

Protocol with Statistical Analysis Plan Cover Page:

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Title: **Non-myeloablative Phase I/II Haploidentical HCT Study for Patients with Sickle Cell Disease, Including Compromised Organ Function**

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Drug Name:	Briquilimab (JSP191)
IND Number:	157,986
Sponsor:	NHLBI, NIH
Manufacturer:	Jasper Therapeutics, Inc (briquilimab)

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. PROTOCOL SUMMARY

1.1 Synopsis

Title:	Non-myeloablative Phase I/II Haploidentical HCT Study for Patients with Sickle Cell Disease, Including Compromised Organ Function
Study Description:	Haploidentical hematopoietic cell transplantation offers a widely available curative option for individuals with sickle cell disease. The goal is to reverse SCD while avoiding unacceptable graft rejection, graft-versus-host disease, infectious complications, and hyperinflammatory responses. We hypothesize that a moderate amount of immunosuppression will maximize efficacy while avoiding unacceptable toxicity.
Objectives:	<p><i>Primary Objective:</i></p> <p>Evaluate the regimen success rate where success is defined as successful engraftment (persistent donor chimerism and free of acute SCD complications) and absence of acute grade 3 or higher GVHD or moderate to severe chronic GVHD evaluated at 1 year post-transplant.</p> <p><i>Secondary Objectives:</i></p> <ul style="list-style-type: none">– Event-free survival and overall survival.– Incidence of recipient-type hemoglobin defined as HbS $\geq 10\%$ when donors have HbAA and HbS $\geq 50\%$ when donors have sickle cell trait (HbAS).– The proportion of patients with myeloid chimerism $\geq 95\%$ at 1 and 2 years post-HCT.– Incidence of acute and chronic GVHD.– Prevalence of donor type hemoglobin at 1-year post-transplant in SCD patients who have not been transfused in the previous 3 months.– Incidence of viral reactivation and disease.– Incidence of autoimmune and hyperinflammatory complications.– Incidence of hematologic malignancies.– Transplant-related mortality. <p><i>Exploratory Objective:</i></p> <ul style="list-style-type: none">– Perform gene therapy research involving cell culture or genetic manipulation to produce normal or therapeutic hemoglobin on excess autologous CD34+ cells collected from recipients.– Evaluate the impact of this non-myeloablative conditioning regimen on organs including the heart, lung, kidneys, liver, brain, neurocognitive function, and endocrine organs– Evaluate the impact of this non-myeloablative conditioning regimen on quality of life.
Endpoints:	<p><i>Primary Endpoint:</i></p>

The percentage of SCD patients at 1 year (+/- 3 months) post-transplant who have not experienced graft failure and who are without severe graft-versus-host disease (defined as grade 3 and higher acute GVHD and moderate to severe chronic GVHD).

Secondary Endpoints:

Total hemoglobin and percent HbS levels
 Percent donor myeloid chimerism and donor CD3 chimerism
 Day of neutrophil engraftment
 Day of platelet engraftment
 RBC transfusion requirement
 Rates of acute and chronic GVHD
 Rates of viral reactivation and disease
 Rates of autoimmune and hyperinflammatory complications
 Transplant-related mortality
 Non-transplant-related mortality
 Rates of graft failure
 Rates of leukemia and related disorders

Exploratory Endpoint:

Completion of gene therapy research involving cell culture or genetic manipulation to produce normal or therapeutic hemoglobin on excess autologous CD34+ cells collected from recipients.
 Organ function and quality of life

Study Population:

Sample size: 30 recipients (15 per cohort) and 30 donors (15 per cohort). Up to 72 subjects may be enrolled to reach the target number of transplanted recipients.

Gender: Both

- Age: ≥ 18 years for recipients (pediatric subjects ≥ 16 years may be enrolled after 6 successful adult transplants); ≥ 4 years for donors

Phase:

Phase I/II

Description of

NIH Clinical Center

Sites/Facilities

Enrolling

Participants:

Description of Study

Non-myeloablative haploidentical peripheral blood stem cell transplantation that includes conditioning with briquilimab and abatacept

Intervention:

Study Duration:

8 years

Participant

5 years post-transplant

Duration:

1.2 Schema

Timeline Diagram for Transplant Recipients

Timing	Scheduling
Autologous hematopoietic stem cell mobilization and collection; timing varies depending on the clinical needs of the patient. The collection of autologous HSC may be performed prior to enrolling in this protocol, at another center, or as part of another protocol.	<ul style="list-style-type: none"> • 2-4 weeks prior to plerixafor: stop hydroxyurea (a) • Within 1 week of plerixafor: apheresis catheter insertion if necessary, red cell exchange or simple transfusion to target HbS of $\leq 30\%$ • Day of HSC mobilization and collection: aspirin (b) 325 mg and plerixafor 240 mcg/kg 4-6 hours prior to apheresis, followed by apheresis to achieve target cell dose of $2 \times 10^6/\text{kg}$ • Repeat aspirin, plerixafor and apheresis if necessary. An alternative to plerixafor may be used when insufficient HSC were collected.
4 to 8 weeks prior to hematopoietic progenitor cell infusion (transplant day)	Optimize hydroxyurea dosing (a), clinic visit(s), pre-transplant testing, pre-transplant consultation(s)
Within 1 week of transplant conditioning	<ul style="list-style-type: none"> • Red cell exchange, or simple transfusion to target HbS of $\leq 30\%$ (j) •
12 days prior to transplant (day -12) (c)	Last dose of hydroxyurea, sickle therapy, or iron chelation
Day -11 (window of day -13 to -6) (d)	Briquilimab infusion, 0.6 mg/kg
Days -7 to -3	Alemtuzumab 0.2 mg/kg/day
Day -1 (e)	<ul style="list-style-type: none"> • 400cGy TBI • Abatacept 10 mg/kg
Before peripheral blood stem cell infusion	Central venous catheter insertion
Day 0 (f)	Transplant Day
Day +3 (g)	Cyclophosphamide 50 mg/kg
Day +4 (h)	Initiate sirolimus

Days +5, +14 (i), +28 (i), +100 (i), and +180 (i)	Abatacept 10 mg/kg/dose
Day +100 to 5 years	Immediate and longer-term follow-up

- (a) May include other sickle specific or relevant therapy
- (b) May be substituted by another agent per PI discretion (e.g., aspirin allergy)
- (c) The last dose of hydroxyurea, sickle therapy, or iron chelation will be given one day before the start of briquilimab or alemtuzumab, whichever is earlier
- (d) One dose of briquilimab infusion to be given between d-13 or -6.
- (e) TBI will be delivered in one fraction of 400 cGy on day -1. Equally weighted opposed lateral beams will be used to encompass the total body with the patient positioned supine. Treatment will be delivered at an SAD of 6 meters (or other distance depending on the treatment room configuration). The prescription point will be the midplane at the maximal hip separation. The dose rate to midplane will be no more than 15 cGy per minute. Head and neck compensation will be used to increase dose homogeneity. Adjustments to treatment technique but not dose prescription may be made at the discretion of the treating radiation oncologist if deemed necessary. Gonadal shielding will be used in males as per Department of Radiation Oncology, unless refused
- (f) Target cell dose is $>10 \times 10^6$ CD34+ cells/kg
- (g) Cyclophosphamide 50mg/kg will be given only on day +3 for the 1st cohort and on days +3 and +4 for the 2nd cohort
- (h) See section 6 for sirolimus loading and maintenance dosing. Target trough levels between 5-15 ng/ml. Sirolimus will be initiated on day +4 for the 1st cohort and on day +5 for the 2nd cohort
- (i) Abatacept will be given on day +14 (+/- 7), +28 (+/- 14), day +100 (+/- 14), and day +180 (+/- 14). If the day +14 dose is delayed, the day +28 dose will be given at least 14 days later.
- (j) An exchange transfusion may not be performed before conditioning per PI/designee when the risks are expected to outweigh any potential benefits, for example in patients with a history of a severe delayed hemolytic transfusion reaction.

1.3 Schedule of Activities (SOA) for Transplant Recipients

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Sign consent	X										
Safety assessments											
Review medical history	X	X									
Review of medications	X	X			X	X	X	X	X	X	X
Review adverse events				X	X	X	X	X	X	X	X
Vital signs	X	X		X	X	X	X	X	X	X	X
Height (cm), weight (kg)	0	X		X	X ²	X ²	X ²	X ²	X	X	X
Efficacy Assessments											

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Physical Exam	X	X		X	X	X	X	X	X	X	X
Assessment of acute and/or chronic GvHD							X	X	X	X	X
ECOG or Lansky performance status		X				X	X	X	X	X	X
Neuropsychologic testing		X					X		X	X	
Reproductive questionnaires		X ¹⁵							X ¹⁵	X ¹⁵	X ¹⁵
PROMIS		X ¹⁵			0	0	X	0	X ¹⁵	X ¹⁵	X ¹⁵
Confirmatory Transplant labs	Screening	Baseline	Transplant Preparation	Inpatient	D+30	D+60	D100	6 months	1 year	2 years	Yearly
HLA A, B, C, DR, DQ(low resolution) ³	X										
HLA confirmatory A,B,C,DRB	X ³	0 ³									

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
HLA antibody screen, class 1	X	X ⁴									
HLA antibody screen, class 2	X	X ⁴									
KIR genotype		X									
Red cell phenotyping ₅	X										
STR Profile		X									
Safety and Efficacy labs	Screening	Baseline	Transplant Preparation	Inpatient	D+30	D+60	D100	6 months	1 year	2 years	Yearly
CBC with differential	X	X	O	X	X	X	X	X	X	X	X
Reticulocyte Count	X	X	O	2x/week	X	X	X	X	X	X	X
Hemoglobin electrophoresis, haptoglobin	X	X	X		X	X	X	X	X	X	X

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Hepatic panel, LDH	X	X	O	X	X	X	X	X	X	X	X
Iron, Transferrin, ferritin ²³	X	X	O		X	X	X	X	X	X	X
Acute Care, Mineral panel	X	X	O	X	X	X	X	X	X	X	X
Plasma cytokine panel		X			X	X	X	X	X	X	X
PT, PTT	X	X	O	2x/week	X	X	X	X	X	X	X
d-dimer	O	X							X	X	X
Total protein, uric acid, creatinine kinase		X			X	X	X	X	X	X	X
β-HCG ⁶	X	O									
Lipid Panel (fasting)	O	X		2x/month	X	X	X	X	X	X	X
Lipoprotein profile, apolipoprotein panel		X			X	X	X	X	X	X	X

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
CMV/EBV PCR ⁷		X		2 times weekly	X	X	X	X	X	0	0
Adenovirus		X		Weekly	X	X	X				
Sirolimus level				At least 2-3 times/week	X	X	X	X	X	0	0
ABO typing, antibody screen	X		X	2 times weekly	0	0	X	X	X	0	0
Transfusion transmitted viruses and pathogen testing ⁸	0	X	0								
Malaria and TB testing (as needed based on travel history)		0									
Cystatin C	X	X	X	0	X	X	X	X	X	X	X
Urine protein/creatinine ratio, albumin/creatinine ratio ²⁰	X	X	0	0	X	X	X	0	X	X	X

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Spot urine studies ²⁰		X									
Timed urine studies ²¹		X									
Osmolality, serum		X									
Endocrine testing ⁹		X					X	X	X	X	X
Hemoglobin genotyping ¹⁰	0										
CRP, pro-BNP		X		0	0	0	X	X	X	X	X
Troponin		X									
DAT	X	X									
ACTH stim test		X									
Oral glucose tolerance test		X									

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Efficacy labs	Screening	Baseline	Transplant Preparation	Inpatient	D+30	D+60	D100	6 months	1 year	2 years	Yearly
Isohemagglutinin titer		X					X	0	X	X	X
WBC chimerism					X	X	X	X	X	X	X
Lymphocyte TBNK subset; immunoglobulins quantitative		X			X	X	X	X	X	X	X
Monoclonal gammopathy of undetermined significance ²²		0	0	0	0	0	0	0	0	0	0
Research Samples	Screening	Baseline	Transplant Preparation	Inpatient	D+30	D+60	D100	6 months	1 year	2 years	Yearly
Research blood		X			X	X	X	0	X	X	X
CD34 samples ¹¹			X								
JSP191 PK samples ¹²				X							

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Alemtuzumab PK samples ¹³				X	X						
RBC Biology Samples to Functional Fluidics and Eaton Lab		0					0	0	0		
Cardiac fibrosis biomarkers		X							X	X	X
Inflammatory and coagulability biomarkers		0							0	0	0
Safety Procedures	Screening	Baseline	Transplant Preparation	Inpatient	D+30	D+60	D100	6 months (optional)	1 year	2 years	Yearly
Chest X-ray		X ¹⁴									
24hr Holter		X ¹⁴									
Autologous HSC backup (see schema)			X								
Consults: • Dental	0		X								

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
<ul style="list-style-type: none"> Infectious disease Transfusion medicine Eye Social worker Nutrition Radiation oncology 											
Efficacy Procedures	Screening	Baseline	Transplant Preparation	Inpatient	D+30	D+60	D100	6 months (optional)	1 year	2 years	Yearly
ECG	X	X ^{14, 15}							X	X ¹⁵	X
ECHO	X	X ^{14, 15}					X	X	X	X ¹⁵	X
PFT, six-min walk test ¹⁶	X	X ^{14, 15}						X	X	X ¹⁵	X
Brain MRI and MRA (non- contrast)	0	X ^{14, 15}							X	X ¹⁵	X ¹⁷

Study visit	Screening ¹	Baseline ¹	Transplant preparation	Inpatient	Post-transplant outpatient visits						
Timing				Day-11 to +28	D 30	D 60	D 100	6mo	1 y	2 y	Yearly
Window	Up to 90 days before baseline	Up to 90 days before transplant preparation	Up to 90 days before inpatient stay	+/- 3 days	+/- 7 day s	+/- 7 days	+/- 7 days	+/- 30 days	+/- 6 months	+/- 6 months	+/- 6 months
Abdominal ultrasound		X ^{14,15}									
Liver MRI		X							0	0	0
Cardiac MRI		X ¹⁵							0	X ¹⁵	0
Bone marrow aspirate and biopsy with research sample ¹⁸		X					X		X	0	0
Dexa scan		X ^{14,15}							X	0	X ¹⁹
Chest CT scan		X									

X: required; O: optional

1. Data from medical records within 6 months may be used.
2. Only weights will be measured.
3. Molecular HLA A, B, C, DR typing of patient and family members to confirm; HLA confirmatory testing of recipient once donor is identified.
4. HLA antibody testing will be repeated within 30 days of conditioning or if a new RBC antibody develops.
5. Extended red cell phenotyping, red cell genotyping, or testing per Transfusion Medicine consult.
6. Urine or blood pregnancy test for females of childbearing potential.
7. CMV and EBV PCR testing will occur at least once a week through at least 100 days post-HCT, then approximately every 2 weeks through day +180, and then approximately every 4 weeks through day +365. More frequent testing may be indicated for some patients.

8. *Labs required prior to autologous PBSC collection: HBsAg, HCV, HTLV-I/II, HbcAb (IgM and IgG), HBs antibody, HIV 1/2 antibody, West Nile Virus, HIV 1/2 antibody/ag combo, CMV IgM and IgG, EBV antibody panel, HAV antibody (IgM and IgG), HIV-1/HCV/HBV NAT, HSV 1/2 antibody, Toxoplasma (IgG and IgM), VZV antibody, Adenovirus PCR, CMV/EBV PCR, T. Cruzi, Syphilis Donor Screening, Babesia, and other transfusion transmitted lab testing. These may need to be repeated within 30 days of transplant date.*
9. *TSH, T3/triiodothyronine (total), FT4/thyroxine, growth hormone, insulin-like growth factor 1, ACTH, morning cortisol, fasting insulin, fasting glucose, serum fructosamine (or glycated albumin), 25-hydroxy vitamin D, FSH (males and females), LH (males and females), testosterone total and free (males only), anti-mullerian hormone (females only), prolactin (females only), estradiol (females only), progesterone (males and females). May use results within 6 months of transplant, or repeat at investigator discretion.*
10. *Confirmatory hemoglobin variant testing by other methods (e.g. alpha or beta globin sequencing, other electrophoresis) for hemoglobin A, F, SS, SC or S β -Thalassemia as appropriate.*
11. *CD34 samples (3 mL EDTA peripheral blood per PI's discretion).*
12. *JSP191 (3-4 mL SST 1 hour after the end of the briquilimab infusion, 24 hours after briquilimab infusion (+/- 4 hours), 72 hours after briquilimab infusion (+/- 4 hours), day of TBI (day -1, may be drawn with morning labs), day 0 (prior to PBSC infusion, may be drawn with morning labs). The timing and type of tubes used for these samples may be adjusted based on the PI's discretion (see section 8.2.2).*
13. *Alemtuzumab (3-4 mL SST at day 0, +28 (+/- 7). The timing may be adjusted based on the PI's discretion (see section 8.2.2).*
14. *May use results within 6 months of transplant, or repeat at investigator's discretion.*
15. *May use results from another NHLBI protocol. Results from this study may be shared with other NHLBI protocols (000479, 000697).*
16. *Spirometry including FEV1, FVC, DLCO. Repeat testing may be at investigator's discretion.*
17. *Will be repeated at 5 years if remains enrolled on the protocol.*
18. *Bone marrow studies include cytogenetics, next generation sequencing (including myeloid neoplasm panel), and flow cytometry with research samples (5mL LAV EDTA). Additional bone marrows (and associated studies) may be performed at the investigator's discretion.*
19. *After 3 years, DEXA scans may be performed as clinically indicated*
20. *Spot urine studies: urinalysis, phosphate, uric acid, osmolality. Urine protein/creatinine ratio and urine albumin/creatinine ratio and other urine studies will not be completed in anuric recipients. Optional for oliguric recipients. Urine protein/creatinine ratio and urine albumin/creatinine ratio may be ordered more frequently per PI/designee.*
21. *Timed urine studies (24-hour urine collection): creatinine, protein, albumin, phosphate, uric acid, creatinine clearance, and urine protein electrophoresis, will not be completed in anuric recipients. Optional for oliguric recipients.*
22. *Serum immunofixation electrophoresis, urine immunofixation electrophoresis, immunoglobulin free light chains.*
23. *Iron studies may be ordered more frequently as per PI/designee.*

2. INTRODUCTION

2.1 Study Rationale

Individuals with sickle cell disease (SCD) experience extensive morbidity, including debilitating painful events, cardiopulmonary complications and stroke, as well as early mortality. Adults with compromised organ function have an increased risk of premature mortality. Further, many patients with severe SCD do not have access to curative therapies for reasons including insufficient or non-existent insurance coverage and older age. Allogeneic hematopoietic cell transplantation (allo-HCT) is the most widely available cure for patients with SCD, but has been infrequently pursued due to its associated complications. The majority of patients who are otherwise eligible for allo-HCT do not have an HLA-matched sibling donor. On the other hand, the vast majority of patients have a haploidentical donor. In addition, the unacceptable risk of death from conventional myeloablative allo-HCT renders many patients, especially those with non-malignant disorders, ineligible for what may otherwise be curative therapy. Recently however, in both malignant and non-malignant disorders, it has been shown that these high intensity regimens are not necessary for engraftment and survival, and many centers are currently exploring non-myeloablative conditioning regimens in order to reduce the toxicity associated with this treatment modality. While successful engraftment has been reported in the majority of patients conditioned with reduced-intensity regimens, these regimens still carry significant toxicity and have not significantly reduced the risk of graft- versus-host disease (GVHD). Both myeloablative and reduced-intensity approaches are prohibitively harmful in patients with severe disease, including subclinical organ damage and compromised organ function. Further, as a non-myeloablative regimen should allow autologous recovery with a low risk of adverse consequences to the recipient if the graft should fail, graft failure is preferable to the development of severe GVHD. Our original non-myeloablative haploidentical HCT protocol was associated with a high incidence of graft rejection and myeloid malignancies, potentially due to insufficient immunosuppression and evolution of somatic mutations in hematopoietic cells exposed to genotoxic conditioning. The most recent non-myeloablative haploidentical HCT protocol with increased upfront immunosuppression led to unacceptable viral disease, Epstein-Barr Virus (EBV)- associated post-transplant lymphoproliferative disorder (PTLD), autoimmune complications, hyperinflammatory responses, and transplant-associated mortality, possibly due to excessive immunosuppression and immune dysregulation. Because of the inclusion of pentostatin, individuals with renal failure were excluded. As such, we propose the development of a non-myeloablative haploidentical HCT protocol with a moderate increase in immunosuppression from our original protocol with a regimen consisting of briquilimab (JSP191), an antibody targeting CD117 (c-Kit), alemtuzumab, abatacept, low-dose Total Body Irradiation (TBI), sirolimus, and post-transplant cyclophosphamide (PT-Cy) in adults with SCD, including those with compromised organ function.

2.2 Background

Sickle cell disease (SCD) is a well described genetic disorder associated with significant morbidity and mortality. It affects one of every 600 African-Americans in the United States alone. The disease is characterized by recurrent vaso-occlusive crises (VOC) as a consequence of abnormal hemoglobin polymerization in areas of low oxygen tension. As a result, patients develop functional asplenicism leading to a high risk of infections from encapsulated organisms, recurrent VOC, acute chest syndrome (ACS), pulmonary hypertension, kidney failure, and neurologic events, as well as sudden death as the most serious consequences of this disease¹. Chronic renal insufficiency is an independent risk factor for death². Those with end-stage renal disease (ESRD) have a particularly high risk of early death³. With a median follow-up of almost 7 years, 24 (75%) deaths were noted in patients with SCD who had ESRD compared to 5 (16%) deaths in patients without ESRD ($p < 0.0001$, age- and sex-adjusted hazard ratio 8.4 (95% confidence interval 3.2-23.5)⁴. Adult sickle cell patients with risk factors such as pulmonary hypertension⁵⁻⁷ and frequent hospitalizations for pain crises^{1,7,8} also experience early mortality. Survival was also decreased in adults with SCD who had more than one organ impaired in patients with heart, lung, and/or kidney damage⁹. Sickle hepatopathy and iron overload have been discovered to increase mortality in patients with SCD, as patients with ferritin > 1000 ug/L or direct bilirubin > 0.4 mg/dL experienced significantly decreased survival compared to patients with ferritin < 1000 ug/L and direct bilirubin < 0.4 mg/dL¹⁰ (see Figures 1-2 and ¹⁰).

