BLOOD AND MARROW TRANSPLANT CLINICAL TRIALS NETWORK

A Multi-Center, Phase 2 Gene Transfer Study Inducing Fetal Hemoglobin in Sickle Cell Disease

BMT CTN PROTOCOL 2001

Version 1.0

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Co-Sponsored by the National Heart, Lung, and Blood Institute, National Institutes of Health, and the California Institute of Regenerative Medicine
IND# 17660

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PROTOCOL SYNOPSIS – BMT CTN 2001 PROTOCOL

A Multi-Center, Phase 2 Gene Transfer Study Inducing Fetal Hemoglobin in Sickle Cell Disease

Co- Principal Investigators: David A. Williams, MD and Mark C. Walters, MD

Study Design: This study is an open-label, non-randomized, multi-center center, phase 2, single arm study involving a single infusion of autologous CD34+ HSC cells transduced with the lentiviral vector containing a shRNA targeting BCL11a.

Primary Objective: The primary objective is to determine if treatment with a single infusion of autologous CD34+ HSC cells transduced with the lentiviral vector containing shmiR targeting BCL11A will lead to a complete absence of severe VOEs (defining VOE as ACS or VOC requiring parenteral opioids) in the period from Month 6 to Month 24 after gene therapy.

Secondary Objectives: Secondary objectives are to assess the following:
1. To determine if treatment with BCL11A shmiR gene therapy will result in an increase total Hb, total HbF, and fraction of F cells post-infusion of gene modified cells.
2. To determine if treatment with BCL11A shmiR gene therapy will result in a reduction in hemolysis.
3. To evaluate the safety of the study treatment, by describing clinically relevant toxicities and adverse events (AEs).
4. To estimate the percentage change in the annualized number of VOEs in the period from Month 6 to Month 24 month after gene therapy as compared to the 24 months prior to consent.

5. To determine if a complete reduction in the number of severe VOEs (defining VOE as ACS or VOC requiring parenteral opioids) is attained if the VOE observation period is limited to the period from Month 6 to Month 18 after gene therapy, as compared to the 24 months prior to consent.

Exploratory Objectives: Exploratory objectives are to assess the following:
1. To evaluate the efficiency of BCL11A knockdown, by estimating the amount of BCL11A protein on peripheral whole blood and sorted erythroid precursors at baseline, 6, 12, and 24 months post infusion.
2. For subjects $\leq$ 14 years old: To determine if treatment with BCL11A shmiR gene therapy will result in stable TCD screening results.
3. To describe the change in SCD-related organ function, including neurocognitive, renal, and cardiopulmonary assessments.

4. To describe the association between baseline socioeconomic status as measured by household material hardship (HMH) and clinical study end-points (reduction in VOE, Hb and HbF, changes in SCD-related organ assessments) and patient-reported outcomes.
5. To describe the impact of the BCL11A shmiR gene therapy as measured by Health-Related Quality of Life (HRQoL) patient reported outcome domains for fatigue, pain interference, and sleep.
6. To describe the proportion of patients who are absent all the following events at Month 24 post-infusion of gene modified cells:
    a. Death
    b. Cerebral vascular events, including strokes
    c. For subjects ≤ 14 years old, significantly worsened annual TCD results leading to institution of transfusion therapy
    d. Lack of engraftment (requiring rescue with back-up CD34 cells)
    e. Lack or loss of engraftment of gene-modified cells as determined by VCN <0.1 copies per cell in peripheral blood MNC at 6 months post-infusion
    f. Development of MDS or leukemia
    g. Presence of replication competent lentivirus

Biologic Objectives: Biologic objectives are to assess the following:
1. To determine if treatment with BCL11A shmiR gene therapy will increase red cell survival and reduce hemolysis as measured by exhaled alveolar end tidal carbon monoxide measurement (ETCO): a) upon entry to the trial; b) as part of the exchange or simple transfusion phase; and c) at Month 6, 12, 18 and 24 post-infusion. Measurements will be performed at regularly scheduled study visits.
2. To determine if treatment with BCL11A shmiR gene therapy will result in a change in the number of RBCs with detectable polymer at venous pO2 (~35 mm Hg) compared to untreated baseline, using an assay of oxygen saturation at the single RBC level.
3. For patients enrolled at selected sites only, to determine if treatment with BCL11A shmiR gene therapy will result in any change in total cerebral blood flow (with phase contrast MRI) and cerebrovascular reserve (with breath hold functional MRI), as well as assessment of oxygen extraction fraction and cerebral blood flow by frequency-domain near-infrared and diffuse correlation spectroscopies (FD-NIRS-DCS) monitoring. Measurements will be performed at baseline, Month 12, and Month 24.

Eligibility: **Inclusion Criteria:** Subjects who have:

1. A diagnosis of sickle cell disease with genotype HbSS and HbS/β0 thalassemia
2. Between the age of 7-40 years
3. Clinically severe disease, defined as at least 4 vaso-occlusive events (VOEs) within the past 24 months prior to consent.
4. Adequate hematologic parameters (regardless of therapy) including white blood cell (WBC) count within the range of 2.5 –

25.0 x 10^9 /L, hemoglobin within the range of 5 – 11 g/dL, and platelet count >150 x 10^9 /L

5. Adequate organ function and performance status:
   a. Karnofsky/Lansky performance status ≥80%
   b. Serum creatinine </= 1.5 times the upper limit of normal for age, and calculated creatinine clearance or GFR >/= 60 mL/min/1.73 m^2
   c. Persistent aspartate transaminase, alanine transaminase, or direct bilirubin value <3× the upper limit of normal,
   d. DLCO, FEV1, and FVC >50% of predicted
   e. Left ventricular ejection fraction >40% or shortening fraction >25%.

6. Patients taking hydroxyurea for > 6 months who still meet all inclusion criteria are eligible for the trial.

7. No HLA-genotypically identical related bone marrow donor available

8. Parental/guardian/patient signed informed consent

Exclusion Criteria: Subjects who have:

1. Concomitant condition or illness including: ongoing or active infection, active malignancy, major surgery in the past 30 days, medical/psychiatric illness/social situations that would limit compliance with study requirements as determined by the treating physician

2. Receiving a chronic transfusion regimen for primary or secondary stroke prophylaxis. (Note: patients with a history of abnormal TCD who have transitioned from transfusions to hydroxyurea for stroke prophylaxis are not eligible for the study.)

3. Patients with history of abnormal TCD (measured with a timed average maximum mean velocity of ≥200 cm/second in the terminal portion of the internal carotid or proximal portion of middle cerebral artery or if the imagining TCD method is used, >185 cm/second plus evidence of intracranial vasculopathy) who were ever on transfusions and subsequently transitioned to hydroxyurea.

4. History of overt stroke or any neurologic event lasting >24 hours. (Note: patients with imaging evidence of silent stroke but not on a chronic transfusion regimen are not excluded.)

5. Isolated recurrent priapism unresponsive to medical and surgical therapies in the absence of other qualifying VOE complications that meet inclusion criteria

6. Contraindication to administration of conditioning medication (busulfan)

7. Prior allogeneic hematopoietic stem cell transplant

8. Known myelodysplasia of the bone marrow or abnormal bone marrow cytogenetics

9. Severe cerebral vasculopathy

10. Liver MRI (≤ 180 days prior to initiation of BU conditioning) to document hepatic iron content is required for participants who have

received ≥20 packed red blood cell transfusions (cumulative); participants who have hepatic iron content ≥ 9 mg Fe/g liver dry weight by liver MRI must have a liver biopsy and histological examination/documentation of the absence of cirrhosis, bridging fibrosis, and active hepatitis (≤ 180 days prior to initiation of transplant conditioning); the absence of bridging fibrosis will be determined using the histological grading and staging scale as described by Ishak and colleagues (1995) as described in the Manual of Operations (MOO);

11. Evidence of HIV infection, HTLV infection, active hepatitis B infection or active hepatitis C infection
12. Known acute hepatitis or evidence of moderate or severe portal fibrosis or cirrhosis on prior biopsy
13. Receipt of an investigational study drug or procedure within 90 days prior to study enrollment
14. Pregnancy or breastfeeding
15. Presence of a genetically-determined hypercoagulable state or personal history of prior VTE (deep vein thrombosis or pulmonary embolism) that would represent a contraindication to proceed with central line placement and study events.

Treatment Plan: After meeting the initial eligibility criteria, patients will be scheduled for mobilization and collection of CD34+ HSPCs. Patients will receive blood transfusions for a period of at least 3 months prior to hematopoietic stem cell collection, with a goal of achieving a HbS level ≤ 30% prior to mobilization. The collected cells of each subject will be split into 2 portions, one portion for transduction *ex vivo* with the BCH_BB694 LCR (-HS4) bGp D12shmiR (hereafter BCH_BB694 or BB694) lentiviral vector / drug product manufacturing and the other portion set aside as a back-up product in the event a rescue treatment is indicated. The backup portion will be cryopresevered then maintained locally for use in case of graft failure. Transduction will be carried out on the selected CD34+ cells and transduced cells will be cryopreserved. Patients will undergo standard work-up for autologous bone marrow transplantation prior to proceeding with conditioning and infusion of gene-modified cells. Patients will receive myeloablative conditioning with busulfan administered on days -5 to -2, prior to infusion of transduced cells. The transduced cells will be infused intravenously over 30-45 minutes after standard pre-hydration and premedication according to institutional guidelines. After infusion of transduced cells, red cell transfusions will be utilized to maintain a hemoglobin > 8 gms/dl.

Accrual Objective: 27 – 30 patients will be enrolled to achieve 25 evaluable patients. To be considered "evaluable", a patient must receive an infusion of transduced CD34+ cells.

Accrual Period: The estimated accrual period is 24 months

Study Duration: Patients will be followed for 24 months post-infusion with the expectation that all patients will be offered enrollment in a long-term study for the mandated 15 years of longitudinal follow-up for gene therapy trials.
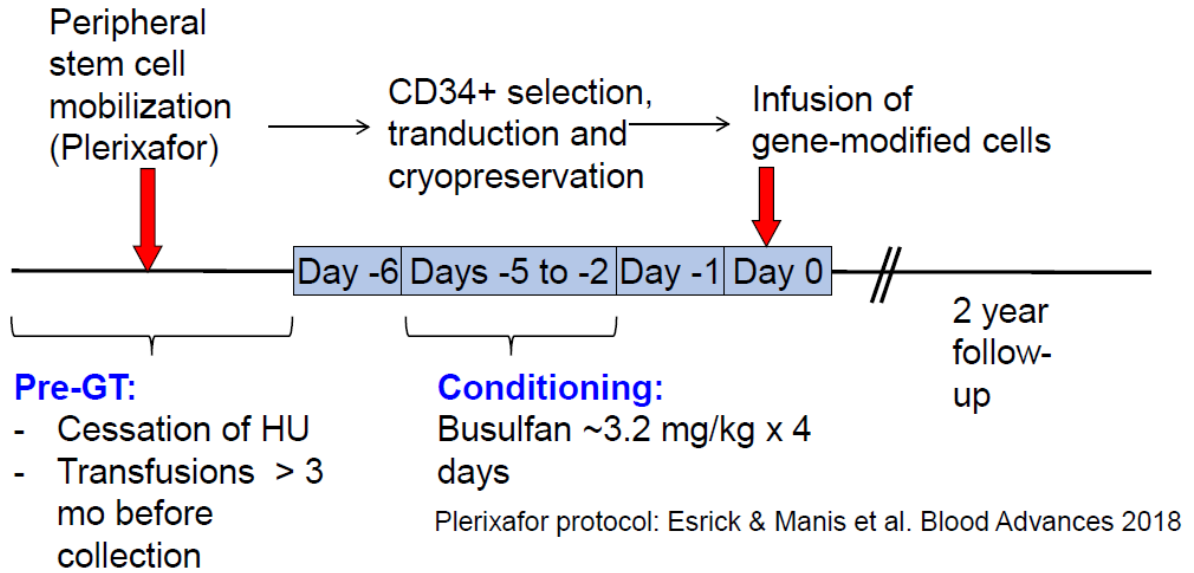
STUDY SCHEMA

Peripheral
stem cell
mobilization   CD34+ selection,   Infusion of
(Plerixafor)   tranduction and   gene-modified cells
                      cryopreservation

| Day -6 | Days -5 to -2 | Day -1 | Day 0 |

2 year
follow-
up

Pre-GT: **Conditioning:**
- Cessation of HU   Busulfan ~3.2 mg/kg x 4
- Transfusions > 3   days
  mo before   Plerixafor protocol: Esrick & Manis et al. Blood Advances 2018
  collection

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ABBREVIATIONS AND DEFINITIONS

Abbreviation	Term
ACS	Acute Chest Syndrome
ANC	Absolute Neutrophil Count
BCH	Boston Children's Hospital
BCL11A	B cell lymphoma 11A
BMT	Bone Marrow Transplant
CBC	Complete Blood Count
cDNA	Complementary DNA
CFCs	Colony Forming Cells
CFR	Code of Federal Regulations
CFU	Colony-Forming Unit
CGD	Chronic Granulomatous Disease
CIBMTR	The Center for International Blood and Marrow Transplant Research
CLIA	Clinical Laboratory Improvement Amendments
CMCF	Cell Manipulation Core Facility
CNS	Central Nervous System
CRICO	Controlled Risk Insurance Company
CRF	Case Report Form
CTC	Common Toxicity Criteria
CTIP	Clinical and Translational Investigator Program
DFCI	Dana-Farber Cancer Institute
DLCO	Diffusing capacity of the lung for carbon monoxide
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein Barr Virus
ER	Emergency Room

