520:1998.
20. Storb R, YU C, Deeg HJ, Georges G, Walters MC, et al: current and future preparative regimens for bone marrow transplantation in Thalassemia. Ann N Y Acad Sic: 850:276-287:1998.
21.Slavin S, Nagle A, et al: Nonmyeloablative Stem Cell Transplantation and Cell Therapy as an Alternative to Conventional Bone Marrow Transplantation With Lethal Cytoreduction for the Treatment of Malignant and Nonmalignant Hematological Diseases. Blood 91(3): 756-63, 1998.
22. C Vermylyn, G Cornu, et al: Hematopoietic stem cell transplantation fro sickle cell anemia: the first 50 patients transplanted in Belgium. Bone Marrow Transplantation (1998) 22, 1-6
23. Lowdell MW, Craston R, Ray N, et al: .The effect of Tcell depletion with Campath-1Mon immunoreconstitution after chemotherapy and allogeneic bone marrow transplant as treatment fro leukemia. Bone Marrow Transplantation (1998) 21,679-686
24. Slavin S, Angler A, Narrate E et al: Nonmyeloablative stem cell transplantation and cell therapy as alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic disease. Blood 1998:91:756-763.

25. Storb R, YU C, Sand Maier BM, et lapsable mixed hematopoietic chimerism after marrow allografts. Transplantation in ambulatory care setting. Ann N Y Acad Sic: 872:372-375:1999.
26. Walters MC. Bone marrow transplantation for sickle cell disease: where do we go from here? J Ped Hemat Oncol. 1999:21:467-474.
27. Giardini C, Lucarelli G. Bone Marrow Transplantation in beta Thalassemia. Hematol Oncol clin N am 1999:13 1059-1064.
28. Champlin R, Khouri L et al; Nonmyeloablative and preparative regimen and induction of graft versus malignancy effect; Oncology: 1999:13:621-628.
29. Craddock C. Nonmyeloablative stem cell transplant. Curr Opin Hemat; 1999:6:383-387.
30. Kottaridis P, Milligan DW, Chopra R, et al: In Vivo Campath-1Hprevents GVHD following nonmyeloabaltive transplantation .Blood; 2000:96:2419-2425.
31. Sullivan KM, Parkman R, Walter MC, Transplantation for non-malignant disease. Hematology (AM Society Hematology edu Program) 2000: 319-338.
32. Migiliacco AR, Adamson JW, Stevens CE, et al: cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitors content is a better predictor than nucleated cell quantity. Blood. 2000:96:2717-2722.
33. Amado RG, Schiller GJ. Nonmyeloablative approaches to the treatment of sickle cell hemoglobinopathies. Semi Oncol 2000:27:82-89.
34. K Van Besien, A Bartholomew et al: Fludarbaine –based conditioning for allogeneic transplantation in adults with sickle cell disease. Bone Marrow Transplantation (2000) 26,445-449.
35. M.C Walters, M.Patience, Storb R, Leisenring W, Taylor T, Sanders JE, Buchanan et al; Impact of bone marrow transplantation on symptomatic sickle cell disease: an interim report: Multicenter investigation of bone marrow transplantation in sickle cell disease-Blood. 2000:1918-1924.
36. Andreani M, Nesci S, Lucacerrili G, Tonucci P, Rapa S, Angeluci E, Persini B, et al: Long term survival of ex-Thalassemia patients with persistent mixed chimerism after bone marrow transplantation. Bone Marrow Transplantation (2000) 25, 401-404.
37. Beatty PG, Boucher KM, Mori M, Milford EL. Probability of finding HLA-mismatched related or unrelated marrow or cord blood donors. Human immunology 2000: 61; 834-840.
38. Krishnamurti L, Blazar BR, Wagner JE -bone marrow transplantation without myeloablation fro sickle cell disease.N.Eng.J.MED (2001):344-368.
39. Krishnamurti L, Blazar BR, Wagner JE, et al; use of non-myeloablative therapy in treatment of severe hemoglobinopathies: National sickle cell disease program meeting: 20001:125.
40. Remberger M, Ringden O, Blau IW, et al: no difference in graft versus host disease, relapse, and survival comparing peripheral stem cells to bone marrow from unrelated donors. Blood (2001): 98: 1739.
41. Champlin R, Khouri L et al; Nonmyeloablative and preparative regimen for allogeneic stem hematopoietic transplantation. Bone marrow transplant; 2001:27 sup 2 S 13-22.

