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HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR PATIENTS WITH SEVERE SICKLE CELL DISEASE USING MYELOABLATIVE CONDITIONING AND αβT-CELL-DEPLETED HEMATOPOIETIC STEM CELLS FROM PARTIALLY MATCHED FAMILIAL DONORS

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TABLE OF CONTENTS

APPENDICES

1.0 EXECUTIVE SUMMARY, HYPOTHESIS & OBJECTIVES

1.1 Executive Summary

Sickle cell disease (SCD) is an autosomal recessive disorder with pathognomonic mutations in the β-globin gene. The resultant 'sickling' of the hemoglobin tetramer in red blood cells leads to vasoocclusive and hemolytic crises, pain, and end-organ damage. [1] SCD has a multi-system pathophysiology that includes life-threatening infection, splenic dysfunction, stroke, neurocognitive deficits and a shortened life span. [2] Despite improvements in medical management of severe SCD, the only known cure for this disease is allogeneic hematopoietic stem cell transplantation (HSCT) and the most experience and success has been demonstrated using matched sibling donors (MSD). Successful outcomes result in retardation of end organ damage, outstanding long-term survival and low rates of chronic transplant-related complications. However, fewer than 15% of patients with severe disease have a matched sibling donor, and an even smaller fraction have matched unrelated donors in the international donor registries. [3, 4] Strategies to expand the donor pool with unrelated cord blood donors have been unsuccessful due to high rates of graft failure, a direct result of the inherent low stem cell doses in these graft products. [5-8] In contrast, early transplantation studies using grafts with large numbers or 'megadoses' of haploidentical parental hematopoietic stem cells depleted of alloreactive T-cells in β-thalassemia, a related hemoglobinopathy, have shown significant promise. [9, 10] In this study, we propose a pilot approach to evaluate a comparable megadose strategy to transplantation of patients with SCD. Specifically, our approach takes advantage of a novel technique that results in extensive depletion of alloreactive T-cell receptor (TCR) αβ+ T-cells ($<1x10^5$ cells/kg), and enrichment of stem cells (CD34, $>10x10^6$ cells/kg). Furthermore, unlike previous graft engineering strategies, we will infuse large numbers of TCRγδ+ T-cells, Natural Killer (NK) cells and other cells that facilitate engraftment and effective immune reconstitution, with the potential of minimizing severe acute graft-versus-host disease (aGvHD) and post- transplantation viral disease.

1.2 Hypothesis

We hypothesize that research subjects with severe SCD who receive a haploidentical CD34+ megadose hematopoietic stem cell graft depleted of αβ T-cells will experience similar outcomes to patients in historical studies of MSD hematopoietic stem cell transplants (HSCT), including similar rates of graft rejection and peri-transplant toxicity.

1.3 Primary Objective

1.3.1 To assess the safety of αβ T-cell depleted haploidentical hematopoietic stem cell transplantation (Hap-HSCT) for children, adolescents and young adults with severe SCD. The treatment plan will be considered safe if it lacks excessive toxicity. Toxicity is defined as failure to engraft (graft failure), grade III/IV irreversible end organ toxicity, grade III/IV aGvHD or death within 100 days post- Hap-HSCT.

Our approach will also provide preliminary observational data on the efficacy of Hap-HSCT for children, adolescents and young adults with severe SCD. Efficacy is defined as stable donor engraftment (>5% total

nucleated cell DNA) and donor erythropoiesis that corrects the SCD hematologic phenotype (<50% HbS in the peripheral blood).

1.4 Secondary Objectives

1.4.1 To estimate 1-year overall and event-free survival after Hap-HSCT.

1.4.2 To observe the incidence and severity of acute and chronic GvHD after Hap-HSCT.

1.5 Exploratory Objectives

1.5.1 To obtain preliminary information regarding relative donor engraftment (chimerism) among different cell subsets, including unsorted mononuclear cell, bone marrow erythroid precursors, and T- and B-lymphoid subsets.

1.5.2 To assess the proportion of research participants requiring stem cell boosts or donor lymphocyte infusions after Hap-HSCT.

1.5.3 To evaluate systemic end organ function and/or complications post Hap-HSCT in a longitudinal manner especially growth and development, neurologic, neurovascular and neurocognitive status, and systemic organ function.

1.5.4 To investigate immune reconstitution after Hap-HSCT.

2.0 BACKGROUND AND RATIONALE

2.1 Introduction

With advances in screening and supportive care, 85-95% of children with SCD survive to their twentieth birthday. However, compared to unaffected African-Americans, overall life expectancy is reduced by 25 or more years.[2, 11] Furthermore, among the approximately 1,000 children with SCD born each year, greater than 20% will develop a severe clinical phenotype with recurrent vaso-occlusive crises (VOC), manifesting as frequent episodes of severe pain, acute chest syndrome (ACS) and/or stroke.[3] Indeed, these individuals have higher rates of mortality than the SCD population as a whole. Scheduled red blood cell transfusions reduce the incidence of recurrent stroke following an initial cerebrovascular event, but there is a significant risk of stroke recurrence (approximately 45%) even with adequate compliance.[12]

Significant morbidity and mortality related to chronic transfusion therapy warrants the investigation of alternate strategies. Agents that induce fetal hemoglobin such as daily hydroxyurea (HU) administration, as well as allogeneic HSCT, have received significant investigatory focus. While the use of HU therapy has increased and demonstrated improvement in many clinical symptoms of disease in some patients with SCD, this is not a curative strategy. [13] Indeed, long term efficacy has only been observed in a subset of patients. Furthermore, effective use of HU has not been associated with retardation in chronic SCD-mediated end organ damage, or prolongation of life. Furthermore, it is frequently associated with poor compliance. [13, 14]

As the SCD patient population reaches young adulthood, the disease phenotype is characterized by complications such as avascular necrosis, priapism, physical growth and sexual development delay, pulmonary hypertension, congestive heart failure and renal dysfunction which are less responsive to current non-transplant modalities. [15] Recent data show that children and young adults have high rates of cognitive dysfunction with lower IQ scoring compared to age matched sibling and peer controls, suggesting an urgent need for novel strategies that correct the disease phenotype effectively. [16]

In contrast to these outcomes, long-term studies of hematopoietic stem cell transplantation (HSCT) from a matched sibling donor (MSD) have demonstrated curative potential with excellent overall and disease-free survival (**Table 1**).[17-21] Impressively, all data to date show a significant amelioration of progressive chronic organ damage after allogeneic stem cell transplantation.

2.2 HSCT for Sickle Cell Disease

The ability of HSCT to cure SCD was first demonstrated in a patient with coincident acute myeloid leukemia in 1984. This patient remains in complete remission of both diseases over 30 years later.[22] Subsequently, over 400 MSD allografts for patients with SCD have been performed. In fact, 327 patients with sickle cell disease received MSD transplantation between 2010-2014. [23] Selection of patients has been agreed by physicians and patient advocates, and is based on the severity of the presenting phenotype including previous strokes, recurrent acute chest syndrome, and/or recurrent vaso-occlusive crises. [24] Summarizing these results, overall survival and disease-free survival range from 91-97% and 73-86%, respectively. [17-21] Peri-transplant toxicity in patients who receive MSD transplantation is acceptable with grade II-IV acute GvHD (aGvHD) occurring in fewer than 20% of transplanted individuals, chronic GvHD (cGvHD) in fewer than 29%, and death in 6% of patients. (See **Tables 1 and 2**) In the most

recent studies, graft failure occurred in 8.6% of patients. Long-term follow-up data demonstrates stabilization of chronic organ damage with no further SCD-related events in the majority of patients. [25-28] Adverse events include transplant-related end organ damage, sterility, and a slightly increased risk of a secondary malignancy.[29]

Table 1: Outcomes Observed in Matched Familial HSCT in SCD using Myeloablative Conditioning (MAC)

Author	Year	Conditioning Regimen	N	OS (%)	DFS (%)	Graft Failure (%)	aGvHD (%)	cGVHD (%)
Walters	1996	Bu Cy ATG	22	91	73	17	NR	NR
Brachet	2004	Bu Cy +/-ATG	24	96	79	17	25	29
Panepinto	2007	Various	67	97	85	13	10	22
Bernaudin	2007	Bu Cy	87	93	86	8	20	13
Dedeken	2014	Bu Cy +/-ATG	50	94	86	8	10	20

Legend: Bu, busulfan; Cy, cyclophosphamide; ATG, anti-thymocyte globulin; NR, information not reported

Our unpublished experience at the University of Chicago, and previously at St. Jude Children's Research Hospital (SJCRH), is consistent with these outcomes. Of 32 patients transplanted, all have full donor engraftment, with 29 (91%) patients demonstrating disease-free survival without long-term SCD-related sequelae (JMC, unpublished). Three patients died of transplant-related complications. Chronic GvHD post transplantation was the major complication precipitating death and occurred in all three of these patients greater than one-year post-HSCT.

Table 2: Outcomes Observed in Matched Familial HSCT in SCD using Reduced Intensity Conditioning

Author	Year	Conditioning Regimen	N	OS (%)	DFS (%)	Graft Failure (%)	aGvHD (%)	cGVHD (%)
Krishnamurti	2008	Bu Flu ATG	7	100	86	14	14	29
Hsieh	2009	Campath TBI	10	100	90	10	0	0
Bhatia	2014	Bu Flu Campath	18	100	100	0	16.7	NR

Legend: Bu, busulfan; Flu, fludarabine; ATG, anti-thymocyte globulin; NR, information not reported

To reduce the incidence of pre- and post-transplant toxicities, MSD HSCT with reduced intensity conditioning regimens has been evaluated. Overall survival in these cohorts approaches 100%, with event-free survival between 86-100% (**Table 2**). [30-32] The University of Chicago Sickle Cell Disease program is currently performing either matched sibling donor or fully matched unrelated donor transplantation for patients with severe phenotype sickle cell disease or using ameliorative medical approaches including chronic hydroxyurea therapy and/or red blood cell transfusions. Individuals who fulfill disease eligibility criteria and have a MSD donor or fully matched unrelated donor would be excluded from this study.

2.3 Reduction in SCD-related Organ Damage in MSD Transplant Survivors

Investigative groups have evaluated not only engraftment and peri-transplant toxicities as outcomes, but also the acute and long-term effects of corrected hematopoiesis on SCD-mediated

end-organ damage. For example, detailed central nervous system (CNS) evaluation by the SJCRH group demonstrated stable neurologic examinations, MRI/MRA scans, and IQ/reading/mathematics scores over a 3-5 year follow-up period when evaluated with longitudinal neuroimaging and neurocognitive testing. [25, 26] Interestingly, most children had clinically non-significant subtle parenchymal changes on MRI. Changes included rare incidences of presumed transplant-related leukoencephalopathy or new vascular lacunae. Other investigators have demonstrated that with successful donor engraftment, patients who had stroke prior to SCT had no recurrent CNS infarction, which is a considerable improvement from the 45% re-infarction rate seen with patients who are cared for with chronic transfusion therapy alone. [12, 27]

In a similar manner, resolution of recurrent vaso-occlusive crises (VOCs), hyposplenism, growth and development delays, chronic pulmonary injury, endocrinopathy and other SCD-related end-organ damage have been assessed longitudinally in post-transplant patients.[27, 28] These studies have demonstrated normalization of hematopoiesis, universal resolution of VOCs in all individuals, reversal of splenic defects, delayed puberty and improvement in other SCD-related damage in many instances. We propose to evaluate organ-specific outcomes longitudinally in a descriptive manner in this study.

2.4 Approaches to Expand the Therapeutic Use of HSCT in SCD

Although results of HSCT for SCD using MSD are encouraging, it is estimated that fewer than 15% of patients will have a non-affected HLA-identical sibling.[3, 4] For example, an unpublished review by our principal investigator of patients treated with chronic transfusions at SJCRH demonstrated that of the 47 patients eligible for HSCT based on clinical features, only four patients had appropriate MSDs. Thus, the lack of availability of MSDs has been a major barrier to transplantation for patients with severe SCD. The goal of our program is to develop a successful transplant strategy for patients who lack a MSD. This strategy must reduce the procedure-related morbidity and mortality to levels that are comparable to those observed in patients receiving MSD grafts.

To date, investigators have considered three potential alternate sources of donor hematopoietic cells which have been evaluated with varying success: matched unrelated donors (MUD), unrelated umbilical cord blood (UCB) grafts, haploidentical unmanipulated bone marrow and peripheral blood stem cell selected grafts with post infusion cyclophosphamide and haploidentical CD34+ selected stem cell grafts. A brief summary of our current understanding of these approaches is provided below:

MUD Donors African-Americans are underrepresented on MUD registries creating substantial difficulties in identifying unrelated donors. [4, 33] Fewer than 60% of African-Americans have a potentially suitable donor compared to 75-80% of Caucasians. As a result, there is minimal data on the outcomes of patients receiving matched unrelated donor transplantation. [34] Although we have had two survivors in our pediatric cohort at Comer Children's Hospital (CCH), we have evaluated innumerable other patients without observing a 10/10 Human Leukocyte Antigen (HLA) match, the currently accepted standard for the use of MUD grafts in non-malignant disease. Thus, this approach is not feasible as an accessible, safe approach to alternate transplantation in children with SCD. We propose that in the unlikely event that a potential research subject has a 10/10 HLA MUD, he/she will be ineligible for this study.

Cord Blood Donors The national and international registries have large banks of unrelated UCB

grafts. Indeed, this graft source has been used successfully to treat children and adults with both malignant and non-malignant diseases. However, the results in patients with hemoglobinopathies have been disappointing. Multiple groups have reported rates of graft rejection ranging from 44-62.5%, with a cGvHD rate of 12-14% (**Table 3**). [5, 6, 8, 35] A recent large Blood and Marrow Transplant Clinical Trials Network trial was closed early to accrual due to graft rejection and related morbidity and mortality. A direct relationship between toxicity to low stem cell dose was observed in these studies. [6] Given the poor outcomes with this approach, we propose that individuals with severe SCD as defined by the eligibility criteria, and who lack a suitable 10/10 MSD or MUD donor, will be eligible for the study regardless of the availability of appropriate cord blood units.

Table 3: Outcomes in Unrelated Cord Blood HSCT in SCD

Author	Year	Conditioning Regimen	N	OS (%)	DFS (%)	Graft Failure (%)	aGvHD (%)	cGVHD (%)
Radhakrishnan	2013	Reduced intensity	8	62.5	50	50	50	12.5
Kamani	2012	Reduced intensity	8	87.5	37.5	62.5	25	12.5
Ruggeri	2011	Both*	16	94	50	44	NR	NR
Adamkiewicz	2007	Both**	7	86	29	57	29	14

Legend: *56% myeloablative, 44% reduced intensity. **57% myeloablative, 43%reduced intensity NR, information not reported

Haploidentical Donors: The effective and safe use of partially matched familial donors for children and adults with hematologic disease has been a strong aspiration since the feasibility of transplantation was first demonstrated. Family members who are heterozygotes for the HbS genotype are highly attractive donors given their accessibility and willingness to donate stem cells. Indeed, haploidentical donors are being used increasingly for children and adults with malignant diseases, a successful expansion of the donor pool. [36, 37]

Table 4: Haploidentical HSCT Transplantation in Sickle Cell Disease

Author	Conditioning Regimen	N	Graft Manipulation	OS (%)	DFS (%)	Graft Failure (%)	aGvHD (%)	cGVHD (%)
Bolanos-Meade	Myeloablative	14	Post Infusion Cyclophosphamide	100	57	43	0	0
Dallas	Myeloablative	8	CD34+ selection	75	37.5	37.5	25	37.5

However, major concerns with this approach include the potential for severe life-threatening GvHD and graft rejection/failure. The haploidentical state is associated with major HLA class I or II mismatches. Although these mismatches may be beneficial for malignant disease control due to the graft versus tumor (GvT) benefit of an allograft, they are unnecessary and potentially detrimental in patients with non-malignant diseases due to their GvHD potential. Approaches to reduce this key toxicity include the use of post-transplant cyclophosphamide as GvHD prophylaxis or allograft engineering with CD34 hematopoietic stem cell selection and associated rigorous and broad T-cell depletion. [38] Both approaches have been associated with very poor engraftment and immune reconstitution rates when used for patients with SCD, the latter being associated with a high incidence of viral reactivation. Interestingly, rates of severe acute and

chronic GvHD were relatively low (<10%) in these studies (**Table 4**). [39, 40]

In summary, these studies show that **a)** new haploidentical stem cell transplant approaches are warranted, and **b)** key components necessary for a successful outcome of alternate donor HSCT for SCD must include: **1)** a reduction in the rate of GvHD and graft failure after haploidentical transplantation; **2)** the ability to achieve rapid myeloid and lymphoid engraftment to reduce the incidence of opportunistic infection and viral reactivation; **3)** the need to use less toxic conditioning regimens to prevent treatment related morbidity.