Abbreviation	Term
FACT	Foundation for the Accreditation of Cell Therapy
FDA	Food and Drug Administration
FD-NIRS-DCS	Frequency-domain near-infrared and diffuse correlation spectroscopies
FEV1	Forced expiratory volume in 1 second
FLT3L	Fms-like Tyrosine Kinase-3 Ligand
FVC	Forced vital capacity
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony Stimulating Factor
GT	Gene Therapy
Glu	Glutamic acid
GMP	Good Manufacturing Practice
GVHD	Graft-versus-host disease
Hb	Hemoglobin
HbF	Fetal Hemoglobin
HbS	Sickle Hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human leukocyte antigen
HPFH	Hereditary persistence of fetal hemoglobin
HPLC	High-performance Liquid Chromatography
HRQoL	Health-related quality of life
HSC	Hematopoietic Stem Cell
HSCT	Hematopoietic Stem Cell transplantation
HSCP	Hematopoietic Stem Cell Progenitor
IL-3	Interleukin-3
IMP	Investigational Medicinal Product
IND	Investigational New Drug
IRB	Institutional Review Board
ISA	Insertion Site Analysis

Abbreviation	Term
IS	Insertion Site
LAM	Linear Amplification Mediated
LCR	Locus Control Region
LDH	Lactate Dehydrogenase
LTR	Long Terminal Repeat
LV	Lentivirus
miRNA	MicroRNA
mL	Milliliter
MRI	Magnetic resonance imaging
MRA	Magnetic resonance angiography
NCI	National Cancer Institute
NIH/OBA	National Institute of Health - Office of Biotechnology Activities
PCR	Polymerase Chain Reaction
PI	Principal Investigator
pO2	Partial pressure of oxygen
PRO	Patient reported outcome
PROMIS	Patient Reported Outcome Measurement Information System
QACT	Quality Assurance for Clinical Trials Office
QC	Quality Control
qPCR	Quantitative PCR
RBC	Red blood cell
RCL	Replication Competent Lentivirus
RIC	Reduced intensity conditioning
RNA	Ribonucleic acid
RNAi	RNA interference
RT-PCR	Reverse transcription PCR
SAE	Serious Adverse Event
SAS	Statistical Analysis Software

Abbreviation	Term
SCD	Sickle Cell Disease
SCF	Stem Cell Factor
SCID	Severe Combined Immunodeficiency
SD	Standard Deviation
SFFV	Spleen Focus Forming Virus
shRNA	Short hairpin RNA
SIN	Self-inactivating
SOPs	Standard Operating Procedures
TCD	Transcranial Doppler Ultrasound
TCR	T-cell Receptor
TPO	Thrombopoietin
TRM	Transplant-related mortality
UCB	Umbilical cord blood
URD	Unrelated donor
Val	Valine
VOC	Vaso-occlusive pain crisis
VOE	Vaso-occlusive event
VPF	Vector Production Facility
WBC	White Blood Cell

CHAPTER 1

1   BACKGROUND & RATIONALE

1.1   Overview

Sickle cell disease (SCD) is an inherited blood condition resulting from abnormal hemoglobin production. It is one of the most common genetic diseases in the world. The clinical manifestations are variable and range from recurrent acute and debilitating painful crises to life-threatening pulmonary, cardiovascular, renal and neurologic complications. Some of the variability in severity of disease between individuals is related to the amount of fetal hemoglobin they make after the newborn period. In general, those who make more fetal hemoglobin have milder manifestations of their disease and improved survival. Improvements in supportive care, infection prophylaxis, and screening for stroke have increased survival in childhood, however these interventions do not correct the underlying disease. Current therapies that are directed at decreasing the amount of sickle hemoglobin include a daily oral medication called hydroxyurea, or monthly blood transfusions. Both therapies have significant potential side effects and are not curative. Regardless of these therapies, many patients continue to have significant morbidity and increased mortality as they age.

The only curative treatment of SCD at this time is bone marrow transplantation (also called hematopoietic stem cell transplantation, or HSCT) using healthy blood stem cells from an unaffected brother or sister, or from an unrelated donor if one can be identified who is a match in tissue typing. Unfortunately, only a minority of sickle cell patients has such a donor available. Typically, patients undergoing a bone marrow transplant have their own bone marrow eliminated using chemotherapy (this treatment is commonly called 'conditioning'), and new blood stem cells from the donor are then given as an infusion into the blood. Bone marrow transplantation is associated with short and long-term consequences. Sometimes the transplant is not successful because the patient's immune system rejects the donor bone marrow (called graft rejection). Other times the transplant is not successful because patients develop graft versus host disease (GVHD), a condition in which some of the immune blood cells from the donor attack the body of the recipient patient and cause severe damage. Treatment for GVHD includes long-term use of powerful immunosuppressive drugs which can increase the risk of serious, even fatal, infections. Both rejection and GVHD are more common when the donor is not a matched sibling but can occur any time a transplant is done using cells from another person. These complications also seem to occur more frequently in patients with SCD.

An alternative promising approach for the treatment of genetic diseases is called gene therapy. Gene therapy is a relatively new field of medicine that alters genetic material (mostly DNA) within the patient to treat his or her own disease. In gene therapy, we introduce new genetic material in order to fix or replace the patient's disease gene or otherwise alter the disease phenotype, with the goal of curing the disease. The procedure is similar to a bone marrow transplant, in that the patient's malfunctioning blood stem cells are reduced or eliminated using chemotherapy, but it is different because instead of using a different person's (donor) blood stem cells for the transplant, the patient's own blood stem cells are given back after the new genetic material has been introduced into those cells. This approach has the advantage of eliminating any risk of GVHD, reducing the risk of graft rejection, and using a single drug for the conditioning portion of the transplant procedure. The method we use to introduce the gene into the patient's own blood stem cells is to engineer and use a modified version of a virus (called a 'vector') that efficiently inserts the "correcting"

genetic material into the cells. The vector is a specialized biological medicine that has been formulated for use in human beings.

We have recently discovered a gene that is very important in the control of fetal hemoglobin expression. In a pilot study, decreasing the expression of this gene in sickle cell patients increased the amount of fetal hemoglobin while simultaneously reducing the amount of sickle hemoglobin in their blood, therefore reducing the severity of the disease. The gene we are targeting for change in this study that controls the level of fetal hemoglobin is called BCL11A. In summary, the advantages of a gene therapy approach include: 1) it can be used even if the patient does not have a matched donor available; and 2) it eliminates the risk of GVHD and graft rejection and thus avoids the potential use of strong medicines often required to prevent and treat these conditions. Our preliminary studies in SCD patients shows this approach is very effective in reducing the amount of sickle hemoglobin in many red cells to much less that 50% which appears to prevent the sickling of these cells in the body. Our goals in the current study are to test in a larger number of patients whether using gene therapy to change the expression of BCL11A will lead to a sustained increase in fetal hemoglobin production and reduction in some of the specific complications of SCD in the treated individuals.

1.2   Genetic and molecular pathophysiology of SCD

In the normal adult, hemoglobin A1 (HbA1) is the most abundant form of hemoglobin, comprising about 95% of all hemoglobin in circulating erythrocytes. HbA1 is a heterotetramer made up of two α globin and two β globin proteins ($\alpha_2\beta_2$). The hemoglobin S mutation (HbS) results in a single amino acid substitution in the β globin protein (HBBGlu6Val). SCD refers to a group of inherited hemolytic anemias in which hemoglobin S (HbS) is present in either a homozygous state (HbSS) or in a compound heterozygous state, such as when combined with hemoglobin C (HbSC), and β-thalassemia (HbS-β thalassemia). The HbS mutation creates a hydrophobic region on the molecule that, in the deoxygenated state, facilitates a non-covalent polymerization of HbS molecules into rigid fibers within the erythrocyte. These HbS polymers damage the erythrocyte membrane and change the rheology of the erythrocyte in circulation, resulting in hemolytic anemia and vaso-occlusion at different sites throughout the vascular bed, particularly in post-capillary venules. These two sequelae of abnormal hemoglobin polymerization lead to acute debilitating symptoms, the inexorable accrual of chronic organ damage, and premature death.

Hemoglobin F (HbF), or fetal hemoglobin, is composed of two alpha globin proteins and two γ globin proteins ($\alpha_2\gamma_2$). HbF is the most abundant hemoglobin produced by the fetus *in utero*, but HbF usually comprises less than 2% of the total hemoglobin in a normal adult. Shortly before and after birth, the production of γ globin (and therefore HbF) "switches off" and β globin "switches on," and HbA1 ($\alpha_2\beta_2$) becomes the dominant hemoglobin by approximately 1 year of age **(Figure 1-1)**. Although the underlying mechanisms mediating this switch have been investigated for decades, the molecular basis remains to be fully elucidated.
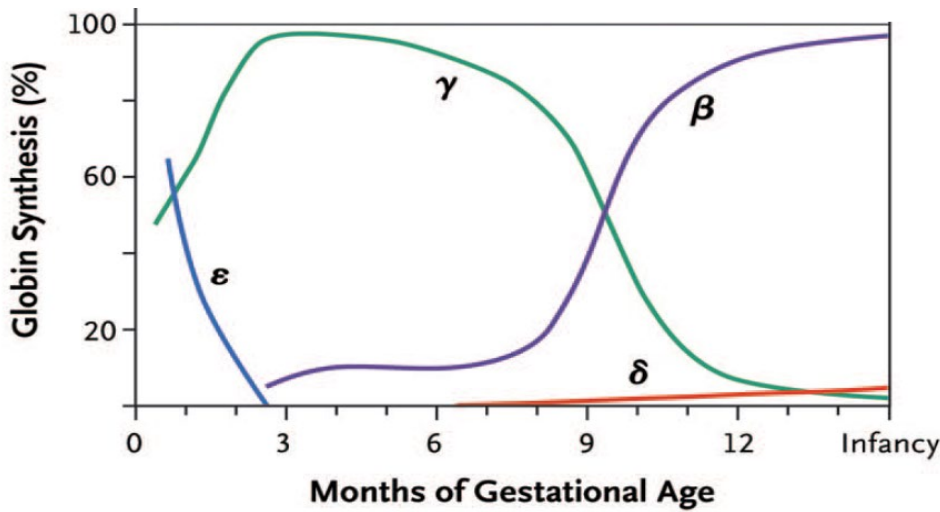
Figure 1-1. Relative globin synthesis during fetal development and infancy.[2]

In homozygous SCD, the hemoglobin switch during infancy is from HbF to HbS. Many clinical observations have revealed that persistently elevated levels of HbF beyond the newborn period can ameliorate the severity of SCD and decrease mortality. These observations have led to attempts to increase the HbF expression for therapeutic benefit using pharmacological strategies; however they have been hampered by the fact that until recently the molecular mechanism regulating globin switching has remained unclear. With the notable exception of hydroxyurea, few medications have successfully modified HbF expression without significant toxicity.

1.3   Clinical features and current medical management of SCD

The clinical manifestations of SCD are protean and related to the pathophysiologic sequelae of chronic hemolytic anemia and acute/recurrent vaso-occlusion. Acute complications can be divided into those associated with vaso-occlusion and those related to hemolysis. Chronic complications, often more severe in adolescents and adults, are related to progressive tissue damage and organ dysfunction, as well as sequelae of chronic anemia **(Table 1-1)**.

Table 1-1. Clinical manifestations of sickle cell disease.

ACUTE	CHRONIC
Vaso-occlusive:	Constitutional: Decreased stamina
Dactylitis (Hand Foot syndrome)	Cardiovascular: Pulmonary hypertension, cardiomegaly
Splenic sequestration	Renal: Hyposthenuria, hematuria, nocturnal enuresis
Priapism	Eyes: Proliferative retinopathy
Pain crises	Lungs: Chronic lung disease

ACUTE	CHRONIC
Acute chest syndrome	Skin: Leg ulcers
Stroke/Cerebrovascular accident	Musculoskeletal: Osteonecrosis, avascular necrosis
Non-Vaso-occlusive:	Endocrine: Growth failure, delayed puberty
Cholelithiasis/ Cholecystitis	Neurologic: Learning disability, motor deficits
Aplastic Crisis	Psychiatric: Poor self-image, depression
Bacteremia	

From Heeney MM. Management of Sickle Cell Disease. In: Comprehensive Pediatric Hospital Medicine (Zaoutis LD, Chiang VW. Editors). Philadelphia: Elsevier. 2007; 731-737

Although the causative Glu → Val amino acid substitution in β globin is the same in all SCD patients, affected individuals exhibit remarkable variability in the severity and spectrum of their clinical manifestations of disease. Some patients are almost entirely asymptomatic, whereas other patients are constantly plagued by painful episodes. Using retrospective data, many investigators have tried to evaluate epidemiologic, laboratory and genetic features to predict severity of disease, but these tools remain imprecise **(Table 1-2)**[3, 4]. As a result, the optimal management of many patients suffering from this disease and specifically those who may benefit most from innovative therapies remains unclear.

Table 1-2. Predictors of adverse outcomes in the Cooperative Study of Sickle Cell Disease.[4]
Predictors of adverse outcomes in the Cooperative Study of Sickle Cell Disease

Predictors	Outcomes
Low Hb concentration☐	Death, stroke, leg ulcers
High Hb concentration	Pain, ACS, AVN
Low Hb F concentration	Death, ACS, pain, leg ulcers
High steady-state WBC	Death, ACS
α-thalassemia present	AVN
α-thalassemia absent	Stroke
High pain rate	Adult death, AVN
Acute anemia	Death, stroke

In the past half-century, SCD patients in resource-rich regions have seen a significant improvement in early childhood morbidity and mortality. The introduction of universal newborn screening, penicillin prophylaxis in early childhood, and the widespread use of conjugated vaccines for encapsulated bacterial pathogens have significantly impacted early childhood mortality **(Figure 1-2)**. However, despite these advances,

teenagers and young adults still remain at high risk for acute complications, associated morbidity, and premature death[1]. Cerebrovascular complications, including cerebral infarction and cognitive deficits, represent a particularly morbid clinical feature of sickle cell disease. Since the late 1990s, the ability to screen for primary stroke risk using transcranial doppler (TCD) ultrasound and the availability of safe blood products for chronic transfusion programs has reduced the incidence of overt strokes in young SCD patients. However, even patients enrolled in intensive screening and intervention programs do continue to develop abnormal TCD velocities, silent brain infarcts, and vascular stenoses[5, 6]. Data from the Cooperative Study of Sickle Cell Disease showed that children with silent infarcts on MRI performed more poorly than children with no MRI abnormality on tests of arithmetic, vocabulary, and visual motor speed and coordination[7], and evidence continues to accumulate that even subtle brain injury caused by sickle cell related vascular compromise likely plays a role in the development of cognitive defects.