42. Gennery AR, Dickinson AM, Brigham K, et al: Campath-1H T cell depleted BMT for SCID: Long term follow up of 19 children treated in a single centre 1987-98. Cytotherapy 2001:3:221-232.
43. Champlin R, Khouri L et al; non myeloabaltive preparative regimen of allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplant. 2001:27; Suppl 2:S13-22.
44. Hoppe CC, WaltersMC. Bone Marrow Transplantation in sickle cell anemia. Curr Opin Oncol, 2001:13:85-90.
45. Franco Locatelli, Vanderson Rocha, Reed W, Bernaudin F, Grafakos f, Brichard B, Nagler A, et al: Related umbilical cord blood transplantation in patients with Thalassemia and sickle cell disease. American society of hematology 43 rd annual meeting, Orlando Flrida: 2001.
46. Devine S, Sanborn R, Jessop E, et all: Fludarabine and Melphalan based conditioning fro patients with advanced hematological malignancies relapsing after previous hematopoietic stem cell transplant. Bone Marrow transplant 2001: 28:557-562.
47..Fixler J, Vichinsky E,Walters MC. Stem cell transplantation for sickle cell disease; can we reduce the toxicity? Pediatric Path Mol Med 2001:20; 73-86.
48. M.C Walters, M.Patience, et al: Stable Mixed Hematopoietic. Chimerism after bone marrow transplantation fro sickle cell anemia. Biology of blood and Bone Marrow transplantation: 7:665-673(2001).
49. Walters M, Patience M, Leisenring W, Rogers Z, Aquino V, Buchanan et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. Biol Blood and Bone Marrow transplant 2001: 7:665-673.
50. Lucacerrili G, Andreani M, et al: The cure of Thalassemia with bone marrow transplant. Bone Marrow transplant 2001: 28:511-513.
51. Storb RF, Champlin R, Riddell SR, et al: Nonmyeloablative transplant in malignant diseases. Hematology (Am Soc Hemat Edu Program): 2001: 375-391.
52. Wagner JE, Barker JN, Defor TE, et al: transplantation of unrelated cord blood in 102 patients with malignant and non-malignant diseases: influence of CD34 cell dose and HLA disparity on treatment related mortality and survival. Blood: 2002:100; 1611-1618.
53. Ianonone F, Casella JF, Fuchs EJ et al: Failure of minimally toxic nonmyeloablative regimen to establish stable donor engraftment after transplantation for sickle cell anemia and β-Thalassemia. Blood 2002; 11(supplement. 1a); 46a (abst.161).
54.Bernaudin F, Vernant JP,Vilmer E, et al; results of myeloabaltive allogeneic stem cell transplant fro severe sickle cell disease in France. Blood 2002:11(supli. 1a):5a (abst. 4).
55. Michael Scheuling, Oliver stoetzer, et al: Hematopoietic stem cell transplantation after reduced intensity conditioning as treatment of sickle cell disease. Experimental hematology: 30(2002)7-10.
56.Neiderweiser D, Maris M, Shizuru J et al: Low dose total body irradiation and Fludarabine followed by hematopoietic cell transplantation from HLA matched or mismatched unrelated donor and post grafting immunosuppression with cyclosporine and mycophenolate mofetil can induce durable complete chimerism and sustained remission in patients with hematologic diseases. Blood; 2003:101:1620-1629.
57. Robert Iannone, James f, casella et al: Results of Minimally Toxic Nonmyeloablataive Transplantation in sickle cell anemia and B-major. Biology of blood and Bone Marrow transplantation: 9:519-528(2003).

