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PILOT STUDY: CLOSELY MATCHED UNRELATED DONOR PERIPHERAL BLOOD STEM CELL TRANSPLANTATION WITH TCRαβ+ T CELL AND B CELL DEPLETION FOR PATIENTS WITH SICKLE CELL DISEASE AND THALASSEMIA MAJOR

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LIST OF ABBREVIATONS

AA	Aplastic anemia
ABW	Adjusted body weight
ANC	Absolute neutrophil count
ATG	Anti-thymocyte globulin
BM	Bone marrow
BMT	Bone marrow transplant
BTM	Beta thalassemia major
CGTL	Cell and Gene Therapy Laboratory
CHOP	Children's Hospital of Philadelphia
CMV	Cytomegalovirus
DMSO	Dimethyl sulfoxide
EBV	Epstein-Barr virus
EFS	Event free survival
GVHD	Graft vs host disease
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSA	Human serum albumin
HSC	Hematopoietic stem cells
HSCT	Hematopoietic stem cell transplant
HSV	Herpes Simplex virus
IBW	Ideal body weight
LDH	Lactate dehydrogenase
MMUD	Mismatched unrelated donor
MRD	Matched related donor
MUD	Matched unrelated donor
NK	Natural Killer
OS	Overall survival
PCR	Polymerase chain reaction
PNH	Paroxysmal nocturnal hemoglobinuria
PSC	Peripheral blood stem cells
PSCT	Peripheral stem cell transplantation
RIC	Reduced intensity conditioning
SAE	Severe adverse event
SCD	Sickle Cell Disease

SMC	Safety monitoring committee
SOP	Standards of Practice
TCR	T cell Receptor
TRM	Transplant related mortality
URD	Unrelated donor
VOD	Veno-occlusive disease
VZV	Varicella Zoster virus

ABSTRACT

While human leukocyte antigen (HLA) matched related donor stem cell transplantation (MRD-SCT) is a well-established curative therapy for patients with sickle cell disease (SCD) and beta thalassemia major (BTM), most patients unfortunately lack an available matched related donor (MRD). For these patients, unmanipulated bone marrow transplantation (BMT) using closely HLA-matched unrelated donors (URD) has to date been complicated by very high rates of graft versus host disease (GVHD), whereas haploidentical related donor transplantation or unrelated cord blood transplantation for SCD and BTM have been associated with high rates of graft failure. We have previously found that partial T cell depletion of unrelated donor peripheral stem cell (PSC) grafts in patients with malignancies and other hematologic diseases is effective in preventing GVHD, while preserving high rates of durable engraftment and excellent immune reconstitution after transplantation. The purpose of this study is to investigate this partial T cell depletion approach in a prospective clinical trial specifically for patients with SCD and BTM.

1.0. OBJECTIVES AND HYPOTHESES

Overview: This is a single arm pilot study of peripheral stem cell transplantation (PSCT) with *ex* vivo TCRαβ$^+$ T cell and CD19$^+$ B cell depletion of URD grafts using the CliniMACS device in patients with SCD and BTM. Apart from CliniMACS-based cell processing, PSCT will be performed according to current standards of care in the CHOP Cell Therapy and Transplant Section, including the use of a standard chemotherapy conditioning regimen and standard follow-up laboratory assessments.

Accrual: 20 patients
Study duration: patients will be evaluated for 2 years post-PSCT.

Primary Hypothesis: Unrelated donor peripheral stem cell transplantation (URD-PSCT) with *ex vivo* TCRαβ+ T cell/CD19$^+$ B cell depletion of the donor graft will result in lower rates of GVHD compared to historical T replete URD-BMT data, and lower rates of immunologic graft rejection compared to previous studies of haploidentical BMT and unrelated cord blood transplantation for SCD and BTM.

Primary Objectives

- Evaluate efficiency and durability of engraftment in patients with BTM and SCD undergoing URD-PSCT with TCRαβ$^+$ T cell depletion including: frequency of primary non-engraftment (primary graft failure); kinetics of engraftment (time to ANC >500/μL, platelets >50 x 10^3/μL); and frequency of secondary graft failure
- Determine incidence and extent of acute and chronic GVHD, including: incidence of grade II-IV acute GVHD, incidence of severe grade III-IV acute GVHD, and incidence of chronic extensive GVHD

Secondary Objectives

- Evaluate frequency and kinetics of mixed donor hematopoietic/immune chimerism
- Assess kinetics and extent of immune reconstitution
- Assess incidence of viral reactivation requiring therapy and symptomatic viral infections, including CMV, adenovirus, and EBV
- Assess survival outcomes, including 100-day treatment-related mortality (TRM), one-year event-free survival (EFS), and one-year overall survival (OS)
- Assess annual PRBC transfusion volume in 2 years post-engraftment compared to 2 years prior to URD-PSCT.
- For patients with SCD, assess annual rate of vaso-occlusive events (Pain, acute chest, splenic sequestration, priapism) in 2 years post-engraftment compared to 2 years prior to URD-PSCT

2.0. BACKGROUND AND RATIONALE

Sickle Cell Disease (SCD) is a severe multi-organ system disease that impacts over 100,000 Americans, with a global incidence of 300,000 infants born with SCD per year.[1] The etiologic driver of SCD is a hemoglobin variant (HbS) inherited from at least one parent and caused by a point mutation encoding a single amino acid change (glutamic acid to valine) at position 6 in the beta globin chain. Severe phenotypic SCD occurs when an individual inherits HbS from both parents (SCD-SS) or inherits HbS from one parent combined with inheritance of either a loss of expression beta globin variant (Beta Thalassemia variant) or another function-altering variant such as hemoglobin C (HbC). In deoxygenated blood, HbS can polymerize causing red blood cell (RBC) malformation into rigid "sickle" cells. Through alterations of blood flow and upregulation of adhesion factors, these RBC block small blood vessels (vaso-occlusion) and induce cascades of endothelial damage and inflammation that produce severe end organ damage. Patients with severe SCD are at risk for numerous complications and end-organ damage including: cerebrovasculopathy manifested as silent infarctions, hemorrhagic or ischemic strokes, and neovascularization (Moya-Moya); recurrent acute chest syndrome which can result in chronic restrictive lung disease; cardiovascular complications including diastolic dysfunction and pulmonary hypertension; renal disease including papillary necrosis, proteinuria, and hematuria; gall bladder and hepatic disease; priapism; and finally musculoskeletal complications including avascular necrosis, osteomyelitis, and recurrent vaso-occlusive pain episodes. Despite great improvements over the last few decades in supportive care for children with SCD, disease complications continue to cause severe morbidity and early mortality, with median life expectancy still limited to between 40 and 50 years in recent studies[2]. Health care utilization in SCD results in staggering health care costs; consequently high income status is one of the few factors found to prolong life-expectancy[3,4]. Even in surviving patients, disease complications leading to chronic functional impairment negatively impact quality of life, educational achievement and employment. Disease modifying therapeutics including hydroxyurea and several recently developed agents have improved complication rates in SCD, but are not curative and have significant side effects[5,6]. Chronic red blood cell transfusion regimens may also prevent progression of SCD complications, but are associated with high rates of RBC allo-immunization and transfusional iron overload[7].

Beta Thalassemia Major (BTM) is a distinct disorder of hemoglobin caused by homozygous or compound heterozygous point mutations or deletions in the beta globin gene leading to decreased beta globin expression[8]. Imbalance of globin chain production leads to ineffective erythropoiesis, which drives altered iron metabolism and hematopoietic expansion. A BTM phenotype is defined as patients who require chronic RBC transfusions to prevent life-threatening anemia and/or consequences of extramedullary hematopoiesis. Beta globin gene alleles associated with no globin expression are termed Beta0, and patients with two Beta0 alleles invariably require chronic RBC transfusions. A larger subset of patients possess one Beta0 allele and either an allele that leads to decreased but present beta globin expression (Beta+), or the hemoglobin E allele (p.E26K) that causes decreased globin expression through abnormal splicing. Patients with Beta0/Beta+ or E/Beta0 genotypes have widely variable clinical courses, and only those with chronic RBC dependence are classified as having BTM. Traditional therapy for BTM has relied on chronic RBC transfusions and management of severe iron overload with chelation therapy[9], interventions associated with substantial lifelong health care costs. World-wide, over 50,000 infants are born with BTM each year, and while incidence of Thalassemia in the United States is not well defined, the number of patients is growing, particularly in states with increasing numbers of foreign-born residents[8]. New drug therapies to limit transfusion burden are being developed, but these are not curative and long-term side effects remain to be determined[10].

Curative Cell Therapy for SCD and BTM: Historical Perspective and Current Concepts

After over three decades of experience, matched related donor bone marrow transplantation (MRD-BMT) is now considered a standard of care and cost-effective therapy for patients with severe manifestations of SCD and BTM who are fortunate to have a full (10/10) human leukocyte antigen (HLA) matched sibling[3,11]. In SCD, early studies of myeloablative conditioning regimens consisting of busulfan and cyclophosphamide for patients receiving either bone marrow (BM) or cord blood (CB) grafts from HLA identical siblings resulted in excellent outcomes with rates of event-free survival (EFS) ranging from 82-91%, overall survival (OS) rates of 94-96%, rates of graft rejection and transplant related mortality less than 10% and rates of Grade II-IV acute GVHD and chronic GVHD ranging from ~10-20%.[12-16] Subsequent studies of reduced intensity (RI) regimens, geared toward minimizing toxicity in organs already damaged by sickle cell disease, have including combinations such as low dose total body irradiation (LDTBI) and alemutuzumab[17,18], or alternatively alemtuzumab combined with fludarabine and melphalan[19]. The LDTBI-based regimen is associated with overall survival approaching 100%; however rates of graft failure due to immunologic rejection are high and consequently many patients treated with this approach require prolonged immune suppression. The melphalan-based regimen, which is the regimen on which a current clinical trial at CHOP for MRD-BMT in SCD is based (CHP894, IRB# 08-005659) is associated with excellent EFS (91%) and OS (93%), with a graft failure rate of less than 2%, but challenges of this regimen include high rates of CMV reactivation and disease, along with high rates of severe GVHD (39%) in patients age 14 or older[19].

In BTM, early studies of MRD-BMT using busulfan and cyclophosphamide regimens demonstrated low rates of GVHD and excellent event-free and overall survival rates of 88-94% and 91-94%, respectively, in patients deemed low risk by the initial Pesaro Criteria (regular chelation and absence of hepatomegaly and portal fibrosis) or by the updated Modified Pesaro Criteria (age <7 years, absence of hepatomegaly) at the time of transplant.[20,21] For patients deemed high risk by these criteria, survival rates are historically as low as 60%. Subsequent modifications to this protocol for high risk patients included pre-transplant RBC hypertransfusion to a goal of > 14 g/dL, addition of pre-transplant immune suppression including hydroxuyrea and azathioprine for ~30 days pre-transplant, and addition of fludarabine; combined, these interventions have improved event-free survival to 85% and reduced immunologic graft rejection the less than 10%[22]. RI regimens based on alemtuzumab, fludarabine and melphalan conditioning have also been utilized successfully in MRD-BMT for BTM[19], though our experience in one prior patient suggests that graft failure with autologous reconstitution may be more likely with BTM due to enhanced hematopoietic drive, a phenomenon that can be mitigated by addition of pre-transplant hydroxyurea and addition of thiotepa to the graft, which is the basis of the current CHOP trial (CHP894, IRB# 08-005659) for patients undergoing MRD-BMT for BTM.

