COLUMBIA UNIVERSITY MEDICAL CENTER

Herbert Irving Comprehensive Cancer Center Protocol

Siplizumab-based Conditioning for Hematopoietic Stem Cell Transplantation in Patients with Advanced Sickle Cell Disease (CD2 SCD)

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1. BACKGROUND AND RATIONALE

Sickle cell disease is the most common monogenic disorder with high prevalence in sub-Saharan Africa, the Mediterranean basin, the middle east, and India.[1] Sickle cell disease (SCD) represents a global health problem where approximately 300,000 infants are born annually with the disease when defined as homozygosity for the sickle hemoglobin gene.[1]

Clinical outcomes of patients with SCD have improved over the years as a result of supportive and preventative care measures implemented early on after birth as well as therapy with hydroxyurea. Measures like penicillin prophylaxis in children, stroke prevention by transcranial doppler screening and regular transfusions, and hydroxyurea therapy have contributed to decreased burden of disease during childhood and improved survival to adulthood. Although incidence of acute chest syndrome decrease with age, the incidence is estimated to be 8.8/100,000 patients-years in adults,[2] with overall prevalence of 74.5%.[3] Admission rate of greater than 1 per year for veno-occlusive crises can be found in 16% of patients.[4] History of stroke can be found in 15.7% of patients.[3] There is evidence to support increased incidence of organ complications in adults as they age.[4] Surviving adults however continue to experience the negative impact of SCD.[5] Survival estimates for adults with SCD are in the 58 – 67 years range and an additive detrimental effect of comorbidities is observed.[3,6] It has been reported that a majority of patients (48%) will develop some form of irreversible organ damage of the kidneys, lungs, brain, retina or bones by the fifth decade of life.[7] The most common forms of organ damage include gall bladder disease, avascular necrosis, chronic lung disease, leg ulcers, priapism, renal failure, and retinopathy. Clinical cerebral infarction has been noted to precede intracranial hemorrhage which is a leading cause of death in adults. Pulmonary hypertension is prevalent in adults with up to 10% incidence of moderate to severe pulmonary hypertension, which is associated with a high risk of death.[8] Pulmonary hypertension, defined as tricuspid regurgitant jet velocity >2.5 m/s can be found in 32% of patients.[9] Renal failure occurs in 11.6% of patients and mortality is up to 3 fold higher in SCD patients with ESRD when compared to those without SCD.[7,10] The high medical burden of sickle cell disease contributes to the high mortality that increases with each decade of age.[7] SCD also affects the quality of life in patients.[5] Painful crises continue to occur in adulthood with a wide variation among sickle cell patients, but up to 20% of patient have weekly to monthly episodes requiring medical attention and chronic pain syndromes are a well-recognized entity in sickle cell disease requiring medical attention.[11]

1.1 HSCT as Curative Therapy for SCD

HSCT remains the only curative treatment strategy for SCD.[1,12,13] In children, HSCT from HLA-identical siblings resulted excellent survival and event-free survival above 90% in several studies.[14-17] Successful HSCT resulted in resolution of sickle cell disease related complications with cessation of episodes of painful crises, ACS, or stroke.[18] Long term follow up showed stable or improved CNS disease on imaging and improvement in pulmonary function.[14] In adults, non-myeloablative conditioning regimens have been explored with excellent success when an HLA-identical sibling donor is available.[19-21] The main limitation to this approach is the availability of HLA-identical siblings as demonstrated in the aforementioned study when the majority of screened patients did not have an HLA-identical sibling.

Published reports on the use of unrelated donors include a BMT CTN phase II study where alemtuzumab, fludarabine and melphalan were used as a conditioning regimen.[22] The reported 2-year EFS was 69% and OS was 79%. There was a high incidence of acute GVHD (28%) and extensive

chronic GVHD was 38%. There was also a high incidence of PRESS in the first 6 months (34%) and the study authors concluded that this regimen was not sufficiently safe. Major limitation of this approach remains the availability of matched unrelated donors through the registry particularly among patients of African descent who are disproportionately affected by this disease.[23]