58. Franco Locatelli, Vanderson Rocha, et al: Related umbilical cord blood transplantation in patients with Thalasemia and sickle cell disease. Blood 15 March 2003 volume 101, number2137-2143.
59. Ball LM, Lankester AC, Giordano PC, et al: pediatric allogeneic bone marrow transplantation for homozygous beta thalessemia, the Dutch Experience. Bone Marrow transplantation: 31:229-239(2003).
60. Kim H, Park CY, et al: successful hematopoietic stem cell transplantation using triple agent immunosuppression in severe aplastic anemia patients. Bone Marrow transplantation: 31: 79-86(2003).
61. Krishnamurti L, Abel S, et al: Availability of unrelated donor for hematopoietic stem cell transplantation for hemoglobinopathies. Bone Marrow Transplantation (2003) 31,547-550
62. Sodani P, Gaziev d, Polchi P, et al; A new approach to bone marrow transplantation in class 3 Thalassemia patients aged less than 17 years. Blood: 104:1201-1203:2004.
63. TvAdamkiewicz, PS Mehta et al: Transplantation of unrelated placental blood cells in children with high risk sickle cell disease. Bone Marrow Transplantation (2004) 34,405-411.
64. C.Brachet, N.azzi, et al: Hydroxyurea. Treatment for sickle cell disease: impact on hematopoietic stem cell transplantation's outcome. . Bone Marrow Transplantation (2004) 33,799-803
65. Jacobsohn D, Duerst R, Tse m, et al: Reduced intensity hematopoietic stem cell transplantation. Lancet; 364:156-162; 2004
66. Disconescu R, Flowers CR, et al: Mortality and morbidity with nonmmyeloabaltive compared myeloablative to conditioning before hematopoietic stem cell transplantation from HLA- Matched related donors. Blood 104(5): 1550-58, Sept 2004
67. Tansg-her Jaing, Iou-Jih Hung, et al: Rapid and Complete Donor Chimerism after unrelated mismatched Cord blood transplantation in 5 children with B-Major. Biology of blood and Bone Marrow transplantation 11; 349-353(2005).
68. T Horan, JL Liesveld, et al: Hematopoietic stem cell transplantation fro multiply transfused patients with sickle cell disease and Thalassemia after low dose total body irradiation, Fludarabine, and rabbit antithymocyte globulin. Bone Marrow Transplantation (2005) 35,171-177.
69. Giorgio La NASA, Franca Argiolu, et al: Unrelated bone marrow transplantation for B-Major patients the experience of Italian bone marrow transplant group.ANN.n.y.acad.SCI.1054:186-195(2005).
70. Shenoy, WJ Grossman et al: A Novel reduced intensity stem cell transplantation regimen for non-malignant disorders. Bone Marrow Transplantation (2005) 35,345-352.
71. Lakshman Krishnamurthi, Timmothy Carlos, et al: A pilot study of allogeneic hematopoietic stem cell transplant fro patients with high risk hemoglobinopathy using a non-myeloablative preparative regimen to achieve stable mixed chimerism- Oct 2005.
72 Steven C, Scardovou A, et al: An empirical analysis of probability of finding well marked cord blood unit. Implications of national cord blood inventory: Blood; 106 Abs 2047; 2005
73. TvAdamkiewicz, Boyer MW, Bray R, et al: Identification of unrelated cord blood units for hematopoietic stem cell transplantation in children with sickle cell disease. J Ped Hemat Oncol. 2006:28-29.

74.Tsiporrah shore, John Harpel et al: A study of reduced –Intensity conditioning regimen followed by allogeneic stem cell transplantation for patients with hematological malignancies using campath-1H as apart of graft-versus- host disease strategy. Biology of blood and Bone Marrow transplantation: 12:868-875(2006).
75. Melissa Mazur, Joanne kurtberg et al: Transplantation of a child with sickle cell anemia with an unrelated cord blood unit after reduced intensity conditioning. Pediatric Hematology Oncology Vol-28, Number 12 December 2006.
76. Adamkiewicz T, Boyez M, et al.: Identification of unrelated cord blood unit for hematopoietic stem cell transplantation I children with SCD. Journ Ped Hemat Oncol; 28:29-32:2006
77. Anni J Shah, Neena kapoor et al: The effects of camapth-1H upon graft-versus-host disease, infection, relapse and immune reconstitution in recipients of pediatric unrelated transplants. Biology of blood and Bone Marrow transplantation: 13:584-593(2007).
78. S.Shenoy: Has stem cell transplantation come of age in the treatment of sickle cell disease. Bone Marrow Transplantation (2007) 40, 813-821.
79. Julie a. Panepinto, Mark C.Walters, et al Matched related donor transplantation fro sickle cell disease report from the centre fro international blood and transplant research. British journal of hematology, 137,479-485 APRIL 2007:
80. Johan Aschan Risk assessment in hematopoietic stem cell transplantation: conditioning>Best practice research in Clinical hematology, 20(2)295-310, 2007.
81. S.Chakrabarti and D Bare ford: A survey on patient perception of reduced –intensity transplantation in adults with sickle cell disease. Bone Marrow Transplantation (2007) 39,447-451.