As the time course of peak morbidity in SCD shifts to an older age group, we are now in need of novel disease-modifying therapeutic options that will target the underlying pathophysiology of the disease, ideally before chronic complications become severe, debilitating, irreversible, and potentially fatal.
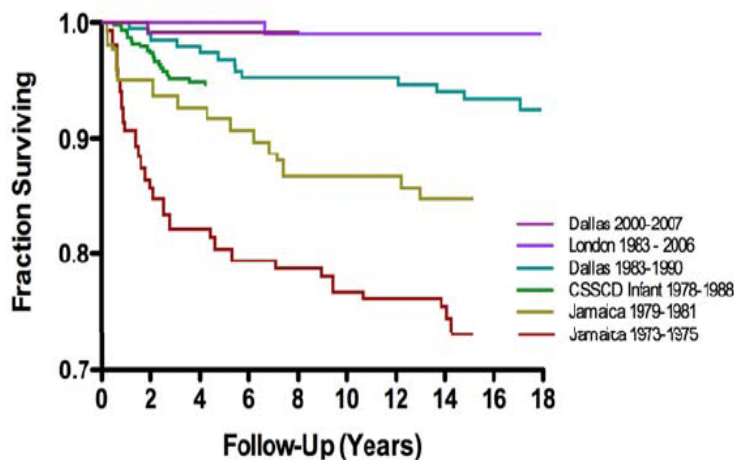
Figure 1-2. Survival in sickle cell disease[1]

Hydroxyurea is currently the longest utilized and until recently the only medicine approved by the US Food and Drug Administration (FDA) for the treatment of SCD. Although its precise mechanism of action is still unclear, data has accrued over the past several decades to show that hydroxyurea can reliably increase HbF, decrease acute vaso-occlusive events, and perhaps protect against or delay chronic organ damage and dysfunction[8]. Furthermore, long-term use of hydroxyurea is relatively safe and decreases disease-associated mortality[9]. However, despite excellent efficacy in many patients, some SCD patients fail to respond to hydroxyurea, cannot tolerate the medication due to adverse effects, or simply do not take the drug as prescribed. In addition, the need for daily medication and frequent laboratory evaluation makes long-term hydroxyurea therapy challenging for some patients. Finally, although data continues to accumulate to support the safety and efficacy of hydroxyurea even in young infants[10,11], several concerns have historically presented additional barriers to the widespread adoption of hydroxyurea[12]. These include the relatively limited scope of long-term data in infants and young children, concerns about effects on fertility, and

concerns about a theoretical (but probably unfounded) risk of malignancy. Several new *non-curative* therapies have been approved by FDA since 2007 to treat sickle cell disease. The chronic use of large enteral doses of L-glutamine has been approved the for the reduction of acute complications of sickle cell disease, particularly pain VOE events and ACS. Another new therapy called voxelotor, is a rationally designed small molecule administered orally that allosterically increases HbS oxygen affinity thus stabilizing the oxyHb state and inhibiting HbS polymerization, RBC sickling and hemolysis. Another new therapy, crizanlizumab, is a humanized monoclonal antibody that specifically inhibits leukocyte adhesion by blocking p-selectin to reduce the frequency of vaso-occlusive events. The long-term effectiveness and benefits of these new disease modifying approaches is still unclear.

1.4   The role of hematopoietic stem cell transplant (HSCT) in SCD

Allogeneic HSCT can provide curative therapy for SCD but there are many obstacles to its widespread implementation, and it is crucial that new strategies continue to be developed to improve long-term outcome for these patients. It is well described in the literature that following successful HSCT, vaso-occlusive pain crises (VOC) remit and neurologic and pulmonary abnormalities stabilize or improve[13] with outcomes notably better using HLA-matched siblings as stem cell donors. Despite the more than 1000 children born annually in the US with SCD, and many more internationally, there were only 528 transplants reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) database during 2010-2014, with the majority of these using a matched family donor. Unfortunately, fewer than 20% of sickle cell disease patients in the United States have such a donor available. The majority of these transplants are fully myeloablative with high doses of chemotherapy given in the preparative regimen to achieve a goal of complete eradication of recipient bone marrow and full reconstitution with donor stem cells. This approach has been successful in high proportions of patients for the past 20 years[13], with ongoing research investigating novel options for alternative donor and conditioning regimens, but allogeneic transplant is still associated with acute and long-term potential toxicities. A small percent of patients experience transplant related mortality (TRM), defined as death unrelated to the underlying disease, usually due to infection or pulmonary, renal, CNS or hepatic toxicity. Virtually all patients require narcotics for mucositis-associated pain, parenteral nutrition, and broad-spectrum antibiotics for fever. Finally, allogeneic transplant patients are at risk of developing graft rejection and/or GVHD. Grade III/IV GVHD occurs in 5-10% of children with a *matched* sibling donor (and higher in matched unrelated donor transplants, see below) and is a serious complication of transplant that can necessitate long-term immunosuppressive therapy, often corticosteroids. The associated complications of long-term steroid use include metabolic syndrome, impact on bone density and growth, cataracts and increased risk of infections. In a small fraction of patients graft rejection can occur resulting in autologous reconstitution with recurrence of SCD phenotype. Patients on chronic transfusion protocols for whom transplant may provide the greatest benefit also have the highest risk of graft rejection due to prior allosensitization. The long-term sequelae of ablative allogeneic transplant are significant. Almost all children experience impaired vertical growth and adults as well as children have gonadal toxicity. Hormone replacement therapy is needed for females and the incidence of infertility is high for both genders.

Recent data on outcome for HSCT comes from the EBMT/CIBMTR, in which a study of 1000 MSD for SCD was conducted encompassing a period of 1986-2013 including adults as well as children. The source of engrafting hematopoietic cells was peripheral blood stem cells (PBSC), cord blood (CB) and bone marrow (BM) grafts. This study showed long term EFS of 91% and OS of 93%[14]. In these studies age strongly influences likelihood of success and risk for CGVHD[14]. Overall, the probability of EFS has improved over time. Secondary malignancies following busulfan/cyclophosphamide are relatively uncommon[15], but the frequency may be found to increase with longer follow-up periods. Transplant-related

complications, especially GVHD and graft rejection, are currently more prevalent in SCD patients undergoing unrelated donor allogeneic transplantation.

Given that HSCT can provide curative therapy for SCD but is only available to the small proportion of affected children that have an appropriate related donor, current efforts have been directed toward the use of alternative donor transplants in this setting. Use of such donors, whether volunteer unrelated donors (URD) or unrelated umbilical cord blood donors (UCB), can also restore normal hematopoiesis. However, the acute complications of URD or UCB transplants, particularly GVHD in URD transplants and graft rejection in umbilical cord blood transplants, have both a higher incidence and greater severity than MSD in SCD. There have been some reports of transplants for SCD using alternative donors and traditional ablative conditioning. Given the increased acute toxicities reported, one current strategy is based on reduced intensity conditioning (RIC). In transplant for malignant diseases, RIC has been definitively shown to decrease acute toxicity and acute GVHD[16], and there is literature suggesting that these advantages extend to childhood nonmalignant disorders[17]. The impact on incidence and severity of chronic GVHD is less clear. In 2006, a multi-institutional trial sponsored by the Blood and Marrow Transplant Clinical Trials Network was opened to investigate outcomes using an alemtuzumab-based reduced intensity regimen in SCD patients. Initially, acceptable donors were fully matched (8/8 loci) unrelated marrow donors and highly matched (5/6 loci) cryopreserved umbilical cord units. However finding an unrelated matched donor for this population proved challenging and is predicted to be possible for well below 1/3 of patients[18]. The cord blood cohort was closed early due to an unacceptable incidence of graft failure. The URD arm has now closed as well. Of 30 patients reported, there were low rates of regimen-related organ toxicity, but high rates of chronic GVHD (62% at 1 year post-transplant), and GVHD was the cause of death in 6 patients[19]. At the National Institutes of Health, a non-myeloablative conditioning regimen consisting of alemtuzumab and total body irradiation has been tested in a group of MSD transplant recipients with SCD. In the group of 30 adult patients, survival outcomes were excellent, but a significant proportion of patients required ongoing immunosuppression[20].

Multiple recent studies and open trials have attempted to expand the donor pool available by including HLA-haploidentical donors for non-myeloablative transplants. Initial results of several haplo-identical trials demonstrate that while GVHD has not been a burden in this group, graft failure occurred in a high proportion of transplanted patients[21, 22].

In summary, allogeneic HSCT is a reasonable therapeutic option for SCD patients who have a perfectly matched sibling donor, with disease-free survival rate of ~90%, and if successful is curative of SCD symptoms, including the reversal of need for chronic transfusion therapies for CNS complications. However up to 10-20% will have chronic GVHD requiring immunosuppressive therapy[23]. For patients without a matched family donor, although promising new strategies are under investigation, significant limitations remain, as outlined above. Thus, the autologous gene therapy approach in this trial would represent a valuable novel option for this patient population.

1.5 The role of hematopoietic stem cell transplant (HSCT) in SCD Increasing fetal hemoglobin through inactivation of BCL11A: a novel therapeutic strategy in SCD

1.5.1 Increasing fetal hemoglobin is known to improve SCD

Through decades of work in basic science, clinical trials, and clinical observations, it is well-understood that increasing levels of hemoglobin F can ameliorate disease severity in patients with SCD. Hemoglobin F is an extremely potent inhibitor of the polymerization of hemoglobin S molecules primarily because a glutamine residue at position $\gamma 87$ acts to prevent lateral contact in the double strand of the sickle fiber [24]. From an epidemiological standpoint, some populations of SCD patients, such as in parts of Saudi Arabia

and India, have higher average baseline levels of fetal hemoglobin than the average levels in other genetic cohorts. These groups also have a milder clinical disease phenotype[25,26]. Similarly, on an individual basis, people who are homozygous for either SCD or severe β-thalassemia, and who also inherit hereditary persistence of fetal hemoglobin (HPFH) have a milder clinical phenotype than their genetic counterparts[27]. A key point in these cohorts of patients is the pancellular nature of the fetal hemoglobin expression, ie a large proportion of the total red blood cells in circulation contain fetal hemoglobin. This characteristic is likely a key to the markedly attenuated phenotype seen in these patients[28]. There have been no deleterious effects reported in patients with high levels of HbF. Indeed, the effects of increasing levels of HbF has recently been studied in two cohorts of patients (personal communication, Vijay Sankaran). A cohort of ~1000 Brazilian sickle cell patients has been examined for adverse clinical effects associated with increasing HbF levels, and none have been identified and no associated morbidities have been found. The HbF range is wide, but the highest levels are ~40%. In addition, a very large cohort of Thai patients is being studied, including individuals with homozygous or compound heterozygous HPFH deletions. There have been no morbidities with increasing HbF levels in any parameter that have been measured. There are many individuals with HbF levels of 98% in this cohort.

Using data from the Cooperative Study of SCD, which was conducted in the United States in the 1980s, the amount of fetal hemoglobin an individual produces was shown to be protective against sickle cell disease severity: low HbF levels were associated with increased VOC and early death whereas those who had HbF levels above the 75th percentile (>8.6%) had a significant improvement in median mortality **(Figure 1-3)**[29].
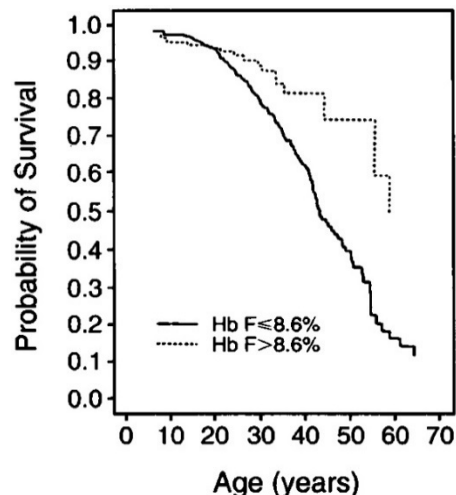
Figure 1-3. HbF levels are associated with survival rates in SCD[29]

Hydroxyurea (HU) is the only FDA approved agent for sickle cell disease that modulates fetal hemoglobin. Multiple studies have demonstrated the effect of elevated fetal hemoglobin on the sickle phenotype and the response of sickle patients to HU[30-34]. These responses are highly variable across the published studies. Overall, patients taking HU demonstrate a 0.6-1.2 gm/dl increase hemoglobin levels concomitant with an increase in Hb F to18-24% with one long-term study showing average Hb F of 15% at 15 years of therapy[35]. Some extraordinary responders have shown an increase up to 40% HbF[36]. The level of HbF correlates with the number of circulating F cells, with patients taking HU showing an increase from baseline to an average of 65% F cells (range of 45-85%). HU treatment has been shown to modestly decrease reticulocytosis, an indicator of hemolytic rate by 0-30% and to reduce incidence of VOE by ~50%.