Other graft sources have been explored, such as unrelated cord blood transplantation in children.[24] In a phase I study where thiotepa was added to the reduced intensity conditioning regimen, there was significant occurrence of autologous reconstitution, GVHD, and viral infection though GVHD was generally mild and patients recovered from viral infections. Haploidentical donor HSCT has improved access to HSCT for patients with hematologic malignancies with outcomes non-inferior to unrelated donor transplant.[25] The most important development in haploidentical donor HSCT is the use of post-transplant high dose cyclophosphamide (PTCy) given on days +3 and +4 after graft infusion. High dose cyclophosphamide is toxic to lymphocytes but spares stem cells as they have high levels of aldehyde dehydrogenase which metabolizes cyclophosphamide.[26] The timing of high dose cyclophosphamide takes advantage of selective elimination of alloreactive lymphocytes over resting non-alloreactive T cells.[27] This strategy was employed in sickle cell disease with the addition of ATG to the standard non-myeloablative regimen used.[28] Fourteen patients were transplanted from HLA-haploidentical donors with minimal GVHD reported and no mortality observed. The main limitation to this approach was the high incidence of graft failure (43%). In the meantime, conditioning protocols based on the PTCy approach have been further refined and shown to achieve higher rates of engraftment of mismatched grafts by intensifying 1. pre-conditioning immunosuppression [29] to minimized HVH-responses and 2. Increasing conditioning intensity [30]. An alternative approach to haplo-HSCT and PTCy is to use combined *in vitro* CD3/CD19 [31] or TCRa/b depletion of the graft [32].

1.2 Barriers to HSCT: Donor Availability

Several barriers exist to the universal application of HSCT therapy for patients with SCD. Most commonly cited barrier to HSCT in sickle cell disease is lack of an HLA-identical sibling.[33] Indeed, in the study by Hsieh et al, 88 of 112 patients whose siblings received HLA typing did not have a sibling match.[21] Although the use of unrelated donors expands access to HSCT for those lacking an HLA-identical sibling, patients of African descent are known to have poor availability of HLA-identical donors giving rise to the utilization of alternative donors in various HSCT settings.[23] The use of haploidentical donors can improve access to HSCT, however published evidence has not demonstrated sufficient efficacy given the high rate of graft failure, up to 43%.[28] Recent studies using pre-conditioning immunotherapy to overcome recipient allo-immunization have garnered encouraging results. Further research is needed to improve the safety and efficacy of HSCT from haploidentical donors. Continued optimization of HSCT with MUD, MMUD or haploidentical donors would allow most patients with SCD to have a donor option that allows them access to HSCT.[34,35] Gene therapy may provide a promising strategy to overcome lack of donor availability in patients with SCD and other hemoglobinopathies without causing treatment-related morbidity or mortality due to the immunogenetic disparity between donor and recipient. Recent progress in gene-therapeutic based on gene-addition or gene-editing approaches has shown great promise and yielded very encouraging results. [36,37]

1.3 Barriers to HSCT: SCD-related Co-morbidities

HSCT carries several risks which contribute to TRM decreased quality of life. These include infection, graft-versus-host disease (GVHD), infertility, and graft failure. This necessitates a risk-benefit

analysis tailored to the burden of disease in each patient. Myeloablative regimens can achieve a long term OS and EFS of 93.1% and 86.1% respectively, however there is a 10-20% risk of GVHD and 6.9% risk of TRM.[38] The increased use of reduced intensity conditioning regimens and nonmyeloablative regimens in HSCT for SCD has encouraged patients and providers alike to consider HSCT for SCD patients which is reflected in results of nonmyeloablative regimens for patients with matched sibling donors.[20,21]

Typical inclusion criteria for clinical trials of matched sibling HSCT reflect a disease burden that is predictive of early mortality. These criteria include evidence of end-organ damage (such as prior cerebrovascular event, sickle cell nephropathy, sickle cell hepatopathy, or tricuspid regurgitant jet >2.5m/s) or complications that affect quality of life and healthcare resource utilization such as frequent veno-occlusive crises, acute chest syndrome, osteonecrosis, or red cell alloimmunization.[15,20,28] Exclusion criteria that prohibit SCD patients from undergoing HSCT are not universally defined and largely determined by individual centers. With improvement in supportive care, these criteria are narrowing, but generally include poor performance status, hepatitis or evidence of portal fibrosis or cirrhosis on liver biopsy, several renal dysfunction, severe cardiac dysfunction, advanced sickle cell lung disease, poor compliance, uncontrolled infections or infection with HIV.[39] These exclusion criteria limit the use of HSCT in the sickest of SCD patients with the highest SCD-related mortality, where the risks of HSCT compare favorably to the risks of SCD disease. Co-morbid conditions, such as renal impairment, liver dysfunction, cardiac dysfunction, and pulmonary disease are widely recognized as risk factors for TRM and routinely assessed by the HCT-CI in most transplant centers.[40] Developing a non-toxic conditioning regimen that allows for safe HSCT in these patients would enable SCD patients with highest mortality risk to access a potentially curative HSCT.

A major advance in addressing this patient population was a study by Fitzhugh et al.[41] In that study, a dose escalation scheme was used to identify the dose of post-transplant cyclophosphamide needed for engraftment. An engraftment rate of 83% was achieved when cyclophosphamide was given on days +3 and +4 however only 50% of patients remained disease free. Nevertheless, donor HLA match ranged from 8/10 to 5/10. Furthermore, the target population for the study was patients with advanced sickle cell disease. Patients had a predicted 4- survival of less than 60% and included patients with cirrhosis, stage IV renal disease, ESRD, cardiac dysfunction and pulmonary hypertension. No stage II-IV GVHD was observed. This approach deserves further study despite failing to meet the investigators pre-specified endpoints.