Figure 1:

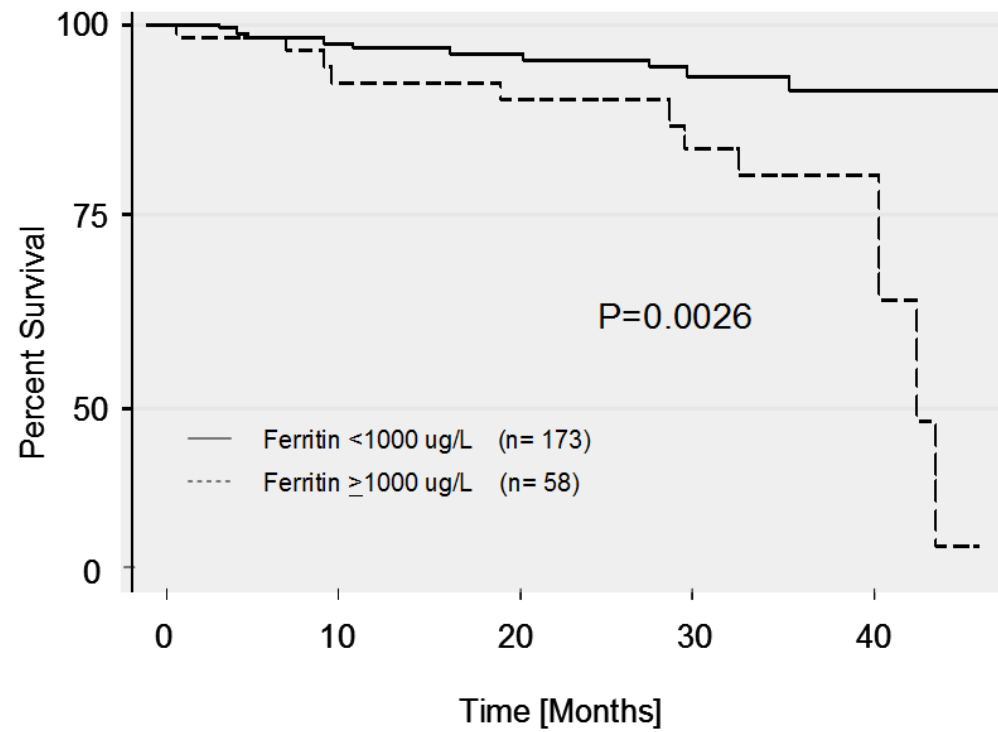
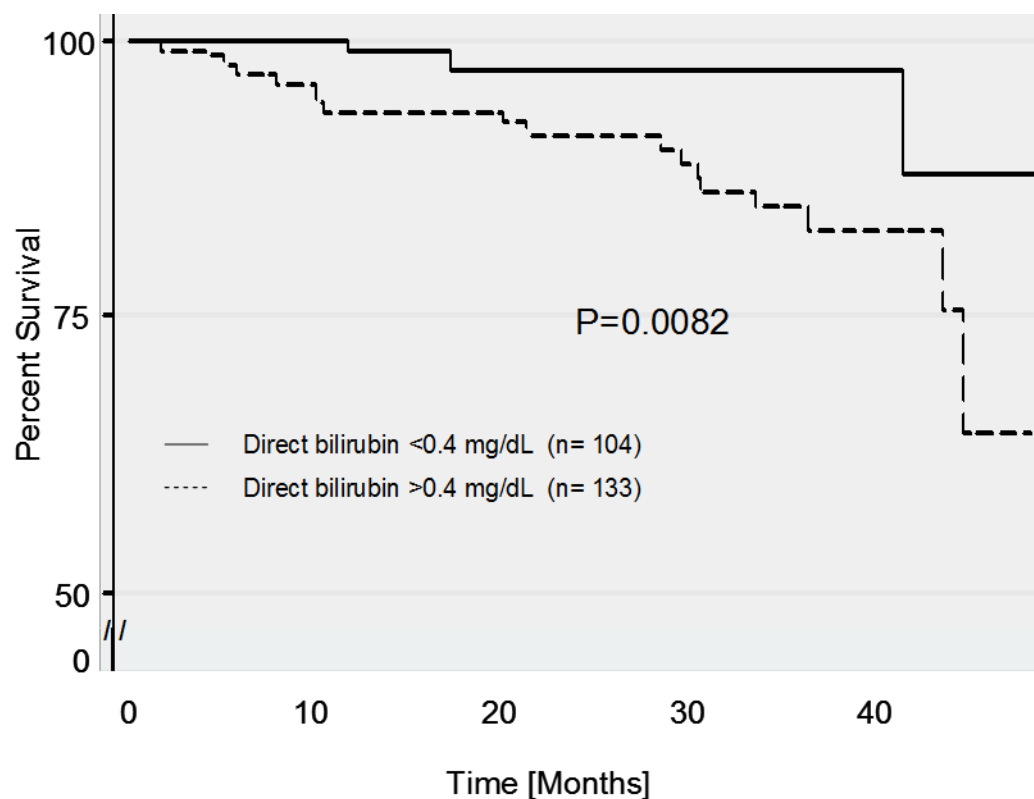


Figure 2:



In addition, patients with platelet counts in the lowest quartile of the cohort (<267,000/ul) had a 2.7-fold increase in direct bilirubin after controlling for WBC, to exclude the effect of generalized bone marrow suppression as a cause for thrombocytopenia (OR 2.70 95% CI 1.11-6.56, p=0.029).

The medical costs of this disease are enormous, with estimates of \$40,000 per patient per year (year 2000 figures) for chronic transfusion therapy and chelation alone, but do not include the impact on quality of life of those with the disease¹¹. More recent estimates ranged from \$275,143 to \$671,321 over a 5-year follow-up, depending on disease severity¹². An overlapping symptom complex also occurs in patients with the double heterozygous forms of SCD, such as sickle-C, and sickle β -thal⁰ disease, and in fact, these patients cannot always be differentiated clinically but only by means of a laboratory test. While transfusions can prevent neurologic events in patients at risk, iron overload is common, resulting in significant end-organ toxicity. The most common form of treatment in SCD had been erythrocyte transfusions, and more recently hydroxyurea¹³⁻¹⁵. Hydroxyurea results in a significant reduction in the number of painful crises per year and a decreased frequency of ACS¹⁴, and has become the treatment of choice for many individuals with SCD. Unfortunately, hydroxyurea is not curative, and does not appear to reverse established end-organ damage, especially in adults.

Several important interventions have led to an improvement in the overall life expectancy of patients with SCD, most notable among these are the use of pneumococcal vaccines and the

prophylactic use of penicillin during childhood. Hydroxyurea has also been suggested to improve survival in patients with SCD^{14,16,17}. However, life expectancy remains significantly shortened compared to the national average with that of an affected male being 47 years versus the national average of 72^{1,14,18}. There are no pediatric factors to predict better survival among patients, further complicating the decision to proceed with higher risk treatments, especially during childhood when such treatments may be better tolerated. In one study of 3,764 patients, 18 percent of the patients died with overt organ failure, and early mortality was highest among patients with symptomatic disease. Importantly however, another 33% who appeared to be clinically free of organ failure died during an acute sickle crisis^{1,14}. Newer therapeutic options, including l-glutamine¹⁹, crizanlizumab²⁰, and voxelotor²¹, also do not offer a cure.

Allogeneic transplantation in SCD

The most widely available cure for patients with SCD remains allo-HCT; however, the procedure has mostly been applied to highly selected children^{14,22-25}. In adults, the higher burden of accumulated end-organ damage would be expected to result in higher transplant-associated morbidity and mortality, beyond that reported in children, including seizures and intracranial hemorrhage. As a result, this method has traditionally only been offered to those patients less than the age of 16 with either end-organ damage or symptomatic disease.

Non-myeloablative conditioning HCT in SCD

We originally had a protocol for adults with severe congenital anemias including SCD and a 6/6 human leukocyte antigen (HLA)-matched sibling donor using a conditioning regimen consisting of alemtuzumab, 300 cGy TBI, and sirolimus for GVHD prophylaxis (protocol 03-H-0170). The graft consisted of immobilized peripheral blood stem cells (PBSCs). For our patients with SCD, we maintained higher platelet counts^{7,26-28}. Patients with SCD who were not routinely transfused for their therapy underwent exchange transfusion prior to transplant to lower their hemoglobin S to less than 30%^{7,22}. The results from this trial were recently published by investigators at the NIH, University of Illinois, Chicago, and King Abdulaziz Medical City in Riyadh, Saudi Arabia, and were very encouraging, with a 93% overall survival, 85% event-free survival, and no grade 3-4 acute or chronic GVHD²⁹.

To reduce the rate of graft rejection and myeloid malignancies³⁰, a new protocol for patients with SCD has recently opened at NIH, which adds briquilimab, an antibody targeting CD117 (c-Kit), to the regimen with the goal of improving donor myeloid chimerism (DMC) levels (protocol 000539). This protocol (as did the prior protocol) allows for enrollment of patients with severe disease and compromised organ function, including renal failure.

However, these protocols have been limited by the availability of HLA- matched sibling donors²⁷. Due to the inheritance pattern of SCD, the chance that an HLA-matched sibling will be SCD-free is low, further limiting the possibility of finding an appropriate donor.

We previously initiated a search to establish the feasibility of matched unrelated donor (MUD) and umbilical cord blood (UCB) HCT. Ten patients who met all study criteria on full screening for our sibling matched HCT protocol but who did not have a suitable donor were selected for alternative donor searching in the National Marrow Donor Program (NMDP) and Bone Marrow Donors Worldwide (BMDW). We found that only one patient had a greater than 1% probability of having a 6/6 HLA match according to haplogenic, a predictive HLA-matching algorithm. Also, only a median of one suitable ($>2.5 \times 10^7$ total nucleated cells per kilogram body weight and

ABO-matched) UCB unit was available per patient³¹. Conversely, the vast majority of patients will have an appropriate haploidentical donor, from a parent, offspring, and/or a haploidentical-matched sibling⁷. Thus, as our HLA-matched sibling HCT protocol has been successful, we are now interested in developing a safe haploidentical protocol for adult patients with SCD to increase the applicability of allogeneic transplant for these patients.

Haploidentical HCT in SCD

Haploidentical HCT is invariably associated with an increased risk of graft rejection and GVHD.³² Recent studies published outside the NIH since 2018 with increased upfront immunosuppression have been more encouraging. Overall survival for adults has ranged from 87-100% and event-free survival from 75-93%³³. However, follow-up was limited, with median follow-up ranging from approximately 17 to 23 months. More recently, Kassim and colleagues reported 39 adults who underwent non-myeloablative haploidentical HCT with anti-thymocyte globulin, thiotepa, fludarabine, 200cGy TBI, cyclophosphamide given before and after HCT, and mycophenolate and sirolimus for GVHD prophylaxis³⁴. With a median follow-up of just over 2 years, overall survival and event-free survival were 95%. 3 patients experienced grade 3-4 acute GVHD and 1 moderate to severe chronic GVHD. However, due to the use of fludarabine and thiotepa, patients with significant kidney and liver damage were excluded. Further, due to factors such as insufficient or non-existent insurance or older age, many patients with SCD do not have access to curative therapies at outside institutions.

In order to develop a regimen with potential application to individuals with compromised organ function such as those with SCD, we have sought conditioning regimens which could be applied in such a context, particularly avoiding renally excreted drugs, and relying on the immunosuppressive effects of TBI as the basis for such an approach. Patients with SCD who have been frequently transfused may be at an increased risk for graft rejection as compared to patients with hematologic malignancies because frequent exposure to blood products may lead to donor HLA sensitization. A modest increase in the dosage of TBI from 300 cGy used in our HLA-matched sibling protocol to 400 cGy may increase both the degree of myelosuppression and immunosuppression without significantly altering the side effect profile.

23 subjects were enrolled on our first haploidentical transplant protocol for patients with SCD or transfusion-dependent β -thal (09-H-0225) as part of 3 sequential cohorts³². Two patients with SCD had cirrhosis, 4 patients were on dialysis, 2 patients had chronic renal insufficiency with baseline creatinine ranging from 2.5 to 5 mg/dL, 5 patients had right heart catheterization-documented pulmonary hypertension, and 8 patients had diastolic and/or systolic dysfunction, with an ejection fraction as low as 36%. All subjects received alemtuzumab, 400 cGy TBI in divided doses 1 and 2 days before transplant, and sirolimus. The first 3 subjects transplanted did not receive cyclophosphamide per protocol. One of the 3 subjects engrafted, and the 3rd subject lost her graft by 7 months post-transplant (see Table 1). No subject experienced viral reactivation or fungal infection peri-transplant. One subject developed a myeloid malignancy and died 5 years post-transplant from an infectious surgical complication. Stopping rules for graft rejection were met and the study was advanced to the 2nd cohort where 1 dose of cyclophosphamide was added at 50 mg/kg IV on day 3 post-transplant, and 8 subjects were transplanted (including 2 with β -thal). Five subjects engrafted, and 2 remained free of SCD long-term. One experienced grade 1 acute GVHD. One subject experienced disseminated adenovirus at approximately 15 months post-transplant with positive nasopharyngeal wash, transaminitis, and detection of adenovirus in

the blood. However, the adenovirus quickly cleared spontaneously. One subject received a platelet transfusion that was contaminated with bacteria and died of sepsis at approximately 6 months post-transplant. Two subjects developed pulmonary lesions that were suspicious for fungus, and they were managed well with anti-fungal therapy. And one subject experienced acute EBV infection and post-transplant lymphoproliferative disorder which was effectively treated with rituximab. Stopping rules for graft rejection were again met, and the study was advanced to the 3rd cohort where 2 doses of cyclophosphamide were given each at 50 mg/kg IV on days 3 and 4 post-transplant. Twelve subjects were transplanted, and 10 engrafted. Six of the 12 subjects remained free of SCD. One subject experienced grade 1 acute GVHD, and one other limited chronic GVHD. Two subjects experienced chronic EBV viremia without symptoms and did not require treatment. Five (including 1 who experienced EBV viremia) experienced CMV reactivation (including 1 with CMV disease) and all were successfully treated with foscarnet. One subject was treated for a presumed fungal lesion. One subject who rejected his graft died 3 years post-transplant from pulmonary hypertension and diastolic dysfunction shortly after being diagnosed with a myeloid malignancy. A third patient who rejected the graft developed a myeloid malignancy 5.5 years post-transplant and subsequently died of relapse. When stopping rules for the 3rd cohort were reached, accrual to the protocol ceased. Importantly, though many of the patients had compromised organ function, the only patient who died before 3 years post-HCT was the one who received the contaminated platelet transfusion.

While the engraftment rate and success rates improved with each successive cohort, the graft rejection rate remained unacceptably high (Table 1). We also observed a higher than expected incidence of myeloid malignancies in 3 of the patients who rejected their grafts^{30,35}. Therefore, we sought to add additional immunosuppressive therapy to decrease the incidence of graft rejection while maintaining a low rate of GVHD.

Table 1

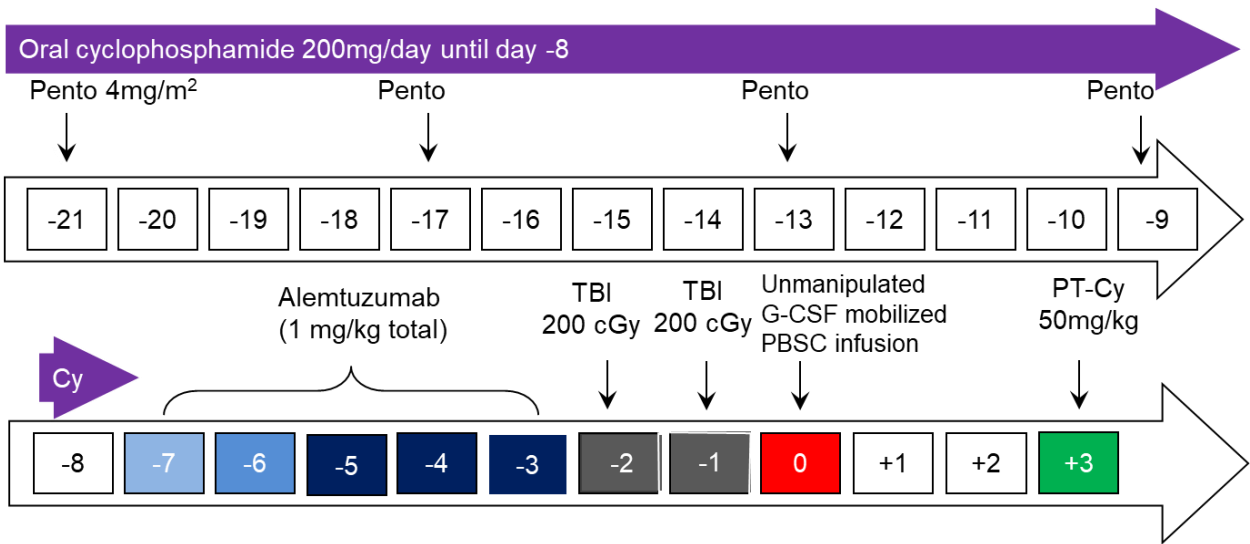
Cohort	Graft rejection	SCD-Free	GVHD		Viral Reactivation	Viral Disease	Myeloid Malignancies	Autoimmune/Hyperinflammatory Complications
			Acute	Chronic				
1 (no PT-Cy)	2/3 (66%)	0/3 (0%)	None	None	0	0	1	0
2 (50mg/kg PT-Cy)	4/8 (50%)	2/8 (25%)	1 (Gr 1)	None	1	1	0	0
3 (100mg/kg PT-Cy)	5/12 (42%)	6/12 (50%)	1 (G 1)	1 (limited)	5	1	2	0

We hypothesized that additional immunosuppression was likely necessary to overcome the stronger host-versus-graft barrier. Based on clinical studies performed at the NIH and elsewhere³⁶⁻³⁸ and a murine study³⁹, an NIH protocol was initiated (14-H-0077) which incorporated intravenous pentostatin (2 mg/m²/day) and oral cyclophosphamide (PC) preconditioning for 14 days starting 21 days before HCT in patients with SCD or β -thal and an HLA-matched sibling donor. Patients also received alemtuzumab, 300cGy TBI, and sirolimus. The addition of PC conditioning led to a decrease in the graft failure rate from 14%²⁹ to 3% (only

1 of 29 patients rejected the graft, data not published). Further, the patients did not experience viral disease, PTLD, or significant hyperinflammatory complications.

Subsequently, we initiated a protocol (17-H-0069) for patients with SCD and haploidentical donors. Because the patients tolerated PC at 2 mg/m²/day well and the target ALC <100/uL was not reached, we increased the pentostatin to 4 mg/m²/day due to the more robust host-versus-graft barrier in the haploidentical setting (Figure 3). Further, as was done in our original haploidentical HCT protocol (09-H-0225), the TBI dose was increased to 400cGy to provide additional myelosuppression and immunosuppression. Lastly, because post-transplant cyclophosphamide increased engraftment in the haploidentical setting and potentially protected the patients from severe GVHD, 50 mg/kg cyclophosphamide was given intravenously 3 days post-transplant. Because of the higher incidence of viral reactivation seen in cohort 3 of protocol 09-H-0225 where a total of 100 mg/kg cyclophosphamide was given post-transplant (Table 1), a second dose of cyclophosphamide was not administered. We anticipated that because alemtuzumab, pentostatin, pre- and post-transplant cyclophosphamide, and sirolimus would be given, the incidence of viral disease and other related complications could potentially be higher as compared to the incidence seen on protocols 09-H-0225 and 14-H-0077. Therefore, stopping rules were included to stop the study if the incidence of viral disease and associated complications was unacceptable. Sirolimus was initiated on day 4 post-HCT.

Figure 3



As of December 9, 2022, 21 patients were initially transplanted on protocol 17-H-0069 with a median follow-up of 3.8 years. Only 1 of the 21 patients failed to engraft and none of the 20 remaining patients experienced acute rejection of their grafts. Of the 21 patients, 18 are alive and 17 are engrafted. However, 4 of the 17 patients have had slowly falling donor chimerism levels over time. We reported that due to vast differences in red blood cell survival between donor and recipient, 20% DMC is sufficient to reverse SCD⁴⁰. One patient with 16% DMC had return of his SCD with jaundice and hospitalization for an acute painful crisis at 2.5 years post-transplant. Another patient was hospitalized for an acute painful crisis and had a DMC level of 15% also about 2.5 years post-transplant. His HbS was 24.2% (donor HbS 0%) and his hemoglobin 9.6 g/dL. The other 2 patients are hovering around the 20% DMC threshold and have not

experienced return of their SCD; both patients are >3 years post-transplant. 15 patients remain alive and free of SCD. Therefore, while acute graft rejection has improved substantially on this protocol, suggesting that acute rejection was indeed due to insufficient immunosuppression on our old haploidentical protocol, late graft failure remains a problem (Table 2).