APPENDIX-A

As part of standard care during and after transplant following measures are considered

Patients Inclusion Criteria for Sickle Cell Disease

Patients at least one year of age to less than or equal to 21 years of age with (Sickle Cell Disease-SS or Sickle Cell -S-β-Thalassemia and with one or more of the following disease complications:

- Development of stroke on chronic transfusion protocol.
- Impaired neuropsychological function and abnormal MRI scan
- Abnormal Transcranial Doppler study
- Acute chest syndrome (2 to 3 episodes of acute chest syndrome in last 3 to 4 years).
- Recurrent painful priapism.
- Visceral infarcts.
- Recurrent vaso-occlussive crisis of at least 3 to 4 episodes/year.
- Osteonecrosis of multiple bones with documented destructive changes.
- Signed informed consent
- Patients physically and psychologically capable of undergoing transplantation and a period of strict isolation.

Patient Inclusion Criteria for β Thalassemia major

Patients less than or equal to 21 years of age with B- Thalassemia major on routine monthly transfusion protocol or with one or more of the following complications:

1. Hepatomegaly.
2. Liver biopsy revealing evidence of portal fibrosis as
   A)-Mild
   B)-Moderate
   C)-Severe.
3. Ferritin level<_1500ng/ml

Patient Exclusion Criteria

1. HIV positive result confirmed by Western Blot
2. Pregnancy (Pregnancy testing for females of child-bearing age will be performed and those with a positive serum β-Human Chorionic Gonadotropin will be excluded) and lactating females
3. Creatinine greater than two times the upper limit of normal for the laboratory,
4. Pulmonary disease with FVC, FEV1 or DLCO parameters < 50% predicted (corrected for hemoglobin) or stage 3 or 4 sickle lung disease.
5. Cardiac insufficiency or coronary artery disease requiring treatment
6. Active infection requiring systemic antibiotic therapy with antibacterial, antifungal or antiviral agents
7. Lansky performance score <70%- (Appendix B)
8. Acute hepatitis/biopsy evidence of cirrhosis.

As a part of standard of care and also a requirement for our study we will obtain the results of the following lab results from the patient chart:

1. This evaluation consists of a complete history and a physical exam.
2. A complete blood count (CBC), chemistry and electrolyte profile (SMAC), blood type, serum ferritin.
3. Peripheral blood for chimerism studies and hemoglobin electrophoresis to look for sickle cell load.
4. Serological testing which includes hepatitis panel, Epstein Barr Virus (EBV), HIV(Human Immunodeficiency Virus) and HTLV(Human T-cell Lymphotrophic Virus) I and II (blood tests for AIDS virus), cytomegalovirus (CMV), syphilis (testing for sexually transmitted disease).
5. Transcranial Doppler (TCD), MRI and MRA of the brain to evaluate if there is disease related ischemia.
6. In addition, chest and sinus x-rays, electrocardiogram (ECG), cardiac echocardiogram (ECHO) and MUGA (Multiple Gated Acquisition Scan) will be obtained for baseline determination.
7. Bone marrow samples and CT (computed tomography) scans and/or MRI (magnetic resonance imaging) studies may be necessary, depending on the patient's clinical condition to assess disease status.
8. MRI of the liver or a liver biopsy to assess for hemochromatosis.
9. To keep pre-transplant hemoglobin S% about 35%, if elevated patients should have exchange transfusion to decrease sickle hemoglobin to $\leq 35\%$
10. 24 hour urine collection (to evaluate kidney function) and pulmonary function tests (PFT) to evaluate lung function.
11. MRI of the hip, knee or spine for evidence of osteonecrosis.
12. Blood tests for pregnancy in women of childbearing age will be done. If the test is positive, subject will not be entered into the study.
13. Sperm Banking will be encouraged as applicable.
14. The above data that we obtain from the subjects medical chart will help us to define our patient criteria for our study, and after the use of our experimental drugs we want to see how long would these subjects be in remission