It would be highly desirable to increase the therapeutic index of allogeneic transplant for patients with SCD by decreasing or minimizing acute (e.g. PRES, VOD, GVHD) and long term (e.g. infertility, secondary malignancies) conditioning toxicity. In this context novel approaches using non-genotoxic conditioning[42] are of highest interest and are entering clinical testing.

1.4 Rationale for Present Study

HSCT can provide durable protection from sickle cell disease and its complications when low toxicity is achieved as in HLA-identical siblings. While studies comparing allo-HSCT to best medical management in patients with SCD are lacking, the long-term benefit of allo-HSCT, and its potential to improve survival, can be inferred when there is documentation of stabilization or improvement in end organ function. Allo-HSCT from an HLA-identical sibling in children has been shown to improve pulmonary function and decrease CNS complications of SCD although gonadal dysfunction was persistent.[14] Successful allo-HSCT resulted in resolution of sickle cell disease related complications

with cessation of episodes of painful crises, ACS, or stroke.[18] In adults undergoing HLA-identical sibling transplant, a decrease in incidence of stroke and improvement in TRJV was documented.[20] Stabilization or even improvement in renal function has been reported as well.[39] HSCT using HLA-identical sibling as graft source has emerged as a standard of care for sickle cell patients with significant burden of disease and who are eligible for HSCT.

Nevertheless, the main limitation to HSCT in sickle cell disease is donor availability and the need for safe, non-toxic HSCT regimens for use when no HLA-identical sibling is available. A major development in addressing this patient population was a study by Fitzhugh et al as described above.[41] A conditioning regimen consisting of alemtuzumab 1 mg/kg total dose in combination with TBI 4Gy and post-transplant cyclophosphamide on days +3 and +4 achieved an engraftment rate of 83% and EFS of 50%. That study was characterized by a high level of donor mismatch, which ranged from 5/10 to 8/10 HLA allele match. Such low HLA match poses higher risk of graft failure especially in the SCD population who are not immune suppressed as patients with hematologic malignancies and are at higher risk of developing alloreactivity to potential donors given frequent transfusions.

In our proposed study, the conditioning regimen backbone will include siplizumab and TBI. This approach allows inclusion of patients with renal dysfunction. [43] Siplizumab is a humanized monoclonal antibody targeting CD2 which is expressed on T and NK cells. Siplizumab has been successfully used in treatment and conditioning of malignant and benign disorders, bone marrow transplantation and tolerance induction in the setting of combined bone marrow and renal transplantation (see 1.5). Administration distal (d-14) and proximal to stem cell infusion of siplizumab together with an anti-CD20 mAb is intended to achieve *in vivo* depletion of host and donor T, NK and B cells. Administration of low dose TBI on d-2, -1 has been included to facilitate donor cell engraftment[21] based on its stem cell but minimal systemic toxicity. [44]

The use of post-transplant cyclophosphamide serves as GVHD and rejection prophylaxis for haploidentical allogeneic stem cell transplant given post-transplant on days +3 and +4.[27,45] This application has been used in patients with sickle cell disease receiving allogeneic transplants from haploidentical donors with varying results.[28,41] Post-transplant cyclophosphamide is thought to exert its beneficial effect by eliminating donor and host allo-reactive T cells while preserving regulatory T cells.[46,47] Post-transplant cyclophosphamide has been mostly studied with haploidentical donors, where the HLA barrier is greatest, but has also been successfully applied to MMUD donor in patients with hematologic malignancies.[48] Post-transplant cyclophosphamide has also been successful as a single agent GVHD prophylaxis in patients with hematologic malignancies receiving grafts MRD and MUD donors.[49,50]

Sirolimus is used for GVHD and graft rejection risk prophylaxis in the HSCT setting. Common toxicities include thrombotic microangiopathy when used in combination with tacrolimus.[51] It has also been associated with a higher risk of VOD when used after myeloablative conditioning regimens, especially those containing busulfan.[52] These risks are minimized with the proposed non-myeloablative regimen and without the use of calcineurin inhibitors. It has been frequently in the setting of hematopoietic stem cell transplant for sickle cell disease due to its property to promote tolerance and sparing Treg cells in contrast to calcineurin inhibitors.[20,21] Furthermore, the use of sirolimus for sickle cell disease transplant has been attractive due to high incidence of posterior reversible encephalopathy syndrome in children treated with calcineurin inhibitors such as tacrolimus,[22] and has been safely used in the setting of non-myeloablative transplants.[20]