2.5 Rationale for a Novel Haploidentical Transplantation Approach: an αβ+ T-cell depleted stem cell infusion with a fixed γδ+ T-cell dose and a megadose of CD34+ stem cells in children, adolescents, and young adults with SCD.

Extensive animal and human transplant studies have demonstrated that two graft characteristics are critical for successful haploidentical hematopoietic stem cell transplantation 1) a high CD34 dose and 2) a low T-cell dose. Murine studies have shown the value of supplementing T-cell depleted bone marrow with megadoses of blood stem cells.[9] *In vitro* studies have demonstrated the presence of tolerizing "veto cells" in mobilized human CD34+ hematopoietic progenitor cells. These cells are capable of inducing immunologic tolerance against donor cells while preserving anti-third party reactivity.[41] In human studies, 'megadoses' of CD34+ cells have been associated with more rapid engraftment and lower probability of graft failure.[9, 41] Megadoses are defined as $\geq 10 \times 10^6$/kg. Other studies have reported more rapid hematopoietic recovery, a reduced incidence of fungal infections, reduced number of febrile days, lower transplantation-related mortality, and higher 180 day survival with higher CD34+ cell doses. [42] In the context of unmanipulated, reduced-intensity, allogeneic matched-sibling donor transplants, higher CD34+ cell doses were associated with more rapid complete donor chimerism in donor T-lymphocytes and a lower relapse rate in high-risk malignancies, but a higher rate of chronic GvHD. [43] However, unlike unmanipulated grafts, CD34-selected grafts have high rates of graft failure, and are associated with viral reactivation and poor immune reconstitution. [44]

To address this deficit, we propose to evaluate the efficacy and toxicity associated with transplantation of αβ+-depleted haploidentical grafts into eligible individuals with severe SCD. These grafts will be composed of megadoses of CD34+ cells with measurable numbers of γδ+ T-cells, NK cells, and other cells that facilitate engraftment. The latter cellular populations, which are depleted in CD34+ selected grafts, are associated with higher rates of myeloid and immune reconstitution in pre-clinical and recently published studies. [45-48]

Donor T-cell identification of host as non-self is the primary insult in the development of GvHD. αβ+ T-cells are the primary culprit in this phenomenon. They constitute the vast majority of T-cells and when biopsied, tissues affected by GvH demonstrate the enrichment of αβ+ T-cells. [49-51] Furthermore, lethally irradiated mice transplanted with bone marrow-depleted of αβ+ T-cells and enriched for γδ+ T-cells were protected from GvHD and mortality when compared to mice transplanted with either unmanipulated or pan T-cell depleted grafts respectively.

These studies have resulted in clinical studies of αβ+-depleted haploidentical stem cell transplantation using the Miltenyi CliniMACS system in patients with hematologic malignancies and non-malignant, non-SCD hematologic disorders. These studies demonstrate favorable morbidity and mortality profiles. Specifically, Bertaina et al. described their experience with 23

children with non-malignant hematologic diseases. In their cohort, only three (13%) developed grade I-II GvHD, with no grade III-IV GvHD, visceral GvHD or chronic GvHD.[40, 48] This strategy therefore is likely to significantly limit the GvHD seen in our study population.

The use of αβ+ T-cell-depleted stem cell haploidentical grafts allows for infusion of megadoses of $CD34^+$ stem cells by removal of T-cells speculated to cause GvHD but retention of supportive cells that help facilitate engraftment. We hypothesize that this strategy will overcome the barriers limiting engraftment for patients with SCD receiving haploidentical grafts. Finally, the rates of viral reactivation were lower (approximately 38%) in γδ+ T-cell enriched grafts when compared to other haploidentical graft strategies that reported rates of viral reactivation as high as 77%. [48, 52] Again, we contend that this apparent advantage should be evaluated in the SCD population at high risk of adverse disease-related morbidity and mortality.

2.6 Justification for the Use of Pediatric Patients

Despite advances in the medical management of patients with sickle cell disease, patients continue to suffer from significant morbidity secondary to disease related end organ damage and early mortality. [1, 2, 53, 54] Hematopoietic stem cell transplantation is the only known cure. The progressive end organ damage patients experience as a result of their sickle cell disease puts them at higher risk of transplant related complications as they age. A recent study evaluating the outcomes of 1000 patients transplanted for severe sickle cell disease demonstrated not only that patients aged <16 years have superior overall and event free survival but that even within these groups increasing age is inversely associated with survival rates suggesting that an older age is a risk for poor outcome and transplantation of pediatric patients yields optimal outcomes. As result, pediatric patients aged 10 years or older will be included in Stage I of this study. Should results of Stage I of this study demonstrate that the strategy is safe in the initial patients, we would extend the lower age limit to two year olds. Additional stopping rules will be put in place for this subset of patients to ensure safety in younger children during the Stage II phase. Please see section 10.0 for details.

In addition to being optimal for individual subject outcomes, the inclusion of pediatric patients in clinical trials of transplantation in sickle cell disease is standard as evidenced by the trials outlined in tables 1-4. In fact, including only adult patients in this study would very likely create a negative selection bias as the trials in the historic literature have been composed largely of pediatric subjects.

2.7 Chemotherapy Considerations

Busulfan pharmacokinetic monitoring: Ablation of host hematopoiesis is critical to successful transplantation for many diseases and particularly when using mismatched donor grafts.
[55] To achieve this goal, we will use a busulfan-containing conditioning regimen given highly successful engraftment rates reported with this approach.

Specifically, busulfan levels will be checked per institutional standard. The goal for the area under the curve (AUC) value will be 1000-1200 μmol/min/L. Previous studies have demonstrated that in patients with sickle cell disease who received transplantation with busulfan, full chimerism was seen more commonly when AUC was higher (median 1018 +/- 122 μmol/min/L). Patients who had a lower AUC (median 863 +/- 72 μmol/min/L) had mixed chimerism or graft loss. In addition, there was no significant difference in rates of toxicity, specifically VOD, between the two groups.[56]

Use of Fludarabine in Sickle Cell Disease: Fludarabine will also be used in this regimen. This agent has previously been described in haploidentical transplantation regimens, including αβ+T-cell and CD19+ B-cell depleted graft transplantation in non-malignant hematologic diseases excluding SCD.[48] Its use in SCD needs to be considered carefully due to its association with neurotoxicity, and the likelihood for SCD patients to have overt or covert neurologic injury prior to, and during, the stem cell transplant procedure. However, the cumulative fludarabine doses of 160 mg/m^2 used in this haploidentical transplant protocol is far below the threshold of 480 mg/m^2 associated with neurotoxicity in adults. Indeed, fludarabine has been utilized recently in the conditioning regimens for patients with SCD. Neurotoxicity was observed in less than 5% of children, with the predominant insult being posterior reversible encephalopathy. It is likely that concurrent calcineurin inhibitor therapy was a factor in the development of neurotoxicity in these patients. Importantly, all events were reversible and did not result in any long-term sequelae. Research subjects on this study will not receive calcineurin inhibitor prophylaxis, reducing the potential risk. [32, 57]

2.7 Prevention and Management of Graft Failure in SCD Patients Undergoing Allogeneic Stem Cell Transplantation

Studies of alternative donor transplant for hemoglobinopathies, including SCD and β–thalassemia, have shown high rates of primary and secondary graft failure. *Sodani* reported on 33 pediatric patients with thalassemia who received MSD BMTs, all were first transplants. All patients had poor prognosis with Lucarelli Class III classifications (severe iron overload, hepatic fibrosis, poor iron chelation) and all received hydroxyurea 30 mg/kg/day, azathioprine 3 mg/kg, G-CSF twice a week, and hypertransfusion with packed red blood cells prior to transplantation. Conditioning consisted of busulfan (14 mg/kg) and cyclophosphamide (120-160 mg/kg). GvHD prophylaxis consisted of cyclophosphamide 7.5 mg/kg IV on day 1, methotrexate 10 mg/m^2 on days 3 and 6, methylprednisolone 0.5 mg/kg/day from day -1 to day +25, and cyclosporine for 1 year. Using this regimen, overall and event-free survival was 96% and 90%, respectively. The rate of rejection was 8% and the death rate was 4%. Previous historical controls at the time demonstrated rejection of 30% when hydroxyurea and azathioprine were not used. [58] Taking a similar approach, others have demonstrated similar improvements in non-engraftment rates in adults with SCD using three months of hydroxyurea and azathioprine prior to transplant. [18]

As a preparative regimen, hydroxyurea and azathioprine will be maintained for 3 months, if tolerated, until the day before the conditioning regimen. In addition, red blood cell transfusions or exchange will be performed to target hemoglobin S levels of less than 30% just prior to initiation of conditioning regimen.

The etiology of graft rejection in haploidentical transplantation is multifactorial, and attributed to a combination of cellular-mediated graft rejection and allo-immunization Strategies proven to improve engraftment secondary to host-versus-graft immune system mediated graft failure include myeloablative chemotherapy and T-cell depletion of the host prior to transplantation. [59] Our preparative regimen will include myeloablative chemotherapy as well as anti-thymocyte globin (ATG) to address both major mechanisms.

Graft failure will be considered donor chimerism less than 5% whole blood DNA or symptomatic cytopenias. We will consider the graft a success if the patient does not have graft failure and achieved a HbS <50%. These criteria were selected specifically given previous data on the kinetics of graft failure in patients with SCD. [60, 61]

Other investigators have demonstrated that in patients transplanted for SCD who develop mixed myeloid and lymphoid chimerism following transplantation, there is enrichment of donor peripheral erythrocytes (predominant donor erythroid chimerism). [61, 62] This state is associated with disease amelioration, even in instances where whole blood DNA chimerism evaluation demonstrates host myelo-lymphopoiesis. [62] This data suggests that non-HbSS donor erythroid precursors outcompete host erythroid precursors. [61]

Poor graft function and graft failure following stem cell transplant are associated with increased morbidity and mortality secondary to infection and hemorrhagic complications. [63] In previous haploidentical studies, patients with graft failure/poor graft function who receive an infusion of a $CD34^+$ enriched stem cell boosts have demonstrated both improved trilineage hematopoiesis as well as decreased GvHD and treatment related mortality when compared to patients who received neither a stem cell infusion or unmanipulated bone marrow boost. [63] Should graft failure occur despite preventative strategies described above, a CD34+ enriched stem cell boost will be infused at the discretion of the principal investigator. Our stem cell boost strategy in the context of graft failure/poor graft function mirrors this approach.

2.8 Special Considerations in SCD Patients Undergoing Allogeneic Stem Cell Transplantation

In the peri-transplant period, several SCD-specific thresholds for transfusion support will be adopted to reduce previously observed cytopenia-related toxicities. Thus, platelet counts will be maintained at $\geq 50 \times 10^9$/L, and hemoglobin will be kept between 9 and 11 g/dL to reduce the risk of stroke. [17, 64] Lastly, penicillin V-potassium will be given twice daily from transplant day +21 or when tolerating PO medications, consistent with institutional standard of care. Treatment will continue until patients have completed pneumococcal vaccinations and developed positive titers, due to the hyposplenic state of all individuals with SCD.

2.9 Justification for monitoring of immune reconstitution and viral reactivation after γδ+ T-cells allografts

Haploidentical hematopoietic stem cell transplantation has expanded the donor pool for patients who lack HLA matched donors. Haploidentical donor transplantation requires T-cell depletion or intensive immune suppression to avoid life-limiting GvHD. Compared to more traditional HLA matched donor transplantation approaches, patients who receive haploidentical transplantation often experience delayed T-cell mediated immune reconstitution when compared to those receiving MSD grafts. [65] This defect can be associated with disease relapse (particularly in malignant diseases) and viral infections. [44] However early engraftment of various lymphocyte subsets predicts both improved survival and decreased treatment-related mortality, including viral infection. [66-71] We anticipate the infusion of large numbers of γδ+ T-cells in our haploidentical stem cell transplantation approach will improve immune reconstitution post-transplant. Support for this conclusion includes a randomized control trial of haploidentical stem cell grafts that compared either **a)** *ex vivo* CD3+T-cell depletion and *in vivo* CD19+ B cells depletion (negative selection), or **b)** CD34+ positive selection grafts. More rapid reconstitution of cytolytic NK cells and enhanced resistance to viral reactivation were demonstrated in patients receiving CD3+ CD19+ depleted grafts. [72]

Viral reactivation following allogeneic stem cell transplantation is a common complication that can contribute to significant morbidity and mortality.[73, 74] Patients are at particularly high risk if they experience delayed immune reconstitution. Major risk factors for viral reactivation, and consequent viral disease include the use of mismatched donors, myeloablative conditioning, T-cell depletion and increased GvHD prophylaxis. The result is not only a slowing in the tempo of T- and B-cell reconstitution, but a reduction in the quality of the response.[73, 75] Previous studies of αβ+T-cell depleted haploidentical transplantation in non-malignant hematologic disease other than SCD have demonstrated low rates of viral reactivation when compared to other alternative donor strategies. This outcome has been attributed to improved immune reconstitution related to the partial T-cell depleted nature of the graft as compared to complete T-cell depletion. [48]

Understanding both the rate and quality of immune reconstitution as well as the rates of viral reactivation following αβ+T-cell-depleted haploidentical transplantation is therefore of critical importance to improving the safety and expected non-relapse related morbidity and mortality risks in these patients. As a secondary goal, we propose to examine defined lymphoid/NK peripheral blood subsets at regular intervals post-transplantation, allowing a comprehensive picture of lymphoid engraftment to be generated. Coupled with these studies, we will examine cytomegalovirus (CMV), Epstein-Barr virus (EBV), Adenovirus and BK DNA titers in the serum to correlate in a descriptive manner the tempo of functional immune reconstitution.

Monitoring of viral reactivation and preemptive treatment has proven to be a cornerstone of successful treatment of viral reactivation events, decreasing morbidity and mortality associated with specifically CMV and EBV related illnesses. [76, 77] The more recent availability of agents active against adenovirus and systemic BK viremia suggests that similar monitoring for these organisms should be contemplated. These approaches are part of the standard of care in the first 365 days following transplantation at the University of Chicago and will be utilized throughout the study.

2.9 The Central Nervous System in SCD

Silent Infarction, Stroke, and Neuropsychological Testing in Children with SCD: Silent infarcts were present in 22% of children with sickle cell anemia who were studied in the Cooperative Study of Sickle Cell Disease (CSSCD). [78] Silent infarcts were predictive of subsequent strokes and new or more extensive silent infarcts in the CSSCD cohort. Importantly, there is a well-documented relationship between decreased performance on neuropsychological testing and the presence of silent infarcts. [79]

Overt stroke occurs in 5-10% of children with SCD who are less than 15 years of age. [80, 81] Chronic transfusion therapy is inadequate to prevent recurrent stroke after transfusions were discontinued. [82] Furthermore, although effective, transfusion therapy is associated with morbidity and does not prevent all recurrent strokes. Indeed, roughly 25% of children receiving chronic transfusion therapy for stroke experienced recurrent overt stroke and 45% suffer either over stroke or additional silent stroke. [12]

Neuropsychological testing is frequently compromised in children with SCD even in patients who have not had an overt stroke or a demonstrable silent infarct on brain MRI. [16] This protocol will include detailed pre- and post-HSCT brain MRI and neuropsychological studies.

Treatment of CNS effects of SCD: Relatively little information is available regarding interventions which prevent, limit, or restore compromised CNS function. Evidence-based conclusions are limited to those of the Stroke Prevention Trial in Sickle Cell Anemia (STOP) trial, in which chronic transfusion was superior to observation in the prevention of primary stroke in high-risk children identified by transcranial Doppler (TCD) studies. [83] The consensus from single institution trials is that chronic transfusion is effective in prevention of secondary stroke in patients who have experienced an initial stroke. [84, 85] Recently, the efficacy of stem cell transplantation in secondary stroke prevention has been demonstrated. [27] We propose to study the CNS outcomes of the patients enrolled in this study in a descriptive manner.