ANCILLARY AND SUPPORTIVE MEASURES

During the patient's hospitalization for transplant, the patient will have blood tests taken each morning to monitor blood counts, electrolytes and vital organ functions. There may be standard x-rays and blood cultures to evaluate infections. These blood samples will be drawn from the central venous catheter. Red blood cell and platelet transfusions will be given to the patient until the stem cells begin to engraft (or grow). The patient will also receive medications such as antibiotics to fight infections and to prevent any infections from occurring (prophylaxis). In addition, the patient will also be given medications to prevent graft versus host disease (GVHD), as well as nutritional support in the form of total parental nutrition or nasogastic tube feedings (preferred) as needed.

Medications

Antiemetics:

Odansetron 0.15mg/kg/dose intravenous every 8 hours, 30 minutes prior to initiation of chemotherapy. Other antiemetics will be given on an as-needed basis.

Antibiotic Prophylaxis:
- Acyclovir prophylaxis (antiviral medication) 500 mg/m^2 if HSV titers (+) or CMV (+), otherwise 250mg/m^2 oral (intravenous if not tolerated) three times a day starting day –1.
- Fluconazole (antifungal medication) will be given at 3-6 mg/kg by mouth or intravenous daily starting day of admission.
- Trimethoprim-sulfamethoxazole for prevention of Pneumocystis Jiroveci pneumonia as a prophylaxis (5 mg/kg/day oral or intravenous in two divided doses will be started upon admission and stopped day –1.

Antiseizure Prophylaxis:
Keppra Prophylaxis 20mg/kg/dose in two divided doses by mouth or intravenous daily starting day of admission

Growth Factor:
A growth factor known as G-CSF will be started 4 hours after the stem cell infusion at a dose of 5 micrograms/kg/day subcutaneously, until the patient's ANC is ≥1500 x 3 days or ≥5,000 x one day

Mucosal Evaluation and Care:
Mucositis will be monitored on a daily basis. Aggressive mouth care will be initiated upon admission. Pain control will be given as required.

Nutrition:
If the patient cannot tolerate nutrition by mouth because of mucositis, intravenous hyper-alimentation or nasogastric tube supplementation of feeds will be initiated upon the discretion of the physician

Blood Component Support:
All blood products will be irradiated to prevent GVHD.
- Red blood cell transfusions (leukocyte-poor) will be used to keep the hemoglobin
- >9 gm/dl, or to relieve symptoms thought to be due to anemia.
- Single donor platelets will be given as needed to keep platelets >50,000/ul to prevent central nervous complications such as hemorrhage.
- HLA-matched platelets will be used for patients who are refractory to platelet transfusions. Leukocyte-poor filtered blood products will be given to all patients.
- If the patient and donor have CMV negative serologies, CMV negative products will be
- Administered

Management of Fever/Infections during Neutropenia:
Patients will be started on empiric broad spectrum antibiotics for fever (>38.0°C) after

Appropriate cultures (blood x 3 sets, urine, and any site with signs or symptoms of infection) have been obtained.

Patients with documented fungal infections or with persistent unexplained fever that is unresponsive to broad-spectrum antibiotics will receive an appropriate antifungal agent.

Management of Fluid Accumulation:
After chemotherapy, patients may experience a "capillary leak" syndrome leading to extra-vascular fluid retention. The peak of weight gain occurs 2 weeks after therapy and resolves. Careful attention to the patient's weight and strict monitoring of input and output for every 24 hours is necessary and intensive forced diuresis will be used to prevent excess fluid retention

EVALUATION DURING THE FIRST 120 DAYS

1. Physical examination, vital signs, strict fluid intake and output and daily weights until discharge, then weekly.
2. Toxicity and GVHD grading weekly.
3. CBC and platelets daily until discharge, then at least weekly.
4. Electrolytes, BUN, creatinine, calcium, phosphorus, magnesium, Cyclosporine and Tacrolimus levels at least twice weekly until discharge, then once weekly or at the discretion of the physician.
5. CMV, EBV and Adenovirus by PCR testing weekly when they are in the BMT unit and as indicated.
6. Peripheral blood for FISH or VNTR study for chimerism, and hemoglobin electrophoresis at least monthly or as indicated.
7. CXR, EKG and Pulmonary Function or Pulse Oximetry as clinically indicated.