1.5 Use of Siplizumab

Siplizumab (TCD601; previously known as MEDI-507) is a non-agonistic, humanized, anti-CD2 monoclonal antibody of the IgG1κ class. Siplizumab binds to a unique epitope on human CD2, distinct from the CD58 binding site, with high affinity (kd – 5 nM), inhibiting co-stimulation and T-cell activation. In addition, the Fc portion of the siplizumab antibody binds to FcγR receptors on NK cells resulting in antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) mediated depletion of CD2+ lymphocytes. Siplizumab has been used as part of the conditioning regimen for HLA-mismatched, haploidentical hematopoietic cell transplantation alone or for combined HLA-mismatched kidney-bone marrow transplantation (CKBMT), a protocol that achieved allograft tolerance in association with transient mixed chimerism induction[13,14]. In recipients of hematopoietic cell transplantation to treat malignancies[15] and CKBMT recipients[16], a marked early enrichment in regulatory T cells (Tregs) was observed during the T-cell reconstitution phase. Considering this activity, siplizumab is expected to modulate T-cell memory and immune reactivity in the setting of HSCT.

The treatment regimen was selected in collaboration with the manufacturer, based on an exposure-response analysis as well as a thorough review of the literature. The goal of the high-exposure regimen is to initiate deep, host, T and NK cell depletion early and maintain adequate siplizumab exposure through the conditioning and peri-HSC infusion period to facilitate durable engraftment and reduce the risk of GvHD in the setting of HLA-mismatched HSCT. This approach is based on the work by Spitzer and colleagues[53] where two siplizumab regimens were investigated in the setting of haploidentical HSCT. The first regimen utilized a treatment regimen of 0.6 mg/kg siplizumab around the day of HSCT (Days -1, 0 and 1). Overall, this regimen resulted in GvHD avoidance and early multilineage mixed-lymphohematopoietic chimerism but was not durable. The second regimen utilized a treatment regimen of 0.6 mg/kg siplizumab on Days -5 and -6 only, resulting in full chimerism and enhanced engraftment, but insufficient donor T-cell depletion (Spitzer et al 2003). Taking this experience and augmenting the pre-transplant conditioning with a dose of siplizumab shown to result in deep T and NK cell depletion, e.g., 4.8 mg/kg, as demonstrated by the experience in the setting of T-cell lymphoma (See TCD601 IB Section 5, Table 5-4) we have developed the regimen presented in the protocol.

Based on the TCD601 IB, it is expected that siplizumab concentrations >1 ug/mL will result in T-cell depletion, but higher concentrations and duration of exposure are needed to facilitate NK-cell depletion due to a lower expression of CD2 and need for NK-NK mediated ADCC. The initial dose of 4.8 mg/kg siplizumab will result in a Cmax concentration of ~80 ug/mL that will quickly drop to approximately 15 ug/mL by Day -6, resulting in in full CD2 saturation during the first week. The single 0.6 mg/kg dose on Day -6 will increase concentrations into the range investigated by Spitzer and colleagues in their pre transplant regimen, maintaining that exposure through Day -1. Following the three-peri-transplant infusions of siplizumab, peripheral concentrations are expected to increase to ~30 ug/mL and persist for about 35 days. This exposure is expected to result in adequate host and donor T-cell depletion while sparing naïve T-cells. Similarly, the administration of PTCy on Study Days 3 and 4 has been included to deplete any remaining alloreactive T-cells not depleted through the ADCC activity of siplizumab.

Regarding the concern for B-cell related LPD, it is recognized that 9 cases are reported in the TCD601

IB (Section 5.2.3.1), occurring in two patient populations at a high risk of LPD, T-cell lymphoma and steroid resistant acute GvHD. It was noted in the IB that these cases have been adjudicated by the manufacturer and the report has been submitted to the IND (per ITB-Med: IND#144519 submission SN0007). Those 9 reported LPD cases are contrasted by several other populations presented in the IB where similar siplizumab exposures were achieved. The largest patient population, psoriasis, spanned several completed trials with cumulative doses and exposures that exceed those in the aGvHD studies. There were no cases of LPD in the psoriasis trials where dosing for up to 16 weeks was well tolerated. Similarly, there were no LPD cases reported in the setting of renal transplant tolerance, where cumulative doses of 1.9 mg/kg siplizumab were administered in combination with non-myeloablative conditioning (cyclophosphamide, thymic irradiation, and rituximab) and an unfractionated, haploidentical, bone marrow cell infusion.