While MRD-BMT is highly successful for SCD and BTM, as few as 18% of patients with SCD have an available MRD[23]. The likelihood of identifying a fully HLA matched (8/8 or 10/10) adult unrelated donor in national and international donor registries depends on ethnicity and is unfortunately only 15-40% for patient groups in which SCD and BTM are most common[24]. Allowance of a single HLA antigen mismatch (7/8 or 9/10) greatly expands the percentage of SCD and BTM patients who have available donor options to over 70%[24], but use of such mismatched unrelated donors (MMUD) has historically been limited in HSCT for non-malignant hematologic diseases due to high rates of GVHD.

For patients with SCD, T cell replete matched unrelated donor (MUD-BMT) studies have been plagued by high rates of GVHD. In the largest published series of 30 patients with SCD undergoing MUD-BMT with alemtuzumab, fludarabine, and melphalan conditioning[25], 2-year EFS

and OS were 69% and 79%, with 28% and 38% incidences of Grade II-IV acute GVHD and chronic extensive GVHD, respectively, leading to 7 GVHD-related deaths. Despite this high amount of GVHD, the immunologic graft rejection rate was also still significant at 10%. Similar findings were seen in a recently reported 5 patient MUD-BMT cohort of the "STRIDE" pilot study using a different conditioning regimen, in which there was 1 death from GVHD and another patient who exhibited graft failure due to immunologic rejection[26]. Studies of T replete MUD-BMT in BTM using various regimens are associated with EFS, OS, and immunologic graft rejection rates similar to those seen with SCD, with slightly lower (though still significant) rates of severe GVHD[27,28]. Studies using unrelated matched and mismatched cord blood as a donor source for HSCT in SCD and BTM have led to poor outcomes primarily because of graft failure rates ranging from 20-50%, but also because of significant rates of GVHD.[27,29-31] Most studies to date of haploidentical related-donor transplantation in SCD have similarly led to very poor disease free survival (~50% or less) due again to high graft failure rates of up to 40%[32,33], though addition of thiotepa to these RI conditioning regimens in haploidentical SCD transplants may improve this graft failure rate[34]. Haploidentical related donor HSCT in BTM has led to similar results[35]. In sum, better strategies are needed to improve disease-free survival after alternative donor allogeneic HSCT for SCD and BTM.

Gene therapy using lentiviral transduction of autologous hematopoietic stem cells (HSC) is now being explored in clinical trials for SCD and BTM[36-38]. To date, results have been encouraging for patients with BTM who have non-β_0/β_0 genotypes, with the majority of these patients achieving transfusion independence, though most patients continue to have moderate anemia with ongoing risk of ineffective erythropoiesis. Results in patients with BTM caused by β_0/β_0 mutations and in patients with SCD have been more mixed, though recent improvements in autologous stem cell collection[39,40] and transduction processes hold promise to increase cure rates using these approaches. However, these therapies to date are currently only available in clinical trials, and thus access to these approaches for patients who need curative therapy now is limited to the few trial slots available. Furthermore, long-term data regarding malignancy risk and durability of vector-derived beta globin production using these strategies is not yet available.

CHOP Experience of Allogeneic HSCT in Patients with SCD and BTM

Since 2008, 34 patients have undergone a first allogeneic HSCT at CHOP for the purpose of curing SCD and BTM, 24 receiving MRD-BMT and 10 receiving either MUD or MMUD HSCT. Of the patients receiving MRD-BMT at present EFS and OS are 79% and 92%, respectively. There have been 2 deaths in the MRD-BMT cohort, one from chronic extensive GVHD and the other from CMV pneumonitis. 2 additional patients exhibited graft failure with autologous reconstitution. One of these patients underwent successful 2nd transplant at CHOP. Another patient required a 2nd transplant/stem cell boost due to persistent thrombocytopenia despite 100% donor chimerism, and is now stably engrafted without GVHD. No patient with BTM has developed GVHD after MRD-BMT at CHOP. GVHD after MRD-BMT for SCD has been seen mainly in patients over age 14 at the time of transplant, with Grade II-IV acute and/or chronic extensive GVHD occurring in 5/8 (62.5%) patients over age 14 years versus 1/10 (10%) in patients 14 years or younger.

Six patients have received T cell replete matched or mismatched URD BMT at CHOP as an attempt to cure SCD. Deaths occurred in 2 of 6 patients. One patient who received an 8/10 donor BMT due to severe progressive cerebrovasculopathy died of severe GVHD, graft dysfunction and infection. The other death occurred in a patient who initially experienced graft rejection with autologous reconstitution after a T cell replete RI transplant, and then died of infection during subsequent HSCT attempts. Of the 4 surviving patients, there was one additional immunologic graft rejection of a 10/10 donor graft, salvaged by subsequent mismatched related donor HSCT.

The remaining 3 patients stably engrafted and remain alive, but all developed both acute and chronic GVHD requiring extended courses of immune suppression.

Four patients with BTM have undergone URD HSCT at CHOP. The first 2 patients received MUD-BMT with RI conditioning: one patient remains stably engrafted with no GVHD, while the other patient exhibited graft failure with autologous reconstitution. Subsequently 2 patients with BTM whose best available donors were 9/10 matches underwent peripheral stem cell transplantation with *Ex vivo* partial $CD3^+$ T cell depletion using the Miltenyi CliniMACs device (as detailed in the next section) per an Expanded Access study at CHOP (IRB #13-010286). The conditioning regimen used for both patients is identical to that described in this protocol (section 4.1.3.) and is derived from a regimen we have utilized successfully for patients with other non-malignant RBC disorders[41], combined with a hydroxyurea prophase used successfully by other groups in transplants for SCD and BTM[22,31]. In addition, this partial T cell depletion strategy enables the use of peripheral stem cells as a donor source, which provide a 2 to 3-fold higher stem cell dose than traditional BM grafts, with the consequent advantage of potentially lowering rates of graft failure. Both patients with BTM exhibited rapid and durable engraftment of their MMUD graft. At last follow-up, both were alive, free of transfusions, and without GVHD. The remarkably positive outcome in these two patients has provided the impetus for conducting this prospective trial of partial T cell depletion in patients with SCD and BTM.

Institutional Experience with Ex Vivo T cell Depletion Strategies in SCT

T cell depletion of unrelated and partially matched related donor (pMRD) stem cell products has been performed at CHOP for nearly 30 years, primarily for patients with hematologic malignancies who have lacked matched related donors[42-46]. The technology used for TCD has changed through the years, as has the stem cell source.

Year	**Stem cell source**	**Technique**	**Extent of T cell depletion (approx)**
1991-2001	Bone marrow	T10B9 Ab + complement	1.7 log
2000-2009	Bone marrow	OKT3 + complement	1.7 log
2000-2009	Mobilized peripheral blood stem cells	$CD34^+$ selection	4.6 log
2005-	Mobilized peripheral blood stem cells	$CD3^+$ depletion +/- $CD19^+$	3-4 log
2014-	Mobilized peripheral blood stem cells	TCR$\alpha\beta^+$ depletion +/- $CD19^+$	4-5 log TCRab depletion

Early T cell Depletion Studies

Ex-vivo partial T depletion of donor grafts first began at CHOP using complement mediated lysis with monoclonal antibody T10B9 obtained through the Medical College of Wisconsin. T10B9 resulted in selective depletion of TCRαβ T cells with sparing of TCR γδ expressing cells. T cell depletion with T10B9 reduced T cells by approximately 1.7 log, which reduced the risk of severe

GVHD, and allowed for engraftment in 95% of patients. However, the continued use of T10B9 and the subsequently utilized antibody OKT3 were limited by availability and the need to concurrently use biological reagents such as rabbit complement. The advent of magnetic bead-based cell sorting devices such as the currently used CliniMACS device, enabled the replacement of these early antibody-based approaches. Our initial studies first utilized a positive selection of CD34+ stem cells to deplete T cells and other immune cells, an approach still utilized by many centers today[43].

CliniMACS Device: Studies with CD3+ Depletion

Subsequently, we transitioned our approach to performing direct CD3+ T cell depletion, as cells that may be important in maintaining a graft vs. leukemia effect and in facilitating durable engraftment, such as NK cells, CD34 negative stem cells, and other elements, are not eliminated with this negative selection method. Depletion of CD3+ cells by the CliniMACS system results in approximately a 3.5 log T cell depletion (range 2.8-4.1) and 74% $CD34^+$ and NK cell recovery[47-49]. To provide opportunity for a residual graft versus leukemia effect and to help prevent immunologic rejection, our prior studies were designed so that patients were given an addback of 1-5 x 10^5/kg CD3+ cells at the time of infusion, unless the donor was haploidentical. CD19 (B cell) depletion, to prevent donor derived Epstein-Barr virus, was added once the appropriate reagents became available, because of the high risk of Epstein Barr Virus (EBV) induced post-transplant lymphoproliferative disorder (EBV-PTLD) associated with methods that remove T cells from donor grafts, but leave behind B cells. This simultaneous removal of donor B cells from T cell depleted products greatly reduces the risk of severe EBV reactivation and EBV-PTLD during the period before T cell immunity recovers[50]. In our hands, the Miltenyi CD19 system results in a 2.98 ±0.4 log reduction in B cells. With this reduction, our group has had several patients develop EBV reactivation, manifested as viremia seen on serum PCR testing, but no patients have developed lymphoproliferative disorder.

While our early *Ex-vivo* T cell depletion studies were performed with bone marrow grafts, technologic advances particularly in stem cell mobilization, led to the use of peripheral blood stem cells (PBSC) as a donor source since the advent of our CliniMACS-based studies. The use of PBSC's has the advantage of providing a 2 to 3 times larger weight-based $CD34^+$ stem cell dose than do BM collections. Previous studies in patients receiving partially matched related donor transplants have shown that such large stem cell doses of up to 20 x 10^6/kg $CD34^+$ facilitate engraftment[51].

From 2005 to 2015, the CHOP CTTS combined with the Medical College of Wisconsin treated 84 pediatric patients with hematologic malignancies with alternative donor PSCT with $CD3^+$/$CD19^+$ cell depletion and partial T cell add back to a target T cell dose of 1-5 x 10^5 CD3+ cells/kg[52]. In this cohort, primary graft failure was a rare event, occurring in only 2.4% of patients, while leukemia relapse occurred in 21% of patients. Three-year overall survival was 61.8%. Day 100 transplant related mortality (TRM) rates were low (7%), though overall TRM was significant (20%), in large part due to GVHD, with rates of Grade II-IV acute, Grades III-IV acute, and extensive chronic GVHD being 39%, 22%, and 12% respectively. Viremia with EBV, CMV, adenovirus, or HHV6 was noted in up to 30% of patients.

We have recently published our experience with $CD3^+$/$CD19^+$ depletion in 12 patients with non-malignant hematologic disorders, including 10 patients with bone marrow failure (BMF) plus the 2 patients with BTM mentioned in the preceding section[41]. Using an Expanded Access protocol (IRB #13-010286), these patients underwent MUD- or MMUD-PSCT from 2014-2017 using $CD3^+$/$CD19^+$ depletion and partial $CD3^+$ cell add-back to a dose of 1 x 10^5 $CD3^+$ cells/kg. This dose of $CD3^+$ addback, the low end of the range administered in the study of patients with hematologic malignancies, was given with the goal of reducing GVHD rates. All 12 patients

demonstrated rapid trilinear engraftment with no patients developing graft failure/rejection. No patients developed Grade III-IV acute GVHD or extensive chronic GvHD. Event-free survival remains at 100% with all patients transfusion-independent and exhibiting stable donor chimerism.