2.10 Community Consensus on use of Allogeneic HSCT for Severe Sickle Cell Disease

To address the issues involved in expanding HSCT to all eligible patients with SCD, the first consensus conference to discuss the medical, psychosocial, safety, and ethical issues associated with alternate donor transplantation was convened at SJCRH in June 2000.[86] This conference was modeled after a similar meeting held prior to the initiation of a multi-institutional study of MSD BMT in SCD. Data was presented on risk factors for severe SCD, the state of allogeneic HSCT, the ethics of experimental therapy in SCD, and the views of patient advocates. Based on the information presented, the participants agreed that pilot trials of haploidentical HSCT for severe SCD were worthy of exploration. All agreed that the criteria for success of these studies with respect to safety, toxicity and long-term donor engraftment should closely match those achieved in the MSD setting. Similar meetings of experts, patient advocates, and ethicists have endorsed and extended these conclusions including the recent Sickle Cell Annual Meeting at the National Institute of Health (NIH) in August 2016. [87]

We have received many inquiries from parents and physicians regarding haploidentical transplantation. We propose to use an approach similar to that used for other allogeneic donor transplants with respect to supportive care. We plan to involve our social workers and Child Life specialists in all patient and family evaluations/discussions, and offer hospital patient advocates and chaplains for families who consider this investigational approach. These efforts will be documented in the clinical record.

2.11 Summary

Although haploidentical HSCT has been historically associated with high rates of morbidity and mortality, the technology and supportive care necessary to achieve a successful outcome has improved significantly over the past several years. The mortality of MSD HSCT for SCD has been approximately 6%. It is our goal to perform haploidentical HSCT with no more than 10% mortality. In studies to date this has been feasible, but the rates of graft failure, GvHD and effective immune reconstitution suggest that significant additional improvements are required. To achieve this goal, we propose that research participants will be treated in a closely monitored two stage design with plans to continue enrollment into the second stage design if strict safety parameters are met in the first phase portion of the study. (See section 10.0)

For this trial, we propose to use:

a) A conditioning regimen that consists of busulfan, fludarabine, low-dose thiotepa, antithymocyte globulin (ATG) and rituximab, to limit toxicity. Busulfan, fludarabine and ATG have been used safely and effectively in children with SCD with matched sibling donors. Low-dose thiotepa and rituximab have been used in haploidentical transplant protocols with modest toxicity. Specifically, the role of the latter agent is to reduce EBV-

related post-transplant lymphoproliferative disease. This is a standard of care approach in the context of haploidentical HSCT.

b) Megadoses of CD34+ stem cells (Target: >10x10^6/kg) for children and young adults with severe SCD. These cells will be purified using the Miltenyi CliniMACS system designed for αβ+ T-cell receptor selection using immunomagnetic beads. A fixed T-cell dose of approximately <1.0 x 10^5 αβCD3+ cells/kg will be infused. No greater than 1.0 x 10^8 γδ+CD3+-cells/kg will be infused.

c) No post-transplant GvHD prophylaxis will be used in an attempt to accelerate immune reconstitution. We will add the use of GvHD prophylaxis should we find we are approaching unacceptable rates of acute GvHD as defined by our stopping rules. See Section 10.0.

d) Close monitoring for toxicity and safety of the protocol.

e) Stem cell boosts to improve engraftment in the context of declining donor chimerism. In general, stem cell boosts will be given for cytopenias or aplasia with or without declining donor chimerism. See **Section 5.9** for more details

We will also study:

a) The kinetics of hematopoietic engraftment and immune reconstitution following stem cell infusion.

b) The effects of haploidentical HSCT on SCD-related organ damage with a particular focus on the central nervous system.

3.0 ELIGIBILITY

3.1 Transplant Research Recipient Inclusion Criteria

3.1.1. Hemoglobin SS, SC, S-β0 Thalassemia, or SO-Arab Sickle Cell Disease.

3.1.2. Age 10-25 years, inclusive (Stage I); 2-25 years, inclusive (Stage II).

3.1.3. Lack of fully matched (10/10) family donor or fully matched (10/10) unrelated donor registered in the National Marrow Donor Program (NMDP).

3.1.4. Partially-matched family member with hemoglobin AA (normal) or hemoglobin AS (sickle trait) phenotype.

3.1.5. SCD with Severe Phenotype, defined by the following criteria:

a) Neurologic manifestations of sickle disease including cerebral vascular accident (CVA), transient ischemic event (TIA) or abnormal MRI findings suggestive of silent infarct.
b) Two or more episodes of acute chest syndrome (ACS) requiring admission for transfusional or respiratory support including supplemental oxygen within [two years] of enrollment in study despite hydroxyurea therapy. Patients who cannot tolerate hydroxyurea and who experience multiple episodes of ACS will also be eligible.
c) History of severe vaso-occlusive (VOC) disease requiring hospitalization and intravenous narcotics on 3 or more occasions

per year over the two years prior to enrollment despite hydroxyurea therapy. Patients who cannot tolerate hydroxyurea and who experience multiple episodes of VOC will also be eligible.

d) Other severe phenotype as evidenced by end organ dysfunction related to sickle cell disease.

3.2 Transplant Research Recipient Exclusion Criteria

3.2.1 Karnofsky or Lansky score < 60%.

3.2.2 Acute hepatitis or evidence of moderate or severe portal fibrosis on biopsy. (Biopsy will be obtained if patient has been on chronic transfusion therapy > 6 months or has a ferritin > 1000 ng/ml) or AST or ALT >5 times the upper limit of normal.

3.2.3 Severe renal impairment (as evidenced by creatinine clearance of <50ml/minute glomerular filtration rate (GFR) < 50% predicted normal).

3.2.4 Cardiac function that demonstrates shortening fraction less than 26% by cardiac echocardiogram or pulmonary hypertension.

3.2.5 Pregnant Female.

3.2.6 Lactating female.

3.2.7 HIV Seropositivity.

3.2.8 Pulmonary function with baseline O2 saturation <85% or Diffusing Capacity for Carbon Monoxide (DLCO) on pulmonary function testing (PFT) with a DLCO <40%.

3.3 Donor Eligibility (Inclusion/Exclusion) Criteria

3.3.1 Is a partially matched family member.

3.3.2 Donor does not have sickle cell disease.

3.3.3 Donor cannot be HIV seropositive.

3.3.4 Donor cannot be positive for anti-Hepatitis C antibodies or anti-Hepatitis B core antibody.

3.3.5 Donor cannot have invasive fungal infection.

3.3.6 Lactating or pregnant females are excluded.

3.3.7 Medical conditions that preclude apheresis.

3.3.8 Donor must be evaluated by their physician and deemed medically safe for apheresis.

3.3.9 If the selected donor is <18 years old, he/she will be meet with the donor advocate to provide additional support to the donor and his/her family as well as to minimize emotional and psychosocial risk to the donor as per institutional guidelines and transplant standard operating procedures.

3.4 Graft Eligibillity (Graft Release) Criteria

3.4.1 Graft must have $>10x10^6$ $CD34^+$ cells.

3.4.2 Graft must have $<1.0x10^5$ $\alpha\beta^+$ T-cells.

3.4.3 Graft must have $>1x10^8$ $\gamma\delta^+$ T-cells.

3.4.4 Graft must have a negative gram stain and culture.

3.4.5 Graft must have an endotoxin test demonstrating less than 5 EU/kg body weight/hour

3.4.6 Graft must have >90% viability testing based on trypan blue test

Note: All graft release criteria will be performed on the final device output product prior to cryopreservation. Graft must meet these criteria prior to initiation of conditioning chemotherapy. Once met graft with by cryopreserved and conditioning chemotherapy will begin.

Gram stain and culture performed on the final device output will be available prior to cryopreservation and be included in the graft release criteria as indicated above. Additional gram stain and culture will be performed after thawing will not be available prior to infusion as the products are thawed at the patient's bedside and infused immediately to ensure the best possible viability. This will be monitored closely and if a positive test is obtained, the subject will initiate appropriate antibiotics immediately in conjunction with guidance from our immunocompromised infectious disease team.

4.0 DESIGN

4.1 Aims of Current Study

4.1.1 Safety

The primary aim of this study is to determine the safety of αβ T-cell depleted haploidentical HSCT in children, adolescents and young adults with severe SCD. The treatment plan will be considered safe if there is no excessive toxicity. Excessive toxicity is defined as failure to engraft/graft rejection or overall grade III/IV aGvHD or death within 100 days post transplantation. Graft failure will

include both primary and secondary graft failure. Primary graft failure is defined as failure to achieve an absolute neutrophil count (ANC) of >500/uL at 28 days post stem cell transplant. Secondary graft failure is defined as loss of neutrophil engraftment (ANC <500/uL for three consecutive days) and <5% total nucleated cell DNA of donor origin despite G-CSF administration and/or platelet <30X10^3/*u*L with <5% total nucleated cell DNA of donor origin despite initial stem cell infusion. [88, 89] We will also consider graft failure if the HgB S percentage is greater than sixty. These criteria are based on standard criteria to define graft failure and an understanding of graft failure specifically in the setting of transplantation for sickle cell disease. [60, 61, 90, 91]

We will evaluate the primary objective of safety of HSCT within the framework of a two-stage design. In the first stage, we will enroll up to eleven research participants to receive HSCT. The treatment will provisionally be considered safe if no more than two of eleven research participants have overall grade III or IV acute GvHD, no more than four of eleven participants have grade II or higher acute GvHD, no more than four of eleven participants have graft failure and no more than two of eleven participants die of transplant toxicity. Once it is determined that the treatment plan is provisionally safe, we will begin enrollment of eligible and consented research subjects into a subsequent second phase (up to twenty-seven) to assess safety as well as the efficacy of the transplant procedure. Further details are given in **Section 10.0** (Statistics).

Contingency plans for the study: Should any of the above defined stopping rules be met during enrollment of the first eleven research participants, study enrollment will be halted and the protocol will require amendment of the conditioning regimen with resubmission of a revised protocol for review by the IRB and the appropriate regulatory agencies.

We will follow the above algorithm with one additional modification. If the rate of aGHVD in the first 11 patients is too high (as defined), we will modify the treatment protocol to include immune suppression prophylaxis and begin anew, enrolling 11 patients in the first stage and up to 26 in the second stage if no further stopping boundary is met.

4.1.2 Mixed Chimerism and Immune Reconstitution

A goal of our study is to monitor hematopoietic chimerism in children and young adults with SCD who receive a haploidentical HSCT. Based on previous experience, we would anticipate at least 80% of patients will have durable (100 days) donor cell engraftment sufficient to correct the erythroid defect of SCD. Our approach will be to determine the donor contribution to hematopoiesis at multiple time points after transplantation. This will include measurements of initial bone marrow chimerism after engraftment, followed by sequential measurement of unsorted peripheral blood chimerism. It is standard of care to measure chimerism following stem cell transplant. This is not experimental.

Donor chimerism analysis will be evaluated from the bone marrow and peripheral blood at the time of full engraftment (roughly between day 21-28 post stem cell Hap-HSCT) and then approximately every two weeks until 100 days

post Hap-HSCT. Chimerism will then be followed monthly until day 180 post Hap-HSCT and at least every three months until one (1) year post Hap-HSCT. Chimerism is measured by a small tandem repeats (STR) PCR technique using total nucleated cell DNA from peripheral blood or bone marrow leukocytes. [92, 93]

Erythroid chimerism will be assessed based on the ratio of β^A to β^S chains by hemoglobin electrophoresis every two weeks post-transplantation until day +100 approximately monthly until day 180, then approximately every three months until one year.

Functional and quantitative studies of T-cells will be performed as part of the standard of care for post stem cell transplant patients. Investigations of immune reconstitution will include characterization of T-cell subsets (CD3, CD4, CD8) at approximately day 28 and day 100 post HSCT, then every three months for two years. Both TCRαβ and γδ+ presentation will be assessed. Additional testing on T-cell number and function including testing T-cell receptor diversity by spectratyping, TCR-rearrangement excision circles (TRECs), and assays of T-cell function including T-cell responses to mitogens may be assessed if clinically indicated.

The tempo of B-cell reconstitution will also be a focus, including enumeration of B-cells and determination of functional activity through measurement of immunoglobin levels. Finally, we will assess NK number. These tests will be performed at approximately day 28 and day 100 post HSCT, then every three months for two years as part of the standard of care for post stem cell transplant patients.

4.1.3 Assessing Effects of HapSCT on End Organ Damage

Previous studies have demonstrated that patients with SCD who undergo successful stem cell transplantation have cessation and even in some instances improvement in their previously documented end organ dysfunction secondary to their SCD that occurred prior to transplantation. We will measure outcomes related to end organ dysfunction including cardiac, pulmonary, liver, renal and CNS both prior to transplantation and at 6 monthly intervals after transplantation. Evaluation of end organ dysfunction as described below is standard of care in the post-transplant setting. These evaluations are not experimental or related to research.

Growth and development: Each subject will have height and weight measured prior to initiation of transplantation. When available, growth curves from the years preceding transplantation will also be evaluated. Height and weight will be measured at every post- transplantation visit to evaluate growth in a longitudinal manner following the intervention.

Cardiac: Echocardiography will be performed at 12 months post-transplantation and then yearly for at least five years following transplantation to evaluate for cardiac dysfunction and evidence of pulmonary hypertension as part of our

standard of care for post-transplantation patients. A cardiac T2* will also be performed to assess for iron overload.

Pulmonary: Pulmonary function testing will occur at approximately day 180 post-transplantation and then at approximately yearly intervals post-transplantation for two years in asymptomatic individuals with normal O_2 saturations at routine visits. Those individuals with aberrant O_2 saturations will be followed more regularly as determined by our pulmonology colleagues.

Renal: Creatinine and BUN evaluation will be performed daily starting from day +1 until discharge from the hospital. These values will be assessed at each clinic visit following discharge, and/or at least every other week through the first 180 days post-transplant. They will be performed at approximately monthly intervals through one year. Creatinine clearance will be assessed at day +180 and 1 year post-transplantation.

Liver: Liver function testing (including AST, ALT, GGT and bilirubin level) will be performed daily starting from day +1 until discharge from the hospital. These values will be assessed at each clinic visit following discharge, and approximately weekly through the first 180 days post-transplant. They will be performed at approximately monthly intervals through one year. Additionally, liver MRI with coronal and axial T2 star sequences will be performed at 12 months post-transplant if clinically indicated.

CNS: Research participants who undergo Hap-HSCT will undergo detailed neurological, neuroimaging and neurocognitive evaluation. Assessment will be at BASELINE, and at approximately 12, 24, 36, and 48 months post-transplantation as determined by our neuropsychology colleagues. We will evaluate sickle vasculopathy and brain parenchyma in SCD research participants by conventional MRI/MRA, as well as arterial spin labeling (ASL) perfusion and diffusion tensor imaging (DTI) of white matter.

Neurocognitive development will be measured by Dr. Scott Hunter and his clinical team. (See appendix II) Full assessments will include evaluation of neurocognitive function, mood/behavior and executive functioning. This testing will occur at baseline and then at one year post-transplantation. Testing at 6 and 18 months post transplantation with focus on behavioral testing. Focused assessment based on individual patient will be assessed yearly starting at two years post transplantation and continue to five years. Many of these testing strategies have been previously used to evaluate neurocognitive development in patients with sickle cell disease post–transplantation, and as part of routine care.[26]

5.0 TREATMENT OR INTERVENTION

The treatment protocol is outlined in the schema below. The treatment of patients with myeloablative chemotherapy followed by stem cell infusion and subsequent clinical monitoring is considered standard of care therapy for patients undergoing stem cell transplantation. The mobilization of peripheral blood stem cells using GCSF and apheresis is standard of care for stem

cell transplant donors. The only experiemental components of treatment is the use of the Miltenyi CliniMACS device for αβ T-cell depletion.