Considering the proposed 7.2 mg/kg cumulative dose and overall exposure in this study, Roswarski and colleagues[54] recently reported on high-dose siplizumab administration in peripheral T-cell lymphoma. In this lymphoma trial, escalating siplizumab doses of 3.4, 4.8, 8.5 and 15 mg/kg were investigated in combination with EPOCH-R therapy. Subjects received up to 6 – 21 day cycles of siplizumab and DA-EPOCH-R with no reports of DLTs or EBV-related LPDs (Roswarski et al 2018). The authors attributed this absence of EBV-related LPD to the inclusion rituximab therefore B-cell depletion is included in this HCST protocol as discussed below

1.6 Use of Rituximab

The administration of rituximab on days -14 and -6 is based on two considerations: The first reason to include the administration of rituximab in the protocol is aimed at preventing the development of EBV-associated PTLD as has been used in other Siplizumab-containing regimen[55]. It has been well established in the general solid organ transplant and BMT field that the administration of rituximab prevents EBV reactivation and development of PTLD[56] [57] . The second reason is to reduce the risk of humoral graft rejection[58] in SCD patients who are known to be frequently presensitized.

2. STUDY OBJECTIVES.

2.1 Primary Objective

The primary objective of this study is a safety assessment by determining the failure rate which is defined as graft failure, grades III-IV GVHD, or death at 100 days.

2.2 Secondary Objectives

The secondary objectives endpoints of this study include survival outcomes as well as transplant-related outcomes which include

- Time to RBC, neutrophil and platelet recovery
- Incidence of GVHD of any grade
- Incidence of grade 3-4 acute GVHD and severe chronic GVHD
- Incidence of VOD, IPS, and CNS toxicities such as PRES, hemorrhage and seizures
- Incidence of virus infections and reactivation which include CMV, EBV, and adenovirus as well as EBV-PTLD. We will also monitor fungal infections
- Donor chimerism at 100 days post-transplant and 1 year post-transplant
- Immune reconstitution following allogeneic HSCT

3. STUDY DESIGN/INVESTIGATIONAL INTERVENTION

3.1 General Design

The presented study is a phase I/II single center study to examine the safety and feasibility of HSCT for SCD subjects with advanced SCD-related co-morbidities.

This is a Phase I/II study. It is a three-stage design with a target sample size of at most 18 patients. At the first stage, a total of 6 patients will be recruited, the study will be stopped, if 2 or more of patients experience any of the following events: graft failure/Grade 3-4 acute GVHD/death in the first 100 days. If not, the study will proceed to the second stage and recruit additional 6 patients. The study will be stopped if 4 or more patients experience an event as specified above among the 12 patients. If not, the study will proceed to the third stage and recruit additional 6 patients, the study will be stopped any time if 6 or more patients experience an event among the 18 patients. The study will be stopped at any time if a patient death occurs. If the event rate is:

- $\leq$ 10%, then the probability of the study to stop early will be less than 0.12.
- $\leq$15%, then the probability of the study to stop early will be less than 0.24.
- $\leq$ 30%, then the then the probability of the study to stop early will be less than 0.67.
- >33%, then the then the probability of the study to stop early will be greater than 0.74

Data related to transplant-related outcomes will be collected for at least 1 year post-HSCT however long-term evaluations will be planned as per standard institutional guidelines.

3.2 Dose Limiting Toxicities

Dose limiting toxicities that would cause study cessation include graft failure, grade III-IV acute GVHD (as per CIBMTR criteria, detailed in appendix B), or death in the first 100 days. The study will be stopped any time if 5 or more patients experience an event among the 18 patients.

3.3 Number of Patients

It is a three-stage design with a target sample size of at most 18 patients. At the first stage, a total of 6 patients will be recruited, the study will be stopped, if 2 or more of patients experience dose limiting toxicities detailed above. If less than 2 patients experience the events mentioned above, the study will proceed to the second stage and recruit additional 6 patients. The study will be stopped if 3 or more total patients experience an event as specified above among the 12 patients. If not, the study will proceed to the third stage and recruit additional 6 patients. The study will be stopped any time if 5 or more patients experience an event among the 18 patients.

4. SUBJECT SELECTION AND WITHDRAWAL

4.1 Inclusion Criteria

4.1.1 Patient Inclusion Criteria

1. Patients with sickle cell anemia (Hb SS, Sβ0 thalassemia or severe SC) who are 18 – 50 years of age inclusive AND who have 1 or more of the following
    a. Clinically significant neurologic event (stroke) or any neurological deficit lasting at least 24 hours. Stroke will be defined as a clinically significant neurologic event that is accompanied by an infarct on cerebral MRI or cerebral arteriopathy requiring chronic transfusion therapy.

b. History of two or more episodes of ACS despite supportive care measures (i.e. asthma therapy and/or hydroxyurea).
c. History of two or more severe vaso-occlusive pain crises per year in the 2-year period preceding enrollment despite the institution of supportive care measures (i.e. a pain management plan and/or treatment with hydroxyurea).
d. Administration of regular RBC transfusion therapy, defined as receiving 8 or more transfusions per year for 1 year or more to prevent vaso-occlusive clinical complications (i.e. pain, stroke, and ACS)
e. An echocardiographic finding of tricuspid valve regurgitant jet (TRJ) velocity > or equal to 2.7 m/sec or pulmonary hypertension diagnosed by right heart catherization.
f. Chronic kidney disease including patients on hemo-dialysis
g. Recurrent tricorporal priapism defined as at least 2 episodes of an erection last ≥4 hours involving the corpus cavernosa and corpus spongiosa.