The Multicenter Study of Hydroxyurea (MSH) in SCD trial demonstrated early data that SCD patients taking hydroxyurea had decreased frequency of VOC, acute chest syndrome, and transfusions[31]. Longer term benefits of HU have also been reported, including reduced mortality among longterm follow up of adults in the MSH trial[9] and in a study of Greek adults[37].

However, the clinical benefit of hydroxyurea is not universal. A significant number of patients cannot tolerate hydroxyurea due to adverse effects including myelosuppression and leg ulcers. In addition, even among patients who do respond to HU, the %HbF achieved at maximum tolerated dose is quite variable, as is the dose that is maximally tolerated in different individuals[38]. Novel strategies for enhancing fetal hemoglobin production are needed.

1.5.2 Preclinical evidence demonstrates that knockdown of BCL11A will cause an increase in fetal hemoglobin

In recent years, the protein BCL11A (B cell lymphoma/leukemia 11A) has emerged as an important developmental regulator of both the initial "hemoglobin switch" and of the persistent silencing of γ-globin expression in adults. The BCL11A protein, which is a zinc-finger protein encoded by a gene on chromosome 2p15, was previously implicated in hematologic malignancies where it may be over-expressed but was not a known regulator of globin expression. In 2007-2008, two independent genome-wide association studies took advantage of the natural variation in fetal hemoglobin levels in humans, and discovered that variation at the BCL11A locus is responsible for a significant degree of the variation in HbF levels[39-41]. Of note, this result was validated in a variety of racial and ethnic groups, and included populations of SCD patients, thalassemia patients, as well as subjects without known hemoglobinopathies.

To further investigate how BCL11A impacts globin regulation, a series of experiments was conducted by Orkin and colleagues. First, in in vitro experiments, down-regulation of BCL11A expression in adult human erythroid precursor cells led to robust induction of fetal hemoglobin[42]. Second, experiments with humanized transgenic *Bcl11a* knockout mice showed that in the absence of Bcl11a, developmental silencing of the human γ-globin genes is markedly impaired in the definitive erythroid lineage[43]. By serving as a repressor of the γ-globin gene, BCL11A is an important developmental regulator of the hemoglobin switch. BCL11A and partner proteins appear to act directly within the β-globin locus on chromosome 11 **(Figure 1-4)**.
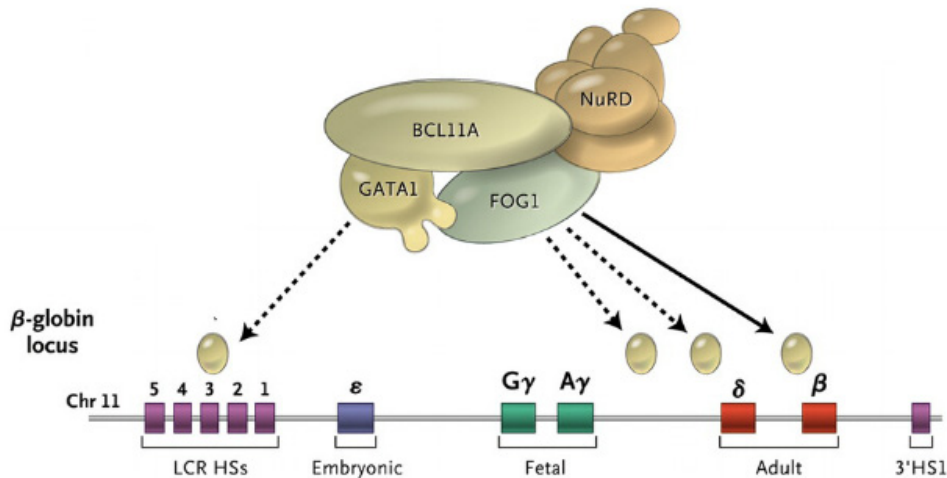
Figure 1-4. BCL11A at the β–globin locus[2]

Finally, in a transgenic humanized *sickle cell* mouse model, when BCL11A is inactivated (by genetically crossing the *Bcl11a* conditional knockout mouse with either the "Berkeley" or "Townes" sickle cell mouse models), fetal hemoglobin levels are increased and pancellular, and the hematologic and pathologic defects associated with SCD are corrected[44]. In an ongoing pilot study (Pilot and Feasibility Study of Hematopoietic Stem Cell Gene Transfer for Sickle Cell Disease; NCT03282656, hereafter referred to as "the Pilot Study"), preliminary data using a lentiviral gene therapy vector to downregulate BCL11A in HSC-derived erythroid precursors via shRNA knockdown validates in humans that reduction of BCL11A induces significant increases in fetal hemoglobin and concomitantly reduces sickle hemoglobin in red blood cells[45, 46].

Therefore, inactivating BCL11A in patients with SCD holds great promise as an effective way to permanently increase levels of fetal hemoglobin, and thus to decrease disease severity. A direct pharmacologic approach is currently not an option, as no BCL11A inhibitor compounds are available. In this trial the same lentiviral gene therapy vector that was effective and safe in the Pilot Study mentioned above to downregulate BCL11A in HSC-derived erythroid precursors via shRNA knockdown will be utilized.

1.6   Justification for a novel gene transfer approach in SCD

Genetically engineering autologous cells offers two major benefits over HSCT: it eliminates the need to find a HSCT donor, and it eliminates the risks of GVHD and immune-mediated graft rejection. Gene therapy has been used to successfully treat multiple but very rare genetic conditions, including adenosine deaminase deficiency[47], X-linked severe combined immunodeficiency (SCID)[48-50], chronic granulomatous disease[51], Wiskott-Aldrich syndrome[52, 53], childhood cerebral adrenoleukodystrophy[54], metachromatic leukodystrophy[55], and, more recently in hemoglobinopathies as well (discussed below). The common feature of these approaches to human disease treatment is the genetic alteration of the blood stem cell and subsequent long-term repopulation of the blood and immune system with progeny of these cells that carry and express the disease-correcting gene.

Gene therapy for the β-hemoglobinopathies has been explored as a promising avenue in multiple pre-clinical and a few clinical studies. Murine models of β-thalassemia and SCD have been corrected by using lentiviral vectors encoding either a human γ-globin gene[56], or a normal or modified β-globin gene designed for anti-sickling activity[57-59].

Hemoglobinopathies are well-suited for gene therapy because a partial correction of the defect should be sufficient to yield full clinical improvement. After HSCT transplant for β-thalassemia or SCD, some patients maintain a persistent mixed chimerism state. Even patients with a relatively small proportion of donor engrafted myeloid cells (as low as 10-20% myeloid marrow chimerism) are capable of producing much higher percentages of donor-derived erythrocytes, and of achieving clinical control of the disease due to the significant prolongation of red cell life-span in the peripheral blood[60, 61]. Preclinical data[62] and mathematical modeling[63] support these clinical observations, showing that ~20% "normal HSCs" engrafted with 80% HSCs derived from a sickle mouse model lead to >80% "normal" red blood cells in circulation.

Several clinical trials are treating hemoglobinopathy patients with lentiviral gene therapy using a gene addition approach. The first hemoglobinopathy patient reported was an 18-year-old individual with HbE/β^0 thalassemia who was treated in 2008 in a clinical trial in France[64, 65]. The vector used in that trial, HPV569, delivered an anti-sickling β-globin gene (β$^{A(T87Q)}$) that was designed to inhibit HbS polymerization[58]. Subsequently, several modifications were made to the β$^{A(T87Q)}$ lentiviral vector, resulting in the vector LentiGlobin BB305[65]. Two clinical trials using the LentiGlobin BB305 vector in SCD patients have reported results: HBG-205 and 206. Clinical trials using LentiGlobin BB305 in thalassemia patients (HBG-204, 207, and 212) have also been completed or are underway. Each of these trials utilizes a myeloablative conditioning regimen consisting of busulfan as a single-agent. The HGB-205 trial (NCT02151526) was open to β-thalassemia and SCD patients in France, including the first SCD patient who was reported to be free of all sickle-related symptoms 15 months after transplantation[66]. The HGB-206 trial (NCT02140554) is open to SCD patients in the U.S, with interim results reported as abstracts at major hematology meetings[67-70]. In the initial cohort of 7 patients reported, the subjects all engrafted, but procurement of adequate numbers of autologous CD34+ cells was challenging, and peripheral vector copy number after product infusion was low[68]. The study was then modified in several ways (requiring pre-harvest transfusions, altering the transduction protocol in an attempt to increase vector copy number, and exploring the use of peripheral blood mobilization after plerixafor), and the most recent data presented in December 2019 reported 13 infused patients and demonstrated an improvement in transduction efficiency[70].

Additional SCD lentiviral gene therapy trials that are using gene addition strategies are open as well. One trial at the University of California, Los Angeles (NCT02247843) utilizing a vector encoding a modified β globin gene has not yet reported data. Another study at Cincinnati Children's Hospital Medical Center (NCT02186418) utilizes a lentiviral vector with a modified gamma globin transgene after melphalan conditioning. Early data presented at the American Society of Hematology meeting in 2018 described two patients who tolerated the treatment without short-term safety concerns and had HbF levels in the ~20% range at 6-12 months of follow-up[71]. Several features of the above-mentioned gene addition trials are of relevance to the current study. The vector employed in the current pilot study and proposed trial (BCH_BB694) was used for the development of much of the preclinical data reported by our group targeting BCL11A (see below and refs. Guda et al.[72] and Brendel et al[62]). Since the early reports cited above, bluebird bio has developed a proprietary method of enhancing gene transfer that effectively increases VCN at any given vector titer or multiplicity of infection. We have confirmed that this method enhances transduction of BCH_BB694. We will utilize this new method of transduction in the current trial with permission from bluebird bio and authorization by the FDA.

In contrast to the gene addition approaches described above, the current protocol involves introduction of a shRNA targeting BCL11A expression in erythroid cells derived from hematopoietic stem cells for the purpose of concurrently increasing γ-globin and reducing mutant $β^S$ globin expression by knockdown of BCL11A. RNA interference (RNAi) is a post-transcriptional silencing mechanism in which a small double-stranded RNA molecule (in this case, a short hairpin, or "shRNA") acts to suppress expression of a target gene (in this case, the *BCL11A* gene) with sequence homology. Initial studies showed that knockdown of BCL11A using a lentiviral vector shRNA approach causes induction of HbF in human erythroid progenitor cells[42, 72], and subsequent preclinical work is described below in **Section 3**. In addition, Wilber and colleagues demonstrated that, even without the use of drug selection to enrich for vector-transduced cells, significantly increased levels of HbF are produced in both normal and β-thalassemic erythroid cells derived from shBCL11a-lentiviral vector-transduced CD34+ HSCs[73]. Finally, as noted above, in our Pilot Study, the BCH_BB694 was shown to effectively knockdown BCL11A in erythroid precursors in vivo resulting in a substantial increase in fetal hemoglobin in a nearly pancellular distribution[45, 46].

The BCL11A knockdown approach takes advantage of the physicological regulation machinery that controls globin expression by reversing a portion of the hemoglobin "switch" that occurs in hematopoietic development. This approach has the advantage of increasing the anti-sickling fetal hemoglobin and concurrently and coordinately reducing the red cell content of $β^s$ sickling globin. In contrast, the gene addition strategies currently being pursued for treatment of β-hemoglobinopathies require expression of the transgene to compete with the endogenous mutant β-globin production. At a similar rate of successful gene transfer, we hypothesize that the BCL11A knockdown approach has the potential for greater clinical impact by simultaneously harnessing the anti-sickling effects of HbF and silencing the mutated β-globin.

In summary, the aim of this study is to use a lentiviral vector shRNA approach to decrease expression of the BCL11A gene. Since BCL11A is a repressor of gamma globin expression, knocking down the expression of BCL11A will de-repress gamma globin expression and thus allow increased production of HbF. Increasing HbF is a promising strategy for significantly alleviating the clinical manifestations of sickle cell disease.

1.7   Requirement for busulfan myeloablative conditioning regimen

The purpose of patient conditioning prior to infusion of genetically modified autologous bone marrow-derived HSCs is to reduce the risk of primary graft failure and favor the engraftment of genetically treated hematopoietic stem cells as much as possible. In SCD, engraftment of transduced erythroid cells at high level is desirable.

Allogeneic HSCT requires conditioning therapy prior to HSC infusion to establish reliable donor engraftment. With allogeneic HSCT, myeloablative doses of busulfan are most commonly combined with one of two immunosuppressive chemotherapies, fludarabine or cyclophosphamide[74], to prevent immunologically driven graft rejection. In GT, where there is no risk for rejection, myeloablative busulfan alone is used. In the current trial patients will receive busulfan alone targeted to a total exposure (AUC) of ~20,740 - 23,180 micromole-min/L, or 85-95 mg*h/L, given over 4 days. Although this range is somewhat higher than that being used in the busulfan and fludarabine regimen in the BMT CTN 1503 trial (STRIDE2, NCT02766465) of allogeneic HSCT for SCD, it is an exposure that is well within the typical range employed for busulfan/fludarine regimens in HSCT for myeloid malignancies[75, 76] and it is an exposure that is associated with a low risk for serious regimen related toxicities[77].The higher dose of busulfan used in this protocol is an attempt to prevent recovery of any host, non-modified HSC present in the patient after

conditioning and outside the graft itself. As demonstrated in other gene therapy trials employing a similar conditioning regimen, this busulfan regimen should promote long-term efficacy and safety by facilitating the engraftment of gene-modified HSCs with minimal therapy-associated acute and long-term sequelae.