Table 2

Patients	Graft Rejection	SCD-Free	GVHD		Viral Reactivation	Viral Disease	Myeloid Malignancies	Autoimmune/Hyperinflammatory Complications
			Acute	Chronic				
21	1/21 (5%)	15/21 (71%)	2 (Grade 2 & 4)	None	13	4 (2 uncontrolled)	0	3

As some of the patients on protocol 17-H-0069 experienced slowly falling donor chimerism levels over time, inadequate myelosuppression may be contributing. Indeed, 3 patients transplanted on our old haploidentical protocol (09-H-0025) or our HLA-matched sibling protocol (03-H-0170) and had falling donor chimerism levels with return of their SCD were re-transplanted with the same donor, under protocols 14-H-0111, 14-H-0130, and 03-H-0170 using busulfan (~10 mg/kg) to increase myelosuppression and half the dose of alemtuzumab (0.5 mg/kg). All 3 patients engrafted and with a follow-up of > 6 years, all 3 patients have DMC >90%, close to 40% HbS (donors have sickle cell trait), and are free from SCD. Patients with insufficient donor chimerism and return of SCD have the option to enroll on a repeat transplant protocol (19-H-0118) with the busulfan-containing regimen.

As mentioned above, 3 patients have died on protocol 17-H-0069 as of December 9, 2022. One of the deaths occurred in a patient with history of life-threatening acute right ventricular failure and chronic thromboembolic pulmonary hypertension who was anticoagulated peri-transplant. She died of an intracranial hemorrhage approximately 60 days post-transplant. Two patients developed Evans syndrome that was refractory to multiple immunomodulatory agents and splenectomy. Both patients developed a severe hyperinflammatory reaction that led to fatal multi-organ failure. Nonmyeloablative conditioning and mixed chimerism was believed to contribute to the development of Evans syndrome.

Protocol 17-H-0069 originally included 400cGy TBI given in 2 fractionated doses (Figure 3). In order to increase the donor stem cell competitive advantage and to inhibit recipient stem cell recovery, we amended the protocol to give 400cGy TBI in 1 fraction instead of 2. Increased myelosuppression may increase the frequency of full donor chimerism, thereby decreasing the incidence of Evans syndrome and late graft failure. Recently, Bolanos-Meade and colleagues published a manuscript where they used the same regimen for patients undergoing haploidentical transplant except that they increased the TBI dose from 200cGy to 400cGy as a single dose in adults and children with SCD⁴¹. The graft rejection rate decreased from 43% with their original regimen to 8%^{7,41}. The Vanderbilt Haploidentical Consortium also uses a 400cGy single dose of TBI in patients with β -thal with similar efficacy⁴².

As of December 9, 2022, 2 patients have been transplanted with the single fraction of 400cGy TBI. With a follow-up ranging from 4 to 8 months, both patients achieved full donor chimerism.

With the 21 patients who have been transplanted, while stopping rules have not been met, the frequency of viral reactivation and PTLD has increased on protocol 17-H-0069 (Table 2). 10

patients experienced cytomegalovirus (CMV) reactivation that was treated with appropriate therapy; none experienced CMV disease. 7 patients developed EBV reactivation; 3 resolved with reduction of immunosuppression. 4 patients with EBV reactivation progressed to PTLDPD. 2 were successfully treated with rituximab. The other 2 required chemotherapy with or without viral-specific T-cells and were characterized as uncontrollable PTLD. Starting with the next patient transplanted on 17-H-0069, we will treat any detectable EBV pre-emptively with rituximab, as is consistent with NIH Transplant Consortium EBV Management Guidelines, with the goal of minimizing the risk of PTLD. One patient developed grade 4 acute GVHD that was believed to be related to lenalidomide that was administered with chemotherapy for PTLD. That patient also experienced a severe hyperinflammatory reaction thought due to EBV. With a median follow-up of 3.5 years, no patient has developed a hematologic malignancy. Therefore, 13 patients experienced CMV reactivation, EBV reactivation, or both.

We hypothesize that the higher rate of viral and immune complications seen on protocol 17-H-0069 is due to too much recipient immune suppression and immune dysregulation. Further, with the addition of pentostatin, we had to exclude adults with significant kidney damage, who are at higher risk of early mortality from SCD. We therefore seek to develop a protocol with immunosuppression intermediate between protocol 09-H-0225 (with a high risk of graft rejection) and protocol 17-H-0069 (with a high risk of PTLD and immune complications) that can include not only patients with kidney damage but also those who are otherwise excluded from other haploidentical protocols for reasons such as other significant organ damage, age, or insufficient insurance coverage.

Costimulation (signal 2) blockade has been shown to prevent T cell-dependent antibody production and lead to the development of tolerance. Kim and colleagues showed that belatacept (CTLA4-immunoglobulin), a drug which inhibits the costimulatory interaction between CD80/CD86 on antigen presenting cells and CD28 on T cells, prevented kidney allograft rejection and the development of alloantibody production in a rhesus macaque model⁴³. Further, patients who received kidney transplants were conditioned with alemtuzumab, daily sirolimus, and monthly belatacept⁴⁴. Of 10 patients who were weaned off sirolimus after one year and maintained on belatacept, 7 remained free from graft rejection. Ten additional patients remained on sirolimus and belatacept, and none experienced graft rejection. Further, mixed chimerism was maintained in the rhesus macaque model in the presence of sirolimus, belatacept, and a nondepleting CD40 monoclonal antibody which inhibits CD154 from binding to CD40⁴⁵. In addition, belatacept and sirolimus decreased the incidence of GVHD and improved survival compared to either agent alone⁴⁶. Belatacept's sister drug, abatacept, also prevents the interaction between CD80/CD86 and CD28. In one study, 10 patients with history of hematologic malignancies underwent unrelated donor HCT⁴⁷. All patients received standard immunosuppression with cyclosporine and methotrexate as well as 4 doses of abatacept between one day before and 28 days after transplant. There was a low rate of acute GVHD and no deaths related to infection or transplant-related mortality by 100 days post-transplant. However, 2 patients developed severe chronic GVHD. The authors surmised that extending the duration of abatacept past 28 days post-transplant may be beneficial.

More recently, abatacept has been used to decrease the incidence of graft rejection and GVHD in patients with SCD. Shenoy and colleagues reported 29 children, median age 14 years, who underwent reduced-intensity matched unrelated donor HCT⁴⁸. 1 and 2 year overall survival were 86% and 79%, and 1 and 2 year event-free survival 76% and 69%, respectively. 28% developed

grade 2-4 acute GVHD (17% grade 3-4 acute GVHD) and 62% chronic GVHD (38% extensive). In an attempt to decrease the incidence of GVHD, abatacept 10 mg/kg/dose was added on days -1, +5, +14, +28, +100, +180, +270, and +365⁴⁹. 14 children underwent unrelated donor HCT with a median age of 13. 2 year overall survival and event-free survival increased to 100% and 92.9%, respectively. While 28.6% developed grade 2-4 acute GVHD, 7% was grade 3-4 acute GVHD. 57% experienced chronic GVHD, 14% severe. Furthermore, Jaiswal et al. reported 5 patients who received a myeloablative conditioning regimen that included alemtuzumab, 100mg/kg PT-Cy, sirolimus, and abatacept 10 mg/kg/dose given on days -1, +5, +21, +35, +60, +90, +120, +150, and +180⁵⁰. At a median follow-up of 28 months, overall survival and event-free survival were 100%. No patient experienced acute or chronic GVHD. 2 patients had CMV reactivation, no CMV disease, and no PTLT. We therefore hypothesize that adding abatacept to sirolimus before lymphocytes have begun to recover will improve tolerance induction and therefore decrease the rate of graft rejection and/or GVHD. Because the above study suggests that 28 days may not be sufficient⁴⁷, abatacept will be continued until 6 months post-transplant. Since acute graft rejection, which occurs by 100 days post-HCT, not GVHD, has been the challenge in our haploidentical studies, abatacept will not be continued past 6 months post-transplant.

Because the goal of our study is to increase the chance of full donor chimerism to decrease the risk of late graft failure, immune complications, and malignancy^{30,51}, this regimen will also employ nongenotoxic conditioning with a CD117 (c-Kit) antibody. c-Kit, is the tyrosine kinase transmembrane receptor for stem cell factor (SCF) that is involved in hematopoietic stem cell (HSC) self-renewal and differentiation⁵². CD117 is expressed on normal HSCs and progenitor cell populations (CD34+). Immunodeficient mice with loss-of-function c-Kit mutations⁵³ or those treated with a neutralizing anti-mouse c-Kit antibody⁵⁴ provide a competitive advantage for donor cells by rendering mouse HSCs uncompetitive in occupying the bone marrow niche. Such mice therefore accept human CD34+ HSCs without the need for conditioning, and give human CD34+ cells with a full c-Kit function a proliferative advantage over mouse HSCs.

The investigational product (IP), briquilimab, is a monoclonal antibody (mAb) that targets human CD117 with high affinity and blocks the binding of SCF. SCF signaling through CD117 is an essential pathway for the survival, maintenance, and proliferation of HSCs. Briquilimab has been shown to transiently deplete mouse HSCs allowing up to 90% donor chimerism after transplantation⁵⁴, safely deplete non-human primate HSCs⁵⁵, results in long-term myeloid chimerism in severe combined immunodeficiency (SCID) patients when used as the sole allogeneic HCT conditioning agent⁵⁶, and displays potent synergy with TBI in pre-clinical models. Briquilimab is being evaluated for clinical safety and utility as the sole transplant conditioning agent in children with SCID, a non-malignant disorder (clinicaltrials.gov study NCT02963064). Murine experiments demonstrated proof of concept that anti-CD117 antibody can deplete HSCs and can be used as a sensitizer to TBI (Figure 4). Administration of ACK2 to T and B cell deficient mice led to the removal of >98% of endogenous HSCs⁵⁴. ACK2 appeared to function by depriving HSCs of SCF, augmenting the effects of cytotoxic intervention. After murine anti-CD117 injection, reduction in HSCs was transient and endogenous HSCs fully recovered in two weeks. Infusion of purified donor HSCs into recipient mice during a window of time, when the serum levels of anti-CD117 antibody were below 2 mcg/mL and before recovery of endogenous hematopoiesis, resulted in robust and durable DMC levels. In another set of murine transplant experiments, anti-CD117 antibody was combined with other agents to formulate conditioning regimens ([jas-bmt-cp-001-clinical-protocol v7](#)).

Figure 4

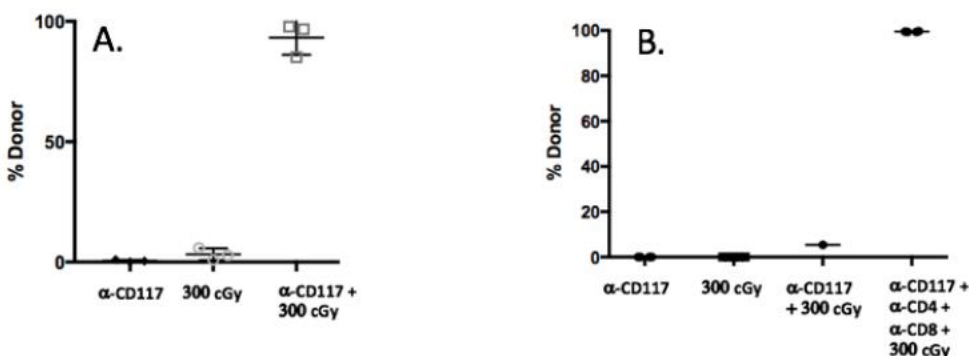


Figure 4A (murine congenic HCT) experiment showed that anti-CD117 antibody sensitizes murine HSCs to low dose irradiation, i.e. ‘creating space’ in the marrow, which is necessary for myeloid engraftment. Figure 4B (murine allogeneic model) experiment showed that anti-CD117 antibody can be combined with other antibodies for allogeneic HSC engraftment. This block on SCF binding took 3-7 days to reach maximal effects in in vitro cultures⁵⁷ and up to 2 weeks in non-human primates⁵⁵. Thus, the window of time between CD117 antibody and radiation is up to 2 weeks.

NIH protocol 000539 adds briquilimab, to the established and well tolerated non-myeloablative immunosuppressive regimen of low dose radiation (300 cGy), alemtuzumab and sirolimus as a strategy to improve event-free survival in patients with severe SCD who have HLA-matched sibling donors. The goal of the combination of briquilimab - alemtuzumab – TBI - sirolimus regimen is to further deplete host lymphoid and myeloid cells to achieve a higher percentage of donor leukocyte engraftment without increased toxicity. The protocol also changes the route of administration for alemtuzumab. It was administered in the 03-H-0170, 09-H-0225, and 17-H-0069 protocols as a 2 hour intravenous infusion daily for 5 days in a stepped dose escalation approach to reduce the risk of infusion related toxicities. The test dose started with 0.03 mg/kg on day -7, 0.1 mg/kg on day -6, and 0.3 mg/kg daily for 3 days on days -5, -4 and -3; total dose of 1.03 mg/kg. The Alberta Children’s Hospital group replicated the 03-H-0170 protocol in children and adolescents with SCD but modified the alemtuzumab dosing regimen to 0.2 mg/kg and by subcutaneous injection daily for 5 days from days -7 to -3. They reported no grade 3 or 4 reactions to alemtuzumab and in a cohort of 16 patients, found the regimen to deliver similar outcomes, donor chimerism and rates of graft failure, GVHD, and survival as the prior published experience from the 03-H-0170 protocol⁵⁸.

Subcutaneous administration of alemtuzumab has been evaluated in other settings. For example, the subcutaneous route has been shown to reduce the incidence of infusion-related reactions compared to the intravenous route of administration, while maintaining the same efficacy when used for treatment of chronic lymphocytic leukemia⁵⁹. In the HCT setting, Patel et al. in a retrospective study of 46 patients, compared subcutaneous versus intravenous alemtuzumab when utilized for GVHD prophylaxis within the preparative regimen for matched unrelated donor HCT⁶⁰. Patients received alemtuzumab 20 mg daily for 5 days from days -7 to -3 by either an IV infusion over 4 hours or by SC injection. All patients were pre-medicated with

acetaminophen and diphenhydramine. Patients in the IV dosing cohort also received methylprednisolone 2 mg/kg pre-alemtuzumab and another 1 mg/kg halfway through the infusion on each day of alemtuzumab. Patients in the SC injection cohort received 100 mg of hydrocortisone pre-alemtuzumab each day. Infusion-related reactions of grade 2 or higher occurred in 8% in the SC dosing cohort versus 26% in the IV dosing cohort ($p = 0.001$). There were no significant differences between the cohorts in CMV/EBV viral reactivation, CMV disease, PTLT, fatal infections, GVHD, relapse or survival. These small trials, despite their limitations in design and size, indicate that alemtuzumab administered at a similar total dose as 03-H-0170 by the SC route of administration will provide similar transplant-related outcomes with less infusion-related toxicity risks. For these reasons, the alemtuzumab dosing regimen was changed to a 0.2 mg/kg daily dose for 5 days (1 mg/kg total) using the SC route of administration in protocol 000539.

With the goal of maximizing efficacy while minimizing toxicity in the haploidentical setting for patients with significant kidney damage, this protocol employs briquilimab, SC administration of alemtuzumab, 400cGy TBI, abatacept, PT-Cy, and sirolimus. As long as uncontrolled viral disease or PTLT safety stopping rules are not met, this study will include a dose escalation of 2 cohorts. The 1st cohort will receive one dose of PT-Cy on day +3 post-HCT. If efficacy or GVHD stopping rules are met, the study will advance to the 2nd cohort where an extra dose of PT-Cy will be given on day +4 post-HCT (see section 6.1).

Patients with donor specific HLA-antibodies (DSA) have traditionally been excluded from our protocol because of the known increased risk of graft rejection in the haploidentical setting. Primary graft failure and delayed engraftment have been associated with DSA⁶¹. The incidence of primary poor graft function is higher in patients with DSAs $\geq 2,000$ MFI compared to those with a DSA $< 2,000$ MFI^{61,62}. One hundred percent neutrophil engraftment was noted among the 316 transplant recipients with either negative DSA or DSA $< 2,000$. Transplant recipients with $< 2,000$ MFI DSA may therefore proceed with transplant without desensitization.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Known Potential Risks from TBI-Alemtuzumab conditioning regimen

At the NIH, the alemtuzumab-TBI backbone for conditioning regimen is considered as a standard non-myeloablative HCT regimen in adults with beta-globin disorders. Risks of alemtuzumab include infusional reactions (such as fever, rigors, fatigue, bone pain, blood pressure changes, rash, or breathing difficulties), hematologic toxicities (leukopenia, lymphopenia, or autoimmune cytopenia), and opportunistic infections (viral, bacterial, pneumocystis, candida, and other pathogens) (FDA-approved alemtuzumab prescribing info).

TBI-specific risks at this low dose (400 cGy) include nausea, parotitis, mucositis, rash, cytopenias, and infections associated with leukopenia²⁹ (and data from 17-H-0069). There are rare delayed/late complications of alemtuzumab and TBI, which were difficult to attribute to alemtuzumab, TBI, or other aspects of transplant; those include bleeding disorder⁶³, hypothyroidism, cataracts, interstitial pneumonitis, nephropathy, sterility, and solid organ and hematologic malignancy.

Risks Related to Radiation Exposure

This research study involves exposure from total body irradiation and DEXA scans. Due to the known radiosensitivity of reproductive organs, subjects will be asked to use birth control and will be monitored after the last injection in order to observe the effects, if any, of radiation. The NIH Radiation Safety Committee has reviewed the use of radiation in this research study and has approved this use as involving acceptable risk and necessary to obtain the research information desired.

Known Potential Risks from Post-Transplant Cyclophosphamide

Potential risks include anorexia, nausea, vomiting, diarrhea, mucositis, myelosuppression, gonadal dysfunction, alopecia, and immunosuppression. Less commonly, they may develop hemorrhagic cystitis, nasal stuffiness with rapid administration, flushing, rash, kidney tubular necrosis (which usually resolves with drug discontinuation) or syndrome of inappropriate antidiuretic hormone (SIADH). Rarely, patients may experience transient blurred vision, cardiac toxicity with arrhythmias, hyperpigmentation, impaired wound healing, myocardial necrosis, hepatotoxicity, weakness, hemorrhagic colitis, nail changes, bladder fibrosis, pulmonary fibrosis, and secondary malignancies.

Known Potential Risks from Sirolimus

Sirolimus is an FDA-approved drug indicated for the prophylaxis of organ rejection in patients receiving renal transplant and for the treatment of patients with lymphangioleiomyomatosis and has a well-established safety profile. Based on the available prescribing information, the most common side effects (>20%) are: peripheral edema, hypertriglyceridemia, hypertension, hypercholesterolemia, increased creatinine, proteinuria, constipation, abdominal pain, diarrhea, headache, fever, urinary tract infection, anemia, nausea, thrombocytopenia, pain, acne, rash, and edema. Other risks may include rhabdomyolysis (data from 03-H-0170), mucosal (including mouth, gastric, small bowel, or large bowel) ulcers, which may bleed, stroke, thrombotic thrombocytopenic purpura/hemolytic-uremic syndrome, posterior reversible encephalopathy syndrome, and angioedema (data from 17-H-0069).

Known Potential Risks of Adding Abatacept to HCT for SCD

Risks include nausea, headache, infections, back, limb pain, acute infusion-related events (mostly mild to moderate) such as hypotension, hypertension, dyspnea, hypersensitivity, and rash, dizziness, and headache. Rarely, patients can develop an anaphylactic or other serious allergic reaction. The incidence of EBV infection and PTLT was noted to be higher in patients who receive belatacept, and in particular in patients who were seronegative for EBV who received an allograft from an EBV seropositive donor⁶⁴. Therefore, EBV seronegative recipients with a seropositive donor will be excluded from this study. Studies performed in individuals with SCD who underwent HCT did not reveal an increased risk related to abatacept in that population^{49,50}.

Known Potential Risks of Adding Briquilimab to HCT in SCD

Previous presentations to annual hematology meetings and the available investigator brochure indicated a favorable side effect profile for briquilimab⁶⁵. To date, 21 patients with severe combined immune deficiency (NCT02963064) and 32 patients with myelodysplasia or acute myeloid leukemia (NCT04429191) have received this antibody as part of the conditioning regimen in separate ongoing phase 1 studies (personal communications with Dr. Wendy Pang, Jasper Therapeutics). With the usual premedications (acetaminophen, diphenhydramine, with or

without hydrocortisone), the briquilimab infusions have been well tolerated, without the expected hypersensitivity side effects (such as fever, nausea, transient rash). There has been a single report of Grade 3 hypersensitivity (verbatim “allergic reaction”) reported on the day of the second dose of 14 mg briquilimab SC. It is noteworthy that symptoms included urticaria and facial angioedema, with an absence of respiratory or cardiovascular symptoms. Other than expected cytopenias (which is desired for this antibody and the conditioning regimen), there have been no grade 3 or higher infusional toxicities or briquilimab--related SAEs in these studies. There were also no significant changes in liver function tests (LFTs) or renal function associated with briquilimab, thus those with abnormal LFTs or GFR <60 mL/min/1.73m² would also expect to tolerate this antibody infusion well. No long-term organ toxicities have been observed to date. In the First in Human single ascending dose study (A Phase 1, Placebo-controlled, Randomized, Double-blind, Ascending Single-dose, Safety, Tolerability and Pharmacokinetic Study of Subcutaneous Injection and Intravenous Infusion of AMG 191 in Healthy Subjects), AEs were reported for 94 of 96 participants (98%); no deaths or SAEs were reported. No treatment-emergent events worse than moderate severity (Grade 2) were observed in any participants that received investigational product. Five AEs (nausea, upper respiratory tract infection, dizziness, back pain, and lethargy) reported for ≥ 10% of participants receiving briquilimab were more than twice as frequent following briquilimab vs placebo, although there was no clear association with briquilimab dose. Based on the dose escalation infusion of briquilimab in patients with SCID, the dose for this study has been determined at 0.6 mg/kg. The low but detectable briquilimab antibody level within a few days of the donor hematopoietic cell infusion has not interfered with donor cell engraftment (personal communications with Dr. Pang). Therefore, adding briquilimab to alemtuzumab, 400cGy TBI, abatacept, and PT-Cy increases the overall risk by a small/modest increment.