CliniMACS Device: Studies with TCRαβ Depletion

Two distinct T cell lineages are generated in the thymus, defined by expression of two different forms of the T cell receptor, TCRαβ or TCR γδ. TCRαβ T cells are the principal T cell population responsible for driving the allogeneic immune responses contributing to GVHD. In contrast, TCR γδ cells appear to be tolerant of host antigens and do not contribute to GVHD, but do play critical roles in facilitating engraftment across HLA barriers, directing immune responses against specific infection pathogens, and mediating anti-tumor responses[53-59]. In a previous study of partially matched related donor transplant for leukemia, patients who recovered higher numbers of circulating TCR γδ T cells in the first 100 days after a T depleted graft had a significantly higher disease-free survival following HSCT compared to patients with poorer TCR γδ T cell recovery (54% vs 19%)[60].

Subsequently, a large-scale method using the CliniMACS device for selective depletion of TCRαβ T cells from mobilized PBSCs was developed and found to be as effective in reducing the risk of GvHD as $CD34^+$ cell enrichment[61]. The final product retains NK cells, dendritic cells, and γδ T that may synergistically exert pro-engraftment and anti-viral effector effects. In mice, these grafts resulted in rapid engraftment of myeloid and lymphoid cells. Pilot studies in haploidentical PSCT, including children, have been encouraging. An initial study of the efficacy of TCRαβ depletion of 102 mobilized PBSC products conducted in Tubingen Germany noted a 4.7 log depletion of TCRαβ T cells, with 73% recovery of CD34+ cells and 83% recovery of TCR γδ T cells[62].

Results were recently reported from a large Italian cohort of 80 pediatric patients with acute leukemia who underwent haploidentical PSCT with $TCR\alpha\beta^+$ T cell depletion from 2011 to 2014[63]. In this study, the overall efficiency of TCRαβ cell depletion was excellent, with all patients receiving a $CD34^+$ stem cell dose greater than 6 x 10^6/kg with a TCR$\alpha\beta$ T cell dose of less than 1 x 10^5/kg. All but 2 patients engrafted, and there were no reports of Grade III-IV Acute GVHD or chronic extensive GVHD, though cancer relapse occurred in 24% of patients. In a prior study, the same group investigated the use of TCRαβ T cell depletion of haploidentical PBSC grafts in 23 pediatric patients receiving HSCT for non-malignant diseases including one patient with BTM.[64] With a median follow-up of 18 months, 21 of 23 patients were alive and no patient had developed Grade III/IV acute GVHD or chronic extensive GVHD. For the 21 patients who achieved engraftment, median time to ANC and platelet engraftment was very rapid at 13 and 10 days, respectively. 4 patients experienced graft failure (2 primary, 2 secondary), but were all successfully re-transplanted.

These promising studies and our prior experiences have led to the development of 3 single institution, investigator-initiated prospective clinical trials at CHOP of $TCR\alpha\beta^+/CD19^+$ T cell depletion for unrelated and haploidentical related PSCT for patients with hematologic malignancies (CHP13BT051, IRB #13-010495), primary immune deficiencies (CHP15BT022, IRB #15-011733) and bone marrow failure (CHP16BT052, IRB #16-012881). In addition, an Expanded Access of $TCR\alpha\beta^+/CD19^+$ T cell depleted PSCT is also approved (CHP16BT1211, IRB #16-013527) and currently enrolling. In total, over 85 patients have been treated on these protocols at CHOP. The patient population most similar to SCD and BTM are 13 patients with non-malignant hematologic bone marrow failure conditions treated either on CHP16BT052 (10) or CHP16BT1211 (3). Infused cell doses for these 13 patients included a median $CD34^+$ dose of 14 x 10^6/kg (range 6.7 to 22.6) and post-depletion TCR$\alpha\beta$ T cell dose of 0.5 x 10^5/kg (range <0.1 to 4.2). With median follow-up of 128 days, all 12 patients with BMF receiving MMUD (9/10, n=5) or MUD (10/10, n=7) PSCT are alive, engrafted, and transfusion independent. Only 1 of 12 subjects

developed transient Grade II acute skin GVHD, and no subject developed Grade III-IV acute GVHD or chronic extensive GVHD. Of note, the one subject receiving a maternal haploidentical graft developed primary graft failure, which was fortunately salvaged by a second haploidentical BMT. Due to this rejection event in the haploidentical donor setting, this protocol for patients with SCD and BTM will be restricted to patients with MUD or MMUD donors.

Rationale for the Proposed Study

SCD and BTM are severe hematologic disorders associated with significant morbidity and risk of early mortality that can be cured by allogeneic HSCT. Unfortunately, for patients with SCD and BTM who lack matched related donors, HSCT outcomes have been suboptimal primarily due to the competing risks of graft failure and GVHD. Our experience to date using partial T Cell depletion strategies, including TCRαβ T cell depletion, in the context of unrelated donor PSCT in patients with hematologic malignancies, BMF, and primary immune deficiencies has demonstrated that this strategy is highly effective in markedly reducing incidence of GVHD, while at the same time providing a high donor stem cell dose to facilitate durable engraftment. Thus, we propose to study the safety and efficacy of closely matched URD-PSCT with TCRαβ+ T cell depletion for patients with SCD and BTM, combining this T cell depletion wit $CD19^{+}$ B cell depletion to reduce risk of symptomatic EBV infection.

The transplant protocol incorporates a conditioning regimen that we have used successfully for patients with chronic RBC transfusion-dependent hematologic diseases including BTM[41]. Patients will receive a 30-day hydroxyurea prophase prior to starting conditioning, as this prophase has been reported to decrease rates of graft failure in HSCT for BTM and SCD[27,31]. As in our prior experience with TCRαβ T cell depletion, patients will not be required to receive standard pharmacologic prophylaxis for GVHD following HSCT, though immune suppression treatment for GVHD symptoms or for immunologic rejection prevention is allowed per treating physician discretion. Our primary endpoints will be the evaluation of engraftment efficiency and durability, including rates of primary and secondary graft failure, following PSCT with $TCR\alpha\beta^{+}$ T cell depletion, as well as Day 100 treatment related mortality and rates of acute and chronic GVHD. Secondary endpoints will include assessment of one-year EFS and OS, rates of viral reactivation and/or symptomatic infections related to CMV, EBV, or adenovirus, and assessment of donor chimerism and immune reconstitution. Safety stopping rules will include statistically demonstrable parameters to ensure that exceeding maximal rates for transplant related mortality, severe GVHD (Grade III-IV acute + chronic extensive GVHD), and graft failure will result in study suspension until evaluation and appropriate corrective action for causes leading to excess rates of these outcomes can be performed. These maximal acceptable rates are based on our prior experience in alternative donor HSCT for both malignant and non-malignant conditions.

3.0. PATIENT AND DONOR ELIGIBILITY

3.1. Subject Eligibility

3.1.1. Patient age must be ≥2 years and ≤ 25 years at time of enrollment.

3.1.2. Patients of both genders and all ethnic backgrounds will be eligible

3.1.3 A clinical decision has been made to transplant the subject and an acceptable donor product has been identified and will be obtained as described in section 3.7.

3.2. Disease eligibility

3.2.1. Severe Sickle Cell Disease

- Genotype: Hemoglobin SS, Hemoglobin SC, Hemoglobin SD, SOArab, or Hemoglobin SBeta thalassemia
- Must have at least one of the following disease manifestations
  - Clinically symptomatic neurologic event (stroke) or any neurologic deficit lasting >24 hours at any time prior to enrollment
  - History of two or more episodes of vaso-occlusive events (VOE) per year in the 2 years preceding enrolment. Patients must be refractory to hydroxyurea, defined as developing VOE despite receiving hydroxyurea for at least 6 months. Patients who are intolerant of hydroxyurea may also be enrolled. Vaso-occlusive events include:
    - Acute chest syndrome,
    - Pain episodes requiring intravenous pain management and/or hospitalization
    - Priapism
    - Splenic sequestration (defined as a 2 g/dL drop in hemoglobin in the setting of an acutely enlarging spleen. This will be determined as part of clinical care and prior to the research)
  - Administration of regular RBC transfusion therapy, defined as receiving ≥ 8 RBC transfusions in the year preceding enrollment to prevent sickle cell-related complications of any kind per treating hematologist's judgment.

3.2.2. Beta Thalassemia Major

- Genotype: Confirmed Beta Thalassemia genotype by molecular genetic testing (May include E/Beta0 and Beta0/Beta+ genotypes)
- Must meet clinical diagnosis of transfusion-dependent thalassemia, defined as need for ≥**8** RBC transfusions per year in the two years preceding study enrollment.

3.3. Organ function status

- Renal: Serum creatinine <1.5x upper limit of normal for age and estimated creatinine clearance ≥60ml/mim/1.73m^2
- Hepatic: Transaminases ≤5x upper limit of normal. Serum conjugated bilirubin <2x upper limit of normal
- Cardiac: shortening fraction ≥27% or left ventricular ejection fraction ≥50%.
- Pulmonary: DLCO ≥40% predicted (corrected for hemoglobin) in patients old enough to comply with PFTs or no baseline oxygen requirement for younger patients.
- Lansky or Karnofsky performance ≥60
- Transfusional iron overload: The following assessments apply to patients who have received or are currently receiving a chronic red blood cell transfusion regimen (≥ 8 RBC transfusions per year) or for patients with sickle cell disease who have received > 10 lifetime episodic transfusions.
    - Hepatic
        - Liver iron concentration (LIC) ≤ 12 mg Fe/g dry weight as assessed by Liver MRI within 6 months of study enrollment

        or

        - Absence of cirrhosis and bridging fibrosis on a liver biopsy or on non-invasive imaging modalities (such as fibroscan) obtained within 6 months of study enrollment
    - Cardiac
        - Patients with BTM and age ≥ 10 years must have cardiac T2* MRI performed and T2* must be ≥ 10 ms to be eligible
        - For patients with BTM and age < 10 years, and all patients with SCD, Cardiac T2* assessment need will be based on prior LIC history and will be up to discretion of treating physician.

3.4. Infectious disease criteria

- No active, untreated infections
- Negative for HIV by serology. No evidence of active Hepatitis B and C infection by combination of serologic and nucleic acid testing. Subjects who had past infection but no evidence of active infection will be allowed in the study.
- Patients with likely bacterial infections must be receiving appropriate antibacterial therapy and demonstrating therapy response
- Patients with likely fungal infections must have had at least 2 weeks of appropriate anti-fungal antibiotics and be asymptomatic.
- Patients with symptoms consistent with active viral infection will be deferred until viral symptoms resolve. Patients with evidence of CMV, EBV or other known viremia must receive appropriate therapy to clear viremia prior to initiating study therapy.

3.5. Consent

- Signed consent by parent/guardian for subjects under 18 years of age or patient if ≥18 years.

3.6. Exclusion criteria

- Patients who do not meet disease, organ or infectious criteria.
- Previous HSCT
- Patients with no suitable unrelated donor available. Patients with suitable fully matched related donor are also not eligible.
- Pregnant females. All females of childbearing potential must have negative pregnancy test.
- Participation in a clinical trial in which the patient receives an investigational drug must be discontinued prior to the time of initiation of transplant therapy. Specifically transplant chemotherapy should not begin until at least 3 half-lives after last use of the investigational drug.
- Severe RBC alloimmunization, defined as inability to receive packed RBC transfusion therapy due to anti-RBC antibodies. Patients with high titer anti-donor HLA antibodies detected on screening may be enrolled if they are willing to undergo HLA antibody desensitization therapy.