EVALUATIONS AT DAY 120

1. History and physical examination.
2. Peripheral blood for FISH or VNTR study for chimerism and hemoglobin electrophoresis and or globin chain synthesis ratio.
3. CBC, differential, electrolytes, creatinine, glucose, magnesium and ferritin level.
4. Pulse oximetry at every visit for SSD or as clinically indicated.

EVALUATIONS DURING DAYS 120-365

1. Physical examination and screening labs and also ferritin level at least monthly until day 365.
2. Peripheral blood FISH or VNTR study for chimerism on day 180, 270 and 365 and hemoglobin electrophoresis and or globin chain synthesis ratio.
3. Follow up for patients with chronic GVHD as per GVHD protocol.

ANNUAL EXAMINATION AFTER DAY 365 AND AT THE END OF 2ND YEAR AND 3rd YEARS

1. Review of systems and physical examination.
2. CBC, differential, electrolytes, BUN, creatinine, glucose, magnesium, liver function

3. Tests (LFT), serum ferritin, hemoglobin electrophoresis and or globin chain synthesis ratio and Urine analysis.
4. Peripheral blood for FISH or VNTR study for chimerism, (for first three years only).
5. Thyroid function tests, LH/FSH/Estradiol (in females) testosterone in males.
6. Pulmonary function tests (PFT) and Echocardiogram yearly for three years.
7. MRI of the liver to assess for iron overload if clinically indicated
8. Evaluation of effects of underlying disease with MRI of brain and liver if indicated.
9. T and B cell subsets and immune function and quantitative immunoglobulins (first 2 years only).
10. Lansky/Karnofsky performance status-Appendix B.
11. Neuropsychological testing /quality of life assessment, as clinically indicated.
12. On or around Day 365, TCD or Cerebral MRI or MRA of areas previously involved with complications or as clinically indicated and at the end of 2nd year and 3rd year as clinically indicated..

EVALUATIONS DURING 2ND YEAR POST TRANSPLANT

1. Physical examination and screening labs and also ferritin level at least every 2 months until the end of 2 nd year.
2. Peripheral blood FISH or VNTR study for chimerism and hemoglobin electrophoresis and or globin chain synthesis ratio at every visit.
3. TCD or Cerebral MRI or MRA of areas previously involved as clinically indicated.
4. Follow up for patients with chronic-GVHD as per GVHD protocol.

EVALUATIONS DURING 3RD YEAR POST TRANSPLANT

1. Physical examination and screening labs and also ferritin level at least every 3 months until the end of 3 rd year.
2. Peripheral blood FISH or VNTR study for chimerism and hemoglobin electrophoresis and or globin chain synthesis ratio at every visit.
3. TCD or Cerebral MRI or MRA of areas previously involved as clinically indicated.
4. Follow up for patients with chronic-GVHD as per GVHD protocol.

However if patients have any complications related to BMT, they will be evaluated more often as needed. It is also possible that not all patients will complete the 3 year follow-up. These patients will be returning for follow-up due to the transplant, and the study visits have been timed to coincide with these follow-up visits. However, if patients are lost to follow-up, this will be noted, and analyses will be carried out using the available data from the remaining patients.

OTHER OPTIONS

Patient will as part of standard of care will receive the following supportive therapy in case of sickle cell disease and also in B- Thalassemia- major:
-Intravenous fluids
-Pain medication in case of pain crisis
-Chronic transfusion regimen in case of stroke, spleenic sequestration
-Exchange transfusion in case of acute chest syndrome/ acute stroke
-Iron chelating agent

-Penicillin prophylaxis and folic acid

All the mentioned therapy are only supportive, none of them are curative and can lead to risk of

1. Iron overload in case of chronic transfusion in turn requiring either oral or intravenous iron chelator (medication used to flush out excess iron from your system).
2. Allosensitization
3. Recurrent stroke on transfusion
4. Recurrent acute chest syndrome
5. Chronic end organ damage
6. Decreased patients compliance (agreement in taking medication) while on chelation
7. Multiple days of school absenteeism because of recurrent illness

Patients may choose to receive other supportive therapy that are available, and also may choose not to have treatment at all.