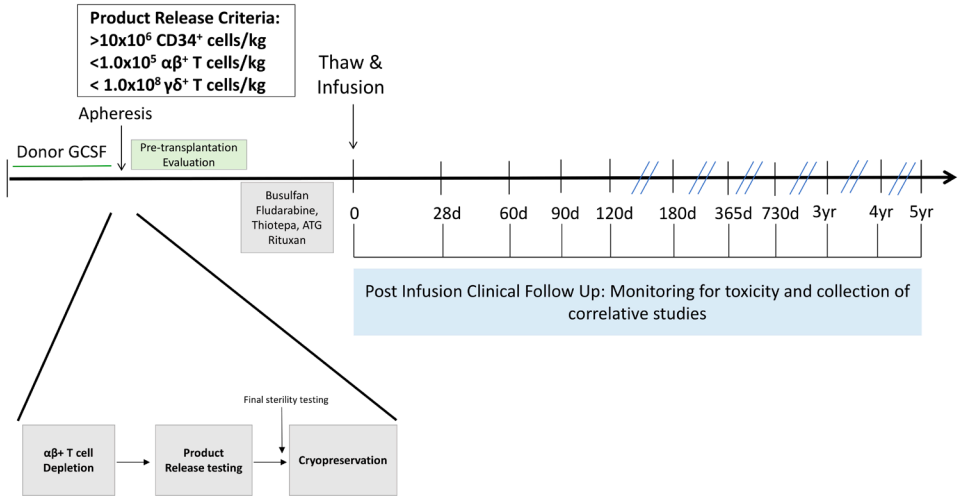

Figure 1. Study Schema
GCSF indicates granulocyte colony-stimulating factor. D indicates days. Yr indicates years.

5.1 Research Participant Selection

Research participants will be recruited from the UCM sickle cell disease joint pediatric and adult cohort. Children, adolescents and young adults followed at other institutions are also eligible. It is anticipated that accrual for Stage I of this study will be achieved within approximately 18-24 months. We currently have eight children/adolescents in the process of evaluation for haploidentical HSCT for SCD. This protocol will treat an underserved and vulnerable (pediatric/adolescent/young adult) population using an investigational approach to HSCT. Eligibility criteria for this protocol are stricter than those for MSD transplants**,** and delineate those children/adolescents with the poorest prognosis for whom therapeutic options are limited. [24]

5.2 Donor Selection:

Donor selection prior to stem cell transplant is standard of care and includes HLA typing of any potential donors and medical evaluation to determine suitability of donor. This study will consider parental donors in addition to sibling donors but each potential donor will be evaluated per standard of care procedures. This is not experiemental or for the purposes of research.

5.2.1 All family members who are at least a 5/10 HLA match will be considered.

5.2.2 Potential donors will be screened for hemoglobinopathy by electrophoresis. Patients with sickle cell disease trait will be considered eligible. Any patient with sickle cell disease will not be eligible to donate.

5.2.3 Donors will be excluded from consideration if any anti-donor HLA antibodies are detected during pre-transplant evaluation.

5.2.4 Donors must be medically fit to undergo therapy with G-CSF and peripheral stem cell mobilization and cleared by their physician to undergo procedure.

5.2.6 If multiple donors are eligible, the transplant physician team will choose the best donor. Priority will first be given to the donor with the most HLA allele matches. Following that preference will be given to maternal donors given improved survival noted in maternal versus paternal haploidentical donors attributed to bidirectional immunological tolerance *in utero*.[94, 95]

5.3 Stem Cell Mobilization/Graft Collection:

Donors will undergo stem cell mobilization per our institutional standard following evaluation by their physician and resultant medical clearance to donate. Donors will receive GCSF 10mcg/kg with close observation of peripheral CD34+ cell count. When the peripheral CD34+ cell count is adequate (20 cells/*u*L), the donor will have a peripheral intravenous line (when possible) or an apheresis catheter placed in conjunction with the blood bank and processing lab, and cells will be collected. Donors may undergo pheresis for two consecutive days if a single day of pheresis does not allow for a sufficient collection. This is standard of care for stem cell mobilization and graft collection. This process is not experiemental or for research.

5.4 Collection of Autologous Back-up Bone Marrow, Pre-Conditioning and Conditioning Therapy

5.4.1 Collection of Autologous Bone marrow:

Each recipient will undergo bone marrow harvest 1-2 months prior to stem cell infusion. This stem cell source will serve as a stem cell rescue if a patient develops primary or secondary engraftment failure after a subsequent stem cell boost. Stem cells will be stored for future use of the patient only. Patient will be admitted the evening prior to their bone marrow harvest. The patient will receive intravenous fluid and transfusion of donor pRBC to reach a goal hemoglobin level of 10 g/dL prior to general anesthesia with the goal of preventing vaso-occlusive manifestations of SCD following anesthesia. This is standard of care. This process is not experiemental or for research.

5.4.2 Pre-conditioning therapy*:*

Hydroxyurea will be given for approximately 3 months prior to the conditioning therapy, (approximately day -102 to day -8). Hydroxyurea will begin with a starting dose 20 mg/kg/day and titrated up to a goal of 30mg/kg/day. The dosage may be adjusted based on the absolute neutrophil count. Doses may be held for an ANC < 500/mm^3. Doses may also be held prior to collection of autologous bone marrow. Dose modifications or changes in doses or days of administration for HU, which are done for safety reasons or as clinically indicated, will be done without causing protocol variations or violations. This is standard of care for transplant in sickle cell disease. This process is not experiemental or for research.

5.4.3 Conditioning Regimen

1. Thiotepa
Dosage and Route of Administration

- **The dose is 5 mg/kg/dose intravenously every 12 hours (2 doses) on Day -8**

2. Busulfan
Dosage and Route of Administration

- **The dose is 0.8 mg/kg/dose intravenously every 6 hours (16 doses) on days -7, -6, -5, -4 and -3**

3. Fludarabine
Dosage and Route of Administration

- **The dose is 40 mg/m^2/dose intravenously every 24 hours (4 doses) on days -6, -5, -4, and -3**

4. ATG
Dosage and Route of Administration

- **The dose is 4 mg/kg/dose intravenously every 24 hours (3 doses) on days -5, -4, and -3**

5. Rituximab
Dosage and Route of Administration

- **The dose is 200mg/m^2/dose intravenously every 24 hours (3 doses) on days -5, -4, and -3**

This conditioning regimen is considered standard therapy in the context of haploidentical stem cell transplant. These agents have also been used safely in patients with sickle cell disease and pediatric patients. This regimen is not experiemental or being used for the purpose of research.

Table 5. Schema of Conditioning Regimen	
Day	Treatment
-8	Thiotepa
-7	Busulfan
-6	Busulfan, Fludarabine
-5	Busulfan, Fludarabine, ATG
-4	Busulfan, Fludarabine, ATG
-3	Busulfan, Fludarabine, ATG
-2	Rest
-1	Rituximab
0	Stem Cell Infusion

For details of source, pharmacology, formulation, supplier, toxicity and route of administration, see Section 6.0. The term “every” used in this table is an approximate term meaning that these medications noted will be administered approximately “every” 12 hours. The drug administration timing in the case of “every 12 hours” may be modified +/- 4 hours as clinically indicated such as in

the case of surgical procedures or to accommodate other necessary medication delivery or procedures (such as an emergency CT scan) without resulting in protocol variation or violation.

All medication dosing may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Specifically, busulfan dosing may be modified to target ideal area under the curve based on patient's pharmacokinetic evaluations per institutional standard. See Section 2.6 for additional details. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

5.5 Research Subject Pre-Transplant Evaluation (see Appendix I for Roadmap)

5.5.1 Time Period

Consent must be obtained any time within 6 months prior to initiation of chemotherapy, specifically day-10. Pre-transplantation evaluations will follow institutional standards for transplantation of patients with SCD as outlined below.

5.5.2 General Assessment obtained for all transplantation candidates

As per our institutional standards, we will evaluate for signs and symptoms of pre- transplant infection with standard pre-transplant infectious disease markers, PPD, CT chest/abdomen/pelvis and sinuses and Panorex in combination with dental evaluation. Respiratory viral panel from nasal secretions will be tested. Blood, nasal, urine and stool cultures will also be sent. Organ function will be assessed with ECHO, PFTs, GFR assessment, liver function studies, audiogram. PTH, TSH, free T4, IGFBP3, IGF1, and 25 hydroxy-vitamin D level and if the subject is greater than 8 years of age testosterone, estrogen, FSH and LH will also be assessed. Lipid panel and coagulation profile will be sent. Consultation with psychology, social work, nutrition, dentistry, child life, and ophthalmology will occur prior to transplantation. This is standard of care. This process is not experiemental or for research.

5.5.3 Organ specific evaluations

These evaluations are standard of care. This process is not experiemental or for research.

<u>Central nervous system:</u> Patients will have MRI/MRA brain as well as arterial spin labeling (ASL) perfusion and diffusion tensor imaging (DTI) of white matter prior to transplantation.

<u>Psychological Assessment:</u> Neurocognitive functioning will be assessed using a comprehensive neuropsychological test battery including measures of general intelligence, academic achievement, attention and concentration, memory, visual-motor and visual-perceptual function, and planning/organization. A listing of the specific neuropsychological measures includes Wechsler Intelligence Scales and Wide Ranging Achievement testing can be seen in Appendix II. These tests will

be performed only if the research participants speak and comprehend English adequately as equivalent test instruments are unavailable in other languages.

Liver: Non-infused Liver MRI will be performed with coronal and axial T2 star sequences to measure iron overload. Optional (to be done at discretion of PI) Liver biopsy with iron quantitation if clinically indicated as judged by the PI (ie. on chronic transfusion therapy for > 6 months or ferritin > 1000 ng/ml).

Cardiac: Cardiac MRI with T2 star sequences will be performed to evaluate for cardiac iron overload. Baseline echocardiogram and electrocardiogram (EKG) will be evaluated to assess for function.

Hematologic and Immunologic: Within four weeks of initiation of conditioning chemotherapy, we will evaluate hemoglobin electrophoresis, ferritin, iron, transferrin, and percent saturation. CBC will be evaluated within one week of conditioning and every two weeks prior to that to allow for titration of hydroxyurea. Lymphocyte subset analysis and quantitative immunoglobins will also be assessed within four weeks of initiation of chemotherapy.

Renal: Kidney function will be assessed through either nuclear medicine Glomerular Filtration Rate (GFR) evaluation or 24 hour urine creatinine clearance. BUN and serum creatinine will also be measured prior to initiation of chemotherapy.

Pulmonary: Pulmonary function testing will be assessed prior to transplantation including DLCO and oxygen saturation testing.

Growth and Development: At a final pre-transplantation visit, height and weight will be measured. When possible, growth curves will be obtained from years prior to transplant presentation from the research subject's primary care provider or hematologist.

5.6 Pre- transplant donor evaluations

If the donor meets criteria as outlined in section 3.3 and is selected as outlined in section 5.2, he/she will undergo the following work up: complete history and physical, donor questionnaire, chest X-ray, EKG and infectious disease evaluation, CBC with differential, reticulocyte count, coagulation studies, LDH, comprehensive metabolic panel with phosphorous, magnesium and uric acid. Urinalysis and urine or serum pregnancy testing (for female participants great than 12 years old) within 7 days of mobilization. This is standard of care. This process is not experiemental or for research.

The infection disease evaluation, in compliance with FDA regulations for all cellular product donors includes screening for HIV-1, HIV-2, Hepatitis B (surface and core antigen), hepatitis C virus (HCV), Treponema pallidium, HTLV-1, HTLV-2 and CMV. The donors will be screened for exposure to Creutzfeldt-Jakob disease and Zika Virus. Additionally, as per institutional standards, we will also test for west nile virus and Chagas disease. Testing is all done at the American Red Cross, National Testing Laboratory which is a CLIA and Centers for Medicare and Medicaid Services (CMS)

certified site. If a donor is found to be "ineligible" based on this testing the subject and/or the guardians will be made aware of testing and eligibility. Should the medical team and the family decide to proceed despite ineligibility the device output will have the following label: "WARNING: Advise patient of communicable disease risk," and when applicable, "WARNING: Reactive test results for (name of disease agent or disease)." This testing will all be completed within 30 days prior to recovery of cellular product.

5.7 *Research Participant Peri- and Post-Hap-HSCT Evaluation (see Appendix I for Roadmap)*

5.7.1 General and Organ Specific Safety Evaluations for Research Participants per Institutional Standard for peri- and post-transplant follow up.

General Physical Evaluations daily while inpatient (typically admitted at initiation of primary conditioning, i.e. day -10) then at least once per week until Day 100 and then at least once per month to one year and then at least annually to five years post-transplantation.

CBC with differential at least daily while inpatient until first hospital discharge post stem cell transplantation then at least weekly until Day 100 then at least once per month till one year and then at least annually till five years.

Comprehensive metabolic panel with magnesium and phosphate at least daily while inpatient until first hospital discharge then at least weekly until Day 100 then at least once per month till one year and then at least annually till five years.

At day 28, day 100 and day 365 evaluations, we will also assess with Urinalysis and CXR. On day 100 and day 365 25-hydroxy vitamin D and PTH will be evaluated. Ferritin, iron at approximately 3, 6, 12, 24 and 36 months post-transplantation.

MRI/MRA using protocol for SCD with perfusion, diffusion, and DTI at approximately 6, 12, 24, 36 and 48 months post-transplantation. TCD study at approximately 6 months, 12 months, and 24 months post SCT at the discretion of the investigator and if technically feasible.

On day 365 dental evaluation, CXR, PFTs, EKG and Echocardiogram, bone density will also be obtained.

This is standard of care. This process is not experiemental or for research.

5.7.2 Infectious disease evaluation and monitoring for viral reactivation

CMV, BK and Adenoviral PCRs will be evaluated weekly starting on day +15 through day 100 and then monthly for one year. Antiviral preemptive or therapeutic interventions will be adjusted accordingly to institutional guidelines. Additionally, should the patient develop fevers, cultures will be drawn and broad-spectrum antibiotics will be initiated. Additional evaluations will be obtained at the discretion of the clinical team and principal investigator.

Research participants will have EBV DNA levels monitored in an effort to detect early viral reactivation as a harbinger of lymphoproliferative disorder (EBV-LPD), allowing for preemptive therapy. Samples of peripheral blood will be drawn weekly from approximately day +15 to day +100 post-transplant and assayed for quantitative EBV DNA levels. This time period may be extended for research participants with delayed immune reconstitution. Research participants with EBV DNA levels > 1,000 copies/mL on two consecutive measurements or those with EBV-LPD may be treated with rituximab (monoclonal anti-CD20) 375 mg/m^2 IV and then as clinically indicated. [96]

This is standard of care. This process is not experiemental or for research.

5.7.3 Measurement of Chimerism

Molecular chimerism: Peripheral blood engraftment analysis will be obtained weekly starting on day +21 and weekly thereafter until day 100. After day 100 these studies will be checked approximately monthly until 1 year post transplantation and then approximately every 3-6 months in the subsequent 5 years. Bone marrow evaluation at day 28, day 100, and day 365 will be obtained to evaluate for marrow engraftment as per current institutional standard.

Erythroid chimerism: Hemoglobin electrophoresis of with quantitation of F, A2, S at approximately two week intervals post transplantation until day 100 and then monthly until one year. It will subsequently be checked yearly through five years post-transplant. If a bone marrow aspirate is clinically indicated in the post-transplant period, it will be tested for erythroid precursor chimerism.

This is standard of care. This process is not experiemental or for research.

5.7.4 Immune reconstitution

Immune reconstitution will be evaluated with a focus on lymphocyte recovery and function. Functional and quantitative studies of T-cells will be performed as part of the standard of care for post stem cell transplant patients. Investigations of immune reconstitution will include characterization of T-cell subsets (CD3, CD4, CD8) at approximately day 28 and day 100 post HSCT, then every three months for two years. Both TCRαβ and γδ+ presentation will be assessed. Additional testing on T-cell number and function including testing T-cell receptor diversity by spectratyping, TCR-rearrangement excision circles (TRECs), and assays of T-cell function including T-cell responses to mitogens may be assessed if clinically indicated.

The tempo of B-cell reconstitution will also be a focus, including enumeration of B-cells and determination of functional activity through measurement of immunoglobin levels. Finally we will assess NK number. These tests will be performed at approximately day 28 and day 100 post HSCT, then every three months for two years as part of the standard of care for post stem cell transplant patients.

This is standard of care. This process is not experiemental or for research

5.7.5 Neuropsychologic Assessment

Neuropsychologic: Evaluations will be performed at approximately 12 months post transplantation and yearly post-transplant where feasible. These tests will be performed only if the research participants speak and comprehend English adequately as equivalent test instruments are unavailable in other languages. See appendix II for additional details.

This is standard of care. This process is not experiemental or for research.

5.8 Supportive Care Guidelines Specific for Patients with SCD Post-Transplant

Anticonvulsants will be prescribed to all research recipients starting on day -10 and to continue for three months post transplant. This approach is standard of care for patients with SCD who have a known risk of neurotoxicity in the peri-transplant period. [17, 64] For safety reasons, research participants' hemoglobin should be kept at approximately 9-11 mg/dl. Platelets should be maintained at approximately >50,000mm^3. Maintenance of these levels has been proven to prevent CNS events in patients previously transplanted for SCD. [17, 64] This is standard of care. This process is not experiemental or for research.