3.7. Donor selection and eligibility

3.7.1. Donor selection will comply with 21 CFR 1271* of the U.S. Food and Drug Administration's Code of Federal Regulations, available at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271

3.7.2. Donor testing

- Unrelated donor meets National Marrow Donor Program criteria for donation
- The following infectious disease testing is standard of care for all donors:
    - CMV Serology
    - HIV 1/2 Antibody and HIV NAT
    - HEP B CORE TOTAL, HEP B SURFACE AB, HEP C
    - SYPHILIS
    - HTLV I AND II

3.7.3. Donor matching

- High resolution HLA typing of patient and unrelated donor at HLA-A, -B, -C, DRB1, and DQB1 loci
- Unrelated donor must be an antigen and allele match at ≥ 8/10 HLA Loci

- In donor with 2 mismatches, only one mismatch involving HLA-A, -B, or -DRB1 will be allowed
- Donor and collection center willing to undergo peripheral stem cell mobilization and collection by apheresis

4.0. THERAPY

All transplant-related care of the recipient including pre-transplant evaluations, conditioning, supportive care and post-transplant evaluations will be performed as per our institutional standard of care practices. In contrast, the stem cell processing and the infusion of the donor cells are considered to be study-related procedures and experimental.

4.1. Standard Therapy (not research)

4.1.1. Pre-transplant evaluations as per our standard practice

- No pre-transplant evaluations are specific to the research objectives; however these evaluations are included in this protocol because they are used to determine eligibility as stated in section 3.0.
- Organ Function Evaluations: Cardiac, pulmonary, renal, and hepatic function will be assessed per standard of practice pre-transplant organ function evaluations defined in the Children's Hospital of Philadelphia's CTTS and in section 3.3 of this protocol. The treating physician may consult with another department to assist in interpreting the results in relation of stem cell transplant..
- In addition, a pregnancy test must be performed on all females > 11 years of age.
- Infectious Disease Evaluation:
  - CMV, EBV HSV, and VZV serology. HIV testing (HIV must be negative to proceed)
  - CT Chest and Abdomen required for all patients with a history of neutropenia, immune suppression treatment, presence of central venous line, or iron overload requiring chelation therapy
  - Patients should not have active untreated infection prior to transplant admission

- Immunology Evaluations: Cellular immunology panel, IgM, IgA, and IgG levels
- Additional evaluations:
  - Anti-HLA antibody testing (all patients)
  - Transfusional iron overload:
    - Ferritin required for all patients.
    - Liver R2 MRI required for patients receiving a chronic red blood cell transfusion regimen (≥ 8 RBC transfusions per year) or for patients with sickle cell disease who have received > 10 lifetime episodic transfusions.
    - Cardiac T2* MRI to assess for cardiac iron loading is required for all patients with BTM age 10 years and older. Cardiac T2* MRI may be considered for other potential subjects based on clinical assessment, but is not required.
    - Additional studies including liver biopsy may be recommended based on clinical assessment, but are not required.
  - Patients with SCD should undergo Brain MRI/MRA within 6 months of transplant admission if they have a known history of cerebrovasculopathy, stroke, or silent infarcts. For patients without a history of these neurologic features, brain

MRI/MRA performed within a year of transplant is acceptable. Transcranial doppler ultrasound (if patient has adequate windows for US) may be considered, but is not required as additional imaging for cerebrovasculopathy.

- Patients with SCD should undergo transfusion therapy (simple or exchange) if needed to decrease the hemoglobin S% prior to initiation of inpatient conditioning regimen. The target goal for the hemoglobin S% prior to conditioning is less than 30%, though clinical judgement may be used as to whether to pursue further transfusion if the pre-transfusion hemoglobin is <50%.
- Patients with BTM should undergo transfusion therapy to maintain Hgb > 9 g/dL for at least 3 months prior to initiation of the inpatient conditioning regimen. For patients with BTM, hypertransfusion to achieve Hgb > 11 g/dL immediately prior to transplant may be considered but is not strictly required.
- Patients receiving chelation therapy with deferasirox or deferiprone should discontinue these medications on the day of hydroxyurea initiation.

4.1.2. Unrelated donor stem cell mobilization

- Peripheral stem cells will be collected as per the standards of the National Marrow Donor Program

4.1.3. Conditioning Regimen

- This protocol will utilize CHOP standard of care conditioning for patients undergoing unrelated donor HSCT, coupled with a 30-day hydroxyurea prophase standardly used for patients with SCD and BTM

Day	Treatment
-40 to -11	Hydroxyurea 30 mg/kg/day orally*
-9, -8, -7[#]	Thymoglobulin (rabbit) 3 mg/kg/day
-8	Busulfan[&$] q6h x 4 doses or Daily x1 with 1st dose PK
-7, -6, -5	Busulfan (adjusted) q6h x 12 doses or Daily x3
-6, -5, -4, -3, -2	Fludarabine 30 mg/m^2/day (<10 kg- 1mg/kg/day)
-3, -2	Thiotepa 5 mg/kg/day^
-1	Rest day
0	Stem cell infusion

*Timing of hydroxyurea may be altered by +/- 1 day. Hydroxyurea may be given on an outpatient basis, with weekly LFT and CBC monitoring. Missed doses of hydroxyurea do not prohibit patients from continuing on study and receiving PSCT. Hydroxyurea may be discontinued by the medical team due to side effect intolerance, LFT abnormalities, or neutropenia, and patients will be allowed to proceed with study therapy.

[#]The timing of thymoglobulin initiation may vary by +/- 2 days based on patient-specific scheduling and logistical considerations.

[&]Busulfan may be given as either every 6-hour or daily dosing

Busulfan Every 6-hour dosing:
< 3 months old: 1 mg/kg/dose IV. as a starting dose q 6 hours x 16 doses
≥ 3 months old but < 10 kg: 0.8 mg/kg/dose IV as a starting dose q 6 hours x 16 doses
> 10 kg but < 4 years old: 1 mg/kg/dose IV as a starting dose q 6 hours x 16 doses
≥ 4 years: 0.8 mg/kg/dose IV. as a starting dose q 6 hours x 16 doses

Busulfan Daily Dosing
<10 kg: 3.2 mg/kg/dose IV daily x 4
>10 kg but <4 years old- 4 mg/kg/dose IV daily x 4
>10 kg and >4 years old: 3.2 mg/kg/dose IV daily x 4

[$]**Dose Modification based on Busulfan Pharmacokinetic Assessments:**
Patients receiving busulfan will have pharmacokinetic testing done after the first dose as per published Children's Hospital of Philadelphia Blood and Marrow Transplant Program Standards of Practice monitoring guidelines. A specimen will be drawn and sent to the Toxicology Laboratory at the University of Pennsylvania.
For every 6-hour dosing, Doses are adjusted to achieve AUC concentration between 900-1500 (micromole/liter)*minute.
For Daily dosing Busulfan, the daily dose targeting AUC 3600-6000 (micromole/liter)*minute

^ Adjusted body weight (ABW) should be used for dosing thiotepa in obese patients, defined as those weighing >125% of Ideal Body Weight (IBW). ABW is determined according to the formula:

$$ABW = IBW + 0.4 (current\ weight - IBW)$$

4.1.4. Prophylaxis and monitoring for infections

- CMV/HSV/VZV prophylaxis: All CMV serology positive recipients will receive CMV prophylaxis according to CHOP CTTS standards of practice. HSV/VZV prophylaxis will also be given according to institutional standards.
- EBV prophylaxis: All EBV serology positive recipients will receive Rituximab 375 mg/m^2 on Day +1
- Monitoring for CMV, EBV, and adenovirus reactivation, along with antibiotic prophylaxis against pneumocystis, fungus, and bacteria and treatment of infection complications will be provided based on institutional standards of practice

4.1.5. Graft versus Host Disease (GVHD) prophylaxis and treatment

- Because of TCRαβ+ T cell depletion of the donor stem cell product, pharmacologic GVHD prophylaxis will not be required following stem cell infusion in the absence of prior GVHD symptoms.
- For patients who develop GVHD, treatment will be administered in accordance with standard institutional practice. GVHD treatment decisions are not considered part of this research protocol.

4.1.6. Prevention of graft rejection

- Cyclosporine/Tacrolimus or sirolimus (in patients with history of renal dysfunction) is the recommended therapy for graft rejection prophylaxis. Administration will be based on institutional standard of care.
- The decision to use pharmacologic agents including cyclosporine/tacrolimus, steroids, or mycophenolate mofetil to prevent immunologic graft rejection is recommended but will be left to the attending transplant physician's discretion based on individual patient risks/factors and will not be considered a violation of protocol therapy. Patients with high titer anti-donor HLA antibodies will be encouraged to undergo desensitization therapy prior to proceeding to study therapy.

4.1.7. Growth factors

- G-CSF or GM-CSF will not be routinely used after stem cell infusion, however use of growth factors will be allowed in individual clinical circumstances and the decision for their use will be left to the inpatient attending physician's discretion.

4.1.8. Clinical and laboratory assessments of subjects during transplant admission and during outpatient follow-up care

- All follow-up care and evaluations will be conducted as part of standard follow-up care for patients receiving allogeneic HSCT at CHOP. Given that much of this information will be accessed as part of study chart review and assessments of endpoints, the pertinent assessments are included in Appendix A.

4.2. Study Procedures/Evaluations (Research)

4.2.1. Stem cell processing and infusion

- The collected peripheral stem cell product will be processed as outlined in section 5.0 using manufacturer's recommendations for CliniMACs procedures
- At least 5 x 10^6/kg CD34+ cells from the peripheral stem cell collection will undergo TCR$\alpha\beta$ depletion. These cells will be infused following conditioning on Day 0.

4.2.2. Table of research-specific procedures and evaluations

Study Procedure	Time of Assessment
TCRαβ+ T cell and CD19+ Depletion Using CliniMACS system (detailed in section 5.0)	Prior to allogeneic stem cell infusion

Flow Cytometry and Cell Quantification Methods to Assess Cellular Content of Infused Graft pre- and/or post CliniMACS selection, including: Viability assessment (Trypan Blue, 7-AAD) CD34 CD3 TCR$\alpha\beta$ TCR$\gamma\delta$ CD19 or CD20 (B cells) NK cells (CD56+)	At time of allogeneic stem cell infusion
Review of Medical Records: review of post-transplant clinical data, performed as standard post-transplant assessments as outlined in Appendix A, in order to analyze study endpoints.	Ongoing through duration of study

4.2.3. Pregnancy considerations:

- In the event of pregnancy occurring prior to the initiation of conditioning and stem cell infusion, subjects will be declared ineligible for study therapy.
- Pregnancy that occurs following conditioning and stem cell infusion, will be monitored with the assistance of a trained obstetrician. In the event this occurs, pregnancy outcome will be monitored. However, given that all post-transplant study procedures are standard of care for follow-up, pregnancy occurring in the post-transplant period will not impact study assessments.

5.0. DONOR STEM CELL PROCESSING (Research procedures)

5.1. Cell Processing

Hematopoietic stem cells (HSC) from apheresis will be processed by the Cell and Gene Therapy Laboratory (CGTL) at the Children's Hospital of Philadelphia (CHOP). CGTL is accredited by the Foundation for the Accreditation of Cellular Therapy (FACT) and maintain complete SOPs and procedure records. Processing of cells using the CliniMACS will occur in accordance with the Investigator Brochure and Technical Manual following the laboratory SOPs and using aseptic technique. This processing and the infusion of this manipulated product is considered experimental and is being performed under an IDE

5.1.1 TCR$\alpha\beta$ and CD19 depletion, including the following main steps:

- HSC product washed to remove platelets
- Biotin-labeled TCRαβ reagent is added for 30 minutes at room temperature

- TCRαβ reagent removed by washing
- Anti-Biotin and CD19 reagents conjugated to magnetic beads are added for 30 minutes at room temperature
- Reagents removed by washing
- Product is applied to the tubing set on the CliniMACS device and depletion program is started
- The TCRαβ/CD19 depleted fraction is collected and adjusted for infusion based on flow cytometry analysis

5.1.2. Cell numbers and volume of reagents used are as per the CliniMACS manual and CGTL SOP. Wash steps use CliniMACS PBS/EDTA buffer supplemented with human serum albumin (HSA).