2. Adequate organ functions as defined as:
    a. ECOG performance status of 2 or better
    b. Cardiac function: LVEF of 40% or greater
    c. Pulmonary Function: Pulse oximetry with a baseline oxygen saturation of 85% or greater and corrected DLCO of 35% or greater
    d. Hepatic Function: Serum conjugated (direct) bilirubin less than 3x upper limit of normal for age as per local laboratory, ALT and AST less than 5 x upper limit of normal as per local laboratory. Patients whose hyperbilirubinemia is the result of hyperhaemolysis, or a sever drop in hemoglobin post blood transfusion are not excluded.
    e. Absence of liver cirrhosis, bridging fibrosis and active hepatitis as documented by liver biopsy for patients with evidence of iron overload by serum ferritin or MRI. The histological grading and scale described by Ishak and colleagues (1995) will be used.

3. Patient must have a matched-or mismatched unrelated donor or mismatched related family donor.
    a. For HLA-matching we will assess 12 HLA-antigens (HLA-A, B, C, DRB1, DQB1 and DPB1).
    b. Fully matched unrelated transplanted are defined as matched at 12/12 HLA-alleles. We will include up to 7/8 (HLA-A, B, C, and DRB1) matched unrelated donors.
    c. One haplotype-mismatched related donors will be included.

4.1.2 Donor Eligibility and Selection Criteria

Please note, donor selection will follow our institutional standard operating procedure (SOP). Key criteria are summarized below for convenience:

1. Donor should be evaluated for eligibility to donate by an independent physician not directly caring for the patient on study protocol
2. Donor is willing to sign informed consent allowing the use of the PBSC product for the HSCT of the recipient
3. Donor must meet HLA match criteria outlined in the inclusion criteria above
4. Donor cannot be pregnant or lactating and must agree to contraception until after the donation procedure is complete

5. Testing negative for HIV and viral hepatitis
6. Free of Hb S (defined as Hb S less than 50%) and other hemoglobinopathies that are symptomatic or of clinical significance
7. Targeted minimum stem cell dose of 5.0 x 10e6 CD34 cells/Kg of recipient weight
8. Fulfills standard criteria for eligibility as a donor for HSCT

Note: HSCT can be deleterious for the developing fetus and pregnant mother due to the conditioning regimen, GVHD prophylaxis and treatment. Agents used in this study such as cyclophosphamide are pregnancy risk factor category D. Sirolimus is pregnancy risk factor category C. Radiotherapy also used (TBI) is a well-known teratogenic agent. Siplizumab is an investigational monoclonal antibody. Non-clinical embryo-fetal development studies have not been conducted with siplizumab, therefore the risk to a developing fetus is unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation (at least 1 year post transplant). Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study and for at least 1 year post transplant.

Finally, pregnancy and lactation restrictions and contraception requirements are also applicable to the donor. Filgrastim or other G-CSF analogues are pregnancy risk factor category C. The restriction lasts for 4 weeks after stem cell donation.

4.2 Exclusion Criteria

1. Pulmonary dysfunction defined as DLCO (corrected for hemoglobin and alveolar volume) < 35% of predicted OR baseline oxygen saturation of <85% or PaO2 <70.
2. Severe cardiac dysfunction defined as ejection fraction <45% or subjects who have been receiving chronic transfusion therapy for > 1 year and have evidence of iron overload (serum ferritin levels >1000 ng/mL), a cardiac MRI is required. Cardiac T2* <10 ms results in exclusion.
3. Liver iron content (LIC) ≥15 mg Fe/g dry weight on R2 MRI of liver, unless liver biopsy within 3 months prior to or at screening shows no evidence of bridging fibrosis or cirrhosis. Presence of bridging (portal to portal) fibrosis or cirrhosis in liver biopsy OR transaminases >5x ULN for age or direct bilirubin >3xULN.
4. Clinical stroke within 6 months of anticipated transplant
5. Karnofsky performance score < 50%
6. HIV infection
7. Uncontrolled viral, bacterial, fungal, or protozoal infection at the time of study enrollment.
8. Patient with unspecified chronic toxicity serious enough to detrimentally affect the patient's capacity to tolerate HSCT in the opinion of the investigator.
9. Patient unable to understand the nature and risks inherent in the HSCT process.
10. History of non-compliance severe enough in the estimation of the treating team to preclude the patient from undergoing unrelated donor transplantation.