Risks Related to Study Procedures

Risks related to blood draws for laboratory testing and research sample collection are minimal and include the discomfort and possible pain of phlebotomy, the potential for bruising and the potential for lightheadedness or fainting surrounding venipuncture, and the potential for mild anemia after blood draws. There are minimal risks associated with electrocardiogram and echocardiogram as these are both relatively safe procedures. While pulmonary function tests are relatively safe, some may feel dizzy or faint from the rapid breathing required for the test. Risk of bone marrow biopsy is generally mild pain but rarely bleeding or infection can occur at the biopsy site. To minimize pain, a local anesthesia will be administered prior to the procedure to numb the area. Potential discomfort from an MRI scan includes the loud noise from the MRI machine and the discomfort of having to lie still for periods of time during the scan. During the MRI, participants may experience nervousness, boredom, anxiety, or claustrophobia. In this study we will be administering neuropsychological tests. These tests may tire or frustrate participants.

2.3.2 Known Potential Benefits

Known Potential Benefits of Adding Briquilimab and Abatacept to HCT in SCD

There is a growing realization that SCD itself may already have an increased risk of leukemia, lymphoma, and other malignancies⁶⁶⁻⁶⁸. The magnitude of this risk ranged from 3.5 times to 10 times for myeloid leukemia. The cause for this risk may include one or more of the following: chronic inflammation from sickling in bone marrow, iron overload and inflammation from

reactive oxygen species, hyperproliferative stress from lifelong anemia, or natural senescence and accumulation of pro-survival mutations in the HSCs⁶⁹. The contribution of hydroxyurea to this increased risk was thought to be negligible based on the data in the myeloproliferative disorders. We recently reported an increased risk of hematologic malignancies in patients with SCD transplanted at the NIH with graft failure or mixed chimerism³⁰. Although the cause for leukemia in those patients with SCD is not completely understood, the clinical characteristics of patients who developed myelodysplastic syndrome or acute myeloid leukemia tended to show poor prognostic features: complex cytogenetics, deletions of whole or partial chromosome 5, 7, and/or 17, gain of pro-survival mutations (e.g. *TP53*, *RUNX1*), and being refractory to standard chemotherapy^{35,70}. We therefore seek to develop a regimen that maximizes full donor chimerism while minimizing the risk of GVHD and uncontrollable viral complications.

While the side effects and risks of briquilimab overlap with those of alemtuzumab, TBI, abatacept, and PT-Cy, the reactions to briquilimab appear less frequently than to alemtuzumab, TBI, abatacept, or PT-Cy. There are other FDA-approved monoclonal antibodies targeting lymphocytes, but none target myeloid or HSCs like briquilimab. Antibodies targeting CD45 or CD47 are being tested in animal models that deplete stem cells as briquilimab, and they are just getting started in clinical trials (Clinicaltrials.gov identifiers NCT04980885, NCT05214482, NCT04083183, NCT03670966), mostly in the setting of hematologic malignancies. Thus in revising this protocol, we reasoned that avoiding additional chemotherapy or up titration of the radiation dose would keep the overall risk from the conditioning regimen low. If this antibody could deplete more host hematopoietic cells for transplantation purpose, the overall non-ablative intent would be preserved, and those with compromised heart, pulmonary, or hepatic function could receive this curative treatment. Further, we have had to exclude many patients from protocol 17-H-0069 because of kidney failure who could enroll on this study. With more antibodies becoming available in the future, radiation could be replaced and overall risk of secondary myeloid neoplasm further reduced. Having a chemotherapy- and radiation-free conditioning HCT regimen would be highly desirable to decrease overall risk of toxicity long-term. Testing this antibody in the haploidentical setting is an important step in achieving this goal. Abatacept also allows avoidance of additional chemotherapy and has been used successfully to decrease the incidence of graft rejection and GVHD in other settings where patients with SCD underwent HCT^{49,50}.

2.3.3 Assessment of Potential Risks and Benefits

Without curative interventions, injuries to one or more organs continues to accumulate in SCD. Life-altering events (e.g. frequent vaso-occlusive crisis, stroke, severe anemia, or kidney failure) or fatal events (e.g. acute chest syndrome or intracranial bleeding from moyamoya syndrome) continue to occur despite being on appropriate sickle-specific therapies. With overall survival of >90% in non-myeloablative and >95% in myeloablative HCT, transplantation offers a viable option to halt and possibly reverse some of the organ injuries. Secondly, risks and complications of HCT have reduced over the last decades where GVHD rates could be very low (<5%) using the NIH regimen or <15% with myeloablative regimens, allowing the majority of patients to discontinue immunosuppression. Halting further disease-related injuries with reduced transplant-related complications offers prospects of assimilating life goals without SCD. Regularly scheduled red cell transfusions can ameliorate symptoms, but there is substantial time lost with the lab testing (type and cross match of red blood cell units or monitoring for iron chelation side effects) and regularly scheduled transfusions. There are economic considerations for lifelong red

blood cell transfusions and iron chelation that would favor a one-time, more intensive, higher upfront cost of HCT. Gene therapy (lovo-cel, marketed as Lyfgenia) and gene editing (marketed as Casgevy) were FDA-approved as potentially curative therapies for SCD on 12/8/23. Other gene therapy and gene editing trials for SCD are ongoing. However, currently the trials include either myeloablative dosing of busulfan or reduced-intensity dosing of melphalan. Thus, most patients with overt organ damage are not eligible for gene therapy or gene editing. Further, the cost of both approaches is expected to be at least \$2,000,000. Therefore, the cost will prohibit many patients with SCD from undergoing gene therapy or gene editing. This protocol targets patients with compromised organ function and who do not have access to autologous or allogeneic HCT options at other centers.

In assessing the overall risk and benefit ratio, adding briquilimab and abatacept are favorable from several important considerations. First, the predecessor 09-H-0225 protocol had a high rate of graft failure; those with SCD that had graft failure all had autologous hematopoietic reconstitution (where autologous stem cell collections were not performed). Adding briquilimab could make TBI more efficient in clearing the marrow niche and improving not only engraftment, but decreasing the long-term risk of graft failure. Protocol 17-H-0069 had a high rate of viral and immune complications, possibly due to the increased immunosuppression provided by high-dose pentostatin and oral cyclophosphamide preconditioning. Abatacept adds an intermediate level of immunosuppression between what was offered in 09-H-0225 and 17-H-0069. Briquilimab does not add additional immunosuppression. There is a small chance of unusually slow clearance of briquilimab, leading to prolonged marrow aplasia or graft failure. We plan to routinely collect autologous stem cells to minimize this risk, in particular because the stress of prolonged autologous recovery may increase the risk for a future hematologic malignancy. The 'naked' briquilimab antibody composition, not conjugated to toxin or drug for direct cytotoxicity, should further minimize the risk of post-infusional marrow aplasia.

Second, briquilimab infusions have been very well tolerated and no grade 3 reactions have been reported to date. When combined with anti-thymocyte globulin infusions so far, reactions were not more frequent, severe, or unexpected. Briquilimab and the first alemtuzumab infusion are several days apart. Further, changing intravenous to subcutaneous administration of alemtuzumab should further minimize any additive infusional reactions from briquilimab or alemtuzumab. Lastly, abatacept has been used safely in patients with SCD undergoing unrelated donor and haploidentical HCT^{49,50}.

Third, we now have a robust long-term dataset from 09-H-0225, with a median follow-up of almost 10 years (range 7.3 to 12.8 years). Identifying complications from briquilimab and abatacept from engraftment, infection, and long-term effects should be straight forward. Adding these well-tolerated agents to the alemtuzumab, 400cGy TBI, PT-Cy backbone has the potential to incrementally improve the event-free survival while including patients traditionally excluded from HCT protocols who are at high risk of mortality from SCD. With briquilimab, the goal is to yield higher DMC levels and reduce the risk of late graft failure, immune complications, and hematologic malignancies. And excluding EBV seronegative recipients with seropositive donors and aggressively using rituximab in patients with detectable EBV levels will decrease the risk for EBV-associated PTLT.

3. OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
Evaluate the regimen success rate where success is defined as successful engraftment (persistent donor chimerism and free of acute SCD complications) and absence of acute grade 3 or higher GVHD or moderate to severe chronic GVHD evaluated at 1 year post-transplant.	The percentage of SCD patients at 1 year (+/- 3 months) post-transplant who have not experienced graft failure and who are without severe graft-versus-host disease (defined as grade 3 and higher acute GVHD and moderate to severe chronic GVHD).	We hypothesize that a moderate amount of additional immunosuppression with abatacept and myelosuppression with briquilimab will lead to a low rate of graft failure and severe GVHD at 1 year post-HCT.
Secondary		
<ul style="list-style-type: none"> - Event-free survival and overall survival - Incidence of recipient-type hemoglobin defined as HbS $\geq 10\%$ when donors have HbAA and HbS $\geq 50\%$ when donors have sickle cell trait - The proportion of patients with myeloid chimerism $\geq 95\%$ at 1 and 2 years post-HCT - Incidence of acute and chronic GVHD - Prevalence of donor type hemoglobin at 1 year post-transplant in SCD patients who have not been transfused in the previous 3 months. - Incidence of viral reactivation and disease - Incidence of autoimmune or hyperinflammatory complications - Incidence of hematologic malignancies - Transplant-related mortality 	<ul style="list-style-type: none"> - Total hemoglobin and percent HbS levels - Percent donor myeloid chimerism and donor CD3 chimerism - Day of neutrophil engraftment - Day of platelet engraftment - RBC transfusion requirement - Rates of acute and chronic GVHD - Rates of viral reactivation and disease - Rates of autoimmune and hyperinflammatory complications - Transplant-related mortality - Non-transplant-related mortality - Rates of graft failure and rejection - Rates of leukemia and related disorders - Organ function and quality of life 	We want to determine if the usual transplant outcomes and complications would differ by the addition of abatacept and briquilimab
Exploratory		

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
-Perform gene therapy research involving cell culture or genetic manipulation to produce normal or therapeutic hemoglobin on excess autologous CD34+ cells collected from recipients. -- Evaluate the impact of this regimen on organs including the heart, lung, kidneys, liver, brain, neurocognitive function, and endocrine organs - Evaluate the impact of this non-myeloablative conditioning regimen on quality of life	-Completion of gene therapy research involving cell culture or genetic manipulation to produce normal or therapeutic hemoglobin on excess autologous CD34+ cells collected from recipients. - Organ function and quality of life	We want to determine if the usual transplant outcomes and complications would differ by the addition of abatacept and briquilimab

4. STUDY DESIGN

4.1 Overall Design

This is a single center, one arm, phase I/II study which consists of 2 cohorts in adults with SCD. All participants will receive the same dose of the investigational briquilimab antibody and abatacept added to the NIH-established regimen of alemtuzumab-TBI-sirolimus and infusion of filgrastim-mobilized peripheral blood hematopoietic cells from haploidentical related donors. The first cohort of patients will receive a single dose of PT-Cy, 50 mg/kg, on day +3 post-HCT. If uncontrolled viral disease or PTLT stopping rules are met, the study will close. If efficacy or other safety stopping rules are met, the second cohort of patients will receive PT-Cy, 50mg/kg, on days +3 and +4 (total 100 mg/kg) post-HCT. The inclusion of SCD is intended to span the common genotypes of SCD - HbSS, HbS/beta-thal (0), HbS/beta-thal (+), HbSC, and other severe phenotypes. We hypothesize that adding abatacept and briquilimab will maximize efficacy while minimizing moderate to severe GVHD, uncontrolled viral disease, uncontrolled PTLT, malignancy, and uncontrolled autoimmune and hyperinflammatory complications.

4.2 Scientific Rationale for Study Design

We previously developed a non-myeloablative haploidentical regimen consisting of alemtuzumab, 400cGy TBI in divided doses, sirolimus, and dose escalation of PT-Cy (09-H-0225). The 1st cohort received 0 mg/kg PT-Cy, the 2nd cohort 50mg/kg PT-Cy, and the 3rd cohort 100mg/kg PT-Cy in divided doses. Stopping rules were built into the study so that if too many patients rejected their grafts or developed grade 3-4 acute or moderate to severe GVHD, the study would advance to the next cohort. While the engraftment rate improved, the incidence of graft rejection decreased, and there was minimal GVHD, there remained a high incidence of

graft rejection and a high incidence of myeloid malignancies. Further, the incidence of viral reactivation increased in the 3rd cohort compared to the other cohorts (Table 1).

We therefore developed a new protocol which included alemtuzumab, 400cGy TBI in divided doses, sirolimus, 50 mg/kg PT-Cy, and pentostatin 4 mg/m² and oral cyclophosphamide preconditioning with the goal of increasing the amount of immunosuppression to decrease the graft rejection rate (17-H-0069). Because patients who rejected their grafts experienced prolonged autologous recovery which may increase the risk for future malignancy, autologous PBSCs are collected prior to conditioning. Further, because of the addition of pentostatin, patients with kidney failure were excluded.

Only one patient acutely rejected the graft (Table 2) and received autologous PBSCs. However, 4 patients experienced falling DMC levels, 2 with return of SCD at 2.5 years post-HCT. Two patients experienced refractory Evans syndrome followed by a severe hyperinflammatory response and multi-organ failure; both patients died. To increase the amount of myelosuppression with the goal of increasing the incidence of full donor chimerism and decreasing the risk of late graft failure and autoimmune complications, the protocol was amended to give a single fraction of 400cGy TBI. Two patients have been transplanted since the TBI adjustment, and the protocol is ongoing. There has also been an increased incidence of viral reactivation and PTLT as described above.

Because of the high rate of viral, autoimmune, and inflammatory complications, we hypothesize that a moderate amount of immunosuppression (more than 09-H-0225 but less than 17-H-0069) will maintain a low rate of graft rejection while avoiding unacceptable toxicities. Abatacept has a low rate of toxicities in children with SCD who have undergone MUD and haploidentical HCT while decreasing the rate of graft rejection and GVHD^{49,50}. Briquilimab also has been shown to have a low risk of toxicities while providing additional myelosuppression to maximize DMC levels. Because abatacept has been associated with PTLT, the first cohort will receive one dose of PT-Cy. If efficacy or safety stopping rules are met (other than the uncontrolled viral disease or PTLT stopping rule), the study will advance to the second cohort where patients will receive 2 doses of PT-Cy. PTLT was not reported in a recent study of children with SCD who underwent a myeloablative conditioning regimen that included abatacept, 2 doses of PT-Cy, and sirolimus⁵⁰. Aggressive preemptive rituximab monotherapy will also be employed for this protocol.

4.3 Justification for Dose

Briquilimab:

Current available pre-clinical and clinical data indicate 0.6 mg/kg briquilimab given within two weeks of stem cell infusion is feasible

In cynomolgus macaques, anti-CD117 antibody levels were after administration of various dose levels (Table 3). Reticulocyte recovery occurred between 15 and 21 days after anti-CD117⁵⁵.

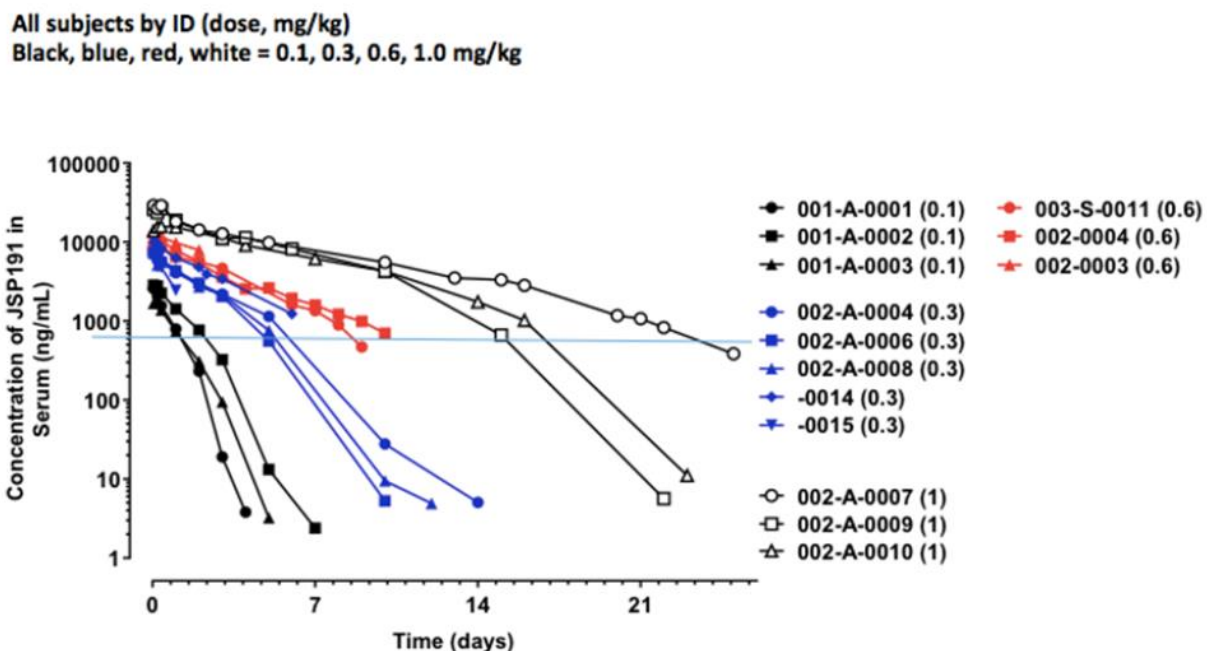
Table 3

2 cynomolgus macaques per dose	T $\frac{1}{2}$ (days)	Days needed for antibody level to reach <1000 ng/mL	Days needed for marrow CD34+ recovery to baseline
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0.1 mg/kg	Not reported: CD117 Ab reached 2000- 3000 ng/mL by day 1	Not reported	21, 42
1 mg/kg	1.88	10	21, 21
5 mg/kg	2.77	21	21, 42
25 mg/kg	4.3	>42	21, >42

Briquilimab is currently being used as a sole conditioning agent for young patients with severe combined immune deficiency (SCID) who may have received an allo-transplant (MRD, haploidentical, or MUD) as infants. In that multi-center study, in which the NIAID (PI Harry Malech) is one of the centers, briquilimab was administered at 0.1, 0.3, and 1.0 mg/kg (Figure 5 below). Allogeneic HSCs were infused when briquilimab level reached below 500 ng/mL, which occurred <7 days for the 0.1 mg/kg, near 7 days for 0.3 mg/kg dosing, and 14-21 days for the 1.0 mg/kg dosing; the extended window at the highest dose prompted an intermediate dose level of 0.6 mg/kg.

Figure 5



The 0.6 mg/kg group appeared to reach below 500 ng/mL near day 10, as predicted in between the 0.3 and 1.0 mg/kg. This dose and timing fit well with the first dose of alemtuzumab at day -7. The myeloid chimerism for the SCID recipients were <10% for 3 patients in the 0.1 mg/kg, <10% for 2 of 3 patients in the 0.3 mg/kg (the 3rd patient had ~30% at 1 year post-transplant),

and <10% for the one patient in 1.0 mg/kg. These low chimerism results are similar to the murine results and support its use in combination with our regimen of alemtuzumab, low dose TBI, sirolimus, and PT-Cy. There is a parallel study where briquilimab is incorporated into non-myeloablative allogeneic transplant for patients with MDS/AML (NCT04429191).

Abatacept:

The abatacept dose is based on the dose used in patients with hematologic malignancies⁷¹, aplastic anemia⁷², and other patients with SCD undergoing HCT^{49,50}. Because the incidence of GVHD has been low on our previous protocols, abatacept will be discontinued after the 6-month dose⁵⁰ rather than being continued until 1 year post-HCT⁴⁹. Because abatacept has been associated with PTLT, the first cohort will receive one dose of PT-Cy. If efficacy or safety stopping rules are met (other than the uncontrolled viral disease or PTLT stopping rule), the study will advance to the second cohort where patients will receive 2 doses of PT-Cy. Abatacept has been used safely in children with SCD who underwent a myeloablative conditioning regimen that included abatacept, 2 doses of PT-Cy, and sirolimus⁵⁰.

5. STUDY POPULATION

5.1 Inclusion Criteria

5.1.1 Recipient:

Participants must fulfill one disease category in section 5.1.1.1, either at least one organ damage or no other access to curative therapy in section 5.1.1.2, and all of section 5.1.1.3.