5.2. Final product formulation and infusion information

- The preferred method will be to provide fresh (non-cryopreserved) product for infusion. The final product will be concentrated and resuspended in 0.9% Sodium Chloride Injection USP supplemented with human serum albumin.

- In rare instances, donor timing will require cryopreservation of the cell product. In these cases, the product will be cryopreserved after processing according to relevant CGTL SOPs and thawed at the time of infusion. For cryopreservation, the TCRαβ/CD19 depleted products are combined with equal volumes of a cryoprotectant containing 20% Dimethyl Sulfoxide (DMSO) in 5% HSA, according to Lab SOPs . After the addition of the 2X cryoprotectant to an equal volume of the cell suspension (1:1) the final concentration of DMSO will be 10%. The products then undergo automated controlled rate freezing with recording of the freezing curves and are stored in the vapor phase of liquid nitrogen in a monitored and alarmed freezer.

- Once release testing is completed the product label is completed and the product is transported by the laboratory to the infusion site in a validated transport container. Accompanying forms are those specified by CGTL SOPs and in accordance with FACT requirements, including a summary of records used for donor eligibility determination.

- Prior to transfer to the infusion team the product is to be examined by an infusion team member and the processing laboratory staff member to confirm identity of the recipient and the product, observation of the product and product container for appearance, and confirmation of all information on the product label. This transfer must be documented.

- The stem cell product will be infused as per institutional practice.

- Infusion information (date and times, identity of infusion team member, etc.) must be recorded on forms as provided by the laboratory, or in the electronic chart record and must include any adverse events associated with infusion.

5.3. Management of infusion-related side effects

- Our transplant center has performed a large number of allogeneic stem cell infusions after CliniMACs cell processing. Grade 3 or Grade 4 infusion reactions after CliniMACS selection are very rare, and not more common than reactions seen following infusions of unprocessed grafts.

- Standard prevention and treatment for allergic reactions following stem cell infusion are part of the CHOP CTTS's SOP's.

5.4. Product quality assessment overview

5.4.1. Overview: The CTGL shall be responsible for product quality assessment. Testing prior to product release for infusion must include:

- Total nucleated cell counts (before, during, and after processing)
- Viability assessment (Trypan Blue and/or 7-AAD) (before and after processing)
- Gram stain prior to product release
- Sterility testing prior to frozen product release
- Flow cytometry assessment by validated methods to include at minimum: CD34, CD3, TCR$\alpha\beta$, TCR$\gamma\delta$, B cells (CD20 post processing, CD19 or CD20 prior to processing), and NK cells (CD56+). The laboratory follows the ISHAGE gating protocol and laboratory staining and analysis SOPs
- Removal of samples for sterility cultures (before and after processing)
- Removal of samples for endotoxin assessment (after processing)

5.4.2. Sterility testing assessments and notification procedures

- Sterility testing samples are sent to LABS, Inc for a 14 day culture using FDA approved methods, including bacterial (aerobic, anaerobic) and fungal cultures. If a culture is positive, the lab. notifies the CGTL Director or his designee who informs the attending transplant physician. This notification is documented on the hard copy of the test results, and it will include the physician's name, and the date and time of the communication.

- If the initial sample (before processing) from an HSC product is found to be contaminated, possibly during collection, the CGTL informs the collection facility. This notification is also documented on the hard copy of the results and it will include the collection center staff member contacted and the date and time of the communication. The collection facility is requested to undertake a review of the collection procedure. The sterility test results for each product are filed with the processing records and kept according to SOPs.

- Positive cultures will be reported to the IRB and the FDA.

- Stem cell products will already have been infused, and the treating physician, working with the Medical Director, will respond accordingly, including monitoring of the patient, follow up cultures and treatment as clinically indicated.

5.4.3. Release criteria for stem cell products

- Viability of Fresh products ≥70%. For viability <70%, the PI and the transplant physician must be notified
- Negative gram stain.
- Cumulative cell doses include:
    - $CD34^+$ Cells- Minimum 2.0 x 10^6 per kg, Target 15.0 x 10^6 per kg
    - $TCR\alpha\beta^+$ T cells-No minimum, maximum 5.0 x 10^5/kg
    - $CD20^+$ B cells- None specified.
    - If the defined doses cannot be met due to processing issues or characteristics of the product, approval by the institutional PI or, in their absence, by one of the co-investigators is required for release.
- Additional required test
    - Endotoxin testing is performed on a sample of the final infusion product. We will obtain endotoxin testing results before product administration. If the endotoxin level result is >5 EU/kg, the laboratory shall inform the PI, BMT attending on service, and Lab Medical Director. The BMT attending on service will:
        - Appraise the risks and benefits of infusing the product, which will depend on the individual clinical situation.
        - If the decision to infuse the product is made, monitor the patient for signs or symptoms related to infection. The patient would already be on broad spectrum antibiotic treatment if this were the case, but we would concur in this treatment. If appropriate, blood culture and an empiric rule-out course of an appropriate antibiotic with gram negative coverage would be initiated.
    - The CGTL will investigate, to be overseen by the Scientific Director and reviewed by the Medical Director. FDA will be notified within 30 days.

6.0. STATISTICAL CONSIDERATIONS AND DATA ANALYSIS PLAN

6.1. Overview

This study is a pilot trial, designed to evaluate the efficacy of selective TCRαβ T cell depletion of donor peripheral blood stem cells as a method to minimize rates of GVHD while maintaining high rates of efficient and durable engraftment in patients with SCD and BTM undergoing closely matched unrelated peripheral blood stem cell transplantation.

6.1.1. Accrual

- We anticipate recruiting 4-5 patients per year to a total of 20 patients.

6.1.2. Study Duration

- Study assessments will continue through 2 years post-SCT.

6.2. Study endpoints

6.2.1. Definitions

6.2.1.1. Engraftment and Graft Failure

- Stable engraftment defined as: donor chimerism > 20% following initial neutrophil engraftment.
- Time to neutrophil engraftment defined as: first day of ANC >500/μl for the first of 3 consecutive days
- Time to platelet engraftment defined as: first day of three measurements that patient has platelet count >20,000/μl AND is platelet transfusion independent for a minimum of seven days following conditioning-induced nadir
- Primary graft failure: primary non-engraftment is defined as no evidence of neutrophil engraftment by day +30 after stem cell infusion
- Secondary graft failure: ANC <500 for at least 7–10 days after initial engraftment occurs in the absence of known infection or drug-mediated suppression, and confirmed by hypocellular bone marrow biopsy and/or total donor chimerism percentage from blood or bone marrow < 10%.

6.2.1.2. GvHD

- Acute GvHD will be graded according to the current guidelines for reporting by the Center for International Bone Marrow Transplant Registry www.CIBMTR.org/manuals.
- Chronic GvHD will be graded as limited or extensive according to standard criteria.

6.2.1.3. Mortality and Survival

- 100 Day treatment-related mortality (TRM) — Defined as death from non-disease related causes in the 100 days from stem cell infusion
- One-year event free survival (EFS). Events include death, primary or secondary graft failure, or the development of hematologic malignancy.
- One-year overall survival (OFS).

6.2.1.4. Infections, Immunity, and Chimerism

- Viral reactivation defined as: presence of viremia for Adenovirus, CMV, or EBV detectable in serum by PCR and requiring antiviral therapy per clinician discretion
- Viral infection defined as: the presence of specific viremia combined with symptoms most likely attributable to adenovirus (pneumonitis, septicemia, colitis), CMV (retinitis, pneumonitis, colitis), or EBV (evidence of lymphoproliferation).
- Immune reconstitution: defined based on evaluations outlined in Appendix A.
- Full Donor Chimerism: defined as ≥ 99% donor chimerism in all chimerism measurement assessments following BMT.
- Stable Mixed Chimerism: defined as <99% donor chimerism, but with < 15% change in percentage of total donor chimerism over any 3 month period.
- Progressive Mixed Chimerism: defined as >15% decrement in the percentage of overall donor chimerism over any 3 month period following MRD-BMT.

6.2.2. Primary endpoints

- Time to neutrophil and platelet engraftment,
- Incidence of primary and secondary graft failure,
- Incidence of Grade II-IV acute GVHD, Severe Grade III-IV acute GVHD, and Chronic Extensive GVHD,

6.2.3. Secondary endpoints will include:

- One-year event-free survival (EFS)

- One-year overall survival (OS)
- 100 day treatment-related mortality.
- Incidence of viral reactivation and symptomatic viral infection
- Rates of full donor, stable mixed, and progressive mixed chimerism
- Immune reconstitution at 1 and 2 years post HSCT.

6.3. Sample Size Considerations

The primary objectives of the study are to evaluate efficiency and durability of engraftment, as well as rates of severe GvHD, in patients with SCD or BTM who undergo PSCT with TCRαβ/CD19 depleted grafts. Since this is a pilot, single arm study, no formal hypothesis will be tested and the sample size is based on the width of a 95% exact confidence interval (CI) around the estimated rates. The table below provides possible number of events among the 20 patients, event rates and the corresponding 95% CIs. For example, for a primary graft failure rate of 20%, the exact Binomial 95% will be [6%, 44%]. This CI is narrow enough to assess whether this approach offers enough hope to be worth pursuing in additional definitive studies.

Number of events	Event rate	95% CI
2	10%	[1%, 32%]
3	15%	[3%, 38%]
4	20%	[6%, 44%]
5	25%	[9%, 49%]
6	30%	[12%, 54%]
7	35%	[15%, 59%]
8	40%	[19%, 64%]

6.4. Stopping Rules

The early stopping rules are established based on excess rates of 100 day TRM, severe GvHD (grade III-IV acute + chronic extensive), and graft failure (primary+secondary). We assume the maximum acceptable rate for 100 day TRM is 10% based on institutional and published data regarding TRM rates in T cell depleted unrelated donor HSCT. Given the low anticipated GvHD incidence in our institutional experience as well as published experience using TCRαβ depletion strategies, the maximal acceptable rate for grade III-IV acute + chronic extensive GvHD will be 20%. Based on published rates of graft failure and our previous experience with partial T cell depletion strategies for non-malignant hematologic diseases, the maximal acceptable rate for primary + secondary graft failure will be 10%.

The stopping rules are constructed for each type of event separately. We will evaluate these events continuously, beginning after enrollment of the first five patients until the end of the study. The stopping rule for each type of event will be triggered if there is significant evidence that the event rate exceeds the maximum acceptable rate, that is, if the lower bound of the one-sided 95% CI exceeds the maximum acceptable rate. If the number of patients with a particular type of event equals or exceeds the number in the tables below, then the study should be suspended pending further evaluation.

6.4.1. Stopping Rules for Day 100 Treatment-related Mortality (TRM)

The detailed stopping rule based on incidence of 100 day TRM is summarized in the following table, for a **maximum acceptable rate of 10%**. For example, if 3 or more out of 10 subjects have TRM in the 100 days from stem cell infusion, the study will be suspended. We would stop the study early with a probability of 0.03 if the true 100 day TRM event rate is 5%, stop early with a probability of 0.17 if the true event rate is 10%, and stop early with a probability of 0.75 if the true event rate is 25%. These probabilities are calculated from a simulation study.