The majority of experience with less intensive regimens has been in the setting of transplants for malignant diseases in the elderly and using these data to predict outcomes in this population of pediatric and young adult patients with a chronic non-malignant condition is challenging. Reported toxicities with RIC or lower intensive regimens are variable and depend on patient comorbidities and prior therapies, as well as individual patient variability. In general, patients receiving busulfan alone still experience mucositis, anorexia requiring parenteral nutrition, nausea and vomiting, and fever with possible bacterial or viral infections. However, these will be expected to be both less frequent and less severe than would occur with a traditional fully myeloablative regimen used in matched sibling allogeneic HSCT and that is what has been our experience in the current pilot study. Trilineage cytopenia is expected in the acute transplant period but should resolve 2-6 weeks following the administration of autologous cells. Patients may require blood product support consisting of red cell and platelet transfusions during this time. If no recovery is observed after six weeks, then a previously-collected unmanipulated back-up source of autologous CD34+ HSC will be administered to restore hematopoiesis. At the discretion of the treating physician, and based upon the clinical status of the patient, infusion of the back-up marrow may be delayed by up to one week (7 weeks after gene transfer).

The risk of graft versus host disease and complications resulting from the use of prolonged immunosuppression required for its therapy will be completely avoided through the use of autologous cells. This along with the fact that the patient serves as her/his own donor represent the major advantages of gene transfer approaches versus correction of SCD using allogeneic donors.

1.8   Peripheral blood mobilization with plerixafor

Successful gene therapy relies on safely and effectively obtaining an adequate yield of autologous HSCs for genetic modification and transplantation. High CD34+ cell numbers are important to ensure a stable donor graft in both allogeneic and autologous settings, as supported by data analysis from several ongoing gene therapy trials. A minimal cell dose is especially unlikely to be sufficient in the hemoglobinopathy setting because in these disorders the genetically corrected stem cells lack a selective advantage, so a graft with low CD34+ cell number would be more likely to fail.

Obtaining adequate CD34+ cell yield is particularly challenging in SCD. Options for autologous HSC collection include bone marrow harvest or peripheral blood HSC mobilization. Bone marrow (BM) harvest is an invasive procedure requiring anesthesia, which can be associated with sickle cell-related morbidities, and may not achieve goal CD34+ cell dose, necessitating repeated procedures scheduled over multiple months. Interim data from the bluebird bio (bbb) HGB-206 LentiGlobin gene therapy trial indicated use of an average CD34+ cell dose of 2.1 x 10^6 cells/kg in the first seven patients (range 1.6-5.1, but only one patient had > 3 x 10^6 CD34+ cells/kg)[68]. In bbb's experience, most subjects who underwent bone marrow harvest required more than one harvest to achieve a minimum cell dose of 1.5 x 10^6 CD34+ cells/kg, with some subjects requiring up to 4 harvests, and unfortunately, the harvest procedures have at times been associated with hospitalization for sickle cell crises (Mohammed Asmal, bbb, personal communication).

In most gene therapy trials, rather than bone marrow harvest, HSCs are obtained through collection after mobilization with granulocyte colony-stimulating factor (G-CSF) followed by peripheral blood (PB)

apheresis. However, this approach is contraindicated in SCD because G-CSF has been reported to cause severe adverse effects in these patients[78]. Even with doses smaller than the dose utilized for standard mobilization of HSCs, G-CSF has been shown to result in VOC, severe acute chest syndrome[79], and in one report, massive splenomegaly and death[80].

Plerixafor is an agent that leads to HSC mobilization by reversibly inhibiting the binding of the chemokine stromal-derived factor 1 (SDF-1/CXCL12) to its receptor CXCR4, which is expressed on the surface of HSCs. Disruption of the SDF-1/CXCL4 interaction results in the rapid release of HSCs from the bone marrow[81]. Short-term administration of plerixafor is safe and well-tolerated in healthy volunteers[82], healthy sibling donors[83], and when combined with GCSF in patients with lymphoma and multiple myeloma[84-86]. It was FDA approved in 2008 for use in combination with GCSF in patients with multiple myeloma and non-Hodgkin lymphoma. Plerixafor is also increasingly being used in the gene therapy setting. In clinical trials of gene therapy for Wiskott-Aldrich syndrome (WAS), chronic granulomatous disease (CGD), and adrenoleukodystrophy (ALD), plerixafor mobilization has been well-tolerated and resulted in good cell procurement.

In the setting of sickle cell disease, the preclinical and early clinical experience supports the safety of plerixafor for PB mobilization. Plerixafor has been compared to G-CSF in a sickle cell mouse model, and results demonstrated effective mobilization of HSC subsets, without neutrophil or endothelial activation, and with lower total WBC and neutrophil counts compared to G-CSF-treated mice[87].

At Boston Children's Hospital a pilot trial was completed to assess safety as well as feasibility of procuring an adequate CD34+ cell dose in subjects with SCD and the results have been reported[88]. Six patients completed treatment with plerixafor, 3 at a lower dose of 180 µg/kg, and 3 at the FDA-approved dose of 240 µg/kg. All of the subjects tolerated the plerixafor and apheresis without any sickle cell-related complications of any grade, and there have been no adverse events of any kind ≥ grade 3. After optimization of the apheresis procedure to allow for sickle cell related cell processing modifications, a successful apheresis collection of > 2 x10^6 CD34+ cells/kg of CD34+ cells was achieved in all subjects mobilized at the full plerixafor dose. Several other small studies have demonstrated the safety and efficacy of plerixafor in SCD patients[89, 90]. Finally, the Pilot Study on which this phase 2 study is based also utilized plerixafor safely and successfully.

1.9   Nephropathy evaluation

SCD patients face multiple renal morbidities, including chronic kidney disease (CKD), hypertension, hyposthenia, and acute kidney injury. The prevalence of albuminuria increases from early adolescence into adulthood. Pediatric patients develop an elevated glomerular filtration rate (GFR) in early childhood (hyperfiltration) that persists during adolescence and young adulthood. In adulthood, patients begin to experience an annual GFR decline[91].

Allogeneic HSCT has well recognized nephrotoxic effects. In addition to nephrotoxicity associated with myeloablative conditioning, which would also of course be relevant in gene therapy, other factors such as the use of cyclosporine and tacrolimus to prevent GVHD and the use of nephrotoxic antibiotics contribute to allo-HSCT-related nephrotoxicity. We are selecting endpoints that will align with ongoing allo-HSCT studies, as well as those recommended in recent ASH/FDA consensus recommendations for endpoints in sickle cell clinical trials[91, 92], in order to facilitate meaningful future comparison. In this study, we will assess

the effects of gene therapy on the kidney by assessing estimated GFR, urine specific gravity and urine albumin to creatinine ratio.

1.10   Work leading up to proposed clinical trial

1.10.1 Description of therapeutic lentiviral vector

The development of the initial lentiviral vector used in this trial, referred to as BSM11-D12G5, is described in detail in Guda et al[72] and Brendel et al., 2016[62]. The features of the vector include:

- It is a vesicular stomatitis virus G (VSV.G) pseudotyped and self-inactivating (SIN) virus vector that contains a shRNA sequence targeting BCL11A embedded in a microRNA and expressed in an erythroid-specific fashion from the locus control region (LCR) of the globin locus as well as the central polypurine tract (cPPT) sequence for efficient transduction of quiescent cells.
- The basic GLOBE vector[93] comprises a LCR-β-globin transcriptional unit containing a 2.7kb fragment encompassing LCR elements HS2 and HS3 which have been reported to have classical enhancer and dominant chromatin-opening functions, respectively[94] coupled with a minimal β-globin promoter and the woodchuck post-transcriptional regulatory element in increase expression.
- The transcriptional unit consists of an artificial microRNA embedded shRNA targeting BCL11A (in this case "D12G5" as depicted in **Figure 1-5** below)[72] cloned into the stem loop of the endogenous miR 223 sequence, as described by Amendola and colleagues[95]. This chimeric miRNA is inserted into a self-inactivating, HIV-derived LV in reverse orientation with respect to the 5'LTR-driven genomic transcript. It can then be expressed by a tissue specific RNA polymerase II promoter such as the erythroid-specific LCR and globin promoter described above.

In preclinical testing, poor vector titers were observed in the scale up of this initial Globe vector construct. Therefore, a second construct was developed in collaboration with bluebird bio, and the resulting lentivirus transfer vector is shown in **Figure 1-5**. This vector, termed **BCH_BB694 (also BB694)**, was utilized in the previous Pilot Study and will be utilized for this clinical trial.

Figure 1-5. BCL11A-LCRshRNAmiR lentivirus vector termed β-globin-m223-shBCL11A (BCH_BB694) which contains the D12G5 shRNA insert. Key: sinLTR: enhancer deleted long terminal repeat; ψ: psi packaging sequence, SD splice donor; RRE; SA: splice acceptor; BGHpA: bovine growth hormone polyadenylation sequence; β-globin pr.: β-globin minimal promoter; HS2 and HS3: hypersensitive sites 2 and 3.

1.10.2 Preclinical data

1.10.2.1 Preclinical efficacy data

Extensive preclinical testing was conducted using the initial lentiviral vector BSM11-D12G5, and is reported in detail in Guda et al.[72] and Brendel et al.[62]. These studies demonstrated success in selectively suppressing BCL11A in erythroid cells via pol II promoter expressed microRNA adapted shRNAs (shRNA[miR]s) resulting in effective knockdown of BCL11A and derepression of *Hbb-y*, a functional homolog of the human β-globin gene in a murine erythroid cell line[72]. Expressing the modified shRNA[miR]s in an erythroid-specific fashion circumvented any adverse effects in other hematopoietic cell lineages and led to efficient BCL11A knockdown and high levels of HbF in primary human CD34-derived erythroid cells. In a series of experiments, G-CSF mobilized CD34+ cells from healthy donors were transduced with a vector expressing a *non-targeting* shRNA (as control) or BSM11-D12G5 and subjected to erythroid in vitro differentiation. These hematopoietic stem and progenitor cells (HSPCs) gave rise to erythroid cells with up to 90% reduction of BCL11A protein. These erythrocytes demonstrated 60-70% γ-chain expression and a corresponding increase in HbF at low vector copy numbers per cell (VCN<1.5).

Preclinical studies also demonstrated induction of HbF in human erythroid cells differentiated *in vitro* after full engraftment of gene modified CD34+ cells in murine xenografts[62]. In these experiments, human CD34+ cells were transduced with controls or BSM11-D12G5 and injected into sublethally irradiated NSG-recipient mice. Bone marrow CD34+ representing engrafted human HSCs were isolated 14 weeks later and subjected to erythroid in vitro differentiation. The resulting induction of γ-globin was similar to the studies described above, with a mean HbF of 70%.

Brendel et al. also assessed the effectiveness of BCL11A knock-down in sickle cell patient cells[62]. Bone marrow CD34+ cells were isolated from four different sickle cell patients and the cells were transduced with control shRNAs or with one of three different shRNAs targeting BCL11A including BSM11-D12G5, and subjected to erythroid differentiation in vitro. The mean γ-globin induction level was 54-68% for the shRNAs targeting BCL11A[62].

Summative data on SCD humanized murine model (BERK-SCD) with the BSM11 vector:

Genetic modification of BERK-SCD HSPCs with the BSM11 vector leads to improvement of disease associated hematological parameters. Engraftment of SFFV-eGFP (control) transduced HSCs derived from BERK-SCD mice was associated with significantly lower red blood cell numbers, hemoglobin and hematocrit levels, and increased reticulocytes and spleen weights indicative of severe hemolytic anemia[62]. These values mirrored the hematologic values of BERK-SCD in a non-transplant setting, supporting the validity of the experimental model. Mice transplanted with HSCs derived from BERK-SCD mice and transduced with BSM11 vector showed improvements in all blood parameters, even at a transduction efficiency of 20% as determined by the surrogate measure of RBC marking. Reticulocyte counts dropped from ~34% (in SFFV-eGFP group) to 11%, indicative of reduced erythropoietic stress as a consequence of reduced hemolysis and improved RBC survival. Consistent with reduced rates of erythropoiesis and lower reticulocyte counts we also observed a striking difference between treatment groups in the frequency of CD71+Ter119+ erythroid precursors (analogous to nucleated red cell counts) in the peripheral blood[62]. In animals which received wild type cells this population is rare (~2%) and similar to untreated healthy mice. This population of stress-induced immature cells was significantly reduced from 33% ± 5% to 6% ± 5% in the BSM11 treated group compared to mice which received SFFV-eGFP transduced HSCs derived from BERK-SCD[62].