5.1.1.1. Adult patients with sickle cell disease at high risk for disease-related morbidity or mortality, according to A, B, C, D, or E or complication(s) not ameliorated by SICKLE CELL-SPECIFIC THERAPIES (F):

- A. Stroke defined as a clinically significant neurologic event that is accompanied by an infarct on cerebral MRI OR an abnormal trans-cranial Doppler examination (≥ 200 m/s); OR
- B. Silent cerebral infarct defined as an infarct-like lesion based on an MRI signal abnormality at least 3 mm in one dimension and visible in two planes on FLAIR or T2- weighted images (or similar image with 3D imaging) and documented neurological examination performed by a neurologist demonstrating the participant has a normal neurologic examination or an abnormality on examination that could not be explained by the location of the brain lesion(s)^{73,74}; OR
- C. Sickle cell-related renal insufficiency defined by a creatinine level ≥ 1.5 times the upper limit of normal and kidney biopsy consistent with sickle cell nephropathy OR nephrotic syndrome⁷⁵⁻⁷⁷;
OR
- D. Tricuspid regurgitant jet velocity (TRV) of ≥ 2.5 m/s at least 3 weeks after a vaso-occlusive crisis⁶ and/or right heart catheterization-documented pulmonary hypertension; OR
- E. Sickle hepatopathy defined as EITHER ferritin >1000 mcg/L OR direct bilirubin >0.4 mg/dL at baseline AND a platelet count <250 K/uL (without vaso-occlusive crisis)¹⁰; OR
- F. Any one of the below complications:

Complication	Eligible for HCT
Vaso-occlusive crises	>1 hospital admission per year while on a therapeutic dose of a SCD treatment/medication ¹
Acute chest syndrome (ACS)	Any ACS while on SCD treatment/medication ⁷⁸

Pediatric patients with sickle cell disease at high risk for disease-related morbidity or mortality, defined as A, B, C, D, E, or F

A. A neurological event resulting in focal neurologic deficits that lasted ≥ 24 hours (classical clinical definition of stroke, not requiring imaging studies of the brain) OR a focal neurological event resulting in abnormalities on T2-weighted or FLAIR images using a MRI scan, indicative of an acute infarct, with no other reasonable medical explanation (definition of a stroke supported with MRI imaging scans of the brain), OR both; OR

B. Abnormal transcranial Doppler (TCD) measurement with a timed average maximum mean velocity of at least 200 cm/sec in the terminal portion of the internal carotid or proximal portion of middle cerebral artery or if the imaging TCD method is used > 185 cm/sec plus evidence of intracranial vasculopathy; OR

C. Silent Cerebral Infarct defined as an infarct-like lesion based on an MRI signal abnormality at least 3 mm in one dimension and visible in two planes on FLAIR or T2-weighted images (or similar image with 3D imaging) and documented neurological examination performed by a neurologist demonstrating the participant has a normal neurologic examination or an abnormality on examination that could not be explained by the location of the brain lesion(s); OR

D. Three acute severe vaso-occlusive pain episodes requiring hospitalization and recalcitrant to maximum medical therapy; OR

E. At least one acute chest syndrome episode resulting in intensive care admission requiring non-mechanical ventilatory support: simple nasal cannula, face mask that requires oxygen content (venti mask, non-rebreather), simple nasal cannula, face mask oxygen (e.g. ventimask, non-rebreather), continuous positive airway pressure, synchronized inspiratory positive airway pressure, bilevel positive airway pressure, high flow nasal cannula or invasive mechanical ventilatory support (delivered by endotracheal tube or tracheostomy); OR

F. Right heart catheterization confirmed pulmonary hypertension defined as pulmonary artery pressure >25 mmHg

5.1.1.2 AND evidence of severe organ damage with at least one of the following who are typically excluded from other HCT protocols:

A. Kidney damage: CrCl <60 mL/min/1.73m² cystatin C-based or iothalamate/iohexol-based or other equivalent GFR testing including patients on hemodialysis or peritoneal dialysis

B. Heart damage: Ejection fraction 35-40%

C. Liver damage: Bridging fibrosis, cirrhosis, direct bilirubin $\geq 2x$ the upper limit of normal and/or ALT $\geq 5x$ the upper limit of normal

D. Lung damage: Adjusted diffusion capacity of carbon monoxide (DLCO) 35-40%

OR who have no other option for curative therapy (including allogeneic HCT, gene therapy, or gene editing studies) on a different SCD HCT protocol.

5.1.1.3 Non disease-specific

- Male or female, age ≥ 18 years (pediatric subjects ≥ 16 years may be enrolled after 6 successful adult transplants)
- Haploidentical relative donor available
- Ability to comprehend and willing to sign an informed consent before the initiation of any study procedures
- Negative serum or urine b-HCG, when applicable
- Agree to use birth control for 12 months after drug product infusion.
 - Female subjects must agree to use a medically acceptable method of birth control such as an oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection from start of screening through 12 months after drug product infusion.
 - Male subjects must agree to use effective contraception (including condoms) from start of screening through 12 months after drug product infusion.
- Stated willingness to comply with all study procedures and availability for the duration of the study

5.1.2 Donor

Haploidentical relative donor deemed suitable and willing to donate, per clinical evaluations. Donors age 4 or older and ≥ 20 kg, eligible to donate hematopoietic stem cells, and who are additionally willing to donate blood for research. Related donors will be evaluated in accordance with existing Standard NIH Policies and Procedures for determination of eligibility and suitability for clinical donation. Note that participation in this study is offered to all related donors, but is not required for a donor to make a stem cell donation, so it is possible that not all related donors will enroll onto this study.

5.2 Exclusion Criteria

5.2.1 Recipient

An individual who meets any of the following criteria will be excluded from participation in this study:

- Available HLA-matched sibling donor
- ECOG performance status of 3 or more (See Appendix A).
- DLCO $< 35\%$ predicted (corrected for hemoglobin).
- Baseline oxygen saturation of $< 85\%$ or $\text{PaO}_2 < 70$ mmHg
- Left ventricular ejection fraction: $< 35\%$ estimated by ECHO
- Evidence of uncontrolled bacterial, viral, or fungal infections (currently taking medication and progression of clinical symptoms) within one month prior to starting the conditioning regimen. HCT candidates with pre-transplant respiratory viral infections will undergo careful clinical assessment with the help of our transplant infectious disease team for lower respiratory tract disease, which may include thorough physical examination, imaging, and/or bronchoalveolar lavage. In case of lower respiratory tract

disease or severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positivity, the transplant will be delayed until resolution.

- Major anticipated illness or organ failure incompatible with survival from PBSC transplant.
- Pregnant or breastfeeding
- Donor specific anti-HLA antibodies (DSAs) ≥ 2000 Mean Fluorescence Intensity (MFI)
- Patients seronegative for EBV who have EBV seropositive donors

5.2.2 Donor

- Pregnant or breastfeeding

5.3 Inclusion of Vulnerable Participants

Inclusion of Children

Once we have demonstrated safety and have not met stopping rules in the first 6 adult patients, we plan to open accrual to adolescents (age 16 and older). The target beta-globin disorders in this protocol are inherited, and disease manifestation begin as soon as fetal hemoglobin has switched to the diseased beta-globin production. Thus including children, using the same criteria in section 5.1, is reasonable. The matched sibling SCD HCT protocol using Briquilimab (000539) includes children as young as 4 years old; it has also been used in young SCID patients with haplo and matched unrelated donors.

Minor donors will only participate in minimal risk activities as their stem cell collection is done as Standard of Care. Therefore, their participation in research is justifiable against the risks of blood collection

Participation of NIH Staff or family members of study team members

NIH staff and family members of study team members may be enrolled in this study if the subject meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable and unidentifiable manner.

The *NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research* will be made available. Please see section 0 for consent of NIH Staff.

5.3.2 Participation of Adult Recipient Subjects Who Become Cognitively Impaired

Cognitively impaired adult recipient subjects are not being targeted on this protocol. Due to the nature of this disease, existing subjects may develop permanent cognitive impairment while the subject is enrolled. Since there is a prospect of direct benefit from research participation, all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. For

those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP Policy 403 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

5.4 Inclusion of Pregnant Women, fetuses or neonates

N/A

5.5 Lifestyle Considerations

Subjects will be advised not to take medication with grapefruit juice and not to take St. John's wort while on sirolimus. Subjects must also be advised about limiting exposure to sunlight and ultraviolet light due to an increased risk of skin cancer.

5.6 Screen Failures

Screen failure is defined as patients who initially sign on this study, but could not meet inclusion criteria, met exclusion criteria, or could not proceed with necessary pre-HCT testing. They may be re-screened at a later time if clinical scenarios have changed or are appropriate, and a different unique patient identifier would be assigned.

5.7 Strategies for Recruitment and Retention

NHLBI has an active patient recruitment office to bring patients with beta-globin disorders for evaluation. Our transplant group receives referrals from local and out-of-state areas for transplant evaluation. Many patients are seen under the Natural History Protocol (04-H-0161) because they are interested in transplant, and most eligible for transplant are referred to us since approximately 90% have haploidentical donors. Thus we anticipate transplanting approximately 5 patients per year. For those individuals who do not sign on to curative intent studies, they may be referred to other NIH studies, or back to their referring physicians.

The study will be listed on the clinicaltrials.gov website, Clinical Center Search the Studies, and the Clinical Center Recruitment website. A recruitment plan will be developed by the NHLBI Patient Recruitment Office to include IRB-approved recruitment materials, (e.g. recruitment flyers/post cards, iStock photos for marketing the study, and a Public Service Announcement to include recruitment language for use with social media (Facebook, Twitter, Instagram, LinkedIn), Office of Patient Recruitment and NIH Listservs, NIH Record and Clinical Center News internal publications and other potential official NIH external publications as available.

There are no restrictions based on gender, race or ethnicity, or non-English speaking subjects.

5.7.1 Costs

The medical costs associated with various parts of the HCT are covered by NIH under this protocol, including clinic visits, medications, inpatient hospital admissions, red cell and platelet transfusions, laboratory and radiologic testing, and consultations for clinical indications.

5.7.2 Compensation

Subjects participating in the quality of life portion of the study may receive up to \$60 USD per year for their time and inconvenience for completion of questionnaires which vary in length (see table below). Payments will be made to the subject by debit card or direct deposit upon completion of questionnaires. If the subject is unable to complete the study, they will still receive compensation for the surveys completed.

Financial Compensation Details				
Procedure	Frequency	Compensation per Test	Male Total	Female Total
PROMIS Quality of Life Questionnaire (male and female)	once / year, (D+100)*	\$20	\$20*	\$20*
Changes in Sexual Function Questionnaire (male and female)	once / year	\$20	\$20	\$20
Fertility Survey (females only)	once / year	\$20	N/A	\$20
Priapism Impact Questionnaire (males only)	once / year	\$10	\$10	N/A
International Index of Erectile Function (males only)	once / year	\$10	\$10	N/A
TOTAL (USD)			\$60	\$60

*In the first year subjects may receive up to \$80 if the Day 100 PROMIS is completed along with the other listed surveys.

Participating donors will receive \$50 total compensation for about 50mL of research blood.

Reimbursement for Travel

This study offers reimbursement to offset travel, transportation, and/or meals. Reimbursement will be consistent with NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects. The amounts reimbursed are as follows:

- *Luggage Fees* – For the patient and the guardian, one checked luggage is approved.
- *Long Distance* (>30 miles from home) –This protocol will cover the train/bus costs; however, this protocol will not cover taxi (or shared-driving) costs.
- *Meals* – For the guardian, the protocol-specific standard rate for meals is \$15.00 per day.
- *Lodging* – For the patient, the protocol-specific standard rate for hotel lodging is \$80.00 per night up to 7 nights, then the rate decreases to \$30.00 per night for subsequent nights.

6. STUDY INTERVENTION

6.1 Study Interventions(s) Administration

Autologous HSC collection

Because the clearance of briquilimab in this population and the duration of its effects are uncertain, autologous hematopoietic stem cells may be mobilized with plerixafor (or equivalent alternative) and collected by apheresis. Hydroxyurea should be discontinued 2-4 weeks prior to plerixafor mobilization. Red cell exchange or simple transfusions to target HbS <30% will be performed within a week of plerixafor mobilization. Aspirin 325 mg may be given with each dose of plerixafor at 240 mcg/kg (capped at 20 mg); the dose may be adjusted based on inadequate response. Leukapheresis will begin 4-12 hours after plerixafor, whole blood is withdrawn at a rate of 60-80 mL/min and conveyed to the separator. Mononuclear cells will be collected with the target CD34 dose of 2.0×10^6 cells/kg (minimum 1.5×10^6 cells/kg). Another day of aspirin, plerixafor (or comparable mobilization agent), and apheresis may be repeated the next day if needed. Whether to proceed with 3 days of mobilization, higher dose of plerixafor, an alternative mobilization agent, or wait for a later time for another cycle of mobilization depends on the side effects from mobilization and apheresis, and/or the number of cells collected; these decisions will be made at PI discretion. The mobilization and collection of autologous HSC may occur outside of the NIH Clinical Center (e.g. more convenient for the patient), prior to enrolling on this protocol, as part of another protocol, or several months or years prior to the planned HCT date.

Prior specific therapy

Hydroxyurea or other sickle-specific therapy should be optimized 4-12 weeks prior to the planned transplant date. They should be discontinued on the day before briquilimab administration. Iron chelation should also be discontinued on the day before briquilimab.

Blood product support

Full or partial red cell exchange or simple red cell transfusions are performed prior to the conditioning regimen to target sickle hemoglobin percent of 30% or lower. Full or partial exchange transfusions may be avoided before conditioning per PI/designee if the risks of the transfusion are expected to outweigh the potential benefits. Once the conditioning regimen has started, target hemoglobin will be ≥ 9.0 g/dL to help suppress autologous hematopoiesis, and platelet count ≥ 50 k/uL to help reduce the risk of intracranial bleeding (as many have subclinical cerebral vasculopathy, even with normal brain MRA). Selection of blood products will be adjusted by Department of Transfusion Medicine, and these transfusion thresholds may be adjusted based on the available product inventory or the clinical situation of the recipient (e.g., history of delayed hemolytic transfusion reaction). Pathogen reduction and irradiation of blood products will be determined by DTM, and the use of leukocyte filters during transfusion as per Clinical Center policies.

Prior red cell antibody and/or alloimmunization

For patients who have a red cell antibody documented in transfusion medicine testing (e.g. presence of alloantibody, positive direct antiglobulin test (DAT)) or prior delayed hemolytic transfusion reaction, rituximab or other antibody-directed treatment may be administered as per PI or designee discretion or in consultation with DTM.

Blood pressure monitoring

Patients with SCD often have relative lower baseline systolic and diastolic blood pressure. While undergoing the conditioning regimen and after stem cell infusion, the target systolic BP is in 110-120's and diastolic BP in 60-70's. Use of intravenous and oral anti-hypertensives is at the discretion of the PI/designee and inpatient teams. Aiming for this target BP range may also minimize the risk of intracranial bleeding during the period of thrombocytopenia.

Conditioning regimen (recipient): (also refer to Schema)

Day -11 (window of day -13 to -6): One dose of briquilimab antibody is to be administered between day -13 and day -6. Premedication with 325-650 mg of acetaminophen and 25-50 mg of IV or PO diphenhydramine (or equivalent, such as cetirizine 5-10 mg PO), then followed 30-60 minutes later by briquilimab 0.6 mg/kg in D5W. For subjects ≤ 83 kg in body weight, briquilimab will be infused at a constant rate over 1 hour through a central venous catheter when possible. For all dose levels for subjects greater than 83 kg, briquilimab will be infused at 250 mL/hour. This will ensure that volumes greater than 250 mL will be infused over longer than 1 hour. The infusion rate of briquilimab may be adjusted based on Jasper Therapeutics recommendations. Hydrocortisone 25-100 mg may be given at the discretion of the PI/designee/medical team as part of the premedication regimen. Briquilimab should be infused via central catheter when possible. Dosing of briquilimab will be based on actual body weight performed within 72 hours of briquilimab administration and will be given after hemodialysis on days participants are scheduled to undergo dialysis.

Day -7, -6, -5, -4 and -3: Premedication with 325-650 mg of acetaminophen and 25-50 mg of IV or PO diphenhydramine (or equivalent). Methylprednisolone (1 mg/kg) or 25-100 mg of hydrocortisone may also be given at the discretion of the PI/designee/medical team. Alemtuzumab 0.2 mg/kg subcutaneously will follow 30-60 minutes later. Dosing of alemtuzumab will be based on actual body weight. Alemtuzumab will be given after hemodialysis on days participants are scheduled to undergo dialysis.

Day -1: Total body irradiation (TBI), 400 cGy given in one fraction. Equally weighted opposed lateral beams will be used to encompass the total body with the patient positioned supine. Treatment will be delivered at an SAD of 6 meters (or other distance depending on the treatment room configuration). The prescription point will be the midplane at the maximal hip separation. The dose rate to midplane will be no more than 15 cGy per minute. Head and neck compensation will be used to increase dose homogeneity. Adjustments to treatment technique but not dose prescription may be made at the discretion of the treating radiation oncologist if deemed necessary. Gonadal shielding will be used in males unless refused.

Abatacept approximately 10 mg/kg/dose will be given by IV infusion over 60 minutes. This dose will be based on a dose calculation weight which is calculated within one week of the Day -1 dose. However, if a weight gain or loss of $>10\%$ compared to the original dose calculation weight has occurred, a new dose calculation weight should be used for drug dosing on days -1, +5, +14, +28. For all calculations, actual weight is used.

Abatacept will be given after hemodialysis on days participants are scheduled to undergo dialysis.

Day 0: Unmanipulated filgrastim - mobilized peripheral blood stem cells collected from the donor will be infused.

Day +3 (and Day +4 in cohort 2 patients): Cyclophosphamide 50 mg/kg/dose will be given on Day +3 by IV infusion over 60 minutes in cohort 1 and cohort 2 patients and on Day +4 by IV infusion over 60 minutes in cohort 2 patients. Dosing will be based on ideal body weight with the exception that if the actual body weight is lower than the ideal body weight, the actual body weight will be utilized for the cyclophosphamide dose calculation. Mesna (which will also be dosed by ideal body weight) and intravenous hydration will be given concurrently with the cyclophosphamide dose as based on current Transplant and Cellular Therapy (TCT, formally BMT) consortium guidelines. The start time of the cyclophosphamide infusion should be within 72 hours of the start time of the stem cell infusion on Day 0. For patients on dialysis, intravenous hydration will not be given; hydration volume may be adjusted as medically indicated per PI discretion. Before cyclophosphamide, a 3 way foley will be placed with irrigation starting 12 hours before and continuing until 24 hours after the last dose of cyclophosphamide. Dialysis will be initiated between 12 and 18 hours after the last dose of cyclophosphamide.

Day +4 (or Day +5 in cohort 2 patients): Sirolimus will be started at 5mg PO q4h x three doses at one day after the completion of cyclophosphamide (on day +4 in cohort 1 patients and on day +5 in cohort 2 patients). The first dose of sirolimus should be at least 24 hours after the completion of the D+3 cyclophosphamide infusion. Sirolimus will be continued at 5mg PO q24h starting on day +5 (or day +6 in cohort 2 patients). Trough levels will be maintained between 5-15 ng/mL. Initial dosing may be adjusted based on concomitant medications and anticipated drug interactions based on consultation with the clinical pharmacist and PI discretion. Initial sirolimus doses may also be reduced in patients with mild to severe hepatic impairment (e.g. Child-Pugh classes A, B and C), based on product labeling recommendations and on consultation with the clinical pharmacist. Subsequent dosing will be adjusted based on serum drug concentrations with a target therapeutic range of 10-12 ng/mL.

Day +5 to Day +180: Abatacept approximately 10 mg/kg/dose will be given by IV infusion over 60 minutes on Days +5, +14 (+/- 7), +28 (+/- 14), +100 (+/- 14), and +180 (+/- 14) (except where noted below regarding abatacept delay in the setting of infection). If the day +14 dose is delayed, the day +28 dose will be given at least 14 days later. This dose will be based on a dose calculation weight which is calculated within one week of the Day -1 dose. However, if a weight gain or loss of >10% compared to the original dose calculation weight has occurred, a new dose calculation weight should be used for drug dosing on days +5, +14, and +28. For all calculations, actual weight is used. Abatacept will be given after hemodialysis on days participants are scheduled to undergo dialysis.

Cases for when to hold abatacept: Abatacept must be held, at least temporarily, for the following infections:

- 1) Uncontrolled serious invasive viral disease (e.g. CMV pneumonitis, enterocolitis or COVID-19 uncontrolled infection).
- 2) Uncontrolled invasive fungal infections, including candida infections, mold infections and pneumocystis.
- 3) Uncontrolled opportunistic protozoal and helminthic infections, such as toxoplasmosis.
- 4) Imminently life threatening bacterial infections, such as septic shock.

While all remaining doses may be withheld for patients with life threatening bacterial, fungal, protozoal, or viral infections, if the infection responds promptly to treatment, abatacept may be

resumed and subsequent doses administered. Dose on Day +14 may be delayed for up to 7 days and doses on Days +28, +100, and +180 may be delayed for up to 14 days to allow infections to clear. The day -1 and +5 doses, however, may only be withheld - they may not be given after a delay.

Abatacept will not be held for less severe infections, including the following:

- 1) CMV or EBV reactivation <1,000 (3 log₁₀) IU/ml. However, appropriate measures to treat CMV and EBV reactivation will be taken, and if CMV or EBV levels are not responding to appropriate therapy, the abatacept may be held after consultation with our Infectious Disease and abatacept experts. If CMV or EBV have reactivated to >1,000 (3 log₁₀) IU/ml and the abatacept is held, subsequent doses may be given once the CMV or EBV viral loads decrease to <1,000 (3 log₁₀) IU/ml.
- 2) Mild, uncomplicated herpes simplex virus (HSV) stomatitis, assuming no other complications of HSV are present. If HSV stomatitis is severe, one or more doses of abatacept may be held, in consultation with our Infectious Disease and abatacept experts.
- 3) Bacteremia when the patient is clinically stable.
- 4) Superficial fungal infections.
- 5) Fever without documented infection.

If **EBV lymphoproliferative disease** is diagnosed, all remaining abatacept doses will be held.

If **CMV invasive disease** is diagnosed, all remaining abatacept doses will be held.

If **adenovirus invasive disease** is diagnosed, all remaining abatacept doses will be held.

If **varicella invasive disease** is diagnosed, all remaining abatacept doses will be held.

If a patient has an anaphylactic reaction, other severe systemic allergic reaction (e.g. severe urticarial rash), or other severe infusional reaction to any abatacept dose, all remaining abatacept doses will be held. Infusion reactions will be managed according to NIH standards.

For patients who reject their grafts or develop GVHD while still receiving abatacept, abatacept administration may be discontinued at the discretion of the PI or designee.

Immunosuppression

The sirolimus goal will be 5-12 ng/mL. Sirolimus may be changed to another immunosuppressant or another immunosuppressant may be added at the discretion of the PI or designee for reasons such as intolerable side effects, falling chimerism levels, or GVHD. Sirolimus or the other immunosuppressant may be weaned and discontinued no earlier than 1 year post-transplant if donor myeloid and donor CD3 chimerism levels are >95% and there is no evidence of GVHD. Donor/recipient chimerism levels will be checked periodically during and after the taper. If lymphoid and/or myeloid donor chimerism levels decrease by >20%, sirolimus and/or other immunosuppressant may be restarted as clinically indicated. Sirolimus or the other immunosuppressant may be discontinued before one year post-HCT in patients with lower chimerism levels at the discretion of the PI or designee for situations such as delayed wound healing or other intolerable side effect.