5.9 Stem Cell Boosts

Donor chimerism studies will be performed by small tandem repeat (STR) analysis on either bone marrow or peripheral blood beginning on or about day 21 after engraftment, approximately weekly thereafter until day 100, and then as indicated in section 5.7.3 above or as clinically indicated.

These studies will determine the need for stem cell boosts. Criteria will generally be the level of donor chimerism (<5%) in whole blood DNA or hemoglobin electrophoresis demonstrating >60% hemoglobin S:

5.9.2 A second peripheral blood stem cell graft will be obtained from the transplant donor and processed using a Miltenyi CD34+ selection column in the Cell Processing Laboratory according to applicable cGMP and cGTP guidelines.

5.9.3 Research participants with low-level donor whole blood or bone marrow chimerism, elevated HbS percentage (>50% on two occasions at least 2 days apart), and/or low peripheral blood counts may receive an infusion of αβ+ T-cell depleted stem cells from the original donor when possible.

5.9.4 Should graft rejection occur, research participants will be reconditioned with anti-T Cell antibody, steroids, cyclosporine and receive a CD34+-selected stem cell boost from the original donor at the discretion of the treating physician and in agreement with the patient/family. A separate informed consent document is required to be signed by the legal guardian for this procedure.

5.9.5 Should graft failure occur following a CD34+-selected stem cell boost, re-infusion of host bone marrow cells will be recommended and the research subject will be taken off study.

This is standard of care. This process is not experiemental or for research.

5.10 Compliance with post-transplantation evaluations

Compliance with post-transplantation evaluations is highly contingent upon the participant's/legal guardian's compliance with returning to the clinic for follow-up appointments. Therefore, once the research participant is four months post-transplant, the required monthly follow-up evaluation schedule may be adjusted by +/-7 days without causing protocol violations/variations; this applies only in the case when the research participant fails to return to the clinic for regularly scheduled clinic visits. If the scheduled weekly or monthly evaluation falls on a holiday or during a holiday time period, a reasonable adjustment in the follow-up clinic visit due to the holiday schedule is expected and would not be noted as a protocol variation or violation. Every effort will be made to encourage and have the research participant/legal guardian return for the scheduled clinic visits.

6.0 DRUG & DEVICE INFORMATION

6.1 THIOTEPA (Thioplex® by Immunex) (TESPA, TSPA)

Source and Pharmacology: Thiotepa is a cell-cycle nonspecific polyfunctional alkylating agent. It reacts with DNA phosphate groups to produce cross-linking of DNA strands leading to inhibition of DNA, RNA and protein synthesis. Thiotepa is extensively metabolized in the liver to metabolites that retain activity, primarily triethylenephosphoramide (TEPA). The main route of elimination is via the urine, mainly as metabolites; the elimination half-life of the thiotepa is 2.5 hours, and that of TEPA is 17.6 hours.

Formulation and Stability: Thiotepa is supplied in single-use vials containing 15 mg of lyophilized thiotepa, 80 mg NaCl and 50 mg $NaHCO_3$. The intact vials should be stored under refrigeration and protected from light. Each vial should be reconstituted with 1.5 ml of sterile water for injection to yield a concentration of 10 mg/ml. Further dilution with sterile water for injection to a concentration of 1 mg/mL yields an isotonic solution; if larger volumes are desired for intracavitary, IV infusion, or perfusion therapy, this solution may then be diluted with 5% dextrose or 0.9% NaCl containing solutions. The 10 mg/ml reconstituted solution is chemically stable when stored in the refrigerator for up to 5 days, however, it is recommended that solutions be prepared just prior to administration since they do not contain a preservative. Reconstituted solutions should be clear to slightly opaque: the solutions may be filtered through a 0.22 micron filter to eliminate haze.

Supplier: Commercially available; manufactured by Immunex.

Toxicity: Dose limiting toxicity is myelosuppression. The leukocyte nadir may occur at any time from 10 to >30 days. Other toxicities include pain at the injection site, nausea and vomiting, anorexia, mucositis, dizziness, headache, amenorrhea, interference with spermatogenesis, and depigmentation with topical use. Allergic reactions, including skin rash and hives, have been reported rarely. Rare cases of apnea, hemorrhagic cystitis, and renal failure have occurred. Thiotepa is mutagenic, carcinogenic, and teratogenic in animals. Pregnancy category D.

Route of Administration: Thiotepa will be administered as an intravenous infusion.

6.2 METHYLPREDNISOLONE (Medrol, Solu-Medrol)

Source and Pharmacology: This is an adrenal corticosteroid, anti-inflammatory, immuno-suppressant. Decreases inflammation by suppression of migration of polymorphonuclear leukocytes and reversal of increased capillary permeability.

Formulation and Stability: Tablet (4mg), Sodium Succinate power for Inj. 125, 500mg.

Supplier: Commercially available.

Toxicity: Toxicities include edema, hypertension, vertigo, seizures, psychoses, headache, pseudotumor cerebri, acne, skin atrophy, impaired wound healing, Cushing's syndrome, pituitary adrenal axis suppression, growth suppression, glucose intolerance, hypokalemia, alkalosis, peptic ulcer, nausea, vomiting, transient leukocytosis, muscle weakness, osteoporosis, fractures, cataracts, glaucoma, and increased risk of infection.

Route of Administration: oral, intravenous infusion.

6.3 BUSULFAN (Myeleran)

Pharmacology: Busulfan is a polyfunctional alkylating agent. It interferes with the normal function of DNA by alkylating intracellular nucleophiles and cross-linking DNA strands. Busulfan is cell cycle phase non-specific. It is well absorbed orally and is metabolized by the liver. Drugs that induce hepatic metabolism (e.g., phenytoin) increase clearance and those that inhibit hepatic metabolism (e.g., itraconazole) may decrease clearance. The plasma half-life is ≈ 2.5 hours in adults, but children may have higher clearances.
Formulation and Stability: Busulfan is available as a 2mg tablet and as a solution or suspension for intravenous administration.

Supplier: Parenteral solutions are commercially available. Parental suspension is investigational.

Toxicity: Acute dose limiting toxicity is myelosuppression including leukopenia, thrombocytopenia and anemia. This effect is delayed with a nadir of 14-21 days. Some patients may develop bone marrow fibrosis. Nausea and vomiting are generally mild. Other GI symptoms include diarrhea, anorexia and associated weight loss. Seizures are associated with high doses. Other side effects include liver dysfunction, skin hyperpigmentation, skin rash, gynecomastia, sterility, cataracts and alopecia. Secondary cancers have occurred. "Busulfan lung", manifested by diffuse interstitial pulmonary

fibrosis, persistent cough, fever, rales and dyspnea may occur, most commonly after high doses or prolonged therapy.

Route of administration: intravenous infusion.

6.4 G-CSF (Filgrastim) (Neupogen®)

Source and Pharmacology: G-CSF (granulocytic colony stimulating factor), is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of *Escherichia coli.* G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.

Formulation and Stability: G-CSF is supplied in vials containing 300 mcg and 480 mcg of G-CSF at a concentration of 300 mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours, but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further diluted for IV infusion in 5% dextrose. Do not dilute in saline---precipitate may form. If the final concentration of this product is < 15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.

Supplier: Commercially available

Toxicity: G-CSF causes marked leukocytosis. Adverse reactions reported commonly include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in patients with allergy to E. coli derived products.

Dosage and Route of Administration: Subcutaneous or intravenous.

6.5 HYDROXYUREA (Hydrea®)

Source and Pharmacology: Hydroxyurea is an antimetabolite. As an antineoplastic, hydroxyurea is considered a cell-cycle specific agent effective in the S-phase of mitosis. Hydroxyurea inhibits the conversion of DNA bases by blocking ribonucleotide reductase. Since the drug causes cells to arrest at the G1-S interface, and irradiation sensitivity is maximum during this time, concomitant hydroxyurea and irradiation result in synergistic toxicity (Gilman et al, 1990).

The known pharmacological effects of hydroxyurea which may be beneficial in sickle cell anemia include increasing hemoglobin F levels in red blood cells (RBCs), decreasing neutrophils, increasing water content of RBCs, increasing deformability of sickled cells, and altering the adhesion of RBCs to endothelium (Prod Info Droxia(R), 2001).

Formulation and Stability: 500 mg capsules. Store in a tightly closed container at room temperature (25 degrees Celsius, 77 degrees Fahrenheit); avoid excessive heat.

Supplier: Commercially available

Toxicity: Toxicities include myelosuppression, anorexia N/V/D, constipation, stomatitis, drowsiness, dizziness, headaches, skin and facial erythema, skin rash, nail changes, alopecia, skin changes, dysuria, abnormal LFTs, jaundice, abnormal BUN and creatinine, seizures and auditory hallucinations.

Dosage and administration: Oral administration; Hydroxyurea will begin with a starting dose 15 mg/kg/day as a single dose. The dosage may increase by 5mg/kg/day weekly if the absolute neutrophil count (ANC) remains > $1000/mm^3$ to a maximum of 30 mg/kg/day. Dose may be held for an ANC < $1000/mm^3$.

6.6 RITUXIMAB (Rituxan ™)

Source and Pharmacology: Rituximab is a murine /human chimeric monoclonal antibody. It is specific for the CD20 antigen located on B-cells. Rituximab has been shown to mediate complement-dependent tumor cell lysis and antibody-dependent cellular cytotoxicity. Direct binding to the CD20 antigen is thought to play a role in inhibition of cell growth. Rituximab is administered intravenously. The mean serum half-life after a single IV dose of 375 mg/m2 is 59.8 hours (range 11.1-104.6 hours).

Formulation and Stability: Rituximab is available as 100 mg/10 ml single-use and 500 mg/50 ml single-use vials. Each vial also contains sodium chloride 9 mg/ml, sodium citrate 7.35 mg/ml, polysorbate 80 0.7 mg/ml and water for injection. Rituximab for injection concentration must be diluted with 5% Dextrose or 0.9% NaCl prior to administration. After dilution, unused drug is stable for 24 hours when refrigerated (2-8 degrees Celsius) and 12 hours at room temperature. Vials should be protected from direct sunlight.

Supplier: Commercially available.

Toxicity: Hypersensitivity reactions may occur; therefore, premedication with acetaminophen and diphenhydramine should be considered before each infusion. The most common toxicities are infusion related and may include chills, fever, headache, nausea, vomiting, angioedema (13%), hypotension (10%), bronchospasm (8%), and arrhythmias. Other possible adverse reactions include thrombocytopenia, myalgias, arthralgias, asthenia, and throat irritation.

Note: Intravenous; Do not administer as an intravenous push or bolus.

6.7 ATG (anti-thymocyte globin)

Source and Pharmacology: Immunosuppressant involved in the elimination of antigen-reactive T lymphocytes (killer cells) in peripheral blood or alteration in the function of T-lymphocytes, which are involved in humoral immunity and partly in cell-mediated immunity.

Formulation and Stability: For IV use only. Infuse over at least 4 hours through a 0.2 to 1 micron inline filter. Allow solution to reach room temperature prior to infusion. Infusion must be completed within 24 hours of preparation. May cause vein irritation (chemical phlebitis) if administered peripherally; high flow veins are preferred to reduce phlebitis (infuse into vascular shunt, arterial venous fistula, or high flow central vein).

Monitor closely throughout infusion for allergic reaction. Appropriate resuscitative equipment should be nearby during administration. May require premedication with an antipyretic, antihistamine, and/or corticosteroid to prevent reactions. Discontinue infusion for anaphylaxis or respiratory distress. Administer epinephrine, corticosteroids, antihistimines, and/or antipyretics as indicated to manage reactions.

Due to possible infusion-related reactions, it may be preferable to avoid initiation treatment late in the day or on weekends; consider withholding beta-blockers prior to administration to avoid suppressing compensatory responses to anaphylaxis.

Toxicity: Chills, headaches, dermatologic reaction, pruritus, skin rash, urticarial, leukopenia, thrombocytopenia, arthralgia, fever, bradycardia, cardiac disease/failure, chest pain, edema, hypertension, hypotension, myocarditis, phlebitis, thrombophlebitis, agitation, burning sensation, dizziness, encephalitis, generalized ache, lethargy, seizure, diaphoresis, night sweats, diarrhea, nausea, stomatitis, vomiting, proteinuria, lymphadenopathy, hepatosplenomegaly, hepatic function tests, anaphylaxis, serum sickness, viral infection, back pain, joint stiffness, myalgia, periorbital edema, renal function abnormality, dyspnea, pleural effusion, respiratory distress

6.8 FLUDARABINE (fludara)

Source and pharmacology: Inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase; also inhibits DNA primase and DNA ligase.
Formulation and Stability: Intravenous. The manufacturer recommends administering over ~30minutes. Continuous infusion and IV bolus over 15 minutes have been used for some off label protocols. Store intact vials under refrigeration or at room temperature, as specified according to each manufacturer's labeling. Reconstituted solution or vials of the solution for injection that have been punctured (in use) should be used within 8 hours.

Supplier: commercially available

Toxicity: Edema, fever, fatigue, pain, chills, rash, nausea/vomiting, anorexia, diarrhea, gastrointestinal bleeding, urinary tract infections, myelosuppression, anemia, neutropenia, thrombocytopenia, weakness, myalgia, paresthesia, visual disturbance, cough, pneumonia, dyspnea, upper respiratory infection, diaphoresis, angina, arrhythmia, cerebrovascular accident, heart failure, MI, supraclavicular tachycardia, deep vein thrombosis, phlebitis, aneurysm, transient ischemic attack, malaise, headache, sleep disorder, cerebellar syndrome, depression, mentation impairment, alopecia, pruritis, seborrhea, hyperglycemia, dehydration, stomatitis, esophagitis, constipation, mucositis, dysphagia, dysuria, hesitancy, hemorrhage, cholelithiasis, liver function abnormality, liver failure, osteoporosis, arthralgia, hearing loss, hematuria, renal failure, renal function test abnormality, proteinuria, pharyngitis, allergic pneumonitis, hemoptysis, sinusitis, bronchitis, epistaxis, hypoxia, anaphylaxis.

6.9 General description of the CliniMACS™System

This system is described in depth in the Investigators Brochure. Below is a brief description of the operation of the system:

The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting. The CliniMACS device is a powerful tool for the isolation of many cell types. Heterogeneous cell mixtures, e.g. apheresis products, can be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, such as CD34+ human hematopoietic cell selection, TCRαβ+ CD3+ T-cell depletion, or CD56+ NK cell enrichment. The cells to be isolated or depleted are specifically labeled with superparamagnetic particles by an antibody directed toward a cell surface antigen. After magnetic labeling, the cells are separated using a high-gradient magnetic separation column. The magnetically labeled cells are retained in the magnetic column while the unlabeled cells flow through. The retained cells are eluted by removing the magnetic field from the column, the washing the desired cells out and collecting them.

The superparamagnetic particles are small in size (about 50 nm in diameter) and are composed of iron oxide/hydroxide and dextran conjugated to monoclonal antibodies. These magnetic particles form a stable colloidal solution and do not precipitate or aggregate in magnetic fields. The CliniMACS device is comprised of a computer-controlled instrument incorporating a strong permanent magnet and a separation column with a ferromagnetic matrix to separate the cells labeled with the magnetic particles. The high-gradient system allows the application of strong magnetic forces and a rapid demagnetization. Small ferromagnetic structures, such as the column matrix, placed in a magnetic field, concentrate this homogenous field and thereby produce high magnetic gradients. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures enabling the retention of magnetically-labeled cells. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells. The CliniMACS device is comprised of a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix and a paramagnetic, cell specific, labeling reagent. The instrument will separate the cells in a fully automated process.

7.0 EVALUATION CRITERIA

7.1 The primary goal of this study is:

To determine the safety of αβ T-cell depleted haploidentical HSCT in research participants with severe SCD. To assess the safety of haploidentical hematopoietic stem cell transplantation (HSCT) for children and young adults with sickle cell disease (SCD) and stroke (persistent neurologic deficit lasting ≥24 hours and present on MRI) or recurrent ACS (≥2 in one year) or recurrent VOC requiring hospital admission ≥3 times in one year. The treatment plan will be considered safe if there is not excessive toxicity. Toxicity for this protocol is defined as failure to engraft/graft rejection, or grade III/IV aGvHD or death within 100 days post transplantation.