11. Patient is pregnant or lactating.
12. Inability to provide adequate transfusion support or increased risk immunohematological complications due presence of anti-RBC antibody against stem cell donor.
13. Presence of donor-specific HLA antibodies

4.3 Inclusion of Women and Minorities

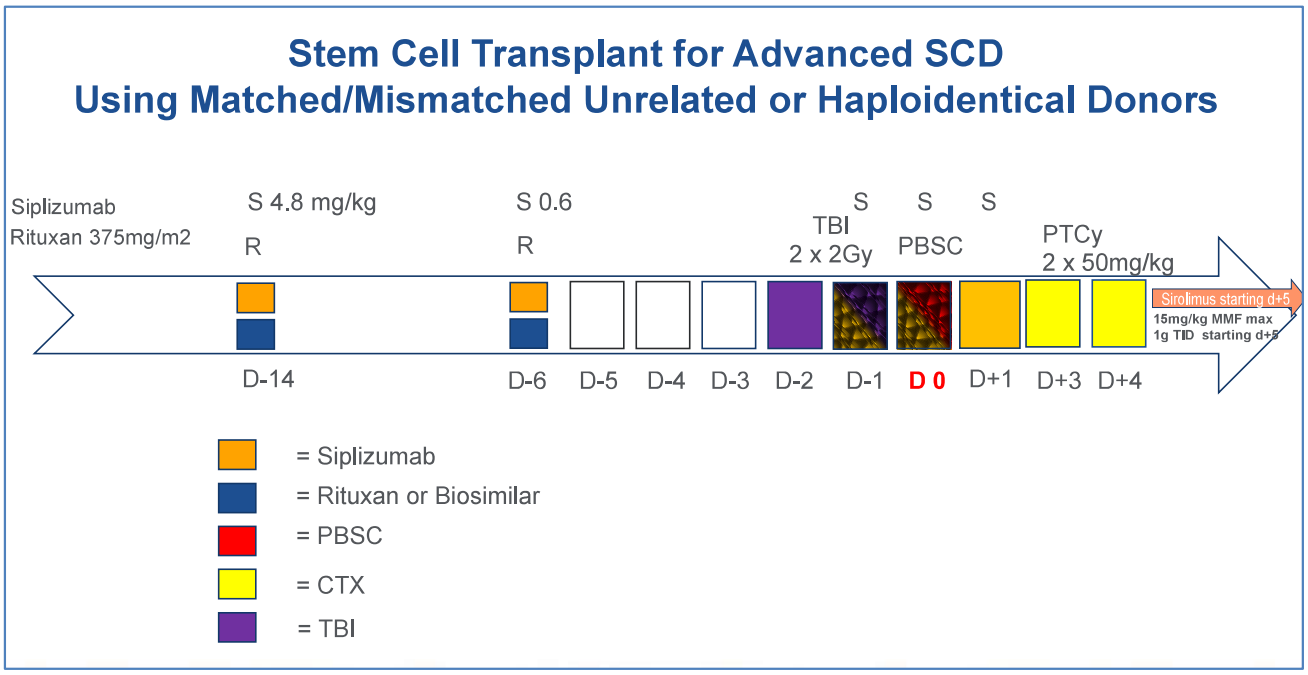
Both men and women of all races and ethnic groups are eligible for this trial. We anticipate higher participation from minority groups due to the increased incidence of sickle cell disease in patients of African or Caribbean origin (Piel et al, 2013). We do not anticipate high participation from white, pacific islander or native American populations due to the disease epidemiology and low prevalence in these populations. As for patients or Asian origin, there is significant incidence of sickle cell disease in India but the population serviced by our medical center is mostly of African or Hispanic origin and identification and hence we do not anticipate the ability to recruit a large number of patients from this ethnic group.

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	3	+	3	=	6
Not Hispanic or Latino	6	+	6	=	12
Ethnic Category: Total of all subjects	9	+	9	=	9
Racial Category					
American Indian or Alaskan Native	0	+	0	=	0
Asian	1	+	1	=	3
Black or African American	6	+	6	=	12
Native Hawaiian or other Pacific Islander	0	+	0	=	0
White	2	+	2	=	4
Racial Category: Total of all subjects	9	+	9	=	18

4.4 Subject Recruitment

Subject will be recruited from the hematology practice at CUIMC. Patients with high burden of sickle cell disease will be referred by our colleagues in the benign hematology practice for evaluation for eligibility to enroll on the study. The study will also be listed on clinicaltrials.gov and outside referral will be accepted. No patient directed advertisements are planned at the moment.

6. TREATMENT PLAN



6.3 Study Interventions and Agent Administration

Exchange Transfusion
Patient will undergo a red blood cell exchange transfusion to achieve a HgbS level < 20% prior to starting therapy to prevent the development of VOC.