Women of childbearing potential will be informed of the potential risks of sirolimus during pregnancy and that they should use effective contraception prior to initiation of the drug.

Engraftment, filgrastim, and the use of autologous rescue hematopoietic cells

Filgrastim may be used to minimize the days of neutropenia, and may be initiated with signs of count recovery (or at the discretion of the PI or designee) at 5 mcg/kg (rounding to the nearest vial) at the discretion of the PI or designee.

If there is insufficient evidence of donor or autologous hematopoietic recovery between day 42 and 45, previously stored autologous rescue hematopoietic cells may be infused. Adding or switching to other immunosuppression may also be considered. These options may be modified at the discretion of the PI or designee.

Hematopoietic cell collection (donors)

The target CD34 dose is 10×10^6 cells/kg, and the minimum is 5×10^6 cells/kg. The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, which will be performed as standard of care.

Regulatory Considerations

Briquilimab is a first-in-class anti-CD117 monoclonal antibody used as a conditioning agent to clear hematopoietic stem cells from bone marrow. It is designed to eventually replace chemotherapy and/or radiation in patients undergoing curative hematopoietic stem cell transplantation and gene therapies. The use of briquilimab for this proposed indication is investigational and the study will be conducted under FDA IND #157986, Sponsored by NHLBI Office of the Clinical Director.

Transplant supportive care and management of complications

A central venous catheter will be placed before peripheral blood stem cell infusion.

Anti-emetics will be administered according to NIH Pharmacy Department guidelines or with consultation of a clinical pharmacist.

Antimicrobial prophylaxis, viral reactivation monitoring, fever monitoring and management, and other aspects of transplants are according to the guidelines in the TCT Consortium. Aggressive rituximab preemptive therapy will be employed to mitigate the risk of PTLT.

Acute and chronic GVHD will be managed as per TCT Consortium guidelines.

Falling chimerism, graft failure, graft rejection: Falling chimerism is when there is decreasing DMC for at least two consecutive dates with DMC <30%. Insufficient chimerism is when DMC is approaching 20% but with detectable CD3 chimerism, with or without SCD-related symptoms or complications. Graft failure is when DMC is >5% with return of SCD. Graft failure that occurs with DMC and donor CD3 chimerism levels <5% is referred to as graft rejection.

For falling chimerism in the myeloid and/or CD3 compartment, several strategies may be considered. Adding or switching to other immunosuppression is one option. Another course of alemtuzumab, as given in the conditioning regimen, has been used in a few patients in London, United Kingdom, and Amsterdam, Netherlands, with subsequent rise in donor chimerism (personal communications, Drs de la Fuente and Nur).

If the patient has graft failure, the patient could be offered our Repeat Transplant Protocol (19-H-0118) using the stem cell product ('boost') from the original donor after preconditioning with busulfan (or melphalan in patients with significant liver damage), alemtuzumab, and sirolimus.

Patients could be offered other NIH transplant protocols as applicable.

For patients who experienced graft rejection or graft failure and return of SCD, there is currently no standard approach. Re-initiation of SCD-specific therapy, another HCT, or other clinical trials are all options to consider. We will discuss these options, refer to applicable clinical trials, or refer them to their local physician.

6.2 Preparation/Handling/Storage/Accountability

Plerixafor

Source: Will be obtained from commercial sources.

Please refer to the package insert.

Briquilimab

Supply: Jasper Therapeutics will supply briquilimab through a collaborative agreement. Clinical Center Pharmacy will acknowledge receipt of briquilimab, keep track of lot numbers, expiration dates, and drug supply.

Product description: Briquilimab (JSP191) is a humanized, aglycosylated IgG1 monoclonal antibody directed against human c-Kit. The clinical material is expressed by Chinese hamster ovary (CHO) cells. The molecule consists of 2 IgG1 heavy chains and 2 kappa light chains covalently linked through disulfide bonds.

Formulation: Briquilimab is a liquid for intravenous (IV) infusion and subcutaneous (SC) administration. Each sterile vial is filled with a colorless to slightly yellow liquid with a deliverable volume of 1 mL. Briquilimab is formulated at a concentration of 50 mg/mL in 10 mM sodium acetate, 263 mM sucrose, 0.02% (w/v) polysorbate 20, pH 5.2. The final container of briquilimab is a 2 cc USP Type-I glass vial. The stock formulation will require dilution prior to injection into final IV syringe or bag for administration. The dilution procedures are described in the **JSP191 Dose Preparation Instructions** and will be executed by the trained pharmacy staff.

Storage and stability: Briquilimab must be stored protected from light and according to the storage and expiration (where required) information provided on the label or Certificate of Analysis. Briquilimab is supplied at 2 different storage conditions, frozen or refrigerated. Storage must be at $-20^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for frozen supply or $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for refrigerated supply until used. Preparation of the drug for injection should follow the instructions outlined in the Pharmacy Manual. Following preparation for administration, the drug can be stored at $2-25^{\circ}\text{C}$ for no more than 4 hours. After this period, the IP syringes must be discarded.

Preparation: Handling of JSP191 requires gloves. On the day prior to study drug administration, for the frozen formulation, briquilimab must be thawed quiescently overnight at 2 to 8°C . Once thawed, the vials should be transferred to room temperature and gently swirled or inverted to ensure mixing. Preparation will be performed using aseptic techniques and under sterile conditions in a laminar-flow hood with controlled room temperature (15 to 25°C). Prolonged exposure to light should be avoided. The pharmacist/staff will prepare the individual subject dose on the morning of infusion and will ensure that the investigational product is prepared and labeled. Briquilimab should be diluted with D5W into final IV syringe or bag (PVC or EVA). The final volume will be determined by the subject's individual dose using the following

equation: $(0.6 \text{ mg} \times \text{body weight in kg}) = \text{total dose in mg}$ divided by $0.2 \text{ mg/mL} = \text{total dose volume in mL}$. Refer to the Pharmacy Manual for dose preparation instructions. The final concentration of the drug to be administered is 0.2 mg/mL . The diluent is 5% dextrose in water. The total dose volume of the intravenous (IV) syringe or bag will be determined by the subject's individual dose. Briquilimab IV infusate is transported and administered at room temperature after preparation by the pharmacist. Investigational Product (IP), as unopened vial, can be stored under refrigerated conditions at $2\text{--}8^{\circ}\text{C}$ for a maximum of 18 hours, or may be kept at room temperature up to 25°C for a maximum of 6 hours. Upon extraction of the IP from the vial, the IP must be administered within 4 hours. **PLEASE NOTE: IP must be used within 4 hours of breaking sterility and dilution.** Briquilimab should be administered by continuous infusion with a Low Sorbing Tubing Set (mandatory) with an in-line $0.2 \mu\text{m}$ PES filtering device (recommended). Pharmacy should prime bag with low sorbing tubing. The line should be completely flushed with D5% dextrose at the end of the infusion to ensure administration of the entire dose. If the investigational agent comes into contact with skin, the area must be washed thoroughly with soap and water. If the agent comes into contact with eyes, the eyes must be thoroughly washed. All unused study drug must be disposed of according to institutional policies for disposal of therapeutic biologics.

Alemtuzumab

Source: Patient-specific supplies will be obtained via the Campath Distribution Program currently administered by the Clinigen Group (www.clinigengroup.com). The investigator team will complete the required patient-specific request forms for delivery to the NIH Pharmacy Department. Prior to submission of a drug request, the patient must provide authorization for the release of medical information (NIH-527).

Please refer to the package insert.

Sirolimus

Source: Will be obtained from commercial sources

Please refer to the package insert for sirolimus tablets and oral solution for oral use (Rapamune®, Pfizer Inc or equivalent generic brand).

Cyclophosphamide

Source: Will be obtained from commercial sources

Please refer to the package insert.

Abatacept

Source: Will be obtained from commercial sources

Please refer to the package insert

6.3 Measures to Minimize Bias: Randomization and Blinding

N/A

6.4 Study Intervention Compliance

N/A

6.5 Concomitant Therapy

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Medications to be reported in the Case Report Form (CRF) are concomitant prescription medications, over-the-counter medications and supplements. Infection prophylaxis, fever management, monitoring for CMV and other viral reactivations are as per NIH TCT Consortium guidelines.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

If a clinically significant finding is identified after enrollment (including, but not limited to changes from baseline), the PI/designee will determine if any change in medical management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

Study procedures or the conditioning regimen may be stopped for SAEs, clinically significant AEs, severe laboratory abnormalities, or any other medical conditions that indicate to the PI/designee that continued dosing is not in the best interest of the patient. Study procedures or the conditioning regimen may resume as per PI/designee.

The data to be collected at the time of study intervention discontinuation may include drug levels or pharmacokinetic monitoring of briquilimab or alemtuzumab, white blood cell count, hemoglobin, or platelet count up to 30 days after briquilimab or alemtuzumab infusion.

Pregnancy shall be reported as below (see Section 8.4.9 below); and the subject will remain on study solely for the purpose of monitoring the safety of the pregnancy or premature termination of the pregnancy in this patient population. Patients who have been transplanted before the pregnancy will continue to be followed on the protocol with resumption of research studies after delivery and after breastfeeding has ceased.

Participants are free to withdraw from participation in the study at any time upon request.

7.2 Participant Discontinuation/Withdrawal from the Study

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study ('off-study') for the following reasons:

- Screen failure (section 5.6)
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation prior to start of intervention
- Significant study intervention non-compliance
- Participating in another treatment study
- Hematologic malignancy
- PI/designee discretion

- Death

The reason for participant discontinuation or withdrawal from the study will be recorded on the study-specific Case Report Form (CRF). Subjects who sign the informed consent form but do not receive the study intervention may be replaced.

7.3 Lost to Follow-up

Participants on this study will not be considered lost to follow-up. If he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff, the staff will continue to reach out to the patient once annually to see if the participant desires to return for follow-up.

8. STUDY ASSESSMENTS AND PROCEDURES

NIH Clinical Center-required testing for protocol activities/events will be completed to include, but not limited to, pregnancy testing for CT, dual x-ray absorptiometry (DEXA), MRI, and PT/PTT for bone marrow biopsies.

8.1 Screening Procedures

8.1.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, prior therapy, etc.
- Review of existing MRIs, x-ray, or CT images or other studies.

8.1.2 Screening activities performed after a consent for screening has been signed

Screening activities that will be performed after the subject has signed the consent for this study are listed in the SOA. Assessments performed at outside facilities or on another NIH protocol within the timeframes listed on the SOA may also be used to determine eligibility once a participant has signed the consent.

8.2 Study Evaluations & Procedures

Medical history and physical examination

A comprehensive medical history will be obtained, including symptoms related to chronic complications of SCD (e.g. pain crises, acute chest syndrome, avascular necrosis, skin ulcers), prior hospitalizations, transfusion history, medications, procedures, surgeries, and infections. The physical examination should include eyes, heart, lungs, abdomen, joints, and skin to document signs of sickle-related complications or prior procedures.

Post-transplant interval history will be obtained at protocol time points as per table of SOA and focus on symptoms of GVHD, infection, or blood count changes. Physical examination will include eyes, oral mucosa, neck, heart, lungs, abdomen, and skin. Performance status will be obtained at protocol time points as per the SOA.

Phlebotomy/Laboratory Testing

Peripheral blood for clinical and research lab testing collected via venipuncture or from an existing IV line per the SOA is approximately 75 mL per blood draw, which is within the limits

of the NIH Clinical Center MAS policy M95-9 guidelines for limits of blood drawn for research purposes in the Clinical Center.

Electrocardiogram (ECG)

Surface ECG will be performed to help exclude cardiac abnormalities in any of the enrolled subjects, during clinically indicated scenarios, and per the SOA. The ECG will take approximately 5-10 minutes.

Echocardiogram

As part of an assessment of cardiovascular phenotype, transthoracic 2D echocardiographic imaging will occur to assess cardiac and proximal vessel structure and function. Doppler and examinations will be performed. The 2D echocardiograms will be performed using a commercially available echo machine. Images will be directly stored in digital format and additional parameters (e.g. chamber volume, function measures, strain, stiffness, flow velocity etc.) may be acquired.

Six Minute Walk (6MW) Test

The 6MW test will be performed in accordance with standard practice. Briefly, site personnel will discuss the 6MW test with each subject and we will ask the subject to complete one practice walk before the 6MW. Distance walked, Borg dyspnea index, New York Heart Association functional class, heart rate, blood pressure, and oxygen saturation may be determined upon conclusion of the test as well as before the test when appropriate.

Pulmonary Function Test (PFT)

Subjects will be asked to breathe in and out in a prescribed manner so that lung function can be assessed with a spirometer.

Radiography

Chest X-rays and CT scans will be performed to screen for lung parenchymal and other disorders.

DEXA Scan

A DEXA scan will be performed to evaluate bone mineral density. The standard sites of DEXA for bone density determination are posterior/anterior and lateral lumbar spine, proximal femur (hip), and forearm (1/3 radius). DEXA is a low radiation x-ray procedure. The radiation exposure is less than 1 rem for the 3 tests combined, which is much less than daily background radiation.

Assessment of Quality of Life

PROMIS (patient reported outcome instrument) is freely available and has been applied in many diseases and conditions, and will be a useful tool to assess the overall physical and mental health perception in our patients with SCD before and after HCT. PROMIS 57 assesses various aspects of quality of life, including physical function, fatigue, social functioning, anxiety, and depression. It takes about 10 minutes to complete. The questionnaire will be administered as per SOA.

Bone Marrow Aspirate and Biopsy

Aspirates and biopsies are obtained from the posterior iliac crest when possible. After preparation of the skin with alcohol and betadine, the epidermis, and periosteal layers are infiltrated with 1% xylocaine. Individual aspirates are obtained through a 16-gauge needle. Up to 30 mL of bone marrow will be collected to rule out other hematologic disorders and for research. The 16-gauge needle will be re-inserted to obtain a tiny piece of bone for clinical assessment.

MRI/MRA (brain, liver, and heart)

MRI/MRA studies investigate the anatomy and function of the organ being scanned and its vasculature. Study specific MRI scans will be performed in the Clinical Center using an FDA-approved MRI scanner in clinical mode. The MRI/MRA study alone does not require IV placement or contrast. Multiple MRI sequences may be performed (45 minutes). Imaging procedures will include localizer scans, two-dimensional or three-dimensional studies of cardiovascular anatomy and function, contrast-enhanced cardiovascular MRI studies, and three-dimensional angiograms. The exact details of the examination performed will depend on individual patient requirements or clinical findings. Gadolinium will only be administered if clinically indicated. The MRIs may also be completed under the NHLBI 000479 protocol for those patients enrolled on both protocols.

Chimerism Analysis

Peripheral blood will be obtained at protocol time points (as per the SOA) and sorted for myeloid (CD14/15) and T cell (CD3) populations. DNA is then extracted and analyzed for donor and recipient short tandem repeats, and expressed as percentage of donor. Whole blood leukocyte donor chimerism may be used when leukocyte subsets cannot be selected.

As in 17-H-0069, engraftment outcomes will be categorized as follows: engrafted and no sickle complications, low DMC and low HbS (<50% if the donor has HbAS and <10% if the donor has HbAA) without return of SCD symptoms or complications, low DMC, high HbS ($\geq 50\%$ if the donor has HbAS and $\geq 10\%$ if the donor has HbAA) without return of SCD symptoms or complications, low DMC with return of SCD symptoms or complications, or graft rejection (<5% for both donor myeloid and T cells).

8.2.1 Biospecimen Evaluations

Biological specimen collection and laboratory evaluations

Research bloods will be processed in a NHLBI research lab. Blood testing for clinical indications will be performed in the Department of Laboratory Medicine (DLM) in the NIH Clinical Center.

8.2.2 Correlative Studies for Research/Pharmacokinetic Studies

Test or assay	Approx. blood volume	Type of tube(s)	Frequency	Location of specimen analysis
RECIPIENTS				
CBC with differential, reticulocyte count,	3-6 mL	EDTA (lavender)	Daily to twice weekly while inpatient, then at D30, 60, 100, 1 year, 2 years, 3 years	NIH DLM

hemoglobin electrophoresis, coagulation (PT/PTT)				
Acute care, LFT, mineral panel	4 mL	Lithium Heparin tube (light green)	Baseline, then at 1 year, 2 years, 3 years	NIH DLM
Chimerism	3-6 mL	EDTA (lavender)	Baseline, then at D30, 60, 100, 1 year, 2 years, 3 years	NIH DLM
Research, briquilimab PK (a)	3 mL	SST (red)	1 hour after the end of the briquilimab infusion, 24 (+/- 4) hours after briquilimab infusion, 72 (+/- 4) hours after briquilimab infusion, day of TBI (D-1), D0 (prior to PBSC infusion)	Jasper Therapeutics and/or Eurofins Bioanalytics
Research, alemtuzumab PK (b)	3 mL	SST (red)	D0 (prior to PBSC infusion), D +28 +/- 7	Fitzhugh Lab, Tisdale Lab, and/or Figg Lab
Research, abatacept PK (c)	10 mL	SST (red)	D-1 (pre and peak), D+5 (pre), D+14 +/- 7 (pre), D+28 +/- 14 (pre and peak), D+100 +/- 14 (pre), D+180 +/- 14 (pre)	Fitzhugh Lab
Research, peripheral blood for biobanking	30 mL	EDTA (lavender)	Baseline, D30, 60, 100, 1 year, 2 years, 3 years (d)	Fitzhugh lab
Research, bone marrow	5-30 mL	EDTA (lavender)	Baseline, D100 and 1 year	Fitzhugh lab
Cardiac fibrosis markers	5 mL	EDTA (lavender)	Baseline, then at 1 year, 2 years, 3 years	Fitzhugh lab
RBC biology biomarkers (e)	3 mL 1 mL	Sodium citrate (blue) EDTA (lavender)	Baseline x2, D100, 6 months, 1 year	Functional Fluidics Eaton Lab
DONORS				

Research, peripheral blood for biobanking	20-50 mL	EDTA (lavender)	Pre-stem cell donation	Fitzhugh lab
RBC biology biomarkers	3 mL	Sodium citrate (blue)	Baseline	Functional Fluidics
	1 mL	EDTA (lavender)		Eaton Lab

a Pharmacokinetic sampling (blood) for briquilimab: blood samples will be batched and stored. The timing and type of tubes used for these samples may be adjusted based on PI's discretion or discussion with Jasper Therapeutics.

b Pharmacokinetic sampling (blood) for alemtuzumab: blood samples will be batched and stored. The timing of blood sample collection may be adjusted based on PI's discretion.

c Pharmacokinetic sampling (blood) for abatacept: pre-infusion blood draw samples should be collected within 30 minutes of the start of the infusion. Post-infusion blood draw samples should be collected within 10 minutes of the end of the infusion. While every effort will be made to draw the blood within these time frames, it will not be considered a protocol deviation as long as the pre-infusion blood draw occurs within 60 minutes of the start of the infusion and the post-infusion blood draw occurs within 30 minutes of the end of the infusion.

d. An additional up to 30 mL of blood will be drawn for newly diagnosed graft rejection, GVHD, EBV reactivation, or other points of interest as determined by the PI or designee, before treatment begins, if possible.

e. 2 samples at baseline; each sample done 2 consecutive weeks after hydroxyurea is stopped prior to exchange transfusion and plerixafor autologous collection.

8.2.3 Samples for Genetic/Genomic Analysis

N/A

8.2.4 Correlative Studies for Research

1. Donor chimerism and lymphocyte subsets
2. Briquilimab levels and leukocyte counts, lymphocyte subsets
3. Alemtuzumab levels and leukocyte counts, lymphocyte subsets
4. Measure plasma cytokine, immunoglobulin, and chemokine levels
5. In vitro immune reactivity such as mixed lymphocyte reaction
6. In vitro cell culture
7. Anti-A, anti-B red cell antibody titer
8. HLA antibody titer
9. Cardiac fibrosis markers and BNP, cardiac MRI/ECHO markers of fibrosis and diastolic function
10. Measure inflammatory and coagulability biomarkers
11. Measure red blood cell biology biomarkers

8.2.4 Autologous Hematopoietic Cells

Following collection of autologous “back-up” hematopoietic cells, at least 2×10^6 /kg CD34 cells or 1×10^8 /kg TNC will be cryopreserved and stored. The rest will be aliquoted and stored for gene therapy research involving cell culture or genetic manipulation to produce normal or therapeutic hemoglobin.

8.3 Safety and Other Assessments

Medical history and physical examination

A comprehensive medical history will be obtained, including symptoms related to chronic complications of sickle cell disease, prior hospitalizations, transfusion history, medications, procedures, surgeries, and infections. Physical examination should include eyes, heart, lungs, abdomen, joints, and skin to document signs of SCD-related complications or prior procedures. Post-transplant interval history will be obtained at protocol time points as per the SOA table and focus on symptoms of GVHD, infection, or blood count changes. Physical examination will include eyes, oral mucosa, neck, heart, lungs, abdomen, and skin. Performance status will be obtained at protocol time points as per the SOA.

Vital signs

Temperature, blood pressure, pulse, respirations, oxygen saturation, and weight will be obtained at baseline and all follow-up visits. Documentation will be made of whether patients are breathing room air or oxygen. Height will be measured and recorded at baseline and as per the SOA.

Electrocardiograms (ECGs):

Surface ECG will be performed at baseline to help exclude cardiac abnormalities in any of the enrolled subjects and during clinically indicated scenarios. Surface ECGs will also be performed annually during the protocol visit to screen for abnormalities such as new arrhythmia or QT prolongation. Our cardiology team reads the ECGs as per Clinical Center policy and will be consulted when necessary for clinically significant abnormalities.

24-hour Holter monitoring

We will ask subjects to use a Holter ECG monitor for 24 to 48 hours at baseline. In this case, subjects will have ambulatory ECG collection for arrhythmia detection, conduction system functional assessment and autonomic nervous system functional assessment. The subjects will have the opportunity to record any symptoms they are experiencing in a diary.