Our approach will also provide preliminary observational data on the efficacy of Hap-HSCT for children, adolescents and young adults with severe SCD. Efficacy is defined as

stable donor engraftment (>5% total nucleated cell DNA) and donor erythropoiesis that corrects the SCD hematologic phenotype (<50% HbS in the peripheral blood).

7.2 The secondary objectives are as follows:

7.2.1 To estimate 1-year overall and event-free survival after Hap-HSCT.

7.2.2 To observe the incidence and severity of acute and chronic GvHD after Hap-HSCT.

7.3 The exploratory objectives are as follows:

7.3.1 To observe the degree of hematopoietic chimerism among different cells subsets after haploidentical stem cell transplantation.

7.3.2 To obtain preliminary information on the degree of engraftment necessary to ameliorate the features of SCD and the proportion of participants requiring stem cell boosts.

7.3.3 To evaluate systemic end organ function and/or complications post Hap-HSCT in a longitudinal manner.

7.3.4 To monitor immune reconstitution after Hap-HSCT.

8.0 OFF-STUDY or OFF-THERPAY CRITERIA

8.1 The following criteria will be utilized to censor stem cell <u>transplant research participants</u>:

8.1.1 Withdrawal of research participation - research participants or guardians may withdraw their consent and/or permission to participate in this study at any time. Research participant and/or legal guardian who withdraw consent and/or permission after initiating conditioning will be counted as failures.

8.1.2 Failure to obtain $\geq$5 X 10^6 CD34+ cells/kg from two successive donors (after a maximum of attempted 2 cycles of G-CSF/apheresis per donor). Cells from only one donor will be infused into the research participant.

8.1.3 Documented graft rejection or graft failure after stem cell infusion (boost).

8.1.4 Research participant death prior to starting conditioning.

8.1.5 Research participant/legal guardian lost to follow-up.

8.1.6 Discretion of the Principal Investigator.

8.2 The following criteria will be used for stem cell donors to be exluded from ongoing medical care:

Stem Cell donors are consented as a standard of care. It is also standard of care for those patients to be followed by an apheresis physician and to have the opportunity to withdraw from care at any time.

8.2.1 Donor participant withdrawal. Donor participants may withdraw their consent to participate as medical stem cell donors at any time.

8.3.2 Donor participant lost to follow-up during their donation of stem cells.

8.3.3 Discretion of the Principal Investigator.

9.0 REPORTING CRITERIA

9.1 Adverse Events[1]

During the first year post initiation of primary stem cell transplant, the Principal Investigator and the clinical research staff assigned to the study will monitor for adverse events and score them according to the criteria specified by the National Cancer Institute's (NCI) Common Toxicity Criteria (CTC) Version 3.0.

For this Phase I study, NCI grade I–IV AEs will be collected for on-study research participants from the initiation of HU/azathioprine until the first 100 days post-transplant. After the day 100 time point, AE data collected will be limited to grade III and IV NCI AEs and only any clinically significant grade I and II AEs (significant as per the judgment of the PI) up to the one-year post-transplant time point.

Post-haploidentical transplant off-study research participants who are off-study due to graft failure/graft rejection after boosts or as per Principal Investigator's discretion will continue to be followed for safety data for 5 years post-initial protocol transplant infusion despite off-study status through the late effects data collection methodology detailed below.

Research participants who are enrolled but go off-study due to inadequate cell collection from 2 successive donors (i.e. research participant enrolled but goes off study and does not receive a transplant on this protocol) will be followed for 30 days post last HU/azathioprine dose only.

Post-one year late effects monitoring: After the first year post treatment, adverse event (AE) data collecting and reporting for on-study participants will be done concurrently with the research participant's annual follow-up visit in the transplant clinic. After conducting a full physical exam, the physician will complete a systems-based late events checklist noting any (significant) new or previously diagnosed medical conditions or

[1] See also Appendix III: Criteria for Adverse Event Evaluation and Reporting

diagnoses that were present during the time frame since the last annual evaluation. Any clinically significant adverse events noted in these annual (approximately annual) follow-up visits that in the opinion of the Principal Investigator warrant expeditious reporting will be reported to the IRB and FDA accordingly. All other late effects noted in this assessment will be summarized in the IRB and FDA annual review reports. Annual reporting of late effects is mandated until five years post-transplant.

Stem cell donor research participants will be followed for grade III and IV NCI adverse event from the time period of the initiation of mobilization treatment to 30 days after the last day of apheresis only. If a complete repeat pheresis procedure is required, the adverse event collection will restart with the time period of the initiation of the second mobilization treatment and will stop 30 days after the last day of the second pheresis procedure. Adverse event reporting for the donor research recipients will be conducted according to the same guidelines set forth for research participants in terms of reporting to FDA and IRB. Adverse event information for research participant donors will be included in the continuing review reports under a separate table.

9.2 Reporting Requirements to the IRB

In addition to the continuing review reports to the IRB, the Principal Investigator is responsible for reporting serious and unexpected adverse events that impact the safety of or risk to research participants. Non-serious adverse events and expected SAEs will be reported to the IRB in the continuing review reports. Serious, unexpected-fatal or serious, unexpected-life threatening events are to be reported to the IRB within 48 hours of notification of the event to the principal investigator or designees. All other unexpected (non-fatal or non-life threatening) SAEs will be reported within 10 working days of notification of the event. The term "life threatening" refers to an event in which the research participant was at risk for death at the time of the event, not an event that hypothetically might have caused death if it were more severe.

9.3 Reporting Requirements to the FDA

Any unexpected fatal or unexpected life-threatening event that occurs during the time period of the initiation of conditioning through the first year post-transplant that is judged by the principal investigator to possibly be due to the investigational treatment, will be reported to the FDA by telephone or fax as soon as possible but no later than 7 calendar days after notification of the event and followed by a complete a written safety report as possible within 8 additional calendar days (i.e. full report 15 calendar days total after notification of event).

Unexpected, non-life-threatening SAEs which occur from the time of the initiation of conditioning through the first year post transplant that are considered to be due to the investigational treatment will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs including expected death, unexpected SAEs even unexpected fatal SAEs considered by the PI to be not related to the study treatment and non-serious AEs will be reported to the FDA in the Annual Review Report.

All FDA correspondence and reporting will be conducted through the BSD Office of Clinical Research (BSD OCR). The Principal Investigator will submit a copy of the Adverse Event Report (AER) form that was submitted to the IRB to the BSD OCR.

9.4 Continuing Review Reports

Reports of progress and summaries of adverse events will be filed with the IRB and the Food and Drug Administration at least annually.

9.6 Data Submission to Miltenyi Biotec

Clinical and safety related data will be provided to Miltenyi Biotec, the manufacturer of the CliniMACS^{TM} system. Data will include but is not limited to variables such as the transplant research participant's age and diagnosis, the relationship of the donor to the transplant recipient (i.e. mother, father, sister, brother) and donor product(s) related information including the stem cell mobilization, processing and infusion procedure. Outcome data including lymphohematopoietic reconstitution, immunological response, disease response and transplant related complications will be shared with Miltenyi Biotec. Representatives from Miltenyi Biotec will be able to review the research participants (donor and transplant research recipient) laboratory and medical record for data verification purposes. Copies of all reports to the governing regulatory bodies will also be accessible to Miltenyi Biotec upon request. Information is submitted using a unique research participant number.

9.7 Reporting to Bone Marrow Transplant Registries

The Transplant Program reports transplant information to the Center for International Bone Marrow Transplant Registry/ Autologous Bone Marrow Transplant Registries (CIBMTR). The CIBMTR is a voluntary research organization of scientists and doctors who study various issues in transplantation. The CIBMTR collects information from more than 450 transplant centers from 48 countries. Information is submitted using a unique research participant number.

9.8 Study Stopping Rules

This study will suspend accrual if a stopping criteria is met as described in section 10.0 below. Should these events occur, this would prompt discussion amongst investigators. Safety analyses will include all subjects receiving any components of the investigational therapy.

10.0 STATISTICAL CONSIDERATIONS

For the primary analysis, we will test the null hypothesis that the GF rate is 35% against the alternative that it is 15%. We will employ a two-stage design to enable stopping for futility if the GF rate is high. [97] Eleven patients will be enrolled in the Stage I and if 4 or more have GF the trial will be terminated. (Accrual will be suspended for 100 days after the 11th patient has been enrolled to await his/her outcome if exactly 3 GFs have been observed up to that point in time.) If 3 or fewer patients have GF an additional 26 patients will be entered for a total of 37. Then if 10 or more GFs have been observed the treatment will be rejected, whereas if 9 or fewer have GF, i.e., $\leq$ 24%, the regimen will be considered sufficiently promising to warrant further trials. This design has an alpha level

(type I error rate) of 10% and 90% power if the true GF rate is 15%. The probability of terminating after the first 11 patients is 0.57 if the true GF rate is 35%.

Three additional stopping rules will be employed in the event of an unacceptable number of deaths, occurrences of aGvHD, or unexpected grade III or higher toxicities deemed related to the therapy. If, at any time, more than two deaths occur, we will suspend the trial and consider early termination pending review of the causes of death. We will also consider early termination if there is evidence that the rate of grade II or higher aGvHD exceeds 25% or the rate of grade III or higher aGvHD exceeds 10%. Specifically, if 5 of the first 11 patients or 13 of 37 develop grade II or higher aGvHD we will consider early termination of the trial. (In both instances, the lower, one-sided 90% confidence bound exceeds 25%.) Similarly, if 3 of the first 11 or 7 of 37 exhibit grade III or higher aGvHD, termination of the study will be considered (in both cases, the lower confidence bound exceeds 10%). Finally, we will consider early termination if there is evidence that the rate of grade III or higher treatment-related toxicities exceeds 25%. Thus if 5 of the first 11 or 13 of 37 patients experience irreversible grade III or higher treatment-related toxicity, the study will be suspended.

We will follow the above algorithm with three additional modifications:

i) As an additional safeguard, the first three patients in Stage I of the study will be enrolled at a rate of one (1) patient per month. Accrual will be suspended for 100 days after the 3rd patient has been enrolled to assess excessive acute and subacute toxicities from the treatment regimen. If excessive toxicity or a stopping rule, is not reached in that time frame, we will proceed to complete enrollment of the additional 8 research subjects to Stage I of the study.

ii) If the rate of aGvHD in the first 11 patients is too high (as defined), we will modify the treatment protocol to include immune suppression prophylaxis and begin anew, enrolling 11 patients in Stage I and up to 26 in the Stage II if no further stopping boundary is met.

iii) If the Stage I data does not meet criteria for stopping rules, we will proceed to Stage II. As Stage I of this study will indicate preliminary safety, we will extend the study to include patients from 2 years to 25 years. The patients who were not previously included (ages 2 to 9 years) will be analyzed both within the entire cohort and as a subset population. Should there be two or more patients age 2-9 who experience death, grade III/IV GvHD, grade III/IV treatment-related toxicity or graft failure, the study will be suspended and a meeting will be convened among the internal Safety Monitoring committee. In addition to stopping rules for this specific cohort, we will also apply a staggering of the enrollment within this subset of subjects. Similar to Stage I, we will stagger the enrollment of patients ages 2-9 for the first three patients in this age range. We will enroll patients at a rate of one (1) patient per month, and accrual will be suspended for 100 days after the 3rd patient has been enrolled to assess excessive acute and subacute toxicities from the treatment regimen.

Since we will employ stopping rules in the event of an unacceptable number of deaths, occurrences of aGvHD, or high-grade toxicity we will stagger enrollment so that, if a signal emerges, we will not have exposed an excessive number of patients. We anticipate that we will see the vast majority of aGvHD develop prior to 60 days post Hap-HSCT. [98-101]

We will continue enrollment at a rate not to exceed 2 patients per month. If we are approaching the boundary of any of the stopping rules (3 deaths; 5 of 11 in Stage I or 13 of 37 $\geq$ grade II aGvHD in Stage II; 3 of 11 in Stage I or 7 of 37 $\geq$ grade III aGvHD in Stage II; 5 of 11 in Stage I or 13 of 37 grade III or higher treatment-related toxicity in Stage II), we will convene a meeting of the principal investigator, co-investigator, and statistician to decide whether curtailment of enrollment or its rate is warranted. Please see **Section 2.5.6** and **Appendix 4** for additional information on the monitoring committee.

Cumulative incidence curves for time to GF will be calculated with treatment-related mortality as a competing risk. [102] Overall and event-free survival will be estimated using the Kaplan-Meier method. [103] Descriptive statistics (mean and standard deviation for continuous variables, frequency summaries for categorical data) will be generated for secondary endpoints (immune reconstitution, end organ function), together with 90% confidence intervals for parameters of interest. Mixed effects regression models will be fit to analyze changes in QOL measures over time. [104]

11.0 HUMAN SUBJECTS

Potential risks of this study include the following:

1. Toxicity from conditioning therapy, including mucosal damage (mucositis), intestinal damage (diarrhea) and liver damage (veno-occlusive disease): Based on the proposed conditioning regimen and preliminary experience using similar regimens, we anticipate a low (<10% incidence of NCI grade IV toxicity).
2. Risk of infection due to low blood cell counts and weakened immune system: We anticipate a fatal infection rate of <5%.
3. Risk of GvHD: Based on our preliminary experience using highly purified stem cell transplantation, we anticipate a risk of severe GvHD (overall grade III/IV acute GvHD or extensive chronic GvHD) of less than 10%.
4. Risk of graft failure, resulting in return of SCD: We anticipate a risk of graft rejection of up to 33%.
5. Death. We anticipate the mortality rate will be $\leq$10%.
6. Late effects such as impaired growth, fertility, or treatment-related malignancy. We anticipate that, due to the absence of irradiation, growth will not be significantly impaired. The risk of treatment-related malignancy with this therapy is likely less than 1% given the absence of cyclophosphamide and irradiation. Fertility is likely to be impaired given the presence of busulfan. Fertility will be serially assessed at the appropriate time. Participants will be educated on this risk prior to transplantation.

12.0 OBTAINING INFORMED CONSENT

Significant safeguards must be put in place prior to proceeding with HSCT in each patient. Education of the patient and their family will be mandatory, which will include the following:

Dr. Cunningham will conduct an initial presentation of the protocol. The transplant procedure as well as alternative forms of therapy will be presented as objectively as possible. The risks and hazards of the procedure are explained to the patient's responsible family members/legal guardians and to the patient. It will be pointed out that patients with sickle cell anemia have a variable course and that there is no clear agreement about the role or optimal time to perform alternate donor hematopoietic stem cell transplantation. There will be ample opportunity for questions related to the protocol, consents, or issues related to stem cell transplantation for sickle cell disease. Patients will also have the opportunity to meet with the social worker and the patient advocate before the signing portion of the informed consent process is initiated unless they decline.

Additional discussions and question and answer sessions between the PI and the family members/legal guardians will be done as needed. A final session, which will be of similar format, at which time the enrollment consent may be signed. Assent will be obtained in all children and adolescents $\geq$ 8 years of age. The social worker, the patient advocate or a designee (in the case where the social worker/patient advocate is not available) must be present as well as a member of the nursing staff. Any person(s) of the patient/parent's choosing will be encouraged to attend.

13.0 DATA ACQUISITION AND QUALITY ASSURANCE MONITORING

13.1 Enrollment on Study

Confirm subject eligibility as defined in Section 3.0 Patient Eligibility using the Case Report Forms (CRFs). Form will be signed by a PI. Complete and sign the 'Patient Eligibility Checklist' and "Donor Eligibility Checklist." Be sure that all required values and dates are filled in.

13.2 Data Submission

The study data manager will review Case Report Forms (CRF) for accuracy and completeness. Data collected using these forms will then be entered into a secure REDCap database accessible to the study investigators and personnel.

13.3 Quality Assurance Monitoring

Monitoring of timeliness of adverse and serious adverse event reporting will be done as events are reported. Monitoring of modified adverse event reporting, data required for primary objectives, and compliance with the conduct of the protocol will be conducted at 3-month intervals.