Number of patients	Stop if Day 100 TRM>=	Number of patients	Stop if Day 100 TRM>=
5	2	12-18	4
6-11	3	19	5

6.4.2. Stopping Rules for severe GVHD (grade III-IV acute + chronic extensive)

The detailed stopping rule based on incidence of severe GVHD is summarized in the following table, for a **maximum acceptable rate of 20%.** For example, if 6 or more out of 15 subjects have severe GVHD, the study will be suspended. Under this stopping rule, we would stop the study early with a probability of 0.02 if the true severe GvHD event rate is 10%, stop early with a probability of 0.19 if the true event rate is 20%, and stop early with a probability of 0.74 if the true event rate is 35%. These probabilities are calculated from a simulation study.

Number of patients	Stop if Severe GvHD>=	Number of patients	Stop if Severe GvHD>=
5-6	3	14-16	6
7-9	4	17-19	7
10-13	5		

6.4.3. Stopping Rules for Primary and Secondary Graft Failure

The detailed stopping rule based on the incidence of graft failure is summarized in the following table, for a **maximum acceptable rate of 10%.** For example, if 3 or more out of 10 subjects have graft failure, the study will be suspended. Under this stopping rule, we would stop the study early with a probability of 0.03 if the true graft failure event rate is 5%, stop early with a probability of 0.17 if the true event rate is 10%, and stop early with a probability of 0.75 if the true event rate is 25%. These probabilities are calculated from a simulation study.

Number of patients	Stop if Graft Failure>=	Number of patients	Stop if Graft Failure>=
5	2	12-18	4
6-11	3	19	5

6.5. Statistical Analyses

The primary analyses will estimate rates and 95% exact binomial CIs, for the endpoints primary and secondary graft failure, grade II-IV acute GvHD, severe (grade III-IV) acute GvHD, chronic extensive GvHD and day 100 TRM. Patients who die will be considered failure for the engraftment success evaluation. Kaplan-Meier (KM) curves will be plotted for EFS and OS, and one-year EFS and one-year OS will be estimated with 95% CIs based the KM methods. Also, cumulative incidence analysis will be used to analyze time to GvHD, time to TRM, time to primary graft failure, and time to secondary graft failure, considering the other events as competing risks. In addition, descriptive statistics will be calculated for kinetics of engraftment, incidence and severity of viral reactivation and infections, and immune reconstitution. Some of these parameters are expected to have skewed distributions. Therefore, we will use box and whisker plots, and non-parametric summaries (e.g., quartiles) to display the data. To assess relationships between various parameters of immune reconstitution and CD34+ and T cell doses, Pearson correlations will be calculated, possibly after transformation, or Spearman's correlations. Toxicities will be tabulated for each toxicity type, per the Common Toxicity Criteria. This study is considered a pilot and these analyses will be descriptive to provide guidance to future studies.

7.0. DATA MONITORING AND REPORTING ADVERSE EVENTS

7.1. Safety Monitoring

- The SMC is comprised of a minimum of two physicians and at least one other staff member (who may also be a physician). The SMC will meet once a year and review enrollment and outcome with regard to the stopping rules. The SMC will meet more frequently if required. Targeted areas of safety monitoring will include the following: treatment-related mortality and engraftment. All SMC communication and meetings will be documented and maintained for this study.

- Before initiation of the clinical investigation, the PI will arrange a pre-trial monitoring visit with the Office of Research Compliance (ORC) to confirm trial readiness. After enrolling and transplanting the first subject on this trial The Office of Research Compliance (ORC) will be contacted to arrange a monitoring visit. Thereafter, ORC will monitor the study at least annually. Interim monitoring activities will include 100% review of regulatory files as well as a percentage of enrolled subject records, source documents, applicable informed consent forms, and case report forms. The percentage of subject data review may be amended throughout the course of the study based on an ongoing risk assessment. A tapered approach to monitoring may be employed, if conduct and documentation of the study reaches a level of reliability that would permit valid conclusions based upon a sampling of data.

7.2. Premature Termination of the Study

If the investigator, the Sponsor or the clinical monitor becomes aware of conditions or events that suggest a possible hazard to patients in case of study continuation, the study may be terminated after appropriate consultation between the relevant parties and the coordinating investigator.

7.3. Adverse Events

7.3.1. Definitions

7.3.1.1. Adverse event (AE)

The term adverse event describes any untoward medical occurrence in a patient or clinical investigation subject administered an investigational medicinal product (IMP). It does not necessarily have a causal relationship with this study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP.

This definition includes:

- Any sign or symptom occurring during the study
- Any event or disease present on the day of inclusion in the study with symptoms that worsen during the study
- Any accident
- Any significant change in laboratory parameters

7.3.1.2. Adverse Event: Study-specific definition

In the context of this study grades III-IV acute GvHD, chronic extensive GvHD, and primary or secondary graft failure will be considered an adverse event.

7.3.1.3. Adverse Reaction (AR)

Adverse reactions (ARs) include all untoward and unintended responses to an IMP related to any dose administered. All AEs judged by either the reporting investigator or the Sponsor as having a reasonable possibility of a causal relationship between the event and the TCR$\alpha\beta$/CD19 depletion qualify as ARs. This means that there are facts (evidence) or arguments to suggest a causal relationship between the event and the depletion. An AR is defined as unexpected when its nature, severity or outcome is not consistent with the information that has been obtained from previous observations and investigational trials.

7.3.1.4. Serious Adverse Events (SAEs)

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening (an event in which the patient was at risk of death at the time of the event; however, it does not refer to an event, which, hypothetically, might have caused death if it had been more serious)
- Requires inpatient hospitalization or prolongation of existing hospitalization with the exception of hospitalizations for elective reasons
- Results in persistent or significant disability/incapacity
- Is another important medical event that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical judgment, is thought to jeopardize the patient or subject or require medical or surgical intervention to prevent one of the outcomes defining an SAE.

- Any pregnancy discovered during the study is considered to be a serious adverse event even if they do not comply with the above definitions.

  Note: Pregnancy is an exclusion criterion. If pregnancy either of a female patient included or the partner of a male patient included should occur during the study the pregnancy has to be followed to term. The outcome for mother and child has to be documented.

7.3.1.5. SAE: Study-specific definition

- In the context of this study any occurrence of acute GVHD greater than grade II has to be reported according to the standard procedure of SAE reporting, irrespective whether or not fulfilling the SAE criteria defined above.

- The assessment of whether there is a reasonable possibility of a causal relationship is usually made by the investigator. The causality assessment given by the investigator should not be downgraded by the Sponsor. If the Sponsor disagrees with the investigator's causality assessment, the opinion of both, the investigator and the Sponsor, should be provided with the report.

7.3.2 Monitoring, Recording and Reporting of Adverse Events

7.3.2.1 General Requirements

During the course of the study, SAEs possibly related to TCR$\alpha\beta$ T cell depletion will be recorded in the patient medical record and will be reviewed by the PI. SAEs will be reported using event terms and severity grading from the NCI Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03 or higher. Serious adverse events (SAEs) will be recorded and reported as per CHOP IRB reporting policy 408 and per FDA guidelines

7.3.2.2. Reporting of Serious Adverse Events

The investigator has to report all unanticipated problems, including AEs that are both serious (SAEs) and related to the study intervention, promptly to the IRB and FDA. The investigator must report these events in accordance with the following timeline:

- All SAEs that are life threatening or which resulted in Death must be reported to the IRB by email, phone or fax within one business day of the initial notification and a full report must be submitted within 48 hours of notification. These SAEs will be reported to the FDA no later than 7 calendar days from the initial receipt of information.
- Events that are not life-threatening but suspected to be related to the study must be reported to the IRB as per CHOP IRB guidelines and to the FDA within 15 calendar days after determining this SAE requires reporting
- All SAEs occurring up to 30 days after receiving the IMP that are considered unexpected and a results of the study drug have to be reported by the investigator

- Where applicable, information from relevant laboratory results, hospital case records and autopsy reports should be obtained. The investigator is also required to follow the subject until the SAE stabilizes or resolves to subject baseline and submit follow up reports.

7.3.2.3 Adverse Events of Specific Interest

Acute GVHD grade 3 and grade 4, Chronic Extensive GVHD, and primary and secondary graft failure are defined as adverse events of specific interest for the purpose of this study and will be monitored in detail. Adverse events of specific interest will be documented and have to be reported according to the standard procedure of SAE reporting described above until Week 12.

8.0. RISK-BENEFIT ASSESSMENT

8.1. Potential benefits of study participation

8.1.1. Decreased risk of GVHD due to TCRαβ T cell depletion

- Decreased rates of GVHD lead to less direct organ damage from GVHD, as well as decreased need for immune suppression after HSCT, which results in fewer side effects from immune suppression medications, lower rates of infection, and improved immune reconstitution after HSCT. Additionally, the lower rates of GVHD provided by T cell depletion enables us to use 1 to 2 antigen mismatched unrelated donors, thus greatly expanding the number of potential patients with an available transplant donor

8.1.2 More rapid engraftment compared to convention bone marrow transplantation

- TCRαβ T cell depletion enables the use of mobilized PSC as a donor stem cell source, which is otherwise limited because PSC without T cell depletion is associated with excessive GVHD. The advantage of mobilized PSC versus conventional BM as a stem cell source is that we are able to collect on average a 2-3 times higher stem cell dose for infusion. This higher stem cell dose leads to more rapid neutrophil and platelet engraftment, decreasing infection risks and decreasing time to transfusion independence

8.1.3. Decreased risk of symptomatic EBV infection due to CD19 B cell depletion

- Depletion of CD19 B cells greatly lowers the risk of EBV reactivation and viremia after HSCT. EBV reactivation can lead to the development of post-transplant lymphoproliferative disease. Thus, CD19 B cell depletion may lower the risk of this complication

8.2. Risks specific to TCRαβ T cell depletion

8.2.1. Possible increased risk of graft rejection

- Graft rejection is more common in fully T cell-depleted transplants compared with transplants where the T cells are not removed. With the TCRαβ T cell depletion strategy, this risk may be decreased due to the presence of TCRγδ T cells in the infused product and the high number of $CD34^+$ cells in the final product that mobilized PSC collection enables.

8.2.2. Risks of stem cell processing

- The blood cells that have been collected will be brought to the CGTL, and processed under sterile conditions. The risks to this procedure include contamination with bacteria and other agents and malfunction of equipment with a loss or decrease of stem cells. These risks are extremely small.
- There is also a very small risk that the TCR$\alpha\beta$ depletion process will result in an incomplete depletion of TCR$\alpha\beta$ T cells, which could increase the risk of GVHD. However, since the TCR$\alpha\beta$ cell count in the final product is known in advance of the infusion, this risk may be mitigated by limiting the infused cell dose.

8.3. General risks of allogeneic hematopoietic stem cell transplantation

Apart from TCRαβ and CD19 depletion of the donor graft prior to infusion, all aspects of HSCT performed for subjects enrolled in this study will be according to standards of care within the the CHOP CTTS. Thus, general risks of allogeneic HSCT are not considered study-specific risks, and are instead considered risks of our standard of care approach to HSCT. Because patients must provide consent to undergo HSCT in order to participate in this study, general risks of allogeneic HSCT are included in Appendix B.

9.0. DRUG INFORMATION

9.1. Conditioning agents

- All conditioning agents are part of established treatment regimens for patients undergoing allogeneic stem cell transplantation for non-malignant hematologic disorders. Thus, the conditioning regimen or individual conditioning agents are not considered investigational. As side effects of these conditioning agents are responsible for many of the risks of HSCT however, these drug-specific side effects are included as Appendix C.

9.2. GvHD and Infection Prophylaxis

- GvHD prophylaxis is not required for this protocol. Medications used for infection prophylaxis and prevention of graft rejection will be left to the treating physician's discretion and will follow standards of practice in the CHOP CTTS.