Comparability data with BCH_BB694:

Preclinical efficacy analysis was likewise carried out with the clinical vector, BCH_BB694. Data was generated from three additional patients with SCD in which bone marrow was sampled at the time of clinically indicated orthopedic procedures. **Figure 1-6** shows the level of induction of HbF after transduction and *in vitro* differentiation. As shown, the amount of HbF was induced to between 66-92% in these three patients with VCN varying from 2.6 to >10, with no apparent toxicities. The higher VCN was attributed to the use of the bluebird bio proprietary enhancer of transduction using a protocol provided by bluebird bio.
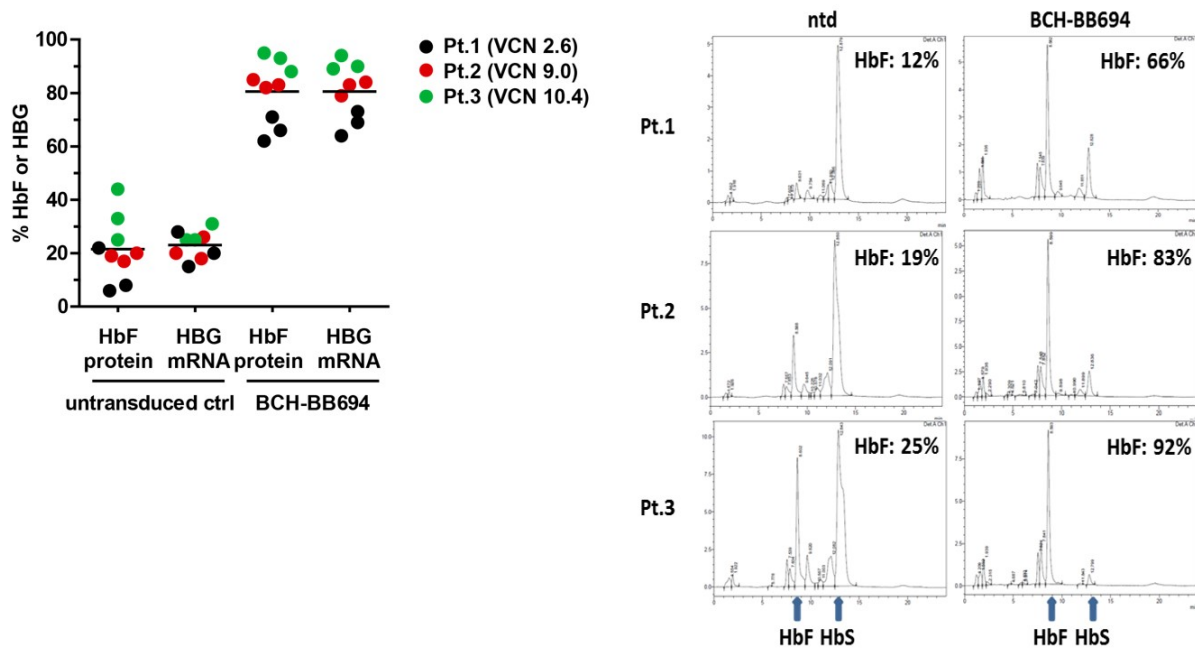
Figure 1-6. Knockdown of BCL11A and induction of fetal hemoglobin in erythroid cells derived from BCH-BB694-transduced CD34 cells from a sickle cell patient. The HbF and HbF peaks are labeled on specimens after 18 days in culture. NT=non-transduced.

Additional comparison analysis performed at bluebird bio showed comparable HbF induction in a healthy donor and a SCD donor. In each case, at the same MOI, BCH_BB694 induced comparable or higher levels of HbF compared to BSM11-D12G5. The addition of an enhancer of transduction significantly increases the transduction efficiency of CD34+ cells with the BCH_BB694 vector.

In the ongoing Pilot Study, clinical data is available on one patient at 18 months post infusion of gene modified cells and on two other patients with shorter follow-up. All of these subjects were adults. **Table 1-3** shows the relevant *in vivo* results, including a high number of circulating F cells, a high calculated F/F cell, a stable level of HbF in the blood (20-30%), reduced hemolysis (low absolute reticulocyte counts, normal LDH), a marked reduction in irreversibly sickled cells on blood smear (data not shown) and a low normal hemoglobin without transfusions. The kinetics of marrow recovery, fetal hemoglobin induction and reduction in hemolysis was similar in all the adult patients treated in the pilot study. More recently, two

adolescent subjects have been treated. While still early in follow-up, data from these individuals are very similar to the results from the adult cohort.

This induction of fetal hemoglobin appears effective in significantly reducing VOEs. In the ongoing Pilot Study no patient has experienced a vaso-occlusive crisis, acute chest syndrome, or stroke since gene therapy (**Table 1-4)**. One patient had severe frequent priapism prior to gene therapy. After gene therapy he had recurrent episodes of priapism between Months 2 and 9. The episodes gradually decreased in frequency and severity and have resolved completely since month 10.

Table 1-3. Hematologic Parameters Post Infusion.

Patient	Months post infusion		Hb (g/dL)	Hct (%)	MCV (fL)	MCHC (g/dL)	F/F cell*** (pg)	ARC (x 10⁹/L) [NL 43-85]	LDH (unit/L) [NL 100-210]
BCL002	25		11.4	34.0	89.2	34.7	9.4	215	217
BCL003⁺	15		11.2	31.1	92.8	36.0	17.2	190	318
BCL004	16		10.8	30.0	86.2	36.0	11.7	195	419
BCL006	12		11.8	34.1	79	34.6	18.6	355	216
BCL007	9		10.4	29.6	78.9	35.1	10.7	100	447

⁺ Hematologic data shown for BCL003 is at 3 months post infusion; latest timepoint prior to resuming transfusions
* % HbF values presented as HbF / (HbF + HbS)
** F cells reported as % of untransfused RBCs
*** calculated: (MCH x HbF)/%F-cell

Table 1-4. Clinical Events Before and After Infusion.⁺

	Months since infusion	No. of transfusions (annualized)		Severe sickle cell clinical events* (total number)		
		Pre-study	Post-GT	Pre-GT	Post-GT (< 5 months)	Post-GT (> 5 months)
BCL002	25	12.5	0	0	0	0
BCL003	15	10.5	4.8	0	0	0
BCL004	16	2	0	13	5	1**
BCL006	12	3	0	6	0	0
BCL007	9	11	0	0	0	0

⁺ Pre-study events represent the 2 years preceding study enrollment. Post-GT events represent the time from engraftment until latest follow-up.
*Events include: VOC pain requiring ED or admission for opioid treatment, acute chest syndrome with admission, and priapism events requiring ED or admission for procedural intervention

** Most recent severe event was an ED visit that occurred 8 months after GT.

1.10.2.2 Preclinical safety data

To date, we have assessed the effect of expression of the transgene on engraftment and reconstitution using a competitive reconstitution assay.

A competitive transplantation experiment was performed to assess potential negative side effects of LCR-vector mediated knock down of BCL11a on engrafting hematopoietic stem/progenitor cells (HSPC) or erythroid lineage development. Murine lineage negative bone marrow cells were isolated and transduced ex vivo with BSM11 -D12G5 (co-expressing the Venus fluorescent reporter) and a neutral control vector only expressing blue fluorescent protein (SFFV-BFP). Six weeks after transplantation into congenic mice the contribution of gene modified cells derived from these two vectors was assessed in total peripheral blood via FACS (**Figure 1-7**). The dashed line at 50% indicates the predicted ratio of gene modified cells derived from both vectors based on the mixture of infused cells (that was 1:1). Deviations towards the lower end indicate lower relative contributions from the BSM11-D12G5 vector, deviations towards the upper end indicate higher contributions, and the data demonstrate a lack of any negativeimpact of the targeting vector compared with the neutral control vector.
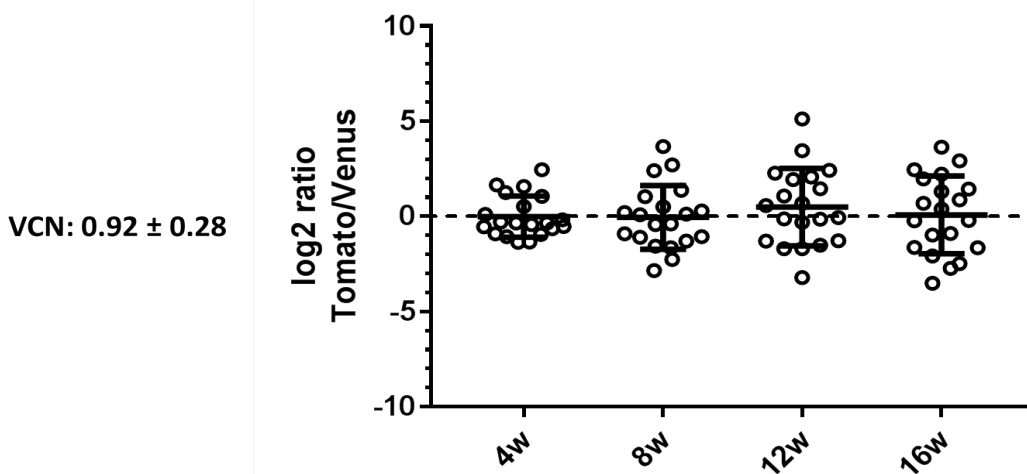
Figure 1-7. Lack of toxicity of BCL11A knockdown in engraftment and differentiation of transduced hematopoietic stem cells. Each dot represents and individual mouse. Weeks post-infusion are shown on X-axis. Y-axis shows ratio of engraftment at each time point.

The data confirm the lack of engraftment toxicity BSM11-D12G5 shRNAs targeting different BCL11A sequences.

An in vitro immortalization (IVIM) assay was performed by the Hannover Medical School group[96-98]. This assay includes a "positive control" vector shown in past assays to induce immortalization and is done with a minimum of three replicates. At MOIs up to 500 and VCN of ~4->11 (mean 7.5 copies per cell) BSM11-D12G5 showed no toxicities at day 1 or day 15 compared to mock transduced cells, and there was no difference in Replating Frequency (ie, immortalization) vs. mock transduced cells. The positive control showed the expected high rate of immortalization in this assay. This data showed that the BSM11-D12G5 vector does not induce immortalization in this assay.

Comparability data with BCH_BB694: Safety

Analysis was likewise carried out with BCH_BB694. IVIM assay confirmed that BCH_BB694 does not induce immortalization in this assay. The data show that even at VCNs as high as 17, BB694 showed no toxicity in cell growth (days 1-6 and 8-15) compared to mock transduced cells and no immortalization was demonstrated. The positive control showed the expected high rate of immortalization in this assay. In summary, in independent IVIM assays, test vectors BCH_BB694 and LCR-V (BSM11-D12G5) showed no signs of vector associated cytotoxicity and a significantly lower mutagenic potential compared to positive control, even at very high vector copy numbers per cell.

In addition, clonogenic assays showed no differences in colony output of progenitors (BFUe, CFU-G/M/GM) or CFU-GEMM) from either normal donor or a SCD donor after transduction with either BCH_BB694 or BSM11-D12G5 versus mock transduced cells.

In the Pilot Study, no adverse events related to use of gene modified cells have been seen, although subjects have had side effects consistent with the use of in-dwelling apheresis catheters and the use of busulfan.

1.11   Justification of Primary Endpoint Criteria and Threshold for Null Hypothesis

Overall, increasing fetal hemoglobin is clearly beneficial in sickle cell: higher HbF is associated with lower mortality, less pain, and lower rates of other complications. Hydroxyurea is successful in increasing HbF and F cells. As noted above, in the few clinical trials reported, the average HbF on HU is 18-24%. However, taking pills daily is a significant burden leading to compliance issues, and optimal prescription practice is not as robust outside of clinical trials. Those factors make real-world response to HU unfortunately lower than the response achieved in clinical trials. We estimate that ~50% of patients on HU do not achieve a sustained level of HbF of 20%. New studies measuring HbF response to optimal PK-based dosing HU suggest that higher HbF levels of 30-40% may be attained in a high proportion of patients[36]. At present, patients with good laboratory and clinical responses to HU are not eligible for this study. Data from our pilot study provide evidence of uniformly high HbF levels attained in both adults and children that are sustained after a one-time GT infusion and no patients to date have experienced any VOEs post-infusion (Table 1-4). These findings support our definition of the primary endpoint, which is a complete absence of severe VOEs from the time of stabilization of HbF after infusion of gene modified cells in the period from Month 6 to Month 24 post-infusion. For the purpose of the primary endpoint analysis the initial 6 months after infusion will be excluded from the VOE observation period. After allogeneic transplant, some sickle cell patients continue to experience pain. However in one study of matched sibling transplant in adults, even patients who required opioid analgesia post-transplant were able to be weaned from the opioids by 6 months post-transplant[20]. In bluebird bio HGB-206 study (group C) preliminarily reported at the 2020 European Hematology Association meeting[153], there were 13/14 patients (92.8%, with 95% CI of [66.3%, 99.8%]) with no VOE post-transplant. These preliminary observations reflect a high rate of complete reduction of severe VOE; however, a slightly lower rate would still be considered clinically important and meaningful. In addition, we considered recent outcome data for allogeneic bone marrow transplant (Eapen et al., Lancet Haematol 2019). Among 910 sickle cell patients who received allogeneic bone marrow transplants between 2008 and 2017, event-free survival (EFS) (defined as survival without graft failure) was 85% in patients < 13 years old and 75.6% in patients 13-49 years old. Analyzed further by donor type, EFS was 90.7% in matched sibling donor (MSD) transplant recipients and 65.3% in non-MSD transplants. Based on these data, for our trial we have chosen an alternative hypothesis of 80.5% and a null hypothesis of 50% of patients with complete absence of VOEs. For Secondary Endpoint 3.2.1, the null hypothesis has

been set at 50% of patients classified as a "success", per the criteria for Secondary Endpoint 1. If this practice is broadly applicable and durable in high percentages of older children and/or adults, the null hypothesis to be tested in Secondary Objective 1, of a 50% success rate, may someday be deemed too low, but that is not the case now.

CHAPTER 2

2   STUDY DESIGN

2.1   Study Overview

This is an open-label, non-randomized, multi-site center, phase 2, single arm study involving a single infusion of autologous CD34+ HSC cells transduced with the lentiviral vector containing a shRNA targeting BCL11a. Accrual of up 27-30 patients may be required in order to obtain a maximum of 25 evaluable patients with SCD. Patients will be age 7-40 years old.

For the purpose of inclusion and endpoints in this study, a VOE is defined as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring parenteral opioids.

2.1.1   Study Objectives

2.1.1.1   Primary Objective

To determine if treatment with a single infusion of autologous CD34+ HSC cells transduced with the lentiviral vector containing a shmiR targeting BCL11A will lead to a complete absence of severe VOEs (defining VOE as ACS or VOC requiring parenteral opioids) in the period from Month 6 to Month 24 after gene therapy. For the purpose of analysis of the primary endpoint, the initial 6 months after infusion will be excluded from the VOE observation period.

2.1.1.2   Secondary Objectives

1. To determine if treatment with BCL11A shmiR gene therapy will result in an increase total Hb, total HbF, and fraction of F cells post-infusion of gene modified cells.