Neuropsychologic testing

Neuropsychologic testing, performed by our licensed psychologists or other members of the neuropsychology group (supervised by a licensed psychologist), may include but not be limited to the Wechsler Abbreviated Scale of Intelligence and several subtests of the Wechsler Adult Intelligence Scale – Fourth Edition or Wechsler Intelligence Scale for Children – Fifth Edition at baseline, 1 year, and 2 years (+/- 6 months) post HCT. The comprehensive evaluation will take about 45-60 minutes to complete. In addition, a brief monitoring battery assessing selective domains such as attention, executive function, and processing speed will be administered at 100 days (+/- 7 days) post HCT. The monitoring battery will take about 25 minutes to complete. The

testing battery or duration at each assessment may be modified by the psychology team. SCD patients will not be tested when they are febrile, and will be given breaks as needed. If responses indicate evaluation from social work, psychology, and/or psychiatry are needed, they will be consulted and will follow the patient until stabilization or resolution.

Patient-reported outcomes

PROMIS 57 is freely available and has been applied in many diseases and conditions, and will be a useful tool to assess the overall physical and mental health perception in our patients with SCD before and after HCT. PROMIS 57 assesses various aspects of quality of life, including physical function, fatigue, social functioning, anxiety, and depression. The PROMIS 57 questionnaire will be administered either electronically or with pencil and paper pre-HCT, day 100, and 1, 2, and 3 years and annually post HCT. If possible, the questionnaire will also be completed on day 30, day 60, and 6 months post-HCT. Compensation for patient's time will be provided if questionnaires are completed.

Patients will also be offered reproductive and sexual health questionnaires. These will be provided to patients at baseline, 1 year, 2 years and annually post-transplant. These are titled: Fertility Survey (female), Changes in Sexual Function Questionnaire (male/female), Priapism survey and the International Index of Erectile dysfunction (See Appendix B). Compensation for the patient's time will be provided if the questionnaires are completed.

For patients that co-enroll onto the 000697 protocol, the questionnaires listed above (PROMIS and reproductive and sexual health) will be completed electronically into the 000697 REDCap system. For patients that do not enroll onto the 000697 protocol, the questionnaires may be completed with pencil and paper.

Autologous Back-Up HSC

Collection of autologous hematopoietic cells (also known as auto back-up or rescue HSC) will be performed prior to the start of conditioning. These cells may be used when there is no or insufficient cell recovery after the conditioning, to prevent prolonged cytopenia.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research. In the context of FDA-required reporting, an AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

An abnormal laboratory value or vital sign will be considered an AE if the abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact

- If any abnormal laboratory result or vital sign is considered clinically significant, the investigator will provide details about the action taken with respect to the research and about the patient's outcome.

8.4.2 Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

This study will utilize the Common Terminology Criteria for Adverse Events version 5.0 (CTCAE v5.0) for toxicity and adverse event reporting except for grading of GVHD (see below). A copy of the CTCAE v5.0 can be downloaded from:
https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

Severity definitions found in the CTCAE v5.0 will be used for grading the severity (intensity) of AEs:

- 1) **Mild (Grade 1):** Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- 2) **Moderate (Grade 2):** Minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental ADL*.
- 3) **Severe (Grade 3):** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
- 4) **Life-threatening (Grade 4):** Life-threatening consequences; urgent intervention indicated.
- 5) **Death (Grade 5):** Death related to AE.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

****Self-care ADL** refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

GVHD Grading

Acute GVHD will be graded by the modified Glucksberg/CIBMTR/Keystone system (NIH consensus 2008 and 2015), evaluating skin, liver, upper GI and lower GI symptoms. Chronic GVHD grading is based on the 2014 NIH consensus system, evaluating skin, nails, scalp and body, mouth, eyes, genitalia, GI tract, liver, lung, muscles/facia/joints, hematopoietic/immune, and other systems. The staging/grading system may be modified based on CIBMTR reporting requirements.

8.4.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study product, sickle cell disease, and/or transplant procedure assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.

- **Possibly Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as “possibly related” soon after discovery, it can be flagged as requiring more information and later be upgraded to “probably related” or “definitely related”, as appropriate.

- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).

- **Not Related** – The AE is completely independent of study intervention administration, and/or

evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.4.3.3 Expectedness

Investigator or designee will be responsible for determining whether an AE is expected or unexpected. An AE (other than allergy or infusion related) will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention (briquilimab mAb, alemtuzumab, sirolimus, TBI, cyclophosphamide, abatacept) in the package insert or investigator's brochure.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

Any medical condition that is present at the time that the participant is screened will be considered as baseline and until start of participant conditioning regimen, and not reported as an AE. AE occurrence will be assessed as any change from participant conditioning regimen in any medical condition that results in worsening in severity and/or frequency of the medical condition, whether or not considered related to study medication, sickle cell disease or transplant procedure.

Briquilimab (JSP191) is a new agent to the alemtuzumab-400 cGy TBI-sirolimus- PT-Cy regimen used in 09-H-0225 and 17-H-0069, and briquilimab (JSP191) clearance may take about 2 weeks after infusion. Similarly, alemtuzumab remains detectable up to 3-4 weeks after infusion.

Abatacept is also new to this protocol the NHLBI transplant regimens, but has been used in the transplant setting in patients with hematologic malignancies, aplastic anemia, and SCD^{49,50,71,72}

Adverse events to be collected at the time of the briquilimab infusion that meet the criteria of CTCAE v 5.0 grade 2 and higher, including laboratory abnormalities that meet the definition of an AE will be captured on the appropriate case report form (CRF) regardless of relationship.

Adverse events prior to briquilimab infusion and after engraftment that meet the criteria of CTCAE v 5.0 grade 3 and higher, including laboratory abnormalities will be captured on the appropriate case report form (CRF) regardless of relationship.

AEs and SAEs whether volunteered by the patient, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, upon review by a study monitor or other means will be recorded. Information to be collected at each study visit includes event description, time of onset, clinician's assessment of severity, relationship to study product, relationship to transplant procedure (assessed only by those with the training and authority to make a diagnosis), and date of resolution/stabilization of the event. These AEs will be followed to adequate resolution when possible or until end of subject study participation.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent may require documentation of onset and duration of each episode per PI discretion.

When possible, signs and symptoms, procedural results and laboratory findings indicating a common underlying pathology or disease, should be recorded as one comprehensive event.

8.4.5 Adverse Event Reporting

8.4.6 Serious Adverse Event Reporting

Any serious adverse events that occur prior to the initiation of conditioning therapy will only be reported if at least possibly related to a pre-transplant procedure.

All SAEs that occur once conditioning therapy has been initiated will be reported by the study investigator to the sponsor and Clinical Director within 10 days, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event.

All SAEs will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by study sponsor and should be provided as soon as possible.

The study sponsor will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information in accordance with 21 CFR 312. In addition, the sponsor must notify the FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

Sponsor Representative will inform Jasper Therapeutics of all SAEs:

1. Copies of all IND Safety Reports will be submitted to Jasper concurrently with their submission to the FDA.
2. All other SAEs will be submitted to Jasper within 10 days of SAE.

The SAEs and IND Safety Reports should be sent to the following email or fax:

Email	safety@jaspertherapeutics.com WILSafety@ppd.com <i>Please email BOTH emails for any new SAEs to be sent to Jasper.</i>
PPD Pharmacovigilance North America Safety Hotline	800-201-8725
PPD Pharmacovigilance North America Facsimile	1-888-488-9697

8.4.7 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem or is new information that might affect the willingness of subjects on the NIH study to enroll or remain in the study will need to be reported to the NIH Intramural IRB in an expedited manner.

8.4.8 Events of Special Interest (briquilimab)

Decreased Hemoglobin and Hematocrit: Decreases in reticulocytes and hemoglobin were observed in subjects receiving briquilimab in cohorts 8 through 10 of the FIH study. No subject had a hemoglobin value less than 10 g/dL. The effects were reversible and not accompanied by clinical signs or symptoms. This finding is consistent with the mechanism of action of briquilimab and the similar findings in cynomolgus monkeys.

Subjects will be monitored for changes in hematologic parameters as a routine part of their care and a component of safety monitoring during clinical studies with briquilimab. Subjects should receive appropriate supportive care as indicated for hematologic adverse events.

Upper Respiratory Infection: A higher incidence of URI was observed in the combined briquilimab group (13 events in 12 subjects, 18.5%, N = 65) compared to 0 in placebo subjects (N = 23) in the first in human trial. All of these events were mild (grade 1) except for one grade 2 event. Clinical information about these events is limited, and the type of infection (viral, bacterial, etc.) is not known; however, no antimicrobial treatment or fever was reported in association with these events. No signs or symptoms of infection were seen in toxicology studies of cynomolgus monkeys. Subjects will be monitored for evidence of infection during briquilimab studies. Subjects should receive appropriate supportive care as indicated for hematologic adverse events.

Hypersensitivity Reactions: Several events consistent with hypersensitivity reactions have been reported following administration of briquilimab. These events have been generally self-limited and non-serious. No relationship was evident between these events and briquilimab dose. Subjects exposed to briquilimab should be carefully monitored for potential hypersensitivity reactions, and clinical study sites should be prepared to recognize and treat any such reaction. Rash, bronchospasm, anaphylaxis, allergy-related edema, hypotension will be graded and monitored until day 28 after infusion, or discharge from inpatient admission (whichever is later).

8.4.9 Reporting of Pregnancy

We typically advise patients not to start having children until one year post-HCT or while on sirolimus. Research studies will not be performed during pregnancy.

Should a participant become pregnant after the transplant has occurred, the investigator, or his/her designee, will follow the subject for safety and pregnancy outcomes. Pregnancy information will be submitted to the NHLBI Clinical Director, NIH Intramural IRB, and Jasper within two weeks of learning of a participant's pregnancy.

Pregnancy per se is not considered an AE unless there is a cause to believe that the study interventions may have interfered with the effectiveness of a contraceptive medication or if the outcome of the pregnancy meets SAE criteria (miscarriage/fetal death or congenital anomaly/birth defect), in which case it should be reported in the same manner and timelines as an SAE. Hospitalization for normal delivery is not an SAE. A spontaneous abortion is always considered to be an SAE.

If a partner of a participant becomes pregnant, the investigator will attempt to request consent from the partner to collect relevant safety information under an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per [Policy 801](#).

9 STATISTICAL CONSIDERATIONS

We propose to test a novel allogeneic PBSCT regimen in high risk patients with SCD. The primary endpoint for each individual is a dichotomous positive/negative outcome where a positive response is defined by absence of graft rejection and absence of severe acute GVHD (grade 3 and higher) or moderate to severe chronic GVHD evaluated 1 year post-transplant. Any other outcome is a negative (i.e. failed) response. Transplanted individuals who do not provide an endpoint outcome (primarily due to dropout or death) will be treated as having a failed response. If an enrolled patient dies before transplant for reasons unrelated to transplant or is otherwise unable to be transplanted, up to 3 such patients will be replaced. Based on historical data for patients with SCD and compromised organ function presented above, we do not believe the primary and secondary endpoints will be impacted by organ function status, and all patients will be analyzed together.

9.1 Statistical Hypothesis

Primary Endpoint:

The percentage of SCD patients at 1 year (+/- 3 months) post-transplant who have not experienced graft rejection and who are without severe graft-versus-host disease (defined as grade 3 and higher acute GVHD and moderate to severe chronic GVHD). Therefore, as seen in the table, regimen failure is defined as the presence of graft rejection and/or the presence of severe GVHD by 1 year post-transplant. Any patient who dies after transplant but before 1 year

will also be counted as a regimen failure. Graft rejection is defined as the absence of sustained donor cell engraftment²⁵ (see section 6.1).

	No graft rejection	Graft rejection
No GVHD	Desired outcome	Regimen failure
Yes GVHD	Regimen failure	Regimen failure

Secondary Endpoint(s):

1. Total hemoglobin and percent HbS levels
2. Percent donor myeloid chimerism and donor CD3 chimerism
3. Day of neutrophil engraftment
4. Day of platelet engraftment
5. RBC transfusion requirement
6. Rates of acute and chronic GVHD
7. Rates of viral reactivation and disease
8. Rates of autoimmune and hyperinflammatory complications
9. Transplant-related mortality
10. Non-transplant-related mortality
11. Rates of graft failure and rejection
12. Rates of leukemia and related disorders
13. Organ function and quality of life

Exploratory Endpoint:

1. Completion of gene therapy research involving cell culture or genetic manipulation to produce normal or therapeutic hemoglobin on excess autologous CD34+ cells collected from recipients.

9.2 Sample Size Determination and Stopping Rules

A sample size of 15 balances the desire for precision in estimating success with practicalities of enrollment. Of particular interest is the lower one-sided 95% confidence interval for the observed proportion of successes, i.e. the proportion without regimen failure as defined in 9.1. As this is a pilot study, with N = 15, there will be some limited power for estimating the true probability of success. The study is designed to determine if the primary outcome success rate shows preliminary efficacy warranting further development.

If an enrolled participant dies before transplant for reasons unrelated to transplant or is otherwise unable to be transplanted, up to 3 recipient subjects will be replaced (and respectively up to 3 donor participants). Therefore, the accrual ceiling for this protocol is up to 72 subjects (36 in each cohort (18 recipients and 18 donors). In the event a second cohort is enrolled (because efficacy or safety stopping rules unrelated to uncontrolled viral disease or PTLD have been met in the first cohort) the data from the two cohorts will not be combined but reported separately. Further, the counts of events contributing to the stopping rules for the second cohort would restart from zero, essentially restarting the analysis for the second cohort with zero patients.

If the true probability of regimen success (engraftment without GVHD) is 85% then there will be 60% power to reject the null hypothesis that the success rate is 60% or lower. If the true success

rate is 90% the power for rejecting the null hypothesis that the success rate is 60% or lower, then power rises to 81% (calculations from PASS software version 20.0.1).

There is 82% and 94% power to rule out relatively poor performance of 50% or lower when the true probability is 85% and 90%, respectively. Rejecting the null hypothesis of 50% or lower true success probability would likely warrant pursuing a larger, confirmatory trial.

The study employs stopping rules for efficacy and safety. Because the 11 subjects enrolled on haploidentical protocol 09-H-0225 who acutely rejected their grafts did so by 100 days post-transplant, stopping rules for graft rejection (regardless of GVHD) will be monitored at 100 days post-transplant. Stopping rules follow a Bayesian design⁷⁹. We feel a successful transplant regimen in patients with severe organ damage should have a successful engraftment probability of at least 70%. Consequently, the stopping rule is designed to halt enrollment when the Bayesian posterior probability indicates the true probability that a patient's graft fails is 30% or more is at least 80%. The prior distribution for the probability of graft rejection is given by a beta distribution with parameters $a = 1$ and $b = 7/3$. For these parameters the prior distribution has a mean of 30% and weight of 3.33 individuals, while the study population will contribute the weight of up to 15 individuals.

The following table summarizes the threshold numbers for the resulting boundary in patients with compromised organ function, which would lead to a recommendation to stop the study due to an excess number of graft rejections.

Number of patients with compromised organ function transplanted	Stop if the number of subjects who have graft rejection is
≤ 3	2
≤ 5	3
≤ 8	4
≤ 11	5
≤ 14	6
15	7

We investigated the performance of the above stopping rule through simulation. In each simulation we generated a set of 15 independent Bernoulli trials, each representing a patient with probability p for graft rejection. For each simulation we determined if the stopping boundary would have been reached with the recommendation to halt the study. We conducted this simulation 100,000 times for each different true value of probability p and recorded the average number of patients treated (it may be less than 15 if the study was stopped early) and the average number of rejections. In addition, we show the proportion of the 100,000 simulations in which the stopping boundary was met. The following table summarizes the performance of the stopping rule under a number of scenarios for rejection probability p :

Probability of graft rejection	0.20	0.25	0.30	0.35	0.40	0.45	0.50
Proportion of Stopped Studies	16%	26%	39%	53%	66%	77%	86%

Average number of subjects	13.3	12.4	11.3	10.0	8.8	7.5	6.4
Average number of graft rejections	2.7	3.1	3.4	3.5	3.5	3.4	3.2

These simulation results suggest that our stopping rule has a low probability of stopping a study when the probability of rejection events is below 30% (e.g. a 26% probability of stopping when the true probability is 25%), and the probability of stopping a study is high when the true probability of rejection exceeds 35% (e.g. a 66% probability of stopping when the true probability of rejection is 40%). Thus, we believe that this Bayesian stopping rule has satisfactory statistical properties.

The study employs a second stopping rule specifically for severe acute GVHD (grade 3 and higher), moderate to severe chronic GVHD, viral disease that is not controllable by anti-viral therapy, or PTLT requiring more than monoclonal antibody therapy (e.g., rituximab).

Uncontrollable viral disease is defined as viral disease requiring more than standard therapy including the following:

- CMV requiring more than standard therapy (e.g., foscarnet, val(ganciclovir))
- Adenovirus requiring more than standard therapy (e.g., cidofovir)
- Varicella-Zoster Virus requiring more than standard therapy (e.g., intravenous acyclovir)
- Any requirement for viral-specific T-cells

Because the majority of viral disease and PTLT occurs by 100 days post-transplant^{80,81} and because acute GVHD is a major risk factor for chronic GVHD^{82,83}, we expect patients that experience GVHD, viral disease, or PTLT will do so by day 100 post-transplant. Thus, these stopping rules will be assessed at 100 days post-transplant. The stopping rule is designed to halt enrollment when the Bayesian posterior probability indicates the true probability that a patient experiences either moderate or severe GVHD (as defined above), uncontrolled viral disease, or uncontrolled PTLT is 15% or more is at least 75%. The prior distribution for the probability of GVHD or uncontrolled viral disease or PTLT is given by a beta distribution with parameters $a = 1/2$ and $b = 17/6$. For these parameters the prior distribution has a mean of 15% and weight of 3.33 individuals, while the study population will contribute the weight of up to 15 individuals.

The following table summarizes the threshold numbers for the corresponding boundary, which would lead to a recommendation to stop the study due to an excess number of GVHD rejections or patients with uncontrolled viral disease or PTLT.

Number of subjects in the study with compromised organ function	Stop if the number of subjects who have GVHD or uncontrolled viral disease or uncontrolled PTLT is
≤ 6	2
≤ 12	3
≤ 15	4

As before, we investigated the performance of the above stopping rule through simulation. In each simulation we generated a set of 15 independent Bernoulli trials; each representing a patient with probability p for GVHD, uncontrolled viral disease, or uncontrolled PTLT. For each simulation we determined if the stopping boundary would have been reached with the recommendation to halt the study. We conducted this simulation 100,000 times for each different true value of probability p and recorded the average number of patients treated (it may be less than 15 if the study was stopped early) and the average number of failures. The following table summarizes the performance of the stopping rule under a number of scenarios for failure probability p :

Probability GVHD or uncontrolled viral disease or PTLT	0.05	0.10	0.15	0.20	0.25	0.30	0.35
Proportion of stopped studies	4.3%	17%	35%	53%	69%	81%	90%
Average number of subjects	14.6	13.6	12.2	10.6	9.0	7.7	6.5
Average number of GVHD or uncontrolled viral disease or PTLT	0.7	1.4	1.8	2.1	2.3	2.3	2.3

These simulation results suggest that our stopping rule has a low probability stopping a study when the probability of GVHD or uncontrolled viral disease or uncontrolled PTLT events is below 15%, and the probability of stopping a study is high when the true probability of failure exceeds 15%. Thus, we believe that this Bayesian stopping rule has satisfactory statistical properties.

Because patients without compromised organ function have a high likelihood of success with haploidentical HCT for SCD at other centers³⁴, a stopping rule based on an engraftment rate of at least 70% is not sufficiently stringent. Therefore, for patients without compromised organ function, we will use the same stopping rule for graft rejection as described above for GVHD or uncontrolled viral disease or uncontrolled PTLT; the study would stop if any of those outcomes reached 15%.

Number of subjects in the study without compromised organ function	Stop if the number of subjects without compromised organ function who have graft rejection, GVHD, or uncontrolled viral disease or uncontrolled PTLT is
≤ 6	2
≤ 12	3
≤ 15	4

Patients will be transplanted at a frequency to ensure that stopping rules are not crossed. For example, with the first 6 patients transplanted, no more than 2 patients will be transplanted every

100 days.

In the event the study enrollment is stopped for the reasons above, a remedial plan will be discussed with the FDA and the NIH IRB and no subjects will be enrolled until that plan is agreed upon and implemented.

We also include a stopping rule for treatment-related mortality. If any patient dies at least possibly as a result of the research, the protocol will pause accrual and discussion with the DSMB and IRB will follow to determine next steps for the protocol. Further, if 2 patients develop a hematologic malignancy, 2 patients experience an uncontrolled autoimmune disease, 2 patients experience an uncontrolled hyperinflammatory disease, or 2 patients experience late graft failure (after 100 days post-HCT), then accrual will be halted and we will discuss with the DSMB and the IRB whether the conditioning regimen should be adjusted.

9.3 Populations for Analyses

9.3.1 *Evaluable for toxicity*

All participants who have received CD117 antibody will be included in the analysis of AEs and evaluated for toxicity from the time of their first treatment with the antibody.

9.3.2 *Evaluable for objective response*

Those patients who have received CD117 antibody and completed a transplant procedure will be evaluated for the primary analysis and secondary endpoints unless otherwise noted.

9.4 Statistical Analyses

9.4.1 *General Approach*

The presentation of descriptive statistics such as baseline characteristics will depend on the data characteristics. Categorical data will be presented as percentages in the categories while continuous data will be described by median, IQR, and range. See below for details of how endpoint data will be analyzed.