10.0. PROTECTION OF SUBJECTS

In the event of publication or presentation, patients will be represented by a unique patient number only. Information regarding this study will be kept in a locked file in the BMT office. As per the Statistical Analysis, stopping rules are in effect for monitoring for severe GVHD, primary and secondary graft failure, and Day 100 treatment-related mortality. All data will be retained as required by institutional and federal regulations.

11.0. INFORMED CONSENT AND DATA CONFIDENTIALITY

11.1. Informed Consent

- Patients ≥ 18 years of age or parents/guardians of patients under 18 who are eligible for enrollment into the study will be informed by the investigator in detail about the study verbally at a scheduled consent meeting and will review the informed consent form. They will be allowed adequate time for consideration and reaching an informed decision. During this period, they will have the opportunity to discuss questions and concerns with their treating physician. If patients are willing to participate in the study, informed consent will be obtained from them or their legally authorized representative according to the regulatory and legal requirements applicable.
- The informed consent form will be retained by the investigator as part of the study records.
- Patients can withdraw their consent at any time during the study without having to give a reason and without prejudice regarding their future medical treatment.
- For patients under age 18, parents/guardians meet with a member of the study team to discuss indications for SCT, potential benefits and risks. The consent form is given at that time, and additional discussion ensues prior to entry on study. The consent form also includes a HIPAA authorization statement. This protocol therapy is very complicated to understand and is intended to be curative in nature. Since subjects under 14 may not fully understand the therapy and its intent we have requested a waiver of assent. We will include subjects in the family meeting and discuss the therapy in an age appropriate discussion. We will obtain assent from subjects 14 years and older.. This discussion may occur with or without parents, depending upon the situation.

11.2. Data Confidentiality

- All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study.
- The anonymity of participating patients will be maintained. Patients will be identified by their unique patient number, not by name. Information related to this study will be kept in a locked cabinet in the BMT office and in a password protected institutional compliant database.
- There is no set time for destroying the information that will be collected for this study. We will continue to analyze data for many years and it is not possible to know when they will be completely done.

11.3. Duration of the Study

- The maximum duration of the study for each patient will be approximately 2 year from day of PSCT. The study will close when all patients have completed 2 year follow-up, have died, or are lost to follow-up.

12.0. REFERENCES

1. Piel FB, Steinberg MH, Rees DC. Sickle Cell Disease. The New England journal of medicine 2017;376:1561-73.
2. Maitra P, Caughey M, Robinson L, et al. Risk factors for mortality in adult patients with sickle cell disease: a meta-analysis of studies in North America and Europe. Haematologica 2017;102:626-36.
3. Arnold SD, Jin Z, Sands S, Bhatia M, Kung AL, Satwani P. Allogeneic Hematopoietic Cell Transplantation for Children with Sickle Cell Disease Is Beneficial and Cost-Effective: A Single-Center Analysis. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2015;21:1258-65.
4. Gardner K, Douiri A, Drasar E, et al. Survival in adults with sickle cell disease in a high-income setting. Blood 2016;128:1436-8.
5. Nevitt SJ, Jones AP, Howard J. Hydroxyurea (hydroxycarbamide) for sickle cell disease. Cochrane Database Syst Rev 2017;4:CD002202.
6. Torres L, Conran N. Emerging pharmacotherapeutic approaches for the management of sickle cell disease. Expert Opin Pharmacother 2019;20:173-86.
7. Chou ST, Fasano RM. Management of Patients with Sickle Cell Disease Using Transfusion Therapy: Guidelines and Complications. Hematology/oncology clinics of North America 2016;30:591-608.
8. Sayani FA, Kwiatkowski JL. Increasing prevalence of thalassemia in America: Implications for primary care. Ann Med 2015;47:592-604.
9. Kwiatkowski JL. Current recommendations for chelation for transfusion-dependent thalassemia. Ann N Y Acad Sci 2016;1368:107-14.
10. Porter J. Beyond transfusion therapy: new therapies in thalassemia including drugs, alternate donor transplant, and gene therapy. Hematology Am Soc Hematol Educ Program 2018;2018:361-70.
11. Gluckman E. Allogeneic transplantation strategies including haploidentical transplantation in sickle cell disease. Hematology Am Soc Hematol Educ Program 2013;2013:370-6.
12. Vermylen C, Cornu G. Hematopoietic stem cell transplantation for sickle cell anemia. Current opinion in hematology 1997;4:377-80.
13. Walters MC, Patience M, Leisenring W, et al. Bone marrow transplantation for sickle cell disease. The New England journal of medicine 1996;335:369-76.
14. Bernaudin F, Socie G, Kuentz M, et al. Long-term results of related myeloablative stem-cell transplantation to cure sickle cell disease. Blood 2007;110:2749-56.
15. Panepinto JA, Walters MC, Carreras J, et al. Matched-related donor transplantation for sickle cell disease: report from the Center for International Blood and Transplant Research. British journal of haematology 2007;137:479-85.
16. Locatelli F, Kabbara N, Ruggeri A, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood 2013;122:1072-8.
17. Hsieh MM, Kang EM, Fitzhugh CD, et al. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. The New England journal of medicine 2009;361:2309-17.
18. Saraf SL, Oh AL, Patel PR, et al. Nonmyeloablative Stem Cell Transplantation with Alemtuzumab/Low-Dose Irradiation to Cure and Improve the Quality of Life of Adults with Sickle Cell Disease. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2016;22:441-8.
19. King AA, Kamani N, Bunin N, et al. Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. American journal of hematology 2015;90:1093-8.
20. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. The New England journal of medicine 1990;322:417-21.
21. Sabloff M, Chandy M, Wang Z, et al. HLA-matched sibling bone marrow transplantation for beta-thalassemia major. Blood 2011;117:1745-50.
22. Sodani P, Gaziev D, Polchi P, et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. Blood 2004;104:1201-3.
23. Mentzer WC, Heller S, Pearle PR, Hackney E, Vichinsky E. Availability of related donors for bone marrow transplantation in sickle cell anemia. Am J Pediatr Hematol Oncol 1994;16:27-9.
24. Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med 2014;371:339-48.

25. Shenoy S, Eapen M, Panepinto JA, et al. A trial of unrelated donor marrow transplantation for children with severe sickle cell disease. Blood 2016;128:2561-7.
26. Krishnamurti L, Neuberg DS, Sullivan KM, et al. Bone Marrow Transplantation for Adolescents and Young Adults with Sickle Cell Disease: Results of a Prospective Multicenter Pilot Study. Am J Hematol 2019.
27. Shenoy S, Walters MC, Ngwube A, et al. Unrelated Donor Transplantation in Children with Thalassemia using Reduced-Intensity Conditioning: The URTH Trial. Biol Blood Marrow Transplant 2018;24:1216-22.
28. La Nasa G, Argiolu F, Giardini C, et al. Unrelated bone marrow transplantation for beta-thalassemia patients: The experience of the Italian Bone Marrow Transplant Group. Ann N Y Acad Sci 2005;1054:186-95.
29. Kamani NR, Walters MC, Carter S, 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;18:1265-72.
30. Ruggeri A, Eapen M, Scaravadou A, et al. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease. Biol Blood Marrow Transplant 2011;17:1375-82.
31. Abraham A, Cluster A, Jacobsohn D, et al. Unrelated Umbilical Cord Blood Transplantation for Sickle Cell Disease Following Reduced-Intensity Conditioning: Results of a Phase I Trial. Biol Blood Marrow Transplant 2017;23:1587-92.
32. Dallas MH, Triplett B, Shook DR, et al. Long-term outcome and evaluation of organ function in pediatric patients undergoing haploidentical and matched related hematopoietic cell transplantation for sickle cell disease. Biol Blood Marrow Transplant 2013;19:820-30.
33. Bolanos-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. Blood 2012;120:4285-91.
34. de la Fuente J, Dhedin N, Koyama T, et al. Haploidentical Bone Marrow Transplantation with Post-Transplantation Cyclophosphamide Plus Thiotepa Improves Donor Engraftment in Patients with Sickle Cell Anemia: Results of an International Learning Collaborative. Biol Blood Marrow Transplant 2018.
35. Sodani P, Isgro A, Gaziev J, et al. T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients. Pediatr Rep 2011;3 Suppl 2:e13.
36. Thompson AA, Walters MC, Kwiatkowski J, et al. Gene Therapy in Patients with Transfusion-Dependent beta-Thalassemia. N Engl J Med 2018;378:1479-93.
37. Cavazzana M, Mavilio F. Gene Therapy for Hemoglobinopathies. Hum Gene Ther 2018;29:1106-13.
38. Ribeil JA, Hacein-Bey-Abina S, Payen E, et al. Gene Therapy in a Patient with Sickle Cell Disease. N Engl J Med 2017;376:848-55.
39. Esrick EB, Manis JP, Daley H, et al. Successful hematopoietic stem cell mobilization and apheresis collection using plerixafor alone in sickle cell patients. Blood Adv 2018;2:2505-12.
40. Boulad F, Shore T, van Besien K, et al. Safety and efficacy of plerixafor dose escalation for the mobilization of CD34(+) hematopoietic progenitor cells in patients with sickle cell disease: interim results. Haematologica 2018;103:1577.
41. Oved JH, Wang Y, Barrett DM, et al. CD3(+)/CD19(+) Depleted Matched and Mismatched Unrelated Donor Hematopoietic Stem Cell Transplant with Targeted T Cell Addback Is Associated with Excellent Outcomes in Pediatric Patients with Nonmalignant Hematologic Disorders. Biol Blood Marrow Transplant 2018.
42. Bunin N, Aplenc R, Leahey A, et al. Outcomes of transplantation with partial T-cell depletion of matched or mismatched unrelated or partially matched related donor bone marrow in children and adolescents with leukemias. Bone Marrow Transplant 2005;35:151-8.
43. Bunin N, Aplenc R, Grupp S, Pierson G, Monos D. Unrelated donor or partially matched related donor peripheral stem cell transplant with CD34+ selection and CD3+ addback for pediatric patients with leukemias. Bone Marrow Transplant 2006;37:143-9.
44. Casper J, Camitta B, Truitt R, et al. Unrelated bone marrow donor transplants for children with leukemia or myelodysplasia. Blood 1995;85:2354-63.
45. Ash RC, Horowitz MM, Gale RP, et al. Bone marrow transplantation from related donors other than HLA-identical siblings: effect of T cell depletion. Bone Marrow Transplant 1991;7:443-52.