2. To determine if treatment with BCL11A shmiR gene therapy will result in a reduction in hemolysis.

3. To evaluate the safety of the study treatment, by describing toxicities and adverse events (AEs).

4. To estimate the percentage change in the annualized number of VOEs in the period from Month 6 to Month 24 after gene therapy as compared to the 24 months prior to consent.

5. To determine if a complete reduction in the number of VOEs (defining VOE as ACS or VOC requiring parenteral opioids) is attained if the VOE observation period is limited to the period from Month 6 to Month 18 after gene therapy, as compared to the 24 months prior to consent. For the purpose of analysis, the initial 6 months after infusion will be excluded from the VOE observation period.

2.1.1.3   Exploratory objectives

1. To evaluate the efficiency of BCL11A knockdown, by estimating the amount of BCL11A protein on peripheral whole blood and sorted erythroid precursors at baseline, Month 6, 12, and 24 post infusion.
2. To assess transcranial doppler ultrasound screening results for subjects ≤ 14 years old at enrollment, from annual clinical TCD with data collected at Month 24 post-infusion of gene modified cells.

3. To describe the change in SCD-related organ function, including neurocognitive, renal, and cardiopulmonary assessments.

4. To describe the association between baseline socioeconomic status as measured by household material hardship (HMH) and clinical study end-points (reduction in VOE, Hb and HbF, changes in SCD-related organ assessments) and patient-reported outcomes.

5. To describe the impact of the BCL11A shmiR gene therapy as measured by Health Related Quality of Life (HRQoL) patient reported outcome domains for fatigue, pain interference, and sleep.

6. To describe the proportion of patients who are absent all the following events at Month 24 post-infusion of gene modified cells:
    a. Death
    b. Cerebral vascular events, including stroke
    c. For subjects ≤ 14 years old, worsened annual TCD results leading to institution of transfusion therapy by institutional standards
    d. Lack of engraftment (requiring rescue with back-up CD34 cells)
    e. Lack or loss of engraftment of gene-modified cells as determined by VCN <0.1 copies per cell in peripheral blood MNC at 6 months post-infusion
    f. Development of clinically-relevant MDS or leukemia
    g. Presence of replication competent lentivirus

2.1.1.4   Biological Objectives

1. To determine if treatment with BCL11A shmiR gene therapy will increase red cell survival and reduce hemolysis as measured by exhaled alveolar end tidal carbon monoxide measurement (ETCO): a) upon entry to the trial; b) as part of the exchange or simple transfusion phase; and c) post-infusion at Month 6, 12, 18 and 24. Measurements will be performed at regularly scheduled study visits.

2. To determine if treatment with BCL11A shmiR gene therapy will result in a change in the number of RBCs with detectable polymer at venous pO2 (~35 mm Hg) compared to untreated baseline, using an assay of oxygen saturation at the single RBC level[99, 100].

3. For patients enrolled at selected sites only, to determine if treatment with BCL11A shmiR gene therapy will result in any change in total cerebral blood flow (with phase contrast MRI) and cerebrovascular reserve (with breath hold functional MRI), as well as assessment of oxygen extraction fraction and cerebral blood flow by frequency-domain near-infrared and diffuse correlation spectroscopies (FD-NIRS-DCS) monitoring. Measurements will be performed at baseline, Month 12 and 24 post-infusion.

2.2   Patient Eligibility

2.2.1   Inclusion Criteria

1. Diagnosis of sickle cell disease with genotype HbSS, and HbS/β0 thalassemia.

2. Age 7-40 years.

3. Clinically severe disease, defined as at least one of the following:

At least 4 VOEs within the past 24 months prior to consent (VOE defined as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring parenteral opioids.)

4. Adequate hematologic parameters (regardless of therapy) including:

    a. White blood cell (WBC) count within the range of $2.5 – 25.0 \times 10^9$ /L
    b. Hemoglobin within the range of 5 – 11 g/dL
    c. Platelet count above 150×10^9 /L

5. Adequate organ function and performance status:

    a. Karnofsky/Lansky performance status ≥80%.
    b. Serum creatinine </= 1.5 times the upper limit of normal for age, **and** calculated creatinine clearance or GFR >/= 60 mL/min/1.73 m2.
    c. Persistent aspartate transaminase, alanine transaminase, or direct bilirubin value <3× the upper limit of normal (ULN).
    d. DLCO (corrected for hemoglobin), FEV1, and FVC >50% of predicted
    e. Left ventricular ejection fraction >40% **or** shortening fraction >25%

6. Failure of hydroxyurea therapy due to lack of clinical improvement or inability to tolerate due to side effects (e.g., myelosuppression, gastrointestinal symptoms, or hepatic enzyme elevations). Clinical criteria (#3 above) must be met despite taking hydroxyurea at an appropriate dose for greater than or equal to 6 months, unless not indicated or not tolerated. Patients taking hydroxyurea who still meet all inclusion criteria are eligible for the trial. Hydroxyurea should be discontinued when transfusions prior to stem cell collection begin.

7. No HLA-genotypically identical suitable related bone marrow donor available

8. Parental/guardian/patient signed informed consent

2.2.2   Exclusion Criteria

1. Subjects who have concomitant condition or illness including, but not limited to:

    a. Ongoing or active infection

    b. Active malignancy

    c. Major surgery in the past 30 days

    d. Medical/psychiatric illness/social situations that would limit compliance with study requirements as determined by the treating physician.

2. Receiving a chronic transfusion regimen for primary or secondary stroke prophylaxis. (Note: patients with a history of abnormal TCD who have transitioned from transfusions to hydroxyurea for stroke prophylaxis are not eligible for the study.)

3. Patients with history of abnormal TCD (measured with a timed average maximum mean velocity of ≥200 cm/second in the terminal portion of the internal carotid or proximal portion of middle cerebral artery or if the imagining TCD method is used, >185 cm/second plus evidence of intracranial vasculopathy) who were ever on transfusions and subsequently transitioned to hydroxyurea.

4. Patients with history of overt stroke or neurologic deficit lasting >24 hours. (Note: patients with imaging evidence of silent stroke but not on a chronic transfusion regimen are not excluded.)

5. Isolated recurrent priapism unresponsive to medical and surgical therapies in the absence of other qualifying VOE complications that meet inclusion criteria.

6. Contraindication to administration of conditioning medication (busulfan).

7. Subjects who have undergone allogeneic hematopoietic stem cell transplant previously.

8. Known myelodysplasia of the bone marrow or abnormal bone marrow cytogenetics.

9. Subjects with severe cerebral vasculopathy (defined by Moya-moya disease or occlusion or stenosis in the circle of Willis)

10. Liver MRI (≤ 180 days prior to initiation of BU conditioning) to document hepatic iron content is required for participants who have received ≥20 packed red blood cell transfusions (cumulative). Participants who have hepatic iron content ≥ 9 mg Fe/g liver dry weight by liver MRI must have a liver biopsy and histological examination/documentation of the absence of cirrhosis, bridging fibrosis, and active hepatitis (≤ 180 days prior to initiation of transplant conditioning). The absence of bridging fibrosis will be determined using the histological grading and staging scale as described by Ishak and colleagues (1995) as described in the Manual of Operating (MOO).

11. Evidence of HIV infection, HTLV infection, active hepatitis B infection or active hepatitis C infection.

12. Known acute hepatitis or evidence of moderate or severe portal fibrosis or cirrhosis on prior biopsy.

13. Receipt of an investigational study drug or procedure within 90 days of study enrollment.

14. Pregnancy, or breastfeeding in a postpartum female, or absence of adequate contraception for fertile subjects. Females of child-bearing potential must agree to use a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection from Screening through at least 6 months after drug product infusion. Male subjects must agree to use effective contraception (including condoms) from Screening through at least 6 months after drug product infusion.

15. Presence of a genetically-determined hypercoagulable state or personal history of prior VTE (deep vein thrombosis or pulmonary embolism) that, in the opinion of the investigator, would represent a contraindication to proceed with central line placement and study events.

2.3   Treatment Plan

2.3.1   Hematopoietic stem cell procedures and processing timeline

Table 2-1. Hematopoietic stem cell procedures and processing

	Day – 54 or earlier	Days -53 to -50 or earlier	Days -49 to -7	Day -6	Days -5 to -2	Day 0
CD34+ collection (including back-up)	X[1]					
CD34 transduction and cryopreservation		X				
Release testing			X			
Subject admitted to transplant unit				X		
Conditioning (busulfan administration)					X	
Infusion of gene-modified cells						X

[1]Multiple collections of CD34+ cells may be required; each collection must be at least 4 weeks apart. A collection is defined as a cycle of peripheral blood mobilization (up to 4 days per cycle).

2.3.2   Transfusions prior to gene therapy

Patients will receive blood transfusions for a period of at least 3 months prior to hematopoietic stem cell harvest with a goal of achieving a HbS level ≤ 30% by the time of mobilization. The timing of transfusions will be coordinated to occur within 7 days prior to any procedures requiring anesthesia. If the patient was previously taking hydroxyurea, it will be discontinued when pre-GT transfusions are initiated, and at least 3 months prior to autologous stem cell collection. Patients should not receive any disease modifying therapy after transfusions begin. The Study Chairs should be contacted if there are any questions about what drugs or therapies can be used.

2.3.3   Collection of autologous cells: mobilization and apheresis

Subjects who enroll and meet all eligibility criteria will be scheduled for mobilization and collection. Mobilized CD34+ HSPCs will be collected from each subject by apheresis after peripheral mobilization. It is anticipated that all cells will be procured by peripheral mobilization and apheresis, however there may be rare circumstances in which bone marrow harvest would be performed at the discretion of the local Investigator, and with approval by the overall study Sponsor/investigator. At the time of anesthesia for line placement, a baseline bone marrow aspirate and biopsy will be obtained. Bone marrow studies will be performed to exclude underlying myelodysplasia or myelodysplastic syndrome (hematopathology, cytogenetics/FISH) and bank bio-sample.

2.3.3.1   Peripheral stem cell mobilization with plerixafor

For plerixafor-mobilized peripheral CD34+ cell collection, Plerixafor (240 µg/kg) will be administered by subcutaneous injection. The use of G-CSF as a mobilizing agent is not allowed. Starting at least 2 hours after the plerixafor dose administration, apheresis will be performed. The target CD34 collection over two days of apheresis is 10×10^6 CD34+/kg, with at least 4×10^6 CD34+/kg after CD34 purification designated for transduction. A backup product can be aliquoted from the two-day collection if the total CD34+ cells/kg exceed 15×10^6 CD34+ or can be collected on a third sequential or alternate day of collection. For backup, 2×10^6 CD34+/kg can be aliquoted from a purified collection if the total cells are greater than 10×10^6 CD34+.

If the dose of CD34+ cells collected by the first apheresis is less than target, a second dose of plerixafor may be administered the next day, followed by a second apheresis collection, and this may be repeated for up to a total of 4 sequential days of plerixafor mobilization. If sufficient cells are collected for both product manufacturing and back-up, the patient will proceed to gene transfer. If sufficient cells are not collected for both backup product and gene transfer, the patient will undergo additional apheresis procedures as outlined below.

2.3.4 Manufactured product and back-up product

The collected cells of each subject will be split into 2 portions, one portion for transduction/ drug product manufacturing and the other portion set aside as a back-up product in the event a rescue treatment is indicated. The back-up product must contain a minimum of 2×10^6 CD34+ HSPCs/kg. The back-up product will be cryopreserved unmodified and stored locally for availability in the event of delayed hematopoietic reconstitution or engraftment failure. A back-up product need not be collected for this study if a previous, clinical-grade product already exists (documentation required), meets the required minimum cell dose of 2×10^6 CD34+ cells/kg, and could be used, if necessary, at the time of gene therapy.

If the first apheresis cycle (despite four sequential days of collection) does not mobilize the minimum number of CD34+ HSPCs required for drug product manufacturing and for rescue treatment (backup), the mobilization procedure may be repeated. Selection of the timing of a second apheresis will be at the discretion of the Investigator based on the subject's clinical status and may occur no sooner than 2 weeks after the initial apheresis. After removal and storage of the back-up product, the remainder of the subject's mobilized cells will be transferred unfrozen to the cell processing facility. A CD34+ cell selection followed by transduction with the BCL11A-targting lentiviral vector will be performed. The drug product will be cryopreserved and stored until the subject is ready for drug product infusion. A subject will not proceed to conditioning with IV busulfan until a sufficient back-up product is obtained and the gene-modified drug product is manufactured and passes quality control and release testing.

2.3.5 CD34 + Cell Processing at Core Manufacturing site

2.3.5.1 CD34+ Cell Purification

All the manufacturing steps are performed in the Connell & O'Reilly Families Cell Manipulation Core Facility at the DFCI. CD34+ cells will be positively selected from the apheresis product using the CliniMACS reagent and instrument. Quality control (QC) samples are taken to assess purity and sterility. Purified cells will be immediately processed for pre-stimulation and transduction.

2.3.5.2 CD34+ Cell Pre-Stimulation and Transaction

Transduction will be carried out on the selected CD34+ cells and transduced cells will be cryopreserved. If a second collection is required, these gene-modified cells will be thawed and prepared for infusion in parallel with the product from the first collection. In this case two separate products would be infused. All cell manipulation procedures and release testing will be performed in the transduction facility in accordance with Good Manufacturing Practice (GMP) following process specific standard operating procedures. Final Drug Product will be accompanied by a Certificate of Analysis, documenting that all release testing is complete and within specification

2.3.5.3 Release Testing Prior to Subject Re-Infusion

At the end of the manufacturing process, quality control samples are taken to assess the product safety and quality. The table below outlines these assays and their release specification criteria. If the product passes all release criteria, it will be thawed and infused.