APPENDIX B

Karnofsky Scale

Adapted from Karnofsky, Abelmann, Araver, and Burchenal 1948

100 Normal: No complaints; No evidence of disease. Able to work

90 Able to carry on normal activity: Minor symptoms. Able to work

80 Normal activity with effort: Some symptoms. Able to work

70 Cares for self: Unable to carry on normal activity. Independent: not able to work

60 Disabled: dependent. Requires occasional assistance: cares for most need.

50 Moderately disabled: dependent. Requires considerable assistance and frequent care.

40 Severely disabled: dependent. Requires special care and assistance

30 Severely disabled. Hospitalized, death not imminent.

20 Very sick. Active support treatment needed

10 Moribund. Fatal processes are rapidly progressing.

Modified Lansky Play-Performance Scale
(For use with people ages 1 through 16 years)

100% Fully active, normal

90% Minor restrictions in physically strenuous activity.

80% Active, but tires more quickly.

70% Both greater restriction of, and less time spent, in play activities.

60% Up and around, but minimal active play: keeps busy with quieter activities.

50% Get dressed but lies around much of the day: no active play: able to participate in quiet activities.

40% Mostly in bed: participates in quiet activities.

30% In bed: needs assistance even for quiet play

20% Often sleeping: play entirely limited o very passive activities.

10% No play: does not get out of bed.

5% Unresponsive.

0% Dead

APPENDIX C

See the attached form

Criteria for grading acute graft-versus-host disease

Stage	Skin	Liver	Intestinal Tract
1	Maculopapular rash<25% of body surface	Bili 2-3 mg/dl. Bili<2mg/dL or alk phos x nl and liver Bx consistent w/GVHD	>500 ml diarrhea/day (300mL/m^2/d^+) Nausea; anorexia**
2	Maculopapular rash 25-50% body surface	Bili 3-6 mg/dl	1000mL diarrhea/day (>600 mL/m^2/d^+) Vomiting food intolerance**
3	Generalized erythroderma	Bili 6-15 mg/dL	>1500 mL diarrhea/day (>1000mL/m^2/d^+)
4	Generalized erythroderma with bullous formation and desquamation	Bili >15 mg/dL	Severe abdominal pain, with or w/o ileus

* Estimated from Burns charts

** Upper GI involvement presents nausea, vomiting, and anorexia and requires UGI biopsies consistent with GVHD

Overall Grading of acute GvHD

Overall Grade	Skin	Liver	Intestinal
O	O	O	O
I (mild)	1-2	O	O
II (moderate)	1-3	1	1
III (severe)	2-3	2-3	2-3
IV (life-threatening)	2-4	2-4	2-4

APPENDIX-D

Schedule of events for study procedure

Study procedure	screen	Day-11	Day-10	Day-9	Day-8	Day-7	Day-6	Day-5	Day-4	Day-3	Day-2	Day-1	Day 0
Review of medical record	X												
Demograp-Hic data	X												
Inclusion/exclusion criteria	X												
Informed consent/ assent	X												
Administ campath -1H		X	X	X	X								
Administ Fludarabine						X	X	X	X				
Administ melphalan										X	X		
Stem cell Transplant (soc)													X
Start of cyclosporine												X	
Start of Tacrolimus												X	
Start of MMF												X	

Schedule of Events: As a part of standard of care, following clinical data will be collected. This data will be retrieved daily for the study during the subjects inpatient stay until the day of discharge, and weekly from the day of discharge until 120 days, monthly for 1 year and every 2 months for 2nd year and every 3 months for 3rd year.

Results of SOC Procedures	Inpatient Stay				Year 1		End of Year 1	Year 2	End of Year 2	Year 3	End of Year 3
	Daily	Bi - Weekly	Weekly	Monthly	Q monthly	Q 3 months		Q2 months		Q 3 montha	
CBC with diff	x				x		x	x	x	x	x
CMP	x				x		x	x	x	x	x
Serum ferritin					x		x	x	x	x	x
UA	x				x				x		
Tacro level		x			x						
CSA level		x			x						
CMV PCR			x				x		x		
Virology Panel			x				x		x		
Hgb Electrophoresis**				x		x	x		x		x
Chimerism studies**				x		x	x				x
TCD*						x					
Cerebral MRA*						x					
Cerebral MRI*						x					x
Echo/EKG							x		x		x
PFT							x		x		x
Lansky/karnofsky status							x		x		x