9.4.2 *Analysis of the Primary Endpoints*

See the description of the primary endpoint above in section 9.1. Of interest are the one-sided 95% lower confidence interval and the 95% two-sided confidence intervals for the population probability of having regimen success. The analyses will be applied to the evaluable for objective response population described in the previous section above. Regimen success is defined as successful engraftment and without severe graft-versus-host disease (defined as grade 3 and higher acute GVHD and moderate to severe chronic GVHD) through the first year after transplant. Calculations will be based on exact tests rather than normal approximations and will use the Clopper-Pearson method for computing confidence intervals.

A secondary, sensitivity analysis will analyze the primary endpoint without including those who die before 1 year for causes unrelated to transplant (but will still count as failures for those who died within 1 year due to reasons related to transplant).

9.4.3 *Analysis of the Secondary Endpoint(s)*

The Kaplan–Meier method will be used to analyze overall survival and disease-free survival patterns following the PBSCT for the population evaluable for objective response described above.

Cumulative incidence functions will be used when treating death as a competing risk for analyses of time to GVHD, viral disease, PTLN, disease relapse, graft rejection, days until neutrophil and platelet engraftment, and transplant-related mortality (for which non-transplant-related causes of death are a competing risk). If there are less than 3 events occurring for a specific outcome (e.g., 2 or fewer instances of GVHD) then the event outcome will be described descriptively (number of events and average time to events) rather than using cumulative incidence curves.

Given the small sample sizes and likely small number of graft failures following PBSCT, the examination of chimerism required to maintain graft survival and hematologic normalcy will be descriptive and exploratory.

Neutrophil engraftment is defined as first of 3 consecutive days with neutrophil count >500 cells/L. Cumulative incidence curves will be computed for the time to engraftment. Death and graft failure will be considered competing risks.

Platelet engraftment is defined as first of three consecutive days with values $\geq 50 \times 10^9/L$ obtained on different days and 7 days from last platelet transfusions. Cumulative incidence curves will be computed for the time to engraftment. Death and graft failure will be considered competing risks.

Graft rejection is defined as DMC (CD14/15) and donor lymphoid (CD3) chimerism <5%. The timing and circumstances of graft rejection will be detailed for any such events. Should more than three such events occur we will present cumulative incidence curves for graft rejection with death not following graft rejection as a competing risk.

9.4.4 *Safety Analyses*

Viral activity will be monitored. Activity will be reported once per person for a specific virus so we will report the observed proportion of the safety population with reactivation/disease of each virus. Two-sided exact 95% Clopper-Pearson confidence intervals will be presented.

GVHD will be reported once per person for acute and chronic GVHD - we will report the observed proportion of the safety population with acute and chronic GVHD. Also we will report the proportion with either acute or chronic GVHD. Two-sided exact 95% Clopper-Pearson confidence intervals will be presented.

The timing, relatedness to transplant, and circumstances of mortality will be detailed for any such events. We do not anticipate enough events to warrant time-to-event analyses but if more than three such events occur we will include Kaplan-Meier (for all-cause mortality) and cumulative incidence curves for transplant and non-transplant related mortality if competing risk events occur.

9.4.5 *Baseline Descriptive Statistics*

Summary statistics of baseline patient characteristics will be provided. For continuous variables the min, max, median, 25th percentile, and 75th percentile will be shown. For categorical variables the observed proportion in each category will be presented. As we are not comparing interventions, we do not anticipate testing for baseline differences between groups.

9.4.6 *Planned Interim Analyses*

None.

9.4.7 *Sub-Group Analyses*

Given the small total accrual for this study, we do not anticipate conducting statistical tests to evaluate differences between subgroups. Descriptive statistics may be presented but any formal or informal comparisons will be presented with a statement of the post-hoc nature of the comparisons.

9.4.8 *Tabulation of individual Participant Data*

The time after transplant at which individuals experienced event endpoints of interest (e.g. graft rejection, viral reactivation, GVHD) will be observable in Kaplan-Meier or cumulative incidence curves. Otherwise, individual data will not be shown but rather presented in summary measures for the transplanted population.

9.4.9 *Exploratory Analyses*

N/A

REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 *Informed Consent Process*

10.1.1 *Consent/Assent Procedures and Documentation*

Informed consent will be conducted following OHSRP Policy 301- Informed Consent.

An IRB-approved consent form will be provided to the participant electronically or by hard copy for review prior to consenting. The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved platforms). The investigational nature and objectives of this trial, the procedures, and their attendant risks and discomforts and potential benefits will be carefully explained to the participant in a private setting. The participant will be given as much time as they need to review the document and to consult with their family, friends, and personal health care providers. In addition, a study team member will be available to answer any questions.

A signed and dated informed consent document will be obtained by any investigator authorized to consent prior to entry onto the study. Consent may be obtained with required signatures on the hard copy of the consent or on the electronic document.

When a document that is in electronic format is used for obtaining consent, this study may use the iMed platform which is 21 CFR, Part 11 compliant, to obtain the required signatures.

During the consent process, participants and investigators may view the same approved consent document simultaneously when participant is being consented in person at the Clinical Center or both may view individual copies of the approved consent document on screens in their respective locations remotely. Signatures may be obtained either by both directly signing on the device that the consenting investigator is using (when in person) or through iMed Mobile Signature Capture

(remotely) which allows texting or emailing a link to the participant. That link allows the participant to review the consent, then proceed to sign on the device they are using.

Whether hard copy or electronic, both the investigator and the participant will sign the document with a hand signature using a pen (if using hard copy), finger, stylus, or mouse (if electronic).

When done remotely, if the participant prefers to sign a hard copy, they may be instructed to sign and date the consent document during the discussion and mail, secure email or fax the signed document to the consenting investigator.

Whether in person or remotely, the privacy of the participant will be maintained.

Finally, the fully signed informed consent document will be stored in the electronic medical record, and the participant will receive a copy of the signed informed consent document.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

For assent of children, if applicable

If the subject is a minor, the parent who signs the consent for the minor must be a legally recognized parent or guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent.

In cases where parents share joint legal custody for medical decisions for a child (e.g., by custody agreement or court order), both parents must give their permission regardless of the level of risk of the research. Exceptions may be made if one parent has since died, becomes incompetent, or is not reasonably available (e.g., incarcerated).

Donors 14 years of age and recipients 16 years of age or older will review and discuss the respective adult consent with the parents and research team, and sign the adult consent in the assent line. If a minor is between the ages of 7 and 13 years of age, then the minor will sign the minor assent form. Minors under 7 years will not provide assent because they are not able to understand the nature of the research. Pediatric donors will be taken off study after they donate PBSCs.

10.1.2 Consent for minors when they reach the age of majority

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

When we have no ongoing interactions with the subjects, including if subjects are lost to follow up or withdrawn from the study, we request waiver of informed consent to continue to use data and/or specimens obtained from those individuals.

Requirements for Waiver of Consent consistent with 45 CFR 46.116(f)(3):

- (1) The research involves no more than minimal risk to the subjects.

- a. Analysis of samples and data from this study involves no additional risks to subjects.
- (2) The research could not practicably be carried out without the waiver or alteration.
 - a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.
- (3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.
 - a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.
- (4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
 - a. Retention of these samples or data does not affect the welfare of subjects.
- (5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
 - a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH staff will be obtained as detailed above with following additional protections: Consent from staff members will be obtained by an individual independent of the staff member's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the staff member.

10.1.4 Consent of Subjects who become decisionally impaired

This protocol will not target decisionally impaired subjects. If there is an unexpected enrollment of a research participant unable to provide informed consent, the subject's legally authorized representative (LAR) will be contacted and assessed for capacity to consent. In doing so, we will follow procedures per NIH HRPP Policy 403. If documentation of decision making capacity is not present in the medical record or the investigator questions the decision-making capacity of the individual, then the Ability to Consent Assessment Team (ACAT) at (301)496-9675 or (301)496-2429 will be contacted to make the determination.

When a subject with capacity consented to the research, and has a temporary loss of capacity (e.g., they are expected to regain capacity), reconsent of the subject by the LAR is not required for the subject's continued participation in the research. However, during the period of

temporary incapacity, the LAR should be engaged to advocate on behalf of the subject, until capacity is regained.

Lastly, Policy 403 specifies that the PI is responsible for engaging with the LAR.

10.2 Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to: study participants, investigator, funding agency, the Investigational New Drug (IND), sponsor, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and, as applicable, the Food and Drug Administration (FDA).

10.3 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NIH CC. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be

identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NIH CC research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NIH CC.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

10.4 Future use of Stored Specimens and Data

The remaining identifiable biospecimens will be stored for future use with IRB approval. Data generated from this protocol will be retained and may be used in the future. Data will be coded and the key will be retained in a secure location.

10.5 Safety Oversight

Safety oversight will be under the direction of a Data and Safety Monitoring Board (DSMB) composed of individuals with the appropriate expertise, including hematopoietic cell transplantation and sickle cell disease. Members of the DSMB should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet regularly to assess safety and efficacy data on each arm of the study. The DSMB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to NHLBI.

10.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/ amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Monitoring of study conduct and clinical investigation progress will be conducted by the NHLBI Clinical Monitoring Program on a regular basis. This monitoring is commensurate with the protocol's degree of risk and the size, complexity and scope of research. Post monitoring "wrap-up" meetings will take place with the study team and formal monitoring follow-up reports will be sent to the PI for filing in the investigator site file.

10.7 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The site will provide direct access to all trial related source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff under the supervision of the principal investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Clinical Trial Database (CTDB) a 21 CFR Part 11- compliant data capture system provided by the NIH CC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. All deviations must be addressed in study source documents, reported to NHLBI Program Official

and NIH Clinical Center. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 Human Data Sharing, Including Genomic Data Sharing, and Publication

10.10.1 NIH Data Management and Sharing Policy and NIH Genomic Data Sharing Policy

This study will comply with the NIH Data Management and Sharing (DMS) Policy, which applies to all new and ongoing NIH-funded research in the IRP, as of January 25, 2015, that is associated with a ZIA, with a clinical protocol that undergoes scientific review and/or will involve genomic data sharing.

The NIH Genomic Data Sharing (GDS) Policy does not apply to this study.

10.10.2 NIH Public Access Policy Compliance

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive [PubMed Central](#) upon acceptance for publication.

10.11 Collaborative Agreements

10.11.1 Agreement Type

Transfers that are associated with correlative studies conducted under an approved protocol:

NHLBI and Jasper Therapeutics have signed a Clinical Trials Agreement. NHLBI will provide the protocol, conduct the accrual and care of the patients, send blood samples to Jasper for pharmacokinetic monitoring, and participate in data safety monitoring of the protocol. Jasper will provide: investigator brochure, relevant material for protocol development, briquilimab antibody for the protocol, and pharmacokinetic data.

10.12 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NHLBI has established

policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

ABBREVIATIONS

6MW	6 minute walk
Ab	Antibody
ACAT	Ability to Consent Assessment Team
ACS	Acute chest syndrome
ADL	Activities of daily living
AE	Adverse event
AI	Associate investigator
Allo	Allogeneic
ALC	Absolute lymphocyte count
ALT	Alanine transaminase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
BMDW	Bone Marrow Donors Worldwide
BMT	Bone marrow transplant
BP	Blood pressure
CC	Clinical Center
CFR	Code of Federal Regulations
cGy	Centigray
CHO	Chinese hamster ovary
CIBMTR	Center for International Blood and Marrow Transplant Research
CMV	Cytomegalovirus
CrCl	Creatinine clearance
CRF	Case report form
CRIS	Clinical Research Information System
CT	Computerized tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTDB	Clinical Trial Database
CTLA4	Cytotoxic T-lymphocyte-associated antigen 4
D5W	5% Dextrose in water
DAT	Direct antiglobulin test
DEXA	Dual x-ray absorptiometry
DIR	Division of Intramural Research
DLCO	Diffusion capacity of carbon monoxide
DLM	Department of Laboratory Medicine
DMC	Donor myeloid chimerism
DPA	Durable Power of Attorney
DSA	Donor-specific human leukocyte antigen antibodies
DSMB	Data and Safety Monitoring Board
DTM	Department of Transfusion Medicine

EBV	Epstein-Barr Virus
ECG	Electrocardiogram
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EDTA	Ethylenediaminetetraacetic acid
EVA	Ethylene vinyl acetate
ESRD	End-stage renal disease
FDA	Food and drug administration
FLAIR	Food and drug administration
GFR	Glomerular filtration rate
GI	Gastrointestinal
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GVHD	Graft-versus-host disease
H&P	History and physical examination
HbA	Adult hemoglobin
HbS	Sickle hemoglobin
HCG	Human chorionic gonadotrophin
HCT	Hematopoietic cell transplant
HLA	Human leukocyte antigen
HRPP	Human Research Protection Program
HSC	Hematopoietic stem cell
ICH GCP	International Conference on Harmonisation Good Clinical Practice
ID	Identification
IND	Investigational new drug
IP	Investigational product
IRB	Institutional Review Board
IV	Intravenous
JSP	Jasper
LAR	Legally authorized representative
LFT	Liver function test
MDS	Myelodysplastic syndrome
MFI	Mean fluorescence intensity
Mos	Months
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
MUD	Matched unrelated donor
NCI	National Cancer Institute
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NMDP	National Marrow Donor Program
OCD	Office of Clinical Director

PaO2	Partial pressure of oxygen in the arterial blood
PBSCs	Peripheral blood stem cells
PBSCT	Peripheral blood stem cell transplant
PC	Pentostatin/cyclophosphamide
PFTs	Pulmonary function tests
PI	Principal investigator
PO	By mouth
PROMIS	Patient-Reported Outcomes Measurement Information System
PT	Prothrombin time
PTT	Partial thromboplastin time
PT-Cy	Post-transplant cyclophosphamide
PTLD	Post-transplant lymphoproliferative disorder
PVC	Polyvinyl chloride
QC	Quality control
RBC	Red blood cell
SAD	Source-axis distance
SAE	Serious adverse event
SC	Subcutaneous
SCD	Sickle cell disease
SCF	Stem cell factor
SCID	Severe combined immunodeficiency
SIADH	Syndrome of inappropriate antidiuretic hormone
SOA	Schedule of activities
SOPs	Standard Operating Procedures
TBI	Total body irradiation
TCT	Transplant and Cellular Therapy
TRV	Tricuspid regurgitant jet velocity
UCB	Umbilical cord blood
UP	Unanticipated problem
URI	Upper respiratory infection
US	United States
VOC	Vaso-occlusive crises
WBC	White blood count

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APPENDIX A: ECOG PERFORMANCE STATUS SCALE

GRADE	DESCRIPTION
0	Fully active, able to carry on all pre-disease activities without restriction.
1	Restricted in physically strenuous activities and able to carry out work of a light or sedentary nature, e.g. light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours.

- | | |
|---|---|
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. |
| 5 | Dead. |

APPENDIX B : Reproductive/Fertility Questionnaires

These questionnaires will be completed before and after transplant (pre-curative/post-curative). The pre and post-curative questionnaires ask the same questions. Examples are provided here; however, they are also available in standalone versions due to formatting inconsistencies.

Changes in Sexual Functioning Questionnaire (CSFQ-F-C) Page 1 **for Females Pre-Curative Therapy**

Please complete the survey below.

Thank you!

This is a pre-curative therapy questionnaire about sexual activity and sexual function. By sexual activity, we mean sexual intercourse, masturbation, sexual fantasies and other activity.

1) Today's Date

2) Compared with the most enjoyable it has ever been, how enjoyable or pleasurable is your sexual life right now?

- ☐ 1-No enjoyment or pleasure
☐ 2-Little enjoyment or pleasure
☐ 3-Some enjoyment or pleasure
☐ 4-Much enjoyment or pleasure
☐ 5-Great enjoyment or pleasure

-
- 9) Are you easily aroused?
- ☐ 1-Never
 - ☐ 2-Rarely (much less than half the time)
 - ☐ 3-Sometimes (about half the time)
 - ☐ 4-Often (much more than half the time)
 - ☐ 5-Always
-
- 10) Do you have adequate vaginal lubrication during sexual activity?
- ☐ 1-Never
 - ☐ 2-Rarely (much less than half the time)
 - ☐ 3-Sometimes (about half the time)
 - ☐ 4-Often (much more than half the time)
 - ☐ 5-Always
-
- 11) How often do you become aroused and then lose interest?
- ☐ 5-Never
 - ☐ 4-Rarely (much less than half the time)
 - ☐ 3-Sometimes (about half the time)
 - ☐ 2-Often (much more than half the time)
 - ☐ 1-Always
-
- 12) How often do you experience an orgasm?
- ☐ 1-Never
 - ☐ 2-Rarely (much less than half the time)
 - ☐ 3-Sometimes (about half the time)
 - ☐ 4-Often (much more than half the time)
 - ☐ 5-Always
-

**Changes in Sexual Functioning Questionnaire (CSFQ-M-C)
for Males Pre-Curative Therapy**

Please complete the survey below.
Thank you!

This is a pre-curative therapy questionnaire about sexual activity and sexual function. By sexual activity, we mean sexual intercourse, masturbation, sexual fantasies and other activity.

- 1) Today's Date
- 2) Compared with the most enjoyable it has ever been, how enjoyable or pleasurable is your sexual life right now?

☐ 1-No enjoyment or pleasure

☐ 2-Little enjoyment or pleasure

☐ 3-Some enjoyment or pleasure

☐ 4-Much enjoyment or pleasure

☐ 5-Great enjoyment or pleasure

3) How frequently do you engage in sexual activity (sexual intercourse, masturbation, etc.) now?

☐ 1-Never

☐ 2-Rarely (once a month or less)

☐ 3-Sometimes (more than once a month, up to twice a week)

☐ 4-Often (more than twice a week)

-
- 9) Do you get an erection easily?
- ☐ 1-Never
 - ☐ 2-Rarely (much less than half the time)
 - ☐ 3-Sometimes (about half the time)
 - ☐ 4-Often (much more than half the time)
 - ☐ 5-Always
-
- 10) Are you able to maintain an erection?
- ☐ 1-Never
 - ☐ 2-Rarely (much less than half the time)
 - ☐ 3-Sometimes (about half the time)
 - ☐ 4-Often (much more than half the time)
 - ☐ 5-Always
-
- 11) How often do you experience painful, prolonged erections?
- ☐ 5-Never
 - ☐ 4-Rarely (once a month or less)
 - ☐ 3-Sometimes (more than once a month, up to twice a week)
 - ☐ 2-Often (more than twice a week)
 - ☐ 1-Every day
-
- 12) How often do you have an ejaculation?
- ☐ 1-Never
 - ☐ 2-Rarely (once a month or less)
 - ☐ 3-Sometimes (more than once a month, up to twice a week)
 - ☐ 4-Often (more than twice a week)
 - ☐ 5-Every day

Priapism Impact Questionnaire Pre-Curative Therapy

Please complete the survey below.

Thank you!

The purpose of this pre-curative therapy questionnaire is to capture data regarding the impact of priapism. This form is for participants 18 years old and older. If the participant's provider is a pediatrician, we defer to the pediatrician's judgment in determining the appropriateness of this form.

Date

Have you ever experienced priapism?

☐ Yes ☐ No

I worry about my overall health has been:

- ☐ 1. None
☐ 2. Minimal
☐ 3. Slight
☐ 4. Moderate
☐ 5. Substantial
☐ 6. Extreme
☐ 7. Very Extreme

The effect of criticism on my relationship with my

Q 1. Mass

Page 1

International Index Of Erectile Function

Please complete the survey below.

Thank you!

The purpose of this questionnaire is to capture data regarding the impact of erectile dysfunction. This form is for participants 18 years old and older. If the participant's provider is a pediatrician, we defer to the pediatrician's judgment in determining the appropriateness of this form.

1) Date

Precede all questions listed below with the phrase, "Over the past 4 weeks,

2) How often were you able to get an erection during sexual activity?

- ☐ 0. No sexual activity
- ☐ 1. Almost never/ Never
- ☐ 2. A few times (Much less than half the time)
- ☐ 3. Sometimes (About half the time)
- ☐ 4. Most times (Much more than half the time)
- ☐ 5. Almost always/ Always

-
- | | |
|---|---|
| 8) When you attempted sexual intercourse, how often was it satisfactory to you? | <input type="radio"/> 0. Did not attempt intercourse
<input type="radio"/> 1. Almost never/ Never
<input type="radio"/> 2. A few times (Much less than half the time)
<input type="radio"/> 3. Sometimes (About half the time)
<input type="radio"/> 4. Most times (Much more than half the time)
<input type="radio"/> 5. Almost always/ Always |
| <hr/> | |
| 9) How much have you enjoyed sexual intercourse? | <input type="radio"/> 0. No intercourse
<input type="radio"/> 1. No enjoyment
<input type="radio"/> 2. Not very enjoyable
<input type="radio"/> 3. Fairly enjoyable
<input type="radio"/> 4. Highly enjoyable
<input type="radio"/> 5. Very highly enjoyable |
| <hr/> | |
| 10) When you had sexual stimulation or intercourse, how often did you ejaculate? | <input type="radio"/> 0. No sexual stimulation/intercourse
<input type="radio"/> 1. Almost never/ Never
<input type="radio"/> 2. A few times (Much less than half the time)
<input type="radio"/> 3. Sometimes (About half the time)
<input type="radio"/> 4. Most times (Much more than half the time)
<input type="radio"/> 5. Almost always/ Always |
| <hr/> | |
| 11) When you had sexual stimulation or intercourse, how often did you have the feeling of orgasm or climax? | <input type="radio"/> 0. No sexual stimulation/intercourse
<input type="radio"/> 1. Almost never/ Never
<input type="radio"/> 2. A few times (Much less than half the time)
<input type="radio"/> 3. Sometimes (About half the time)
<input type="radio"/> 4. Most times (Much more than half the time)
<input type="radio"/> 5. Almost always/ Always |
| <hr/> | |
| 12) How often have you felt sexual desire? | <input type="radio"/> 0. No sexual stimulation/intercourse
<input type="radio"/> 1. Very low/none at all
<input type="radio"/> 2. Low
<input type="radio"/> 3. Moderate
<input type="radio"/> 4. High
<input type="radio"/> 5. Very high |
-