14.0 REFERENCES

1. Ingram, V., *Gene mutations in human haemoglobin: the chemical difference between normal and sickle cell haemoglobin.* Nature, 1957. **180**(4581): p. 326-8.
2. Platt, O.S., et al., *Mortality in sickle cell disease. Life expectancy and risk factors for early death.* N Engl J Med, 1994. **330**(23): p. 1639-44.
3. Mentzer, W.C., et al., *Availability of related donors for bone marrow transplantation in sickle cell anemia.* Am J Pediatr Hematol Oncol, 1994. **16**(1): p. 27-9.
4. Gragert, L., et al., *HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry.* N Engl J Med, 2014. **371**(4): p. 339-48.
5. Radhakrishnan, K., et al., *Busulfan, fludarabine, and alemtuzumab conditioning and unrelated cord blood transplantation in children with sickle cell disease.* Biol Blood Marrow Transplant, 2013. **19**(4): p. 676-7.
6. Kamani, N.R., et al., *Unrelated donor cord blood transplantation for children with severe sickle cell disease: results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).* Biol Blood Marrow Transplant, 2012. **18**(8): p. 1265-72.
7. Ruggeri, A., et al., *Umbilical cord blood transplantation for children with thalassemia and sickle cell disease.* Biol Blood Marrow Transplant, 2011. **17**(9): p. 1375-82.
8. Adamkiewicz, T.V., et al., *Unrelated cord blood transplantation in children with sickle cell disease: review of four-center experience.* Pediatr Transplant, 2007. **11**(6): p. 641-4.
9. Reisner Y, M.M., *Bone Marrow Transplantation Across HLA Barriers by increasing the number of transplanted cells.* Immunology Today, 1995. **15**(9): p. 437-440.
10. Sodani, P., et al., *T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients.* Pediatr Rep, 2011. **3 Suppl 2**(34-37): p. e13.
11. Powars, D.R., et al., *Outcome of sickle cell anemia: a 4-decade observational study of 1056 patients.* Medicine (Baltimore), 2005. **84**(6): p. 363-76.
12. Hulbert, M.L., et al., *Silent cerebral infarcts occur despite regular blood transfusion therapy after first strokes in children with sickle cell disease.* Blood, 2011. **117**(3): p. 772-9.
13. Strouse J, L.S., Beach M, Haywood C, Park H, Witkop C, Wilson R, Bass E Segal J, *Hydroxyurea for Sickle Cell Disease: A Systematic Review for Efficacy and Toxicity in Children.* Pediatrics, 2008. **112**(6): p. 1332-1342.
14. Candrilli SD, O.B.S., Ware RE, Nahata MC, Seiber EE, Balkrishnan R, *Hydroxyurea adherence and associated outcomes among Medicaid enrollees with sickle cell disease.* Am J Hematol, 2011. **86**(3): p. 273-7.
15. Seargent, G., *The natural history of sickle cell disease.* Cold Harb Perspect Med, 2013. **3**(10).
16. Schatz, J., et al., *Cognitive functioning in children with sickle cell disease: a meta-analysis.* J Pediatr Psychol, 2002. **27**(8): p. 739-48.
17. Walters, M.C., et al., *Bone marrow transplantation for sickle cell disease.* N Engl J Med, 1996. **335**(6): p. 369-76.
18. Brachet, C., et al., *Hydroxyurea treatment for sickle cell disease: impact on haematopoietic stem cell transplantation's outcome.* Bone Marrow Transplant, 2004. **33**(8): p. 799-803.
19. Panepinto, J.A., et al., *Matched-related donor transplantation for sickle cell disease: report from the Center for International Blood and Transplant Research.* Br J Haematol, 2007. **137**(5): p. 479-85.
20. Vichinsky, E., et al., *A randomised comparison of deferasirox versus deferoxamine for the treatment of transfusional iron overload in sickle cell disease.* Br J Haematol, 2007. **136**(3): p. 501-8.
21. Dedeken, L., et al., *Haematopoietic stem cell transplantation for severe sickle cell disease in childhood: a single centre experience of 50 patients.* Br J Haematol, 2014. **165**(3): p. 402-8.
22. Johnson, F.L., et al., *Bone-marrow transplantation in a patient with sickle-cell anemia.* N Engl J Med, 1984. **311**(12): p. 780-3.
23. HRSA. *US Transplant Data by Disease Report*. [cited 2017; Available from: https://bloodcell.transplant.hrsa.gov/RESEARCH/Transplant_Data/US_Tx_Data/Data_by_Disease/national.aspx.

24. Walters, M.C., et al., *Indications and Results of HLA-Identical Sibling Hematopoietic Cell Transplantation for Sickle Cell Disease.* Biol Blood Marrow Transplant, 2016. **22**(2): p. 207-211.
25. Steen RG, H.K., Horwitz EM, Benaim E, Thompson S, Bowman LC, Krance R, Wang WC, Cunningham JM, *Improved cerebrovascular patency following therapy in patients with sickle cell disease: initial results in 4 patients who received HLA-identical hematopoietic stem cell allografts.* ann neurol, 2001. **2**(222-229).
26. Woodward P, H.K., Khan R, Hale G, Phipps S, Wang W, Handgretinger R, Cunningham JM, *Brain parenchymal damage after haematopoietic stem cell transplantation for severe sickle cell disease.* British Journal of Haematology, 2005(129): p. 550-552.
27. Walters, M.C., et al., *Pulmonary, gonadal, and central nervous system status after bone marrow transplantation for sickle cell disease.* Biol Blood Marrow Transplant, 2010. **16**(2): p. 263-72.
28. Nickel, R.S., et al., *Improved Splenic Function After Hematopoietic Stem Cell Transplant for Sickle Cell Disease.* Pediatr Blood Cancer, 2016. **63**(5): p. 908-13.
29. Atsuta, Y., et al., *Late Mortality and Causes of Death among Long-Term Survivors after Allogeneic Stem Cell Transplantation.* Biol Blood Marrow Transplant, 2016. **22**(9): p. 1702-1709.
30. Krishnamurti, L., et al., *Stable long-term donor engraftment following reduced-intensity hematopoietic cell transplantation for sickle cell disease.* Biol Blood Marrow Transplant, 2008. **14**(11): p. 1270-8.
31. Hsieh, M.M., et al., *Allogeneic hematopoietic stem-cell transplantation for sickle cell disease.* N Engl J Med, 2009. **361**(24): p. 2309-17.
32. Bhatia, M., et al., *Reduced toxicity, myeloablative conditioning with BU, fludarabine, alemtuzumab and SCT from sibling donors in children with sickle cell disease.* Bone Marrow Transplant, 2014. **49**(7): p. 913-20.
33. Dew, A., et al., *Paucity of HLA-identical unrelated donors for African-Americans with hematologic malignancies: the need for new donor options.* Biol Blood Marrow Transplant, 2008. **14**(8): p. 938-41.
34. Shenoy, S., *Hematopoietic stem-cell transplantation for sickle cell disease: current evidence and opinions.* Therapeutic Advances in Hematology, 2013. **4**(5): p. 335-344.
35. Radhakrishnan K, B.M., Geyer M, del Toro G, Jin Z, Baker C, Harrison L, Morris E, Baxter-Lowe LA, Cairo M, *Busulfan, Fludarabine and alemtuzumab conditioning and unrelated cord blood transplantation in children with sickle cell disease.* Biol Blood Marrow Transplant, 2013(19): p. 676-677.
36. Oevermann, L. and R. Handgretinger, *New strategies for haploidentical transplantation.* Pediatr Res, 2012. **71**(4-2): p. 418-426.
37. Marks D, K.N., Cummins M, Goulden N, Green A, Harvey J, Hunt L, Keen L, Robinson S, Steward C, Cornish J, *Haploidentical stem cell transplantation for children with acute leukemia.* British Journal of Haematology, 2006. **134**(2): p. 196-201.
38. Reisner, Y., D. Hagin, and M.F. Martelli, *Haploidentical hematopoietic transplantation: current status and future perspectives.* Blood, 2011. **118**(23): p. 6006-17.
39. Bolanos-Meade, J., et al., *HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease.* Blood, 2012. **120**(22): p. 4285-91.
40. Dallas MH, T.B., Shook D, Hartford C, *Long Term Outcome and Evaluation of Organ function in pediatric patients undergoing haploidentical and matched related hematopoietic cell transplantationfor sickle cell disease.* Biol Blood Marrow Transplant, 2014. **19**: p. 820-830.
41. Reisner Y, G.H., Reich-Zeliger S, Martelli M, Bachar-Lustig E, *Tolerance Induction by Megadose CD34 Cells and Other Veto Cells.* Ann. N.Y. Acad. Sci., 2005. **1044**: p. 70-83.
42. Bittencourt, H., et al., *Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation.* Blood, 2002. **99**(8): p. 2726-2733.
43. Pérez-Simón, J.A., et al., *Impact of CD34+ cell dose on the outcome of patients undergoing reduced-intensity-conditioning allogeneic peripheral blood stem cell transplantation.* Blood, 2003. **102**(3): p. 1108-1113.
44. Chang, Y.J., X.Y. Zhao, and X.J. Huang, *Immune reconstitution after haploidentical hematopoietic stem cell transplantation.* Biol Blood Marrow Transplant, 2014. **20**(4): p. 440-9.

45. Blazar, B., et al., *Murine gamma/delta-expressing T cells affect alloengraftment via the recognition of nonclassical major histocompatibility complex class Ib antigens.* Blood, 1996. **87**(10): p. 4463-4472.
46. Kongtim, P., et al., *Haploidentical Hematopoietic Stem Cell Transplantation as a Platform for Post-Transplantation Cellular Therapy.* Biology of Blood and Marrow Transplantation, 2015. **21**(10): p. 1714-1720.
47. Drobyski, W.R. and D. Majewski, *Donor gamma delta T lymphocytes promote allogeneic engraftment across the major histocompatibility barrier in mice.* Blood, 1997. **89**(3): p. 1100-9.
48. Bertaina, A., et al., *HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders.* Blood, 2014. **124**(5): p. 822-6.
49. Diamond, D.J., et al., *Immunohistochemical analysis of T cell phenotypes in patients with graft-versus-host disease following allogeneic bone marrow transplantation.* Transplantation, 1995. **59**(10): p. 1436-44.
50. Norton, J., N. al-Saffar, and J.P. Sloane, *Immunohistological study of distribution of gamma/delta lymphocytes after allogeneic bone marrow transplantation.* J Clin Pathol, 1992. **45**(11): p. 1027-8.
51. Roy, J., J.L. Platt, and D.J. Weisdorf, *The immunopathology of upper gastrointestinal acute graft-versus-host disease. Lymphoid cells and endothelial adhesion molecules.* Transplantation, 1993. **55**(3): p. 572-8.
52. Crocchiolo, R., et al., *Infections after T-replete haploidentical transplantation and high-dose cyclophosphamide as graft-versus-host disease prophylaxis.* Transplant Infectious Disease, 2015. **17**(2): p. 242-249.
53. Blinder, M.A., et al., *Age-related treatment patterns in sickle cell disease patients and the associated sickle cell complications and healthcare costs.* Pediatr Blood Cancer, 2013. **60**(5): p. 828-35.
54. Chaturvedi, S. and M.R. DeBaun, *Evolution of sickle cell disease from a life-threatening disease of children to a chronic disease of adults: The last 40 years.* Am J Hematol, 2016. **91**(1): p. 5-14.
55. Slattery, J.T., et al., *Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics.* Bone Marrow Transplant, 1995. **16**(1): p. 31-42.
56. McPherson, M.E., et al., *Safety and efficacy of targeted busulfan therapy in children undergoing myeloablative matched sibling donor BMT for sickle cell disease.* Bone Marrow Transplant, 2011. **46**(1): p. 27-33.
57. Horan J, H.A., Lagerlof Diogaurdi, Brown C, Grizzle A, Shelman C, Kanter J, Hale G, Nieder M, Benton M, Kasow K, Abraham A, Chiang KY, *Using Fludarabine to Reduce Exposure to Alkylating Agents in Children with Sickle Cell Disease Receiving Busulfan, Cyclophosphamide, and Antithymocyte Globulin Transplant Conditioning: Results of a Dose De-Escalation Trial.* Biology of Blood and Marrow Transplantation, 2015. **21**(5).
58. Sodani, P., et al., *New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years.* Blood, 2004. **104**(4): p. 1201-3.
59. Kongtim P, C.K., Ciurea S, *Donor specific anti-HLA antiboday and risk of graft failure in haploidentical transplantation.* Advances in Hematology, 2016. **2016**: p. 1-10.
60. Andreani, M., et al., *Quantitatively different red cell/nucleated cell chimerism in patients with long-term, persistent hematopoietic mixed chimerism after bone marrow transplantation for thalassemia major or sickle cell disease.* Haematologica, 2011. **96**(1): p. 128-133.
61. Hsieh, M.M., C.J. Wu, and J.F. Tisdale, *In mixed hematopoietic chimerism, the donor red cells win.* Haematologica, 2011. **96**(1): p. 13-5.
62. Walters, M.C., et al., *Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia.* Biol Blood Marrow Transplant, 2001. **7**(12): p. 665-73.
63. Larocca, A., et al., *Boost of CD34+-selected peripheral blood cells without further conditioning in patients with poor graft function following allogeneic stem cell transplantation.* Haematologica, 2006. **91**(7): p. 935-940.
64. Walters, M., et al., *Neurologic complications after allogeneic marrow transplantation for sickle cell anemia [see comments].* Blood, 1995. **85**(4): p. 879-884.
65. Chang, Y.J., et al., *Immune reconstitution following unmanipulated HLA-mismatched/haploidentical transplantation compared with HLA-identical sibling transplantation.* J Clin Immunol, 2012. **32**(2): p. 268-80.

66. Savani, B.N., et al., *Absolute lymphocyte count on day 30 is a surrogate for robust hematopoietic recovery and strongly predicts outcome after T cell-depleted allogeneic stem cell transplantation.* Biology of Blood and Marrow Transplantation, 2007. **13**(10): p. 1216-1223.
67. Kim, H.T., et al., *Absolute lymphocyte count recovery after allogeneic hematopoietic stem cell transplantation predicts clinical outcome.* Biol Blood Marrow Transplant, 2015. **21**(5): p. 873-80.
68. Einsele, H., et al., *Lymphocytopenia as an Unfavorable Prognostic Factor in Patients with Cytomegalovirus-Infection after Bone-Marrow Transplantation.* Blood, 1993. **82**(5): p. 1672-1678.
69. Pical-Izard, C., et al., *Reconstitution of natural killer cells in HLA-matched HSCT after reduced-intensity conditioning: impact on clinical outcome.* Biol Blood Marrow Transplant, 2015. **21**(3): p. 429-39.
70. Dunbar, E.M., et al., *The relationship between circulating natural killer cells after reduced intensity conditioning hematopoietic stem cell transplantation and relapse-free survival and graft-versus-host disease.* Haematologica-the Hematology Journal, 2008. **93**(12): p. 1852-1858.
71. Chang, Y.J., et al., *Influence of lymphocyte recovery on outcome of haploidentical transplantation for hematologic malignancies.* Medicine (Baltimore), 2009. **88**(6): p. 322-30.
72. Eissens DN, S.N., Preijers FW, Dolstra H, van Cranenbroek B, Schattenberg AV, Joosten I, van der Meer A, *CD3+/CD19+-depleted grafts in HLA-matched allogeneic peripheral blood stem cell transplantation lead to early NK cell cytolytic responses and reduced inhibitory activity of NKG2A.* Leukemia, 2010. **24**(3): p. 583-91.
73. Chakrabarti, S., et al., *Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery.* Blood, 2002. **100**(5): p. 1619-27.
74. Lin, R. and Q. Liu, *Diagnosis and treatment of viral diseases in recipients of allogeneic hematopoietic stem cell transplantation.* Journal of Hematology & Oncology, 2013. **6**(1): p. 1-14.
75. Xuan, L., et al., *Effects of intensified conditioning on Epstein-Barr virus and cytomegalovirus infections in allogeneic hematopoietic stem cell transplantation for hematological malignancies.* J Hematol Oncol, 2012. **5**.
76. Goodrich, J.M., et al., *Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation.* N Engl J Med, 1991. **325**(23): p. 1601-7.
77. Green, M.L., et al., *Efficacy of a viral load-based, risk-adapted, preemptive treatment strategy for prevention of cytomegalovirus disease after hematopoietic cell transplantation.* Biol Blood Marrow Transplant, 2012. **18**.
78. Pegelow CP, R.G., Moser F, Wang W, Bellow J, Miller S, *Natural history of silent infarct in children with sickle cell anemia (HbSS) [Abstract].* Blood, 1999. **94**: p. 419A.
79. Armstrong, F.D., et al., *Cognitive Functioning and Brain Magnetic Resonance Imaging in Children With Sickle Cell Disease.* Pediatrics, 1996. **97**(6): p. 864-870.
80. Powars D, W.B., Imbus C, Pegalow C, Allen J. The natural history of stroke in sickle cell disease. , *The natural history of stroke in sickle cell disease. .* Am J Med, 1978. **65**: p. 461-471.
81. Balkaran, B., et al., *Stroke in a cohort of patients with homozygous sickle cell disease.* J Pediatr, 1992. **120**(3): p. 360-6.
82. Wang, W.C., et al., *High risk of recurrent stroke after discontinuance of five to twelve years of transfusion therapy in patients with sickle cell disease.* J Pediatr, 1991. **118**(3): p. 377-82.
83. Lee, M.T., et al., *Stroke Prevention Trial in Sickle Cell Anemia (STOP): extended follow-up and final results.* Blood, 2006. **108**(3): p. 847-852.
84. Russell, M.O., et al., *Effect of transfusion therapy on arteriographic abnormalities and on recurrence of stroke in sickle cell disease.* Blood, 1984. **63**(1): p. 162-9.
85. Wilimas, J., et al., *Efficacy of transfusion therapy for one to two years in patients with sickle cell disease and cerebrovascular accidents.* The Journal of Pediatrics, 1980. **96**(2): p. 205-208.
86. Woodard, P., et al., *Summary of symposium: the future of stem cell transplantation for sickle cell disease.* J Pediatr Hematol Oncol, 2002. **24**(7): p. 512-4.
87. NHLBI. *2016 Annual Sickle Cell Disease Clinical Research Meetings*. 2016; Available from: https://www.nhlbi.nih.gov/news/events/2016-annual-sickle-cell-disease-clinical-research-meetings/agenda.