46. Bunin NJ, Casper JT, Lawton C, et al. Allogeneic marrow transplantation using T cell depletion for patients with juvenile chronic myelogenous leukemia without HLA-identical siblings. Bone Marrow Transplant 1992;9:119-22.
47. Gordon PR, Leimig T, Mueller I, et al. A large-scale method for T cell depletion: towards graft engineering of mobilized peripheral blood stem cells. Bone Marrow Transplant 2002;30:69-74.
48. Barfield RC, Otto M, Houston J, et al. A one-step large-scale method for T- and B-cell depletion of mobilized PBSC for allogeneic transplantation. Cytotherapy 2004;6:1-6.
49. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. Annu Rev Immunol 2003;21:139-76.
50. Bethge WA, Haegele M, Faul C, et al. Haploidentical allogeneic hematopoietic cell transplantation in adults with reduced-intensity conditioning and CD3/CD19 depletion: fast engraftment and low toxicity. Exp Hematol 2006;34:1746-52.
51. Handgretinger R, Klingebiel T, Lang P, et al. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. Bone Marrow Transplant 2001;27:777-83.
52. Seif AE, Li Y, Aplenc R, et al. Favorable chronic graft-versus-host-disease, event-free, and overall survival rates following partially CD3-depleted alternative donor peripheral stem cell transplantation for pediatric hematologic malignancies. BMT Tandem Meetings; 2016; Honolulu, HI.
53. Vodanovic-Jankovic S, Drobyski WR. Gammadelta T cells do not require fully functional cytotoxic pathways or the ability to recognize recipient alloantigens to prevent graft rejection. Biol Blood Marrow Transplant 2006;12:1125-34.
54. Drobyski WR, Vodanovic-Jankovic S, Klein J. Adoptively transferred gamma delta T cells indirectly regulate murine graft-versus-host reactivity following donor leukocyte infusion therapy in mice. J Immunol 2000;165:1634-40.
55. Drobyski WR, Majewski D, Hanson G. Graft-facilitating doses of ex vivo activated gammadelta T cells do not cause lethal murine graft-vs.-host disease. Biol Blood Marrow Transplant 1999;5:222-30.
56. Drobyski WR, Majewski D. Donor gamma delta T lymphocytes promote allogeneic engraftment across the major histocompatibility barrier in mice. Blood 1997;89:1100-9.
57. Wilhelm M, Kunzmann V, Eckstein S, et al. Gammadelta T cells for immune therapy of patients with lymphoid malignancies. Blood 2003;102:200-6.
58. Gertner-Dardenne J, Castellano R, Mamessier E, et al. Human Vgamma9Vdelta2 T cells specifically recognize and kill acute myeloid leukemic blasts. J Immunol 2012;188:4701-8.
59. Bonneville M, O'Brien RL, Born WK. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. Nat Rev Immunol 2010;10:467-78.
60. Godder KT, Henslee-Downey PJ, Mehta J, et al. Long term disease-free survival in acute leukemia patients recovering with increased gammadelta T cells after partially mismatched related donor bone marrow transplantation. Bone Marrow Transplant 2007;39:751-7.
61. Chaleff S, Otto M, Barfield RC, et al. A large-scale method for the selective depletion of alphabeta T lymphocytes from PBSC for allogeneic transplantation. Cytotherapy 2007;9:746-54.
62. Schumm M, Lang P, Bethge W, et al. Depletion of T-cell receptor alpha/beta and CD19 positive cells from apheresis products with the CliniMACS device. Cytotherapy 2013;15:1253-8.
63. Locatelli F, Merli P, Pagliara D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after alphabeta T-cell and B-cell depletion. Blood 2017;130:677-85.
64. Bertaina A, Merli P, Rutella S, et al. HLA-haploidentical stem cell transplantation after removal of alphabeta+ T and B cells in children with nonmalignant disorders. Blood 2014;124:822-6.

APPENDIX A

General risks of allogeneic hematopoietic stem cell transplantation:

- Infection: Bacterial, fungal, viral. These can be life threatening. Aggressive antibiotic use, including antifungal and antiviral therapy, will be initiated as needed. Weekly monitoring for CMV, adenovirus and EBV will be done until day +100.

- Acute Graft vs. host disease (GvHD): The target organs of acute GvHD include the skin, liver and gut. Symptoms can be mild or severe, and medication may not always control the symptoms. Severe acute GvHD (grades III and IV) is associated with an increased risk of mortality from infectious complications.

- Chronic GvHD occurs most commonly in patients who have had acute GvHD, but may occur in patients who did not have any acute symptoms. It may be limited to a single organ system or extensive in involvement of multiple organ systems. It usually develops after the third month post-transplant. It is more common in patients who have received peripheral stem cells or marrow from HLA mismatched donors without T cell depletion. Patients can have problems with skin, liver, intestine, joints, mucous membranes, eyes, or other organs. Scarring may result. Medicines can help, but may not completely eliminate all the symptoms. Chronic GvHD may have lingering symptoms for years, or may go away completely. Infection is a major risk for patients with chronic GvHD, as immune systems may not return to normal. Severe extensive chronic GvHD is associated with an increased risk of mortality.

- Graft Rejection: This may be primary or secondary (following initial engraftment) with autologous reconstitution. A second transplant may be required. Risks of graft rejection are increased in patients with bone marrow failure syndromes compared to patients receiving transplant for other conditions. Graft rejection risk is also increased in patients receiving reduced intensity conditioning regimens, patients receiving HLA-mismatched donors, and patients receiving grafts with full T cell depletion.

- Bleeding: Risk is due to thrombocytopenia and mucosal/endothelial barrier disruption caused by transplant conditioning or underlying bone marrow failure

- Mucositis and diarrhea: Transplant conditioning therapy causes mucositis. This can result in painful mouth sores and diarrhea. Narcotic pain medicine is generally required for mucositis, which resolves upon engraftment.

- Capillary leak syndrome: This may occur as a result of chemotherapy and radiation therapy. The blood vessels may become “leaky” and fluid enters the abdominal cavity and tissues. Swelling may result and this may result in or worsen renal failure. Pulmonary capillary leak may cause respiratory failure or death.

- Veno-Occlusive Disease (VOD): VOD can occur as a result of chemotherapy, radiation therapy, or both. Symptoms include jaundice, with liver dysfunction, weight gain, and extra fluid in the abdominal cavity. It may often be managed successfully, and completely resolve. However, complications can arise that can be fatal.

- Unexpected organ damage: This includes unpredictable life-threatening heart, lung, kidney, or liver damage may occur as a result of conditioning and other factors that occur post SCT. Multisystem organ failure usually results in death despite intensive care treatment.

- Late effects from conditioning and/or prior therapy: These risks may include hypothyroidism, sterility, decreased renal function, and decreased heart and lung function. Secondary malignancies may also occur as a result of chemotherapy and/or radiation therapy. The risks of conditioning and prior therapy upon the developing brain are unknown.

APPENDIX B

Drug Information

CYCLOPHOSPHAMIDE (CYTOXAN):

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Loss of appetite (L), nausea (L), vomiting (L)	Metallic taste (L), abdominal pain, diarrhea	Temporary blurred vision, heart damage with abnormal heart rhythms, abnormal hormone function affecting levels of salt in the blood and urine, causing too much or too little urine (SIADH), nasal congestion/discomfort, rash, anaphylaxis
Prompt: Within 2-3 weeks, prior to next course	Decrease in the number of red and white blood cells and platelets made in the bone marrow, hair loss, immune suppression	Bleeding and inflammation of the urinary bladder (hemorrhagic cystitis) (L)	decay of muscle tissue in the heart, impaired wound healing
Delayed: Any time later during therapy, excluding the above conditions	Decreased ability of the body to fight infection or disease, absence of sperm or stopped monthly periods, inability to have children (L), ovarian dysfunction		Damage/scarring of lung tissue [3] (L)
Late: Any time after completion of treatment			A new cancer or leukemia resulting from this treatment, damage/scarring of bladder tissue

(L) Toxicity may also occur later.

FLUDARABINE:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, diarrhea, fatigue, weakness, cough, poor appetite, fever, shortness of breath	Edema, rash, diarrhea, abdominal pain, headache, back pain, flu-like syndrome	Severe allergic reaction, dehydration
Prompt: Within 2-3 weeks, prior to the next course	Depression of bone marrow function causing bleeding, anemia, low blood counts, decreased appetite, increased susceptibility to infection.	Weight loss, gastrointestinal bleeding, lung inflammation, hemoptysis, hearing/vision changes	Opportunistic infections and reactivation of latent viral infections (L), EBV associated lymphoproliferative disorder, pulmonary hypersensitivity and toxicity, pericardial effusion, severe skin toxicity, liver failure, renal failure, hemorrhage
Delayed: Any time later during therapy, excluding the above conditions			Neurotoxicity including seizures, weakness, coma, stroke, death, ventricular arrhythmia, congestive heart failure, autoimmune conditions
Late: Any time after completion of treatment			

THIOTEPA:

	Common Happens to 21-100 children out of every 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Nausea, vomiting, loss of appetite, weakness, fatigue	Pain at the injection site, dizziness, headache, abdominal pain, contact dermatitis/ rash, vision changes	Severe allergic reaction (anaphylaxis, hives, wheezing, difficulty breathing)
Prompt: Within 2-3 weeks, prior to next course	Decrease in the number of red and white blood cells and platelets made in the bone marrow. At high doses used before marrow transplants: mouth sores, inflammation of the passage between the throat and stomach, skin redness, peeling, blisters	At high doses used before marrow transplants: inappropriate behavior, confusion, drowsiness, increased liver enzymes in the blood, increased bilirubin in the blood, darkening of the skin	Sudden high fever conjunctivitis, urine symptoms
Delayed: Anytime later during therapy, excluding the above conditions	Absence of sperm or stopped monthly periods, inability to have children, ovarian dysfunction		Hair loss, secondary cancer

THYMOGLOBULIN (ATG):

	Common Happens to 21-100 children out of every 100 children	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Fever, hives, shortness of breath	wheezing, trouble breathing, pain (chest, back, headache), nausea, vomiting, diarrhea, itching	Severe allergic reaction (anaphylaxis, hives, wheezing, difficulty breathing), hypertension, hypotension, syncope, swelling pulmonary edema, tachycardia, malaise
Prompt: Within 2-3 weeks, prior to the next course	Decrease in number of lymphocytes in blood, thrombocytopenia	increased risk of infections, abnormal renal function	liver abnormalities, hemolysis
Delayed: Any time later during therapy, excluding the above conditions		Serum sickness (L), which can cause renal dysfunction, fever, muscle and joint aches	Increase in viral infections, lymphoma from Epstein Barr virus

(L) Toxicity may also occur later.

RITUXIMAB (Rituxan):

	Common Happens to 21-100 children out of 100	Occasional Happens to 5-20 children out of every 100	Rare Happens to <5 children out of every 100
Immediate: Within 1- 2 days of receiving drug	Fever, chills, weakness, rigors, lethargy, malaise, nausea	Vomiting, pain (headache, throat, abdomen, back, joints), hypotension, hypertension, arrhythmias, diarrhea, rash, hives, itching, cough, nasal congestion dizziness, night sweats	Heart arrhythmia, Fatal infusion reactions, tumor lysis syndrome and associated electrolyte imbalance and renal failure, serum sickness, seizures,
Prompt: Within 2-3 weeks, prior to the next course	Low lymphocytes and increased risk of viral, bacterial and fungal infections, Night sweats, dizziness, high blood sugar	Face swelling (angioedema), decreased white blood cells, sinusitis, high blood sugar	Pancreatitis, ulcerative and other severe skin reactions, autoimmune phenomena causing decreases in blood counts
Delayed: Any time later during therapy, excluding the above conditions	Decreased immunoglobulins		Severe, possibly fatal skin reactions/rashes, bowel obstruction/perforation
Late: Any time after Completion of therapy			New cancer, severe viral infections that can cause liver failure, lung disease, and Inflammation of the brain tissue caused by a virus, chronic lung disease, fatal cardiac failure

Appendix C
Performance Status Scales/Scores

Karnofsky		Lansky	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal
90	Able to carry on normal activity, minor signs of symptoms of diseases	90	Minor restrictions in physically strenuous activity
80	Normal Activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work	70	Both greater restriction of and less time spent in play activity
60	Required occasional assistance, but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
50	Requires considerable assistance and frequent medical care	50	Gets dressed but lies around much of the day; no active play, able to participate in all quiet play and activities
40	Disabled, requires special care and assistance	40	Mostly in bed; participates in quiet activities
30	Severely disabled, hospitalization indicated. Death not imminent	30	In bed; needs assistance even for quiet play
20	Very sick, hospitalization indicated. Death not imminent	20	Often sleeping; Play entirely limited to very passive activities
10	Moribund, fatal processes progressing rapidly	10	No play; Does not get out of bed