Siplizumab
Premedication with diphenhydramine 50 mg PO is to be given 1 hour prior to siplizumab infusion. Premedication with hydrocortisone succinate 100 mg IV infused over 30 minutes and acetaminophen 650 mg PO are to be given at least 30 minutes prior to siplizumab infusion. Siplizumab 4.8 mg/kg total is given on d-14 and 0.6mg/kg is given on days -6, -1, 0, and +1. It will be given as an IV infusion over 1 hour.

TBI
Radiation dose is listed above. Radiation source and dose rates will be according to institutional practice. TBI may be delivered from either linear accelerator or Cobalt sources.

Stem Cell Infusion
Standard institutional procedures should be followed for the processing and administration of stem cell products for infusion. The infused graft under no circumstances is to be irradiated. No in-line leukocyte filter should be used and no medications or fluids should be given piggyback through the catheter lumen used for infusion of stem cells. Vital signs should be monitored before beginning the infusion and periodically during administration and in accordance with institutional guidelines. Pre-medications prior to the graft infusion will be according to the institutions standard practice. Benadryl, epinephrine, hydrocortisone, and oxygen be available at the bedside, as well as an emergency medical code cart available in the vicinity of the patient, for emergency use in case of an infusion reaction.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a Phase I/II study. It is a three-stage design with a target sample size of at most 18 patients. At the first stage, a total of 6 patients will be recruited, the study will be stopped, if 2 or more of patients experience any of the following events: graft failure/Grade 3-4 acute GVHD/death in the first 100 days. If not, the study will proceed to the second stage and recruit additional 6 patients. The study will be stopped if 4 or more patients experience an event as specified above among the 12 patients. If not, the study will proceed to the third stage and recruit additional 6 patients, the study will be stopped any time if 6 or more patients experience an event among the 18 patients. The study will be stopped at any time if a patient death occurs. If the event rate is:

- $\leq$ 10%, then the probability of the study to stop early will be less than 0.12.
- $\leq$15%, then the probability of the study to stop early will be less than 0.24.
- $\leq$ 30%, then the then the probability of the study to stop early will be less than 0.67.
- >33%, then the then the probability of the study to stop early will be greater than 0.74

Data related to transplant-related outcomes will be collected for at least 1 year post-HSCT however long-term evaluations will be planned as per standard institutional guidelines.

13.2 Size/Accrual Rate

Target sample size is 18 patients with an accrual rate of 1.5 patients per month.

13.3 Analysis of Secondary Endpoints

Statistical methods will be formulated in consultation with the study statistician and may be modified to accurately describe study events. Descriptive statistics and probabilities will be used to report incidence of other secondary endpoints

Cyclophosphamide and Mesna

Cyclophosphamide will be given on days +3 and +4 at a dose of 50 mg/kg. Hydration prior to cyclophosphamide should be given according to standard institutional practice. Day +3 dose should occur between 60 and 72 hours after graft infusion and day +4 post-transplant should occur approximately 24 hours after prior dose. Cyclophosphamide will be given as an IV infusion over 1-2 hours depending on volume or as per institutional standard practice. Cyclophosphamide will be dosed according to AIBW in patients weighing > 125% IBW. Mesna is to be given starting 1 hour prior to cyclophosphamide at the same dose (50 mg/kg) as a continuous infusion over 24 hours.

Corticosteroids as an anti-emetic agent is prohibited until 24 hours after the completion of post-transplantation cyclophosphamide with the exception of cases where it is needed for a medical emergency such as adrenal support or anaphylaxis.

Sirolimus

Sirolimus will be given orally with a loading dose of 12 mg, then followed by 4 mg orally daily. Dosage will be adjusted to a therapeutic target of 10 – 15ng/ml in first 3 months post transplant and 3-10 after 3 months. Sirolimus should be started on day +5. It will be continued until at least day 180. Sirolimus can be tapered or continued according to the treating physician discretion and the presence of absence of GVHD and/or degree of donor chimerism. Sirolimus should be continued if donor chimerism is less than 50% to prevent graft loss.

Rituximab or Biosimilar

Rituximab (375 mg/m2/dose) will be administered on Days -14 and -6. In those subjects receiving ongoing renal replacement therapy, rituximab should be administered several hours after hemodialysis. The first rituximab solution for infusion should be administered intravenously at an initial rate of 50 milligrams/hour (mg/hr). The rate may be escalated by 50mg/hr every 30 minutes to a maximum of 400 mg/hr. Subsequent infusions may be started at 100 milligrams/hour (mg/hr) and titrated by 100 mg/hr every 30 minutes to a maximum of 400mg/hr if the subject tolerated the first infusion.

Mycophenalate Mofetil (MMF)

MMF will be given at a dose of 15 mg/kg PO TID (based upon actual body weight) with the maximum total daily dose not to exceed 3 grams (1 g PO TID). MMF prophylaxis will begin on Day 5 post-transplant and will be discontinued after the last dose on Day 35 or may be continued If active GVHD is present.

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