88. Olsson, R., et al., *Graft failure in the modern era of allogeneic hematopoietic SCT.* Bone Marrow Transplant, 2013. **48**(4): p. 537-543.
89. Rondón, G., et al., *Long-Term Follow-Up of Patients Who Experienced Graft Failure Postallogeneic Progenitor Cell Transplantation. Results of a Single Institution Analysis.* Biology of Blood and Marrow Transplantation, 2008. **14**(8): p. 859-866.
90. Olsson, R., et al., *Graft failure in the modern era of allogeneic hematopoietic SCT.* Bone Marrow Transplant, 2013. **48**(4): p. 537-43.
91. Ozdemir, Z.N. and S. Civriz Bozdag, *Graft failure after allogeneic hematopoietic stem cell transplantation.* Transfus Apher Sci, 2018. **57**(2): p. 163-167.
92. Nollet, F., et al., *Standardisation of multiplex fluorescent short tandem repeat analysis for chimerism testing.* Bone Marrow Transplant, 2001. **28**(5): p. 511-8.
93. Kristt, D., et al., *Hematopoietic chimerism monitoring based on STRs: quantitative platform performance on sequential samples.* Journal of biomolecular techniques : JBT, 2005. **16**(4): p. 380-391.
94. Stern, M., et al., *Survival after T cell-depleted haploidentical stem cell transplantation is improved using the mother as donor.* Blood, 2008. **112**(7): p. 2990-5.
95. Tamaki, S., et al., *Superior survival of blood and marrow stem cell recipients given maternal grafts over recipients given paternal grafts.* Bone Marrow Transplant, 2001. **28**(4): p. 375-80.
96. Omar H, H.H., Gustafsson-Jernberg A, LeBlanc K, Mattsson J, Remberger M, Ringden O, Sparrelid E, Sundin M, Winiarski J, Yun Z, Ljungman P, *Targeted monitoring of patients at high risk of post-transplant lymphoproliferative disease by quantitative Epstein Barr virus polymerase chain reaction.* Transplat Infectious Disease, 2009(11): p. 393-399.
97. Simon, R., *Optimal two-stage designs for phase II clinical trials.* Control Clin Trials, 1989. **10**(1): p. 1-10.
98. Holtan, S.G., M. Pasquini, and D.J. Weisdorf, *Acute graft-versus-host disease: a bench-to-bedside update.* Blood, 2014. **124**(3): p. 363-73.
99. Moon, J.H., et al., *Early onset of acute GVHD indicates worse outcome in terms of severity of chronic GVHD compared with late onset.* Bone Marrow Transplant, 2010. **45**(10): p. 1540-5.
100. MacMillan, M.L., et al., *Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems.* Biol Blood Marrow Transplant, 2002. **8**(7): p. 387-94.
101. Lee, S.E., et al., *Risk and prognostic factors for acute GVHD based on NIH consensus criteria.* Bone Marrow Transplant, 2013. **48**(4): p. 587-92.
102. Gooley, T., Leisenring W, Cowley JC, Storer BD, *Estimation of failure probabilities in the presence of competing risks: new intreptation of old estimators.* . Statistics in Medicine, 1999. **18**: p. 695-706.
103. Kaplan, E.L. and P. Meier, *Nonparametric Estimation from Incomplete Observations.* Journal of the American Statistical Association, 1958. **53**(282): p. 457-481.
104. Gibbons, R.D. and D. Hedeker, *Applications of Mixed-Effects Models in Biostatistics.* Sankhya; The Indian Journal of Statistics, Series B (1960-2002), 2000. **62**(1): p. 70-103.

Appendix I: Schedule of Events: Clinical and Laboratory Evaluations

Required Evaluations of End Organ Function							
Observation	Prior to Transplantation	Day 28	Day 100	Day 180	Year 1	Year 2, 4	Year 3, 5
Echocardiogram	X				X	X	X
EKG	X				X		
PFTs	X			X	X	X	X
Chest XR	X	X	X		X		
Cr Clearance or GFR	X			X	X		
Urinalysis	X	X	X		X		
T2 Star MRI (liver & heart)	X				X		
Brain MRI/MRA	X			X	X	X	X⬩
Neurocognitive Testing⊙	X		X	X	X	X	X
Dental Evaluation	X				X		
Bone Density					X		
Endocrine Evaluation❖	X		X		X		
Lipid Panel	X						
Bone Marrow Evaluation	X	X	X		X		
Coagulation Testing (PTT/INR)	X		X				

⊙ See appendix II for specific neurocognitive and psychological testing to be performed at various time points

❖ Endocrine evaluation will include PTH, 25 hydroxy vitamin D level, IGFB3, IGF1 and if >8 years testosterone, estrogen, LH/FSH at initial evaluation. At day 100 post-transplant we will obtain TSH/T4, PTH and vitamin D.

⬩ MRI/MRA will be evaluated at year 3 and year 5 when deemed clinically appropriate.

All of the above evaluations are standard of care. They are not experimental or for research.

Required Infectious Disease Evaluation Prior to Transplantation
CT sinus
CT chest/abdomen/pelvis
Infectious Disease Markers per institutional standards
Panorex
Nasal Respiratory Viral Panel
Cultures (blood, urine, stool, nasal)

Additional studies or repeat studies will be performed during transplantation as deemed clinically appropriate by clinical transplant team. CMV, EBV, BK and adenoviral PCRs will be evaluated approximately weekly starting at day +15 through day 100, then monthly for one year.

All of the above evaluations are standard of care. The evaluations are not experimental or for research.

Appendix II: Neuropsychological Testing

As described previously, neurocognitive and psychological testing will occur prior to transplantation and post transplantation at 12 months and yearly through five years post transplantation. Baseline and one year post transplant evaluations will be performed that will include neurocognitive, mood/behavior and executive functioning assessments. At six and eighteen months post transplantation limited behavioral assessments will be performed. Testing that occurs yearly starting at two years post transplantation will be focused and address patient specific needs as determined by the Dr. Scott Hunter and his clinical team. Below are the evaluations that will be incorporated into these evaluations.

Neurocognitive Functioning
Wechsler Abbreviated Scale of Intelligence, Second Edition (WASI-2)
Wide Range Achievement Test, Fourth Edition (WRAT-4)
California Verbal Learning Test for Children (CVLT-C)
Rey Complex Figure Test (RCFT)
Beery-Buktenica Developmental Test of Visual-Motor Integration
Connors Continuous Performance Test
Delis-Kaplan Executive Function Test: Trail Making Tests, Sorting Test, Color-Word Identification Test

Assessment of Mood/Behavior and Executive Functioning
Behavioral Assessment System for Children, Second Edition (Parent, Teacher and Self-Report forms)
Behavior Rating Inventory of Executive Function (Parent, Teacher, and Self-Report forms)

All of the above evaluations are standard of care. The evaluations are not experimental or for research.

Appendix III:
Criteria for Adverse Event Evaluation and Reporting

Specialized criteria are required for the reporting of adverse events in transplant clinical trials as some medical events, even severe medical and adverse events/side effects are not only anticipated in these research participants but are intended (iatrogenic) events. For example, abnormal hematological values including (moderate to life threatening) levels of neutropenia, leukopenia, anemia and thrombocytopenia are deliberately induced by conditioning regimens in an effort to provide space in the bone marrow compartment for the donor stem cells.

In order to be able to provide the highest quality of accurate and pertinent protocol safety data it is necessary to clearly specify the criteria for capturing and reporting what constitutes an adverse event for this protocol. However, these guidelines are not considered all-inclusive and/or unconditional. Medical and scientific judgment should be exercised in deciding whether particular medical/adverse events may require reporting despite the criteria put forth in this document.

Section 1: General Definitions

Adverse Event (AE)– An adverse event is any unfavorable or unintended symptom, sign, or disease (including an abnormal laboratory finding), temporally associated with the use of a medical device, treatment or procedure that may or may not be considered related to the medical device, treatment or procedure.

Serious Adverse Event (SAE): An SAE is any untoward medical occurrence that:

- Results in death or
- Is life-threatening or
- Requires inpatient hospitalization of greater than 24 hours or causes a significant prolongation of existing hospitalization or
- Results in persistent or clinically significant disability/incapacity, second cancer or
- Is a congenital anomaly/birth defect (in an offspring)

The term "life threatening" refers to an event in which the research participant was at risk for death at the time of the event, not an event that hypothetically might have caused death if it were more severe.

Section 2: Guidelines for Transcription and Reporting of Adverse Events

The following guidelines pertain to adverse event reporting in this transplant protocol:

1. Time period for adverse event reporting will begin at the time of initiation of the conditioning regimen pre-transplantation. The timeframe for the completion of AE reporting and required NCI grades to be reported is specified in the reporting section of this protocol.

2. Information collected concerning AEs will include the name of the event; onset date/ resolution date (if ongoing at time off study, it will be noted only as "ongoing at time off

study" in a specific field in the database with no resolution date; NCI CTC Version 3.0 grade where applicable (example, GvHD will only be graded by Seattle Criteria); SAE determination; Expected/ unexpected; A relationship to research therapy.

3. Any medical diagnoses, diseases, signs and symptoms that are present prior to the initiation of conditioning should not be recorded as adverse events unless they worsen in severity or frequency with causality appropriately identified.

4. AEs will be reported as diseases or syndromes whenever possible (as long as clearly related) instead of reporting as individual component symptoms, signs or laboratory abnormalities. For example in the case of medically diagnosed veno-occlusive disease, the sub-components of this disease such as weight gain, increased bilirubin, abdominal distension, abdominal cramping/discomfort/pain requiring medication, and/or ascites would *not* be reported individually as AEs as they are known symptoms and manifestations of the primary disease.

5. Disease progression and/or disease relapse will not be considered an adverse event, as this is part of the potential natural course of the underlying disease. Any adverse event that is determined to be clearly related to the research recipients underlying disease/disease progression will not be reported as an adverse event. Please note that disease progression and/or disease relapse are reported in other contexts within transplants studies.

6. The following are considered provisions for best clinical management for this patient population and will not be documented and reported as adverse events as specified:

- All blood product infusions including exchange transfusions.

- Loss of appetite requiring initiation of total parenteral nutrition (TPN) or initiation of TPN for nutritional prophylaxis pre-transplant and up to day +100 post-transplant. TPN administration is a standard therapy administered to the majority of transplant recipients regardless of the degree of loss of appetite. If TPN is required to be initiated after day +100 post-transplant (i.e., the AE or causative event leading to initiation of TPN occurs after day 100), then the etiology for the initiation of the TPN (e.g. anorexia/loss of appetite) may be deemed an AE as determined by the PI.

- If the research participant requires an additional infusion of donor stem cell (boost), the guideline for AE reporting of initiation of TPN will resume (restart) from date of boost to day 100 post boost.

7. Due to the deliberate myeloablation/myelosupression required for stem cell transplantation the following criteria will be applicable to abnormal hematological laboratory values in relation to adverse event reporting in this stem cell transplant patient population:

Platelets/Thrombocytopenia: Thrombocytopenia will be reported as an adverse event under the following conditions regardless of study phase/grade of AEs noted to be collected in Reporting section:

If the platelet counts drop to $< 20{,}000/mm^3$ requiring platelet transfusions after the following criteria have been met:

1. If the patient has exhibited post-transplant platelet engraftment as defined as a platelet count of $\geq 20{,}000/mm^3$ with no platelet transfusions within 7 days

AND (both criteria must be met)

2. The research recipient continues to maintain a platelet transfusion independent state* for a minimum of 1 month after this initial (above specified) engraftment time period.

(*Exceptions – prophylaxis platelet transfusions for surgical procedures, BMA, BX, LP, endoscopy, BAL, etc, hemorrhage will not be factored into this time period)

If thrombocytopenia occurs after this time point, an adverse event will be captured for this disorder.

Hemoglobin/Anemia will be transcribed and reported as an adverse event if the patient has exhibited RBC engraftment (Hgb ≥8 without transfusion for at least 30 days) and then would require red blood cell infusion for anemia of ≤8.0 (Exceptions – prophylaxis RBC infusions for surgical procedures, Bone marrow aspirates/biopsies, lumbar punctures, etc. would not be factored into this criteria)

Neutrophils/Neutropenia – neutropenia will be reported as an AE if the patient has exhibited by an absolute neutrophil count (ANC) of > 1,000/mm^3 without granulocyte colony stimulating factors (G-CSF) for at least 30 days and would require further G-CSF treatment for an ANC of ≤ 500/mm^3

Leukocytes/Leukopenia: Leukocyte values will not be evaluated and graded as adverse events in the context of this study as engraftment/related events are based on the corresponding neutrophil count and treat according to ANC (see neutrophil section).

8. Metabolic laboratory and vital sign abnormalities should not be reported as adverse events unless criterion for a SAE is fulfilled or the investigator feels the abnormality should be reported as an AE.

9. Reporting of SAE: Any hospitalizations regardless of duration which meet the following criteria will not be reported as SAEs as they are part of the protocol therapy and/or conditions of best clinical management for this patient population:

- Apheresis, line placement, conditioning /stem cell infusions/boosts/DLI administration
- Disease evaluation procedures such as bone marrow aspirates/ biopsies, liver biopsies, staging/re-staging procedures, or scans.
- Planned hospitalization for administration of medication/ chemotherapy and related disease or protocol required treatment.
- Planned /scheduled treatment (including surgical intervention) for underlying primary disease related interventions.
- Concomitant radiotherapy (if applicable)
- Transfusion support, exchange transfusions, chelation therapy, etc.

10. Every effort will be made to obtain records as soon as possible from outside facilities in the case where a patient may require planned (if applicable) or unplanned medical or surgical intervention at an outside institution. These documents will be reviewed upon receipt to UCM for adverse events and will be reported according to the reporting guidelines specified in this document.

11. Only the primary causative diagnoses/clinical indication(s) for a research participant being admitted to the hospital for > 24 hours will be identified as the SAE in the database and reported to the IRB in the SAE continuing review reports. Other concurrent diagnoses occurring at the time of this hospitalization that - in and of themselves - would not have resulted in hospitalization and therefore were not the reason for the hospitalization (do not meet criteria of SAE), will not be reported as SAEs but will be reported accordingly as adverse events. Although these concurrent events *may* be reported within the context of the describing the research participant's overall clinical situation in an SAE report long form to the IRB/FDA, they will not be reported separately as SAE since they do not meet the criteria of SAEs.

For example, if a research participant is evaluated in the clinic and is noted to have an NCI CTC grade III headache, grade I left foot pain and grade II fever with neutropenia. The Principal Investigator feels the headache and foot pain, although significant, do not warrant hospitalization. However, the Principal Investigator deems that the fever does require hospitalization of greater than 24 hours. In this case, the SAE is the fever with neutropenia. The headache and foot pain would be noted where appropriate as AEs rather than SAEs.