Table 2-2. Final product testing and acceptance criteria

Processing step	Test	Method	Acceptance criteria
CD34$^+$ post-transduction (Drug Substance)	Nucleated Cell Count	Manual or Automated	Cell dose determined pre-freeze $\geq 4x10^6$ CD34+ cells/kg*
	Viability	Trypan Blue exclusion or equivalent	$\geq$80%
	Sterility (pre-freeze, last supernatant)	21CFR610.12	No growth
	Endotoxin	Endosafe PTS	$\leq$5.0 EU/Kg/hr
	Mycoplasma	qPCR	Negative
	VCN assay	qPCR	0.7-5 copies/cell
	HbF Induction in CFU	HPLC	>60% of colonies with >50% Hb F
	Replication Competent Lentivirus (RCL)	PCR	Non detected
	Identity	PCR	PCR band size matches provirus
	Identity	Flow	Greater than or equal to 80% CD34+/CD45+

* Total combination of all collections must be $\geq 4x10^6$

Additional quality control testing including CD34 phenotype, Integration site analysis (ISA), colony forming unit (CFU) assay are also performed on final product but they are not included as release criteria.

Subjects withdrawn from the study prior to administration of transduced CD34+ cells will resume normal clinical care (supportive care and/or allogeneic HSCT). Efficacy and safety assessments will not be carried out from the point of withdrawal and data will not be recorded in the Case report forms (CRFs).

2.3.6 Subject Conditioning Regimen

Prior to proceeding with conditioning and infusion of gene-modified cells, patients will undergo standard work-up for autologous bone marrow transplantation according to FACT regulations and institutional guidelines (listed in section 4.6). Subjects will receive myeloablative conditioning with busulfan (daily intravenous dose, adjusted for weight as described below, given over 3 hours once daily for 4 days) administered on days -5 to -2, prior to infusion of transduced cells. Busulfan levels will be drawn and used per standard protocol to adjust the area under the curve target as stated below, to target an AUC of 85-95 mg*h/L. The number of doses may be adjusted according to the busulfan levels achieved at the discretion of the PIs.

It is recommended that adjusted ideal body weight (AIBW) be used when calculating conditioning regimen chemotherapy doses unless actual body weight (ABW) is below IBW.

Ideal Body Weight (IBW) Formulas:

> Males IBW = 50 kg + 2.3 kg/inch over 5 feet

Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet

Adjusted Ideal Body Weight Formula:

AIBW = IBW + [(0.25) x (ABW - IBW)]

Busulfan Dosing

The following table shows the dose of busulfan to be given according to recipient weight. Dosing for subjects over 100 kg will be discussed with the study PI.

Body weight	mg/kg/day
3 – 14.99 kg	5.1
15 – 24.99 kg	4.9
25 – 49.99 kg	4.3
50 – 74.99 kg	4.5
75 – 100 kg	3.6

Busulfan doses will be adjusted based upon the area-under-the-curve calculations. Busulfan levels will be obtained following the 1st and 2nd doses of busulfan. AUC measurements are generally expressed as micromole-min/L, which can be converted into mg*h/L if divided by 244, a factor derived from the molecular weight of busulfan (246 g/mole). For full myeloablation a total AUC of 23,200 micromole-min/L or 95 mg*h/L (equivalent to 16 doses of every-6-hour busulfan with AUC of 1450 micromole-min/L) is the maximum tolerated with regard to incidence of veno-occlusive disease when busulfan is combined with fludarabine. The goal busulfan area-under-the-curve for this protocol will be 4 doses at ~5500 micromole-min and goal total for all doses combined will be ~20,740 - 23,180 micromole-min/L (~85-95 mg*h/L). If goal busulfan is exceeded, less than 4 doses may be given to achieve the goal total.

2.3.7 Infusion of Transduced Cells

Manufactured cells will be shipped frozen for administration at clinical sites no later than 3 days prior to the date of planned administration. Cells will be thawed according to standard operating procedure outlined in MOO and infused intravenously over 30-45 minutes after standard prehydration and premedication according to institutional guidelines. This standard requires that the patient be on continuous cardiac, respiratory and oxygen saturation monitor throughout the infusion and for 30 minutes afterwards. Vital signs will be measured and recorded pre-infusion, 15 minutes into infusion, and end of infusion. The RN will stay with the patient for the first 5 minutes of the transfusion. If two transduction products are administered, the second transduced product will be administered without delay after the first. Investigational Medicinal Product (IMP)

2.4 Description of the IMP

The investigational medicinal product is patient-specific and corresponds to autologous CD34+ cells transduced ex vivo with the BCH_BB694 LCR(-HS4) bGp D12shmiR (hereafter BCH_BB694 or BB694)

lentiviral vector in final formulation and container closure system, ready for intended medical use. The starting materials used for the production of the IMP consist of BB694 and the patient's CD34+ cells.

2.4.1 Production, Supply and Release of the IMP

The lentiviral vector clinical batch is manufactured by Lonza Houston, Inc. according to current good manufacturing practices (cGMP). Transduction of autologous CD34+ cells with the lentiviral vector BCH_BB694 will be carried out in the Connell & O'Reilly Families Cell Manipulation Core Facility, Dana-Farber Cancer Institute. The transduced cells will be cryopreserved until all quality control (QC) testing results are available and pass specification. The drug product will then be released for infusion and shipped to the clinical site. At that time, the drug product is thawed and for administration.

2.4.2 Description of the IMP

IMP accountability is ultimately the responsibility of the Sponsor. This responsibility however will be delegated to the technologist in charge of the transduction procedure. Detailed records will be kept to allow for accurate accountability of the vector and transduced CD34+ cells. These records will include details of shipping, receipt, storage, use and destruction of the vector. Transfer of the transduced CD34+ cells from the manufacturing laboratory and administration of the transduced CD34+ cells to patients will be recorded.

2.4.3 IMP Treatment and Dosage

The lentiviral vector BCH_BB694 is used to transduce autologous CD34+ cells *ex vivo*. These transduced cells are then cryopreserved. The cell dose is determined pre-freeze and the target is $\geq 4 \times 10^6$ CD34+ cells per kg of body weight. Final product characterization and release specification is show in **Table 2-2.**

2.5 Risks & Toxicities

2.5.1 Venous Central Line Placement and Blood Transfusions

All subjects will require central venous access which will be used for stem cell collection, HSCT admission (busulfan administration, product infusion, hydration, medications, etc), likely for pre-GT transfusions, and other study specific treatments/procedures. If subjects do not already have central venous access in place, a central venous line or port will be inserted under general anesthetic conditions required for this procedure prior to or at the time of the above-mentioned study procedures. The risks associated with vascular access devices include bruising or bleeding which may require hemostatic intervention, infection which may require antibiotics or catheter removal, line-associated venous thromboembolism which may require anti-coagulation, other therapies, and/or catheter removal. Every effort will be made to minimize the risks associated with the central venous access.

Some individuals will receive blood transfusions prior to gene transfer that would not have otherwise been part of that subject's non-research clinical care. The risks of transfusion include infection; transfusion reactions, including allergic reactions, febrile reactions, hemolytic reactions, or other rare reactions; alloimmunization; and other risks, including local tenderness, local infection, loss of red blood cells, anticoagulant discomfort, or air infusion. Blood transfusion or red cell exchange by apheresis may be done by peripheral vein IV access (two large-size intravenous catheters (IVs) in each arm) or use of your existing central venous access device (port or catheter). If there is difficulty with obtaining IV access, an apheresis grade central venous catheter (central line) would be placed temporarily for the procedure.

2.5.2   Plerixafor

Adverse effects associated with plerixafor have been mild and transient, including headache, erythema and stinging at injection site, perioral paresthesias, nausea, and sensation of abdominal distention[82]. Hematologic effects observed have included leukocytosis and thrombocytopenia. Splenic enlargement was observed following prolonged (2-4 weeks) daily plerixafor administrations in rats at doses approximately 4-fold higher than the recommended human dose (drug label information). The most common adverse reactions (>/= 10%) reported in patients who received plerixafor in conjunction with G-CSF regardless of causality and more frequent with plerixafor than placebo during HSC mobilization and apheresis were diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting (drug label information).

In individuals with sickle cell disease, there may be a risk of sickle-related complications with leukocytosis, including VOC, acute chest syndrome, splenic sequestration, or stroke. These risks will be minimized by close monitoring and pre-plerixafor transfusion.

2.5.3   Apheresis

Stem cell collection by apheresis requires an apheresis compatible catheter. If an existing CVL is not apheresis compatible, a temporary apheresis catheter will be placed for the duration of the collection procedure. The apheresis procedure lasts between 4 to 8 hours and has been shown to be safe both in adults and in children. Adverse reactions to apheresis procedures are rare, although vasovagal episodes related to needle insertions or transient volume loss can occur. The former reaction is prevented by lying down and if necessary, fluid administration. Paresthesia or tingling can also sometimes occur due to lowering of calcium levels by the citrate anticoagulant. This is readily relieved by slowing the rate or temporarily interrupting the anticoagulant infusion. The precautions taken to minimize pain, hematoma, risk of infection at the needle site and vasovagal fainting are outlined in the venipuncture section. Leukapheresis can be associated with some loss of red blood cells and plasma equivalent to 50 to 100 ml of whole blood though every effort will be made to keep this volume as low as possible. This is a routine procedure with minimum side effects or complications. Potential complications include formation of hematoma at the site and a small risk of infection as with any intravenous catheter or access of a central line.

2.5.4   Conditioning Regimen

Immediate side effects relating to the use of alkylating agent chemotherapy are mainly limited to the gastrointestinal tract with transient nausea and vomiting followed by stomatitis, esophagitis and diarrhea, which resolve with neutrophil recovery. There is a risk of veno-occlusive disease associated with busulfan. All subjects will receive standard antimicrobial prophylaxis according to institutional guidelines. With these measures, the immediate risk to the subject as a direct result of the conditioning and period of neutropenia will be no more than that associated with standard allogeneic HSCT. If there is a failure of bone marrow recovery after infusion of transduced cells, the backup product collected prior to therapy will be administered. The risk of secondary malignancy associated with the use of this regimen for HSCT in non-malignant disease is very low. Myeloablative busulfan dosing is associated with gonadotoxicity. As is standard prior to HSCT, all patients are offered a consultation regarding fertility preservation, including discussion of sperm or egg preservation, or testis or ovary biopsy when appropriate.

2.5.5   Lentivirus-Mediated Gene Transfer

2.5.5.1   Insertional mutagenesis and testing for presence and integration pattern of vector sequence

Insertional mutagenesis remains a finite risk of gene therapy using integrating vectors. However, modifications to the vector design including removal of LTR enhancer sequences, and utilization of a less potent cellular promoter sequence for transgene expression, have been shown to significantly diminish mutagenic risk in experimental systems. It is therefore probable that the risk of inducing clonal dominance due to insertional mutagenesis will be substantially reduced in this study. Furthermore, there is no evidence for potential cooperating effect between the transgene in use in this study and adverse insertional events.

Testing by PCR for the presence of vector sequences will be performed every 6 months (on this protocol until 2 years post-gene transfer, and then on the long-term follow-up protocol until 15 years post-gene transfer) or until no vector sequences are detected in the sample on consecutive periodic tests. The assay used to determine vector persistence has demonstrated a limit of quantitation of 0.05 copies of vector / 100 ng genomic DNA. If at least 1% of the cells in a sample are positive for vector sequences by PCR, the vector integration pattern will be investigated (as described below) and reported in the study semi-annual report.

Clonal Dominance

Criteria that can Trigger Clinical Work-up for Malignancy:

- Any clinical suspicion of malignancy including leukemia or lymphoma.

- Unexplained white blood cell (WBC) count > 30,000 (cells/μL) on 2 consecutive measurements or other findings suggestive of an oligoclonal expansion.

- After achievement of a WBC count within the normal range post-drug-product infusion and engraftment of gene-modified cells, the development of a WBC < 1000 (cells/μL) on 2 consecutive measurements.

Assessment of Clonal Dominance:

Integration Site Analysis (ISA) will be performed on whole blood (providing that VCN >0.01) at least every 6 months through 2 years post-infusion, and then yearly until 15 years post-infusion (for two years on this protocol, and then on a long-term follow-up protocol). ISA will use Ligation-mediated amplification methods (LAM) for screening but quantitative polymerase chain reaction (qPCR) to validate the screening results, as needed. Of note, only whole blood MNC ISA will be considered for assessment of clonal dominance, not ISA of sorted cell populations. The frequency of the ISA should be increased for subjects with VCN of ≥0.3 if the ISA demonstrates that a mappable insertion site (IS) contributes >30% by of the insertion to the total number of retrieved IS as follows, or at any time at the discretion of the investigator and sponsor:

- If ISA detects an IS contributing >30% to the total IS, ISA should be repeated monthly for 3 months.

- If result is ≤30% clonal contribution on repeat ISA, monitoring of the subject returns to the protocol-defined schedule.

- If result is >30% and ≤90% clonal contribution at the first repeat, and > 50% clonal contribution at any subsequent repeat, clonal dominance criteria are met and clinical work-up for malignancy

should be initiated.

- If ISA result is >90% clonal contribution at any time, clonal dominance criteria are met and clinical work-up for malignancy should be initiated.

- If evidence that multiple insertions are present in the same clone, the clonal dominance is determined by the frequency of the summation of all insertion sites in that clone. The evidence of multiple insertions in the same clone is when at three different time points, the ISs are consistently within 10% of each other. Definitive evidence of multiple insertions in the same clone is derived from ISA of individual bone marrow or peripheral blood isolated progenitor colonies. Additional investigations may be undertaken with the agreement of the study sponsor, including additional (unscheduled) ISA testing.

Clinical Work-up for Malignancy:

If any of the above criteria are met, the PIs will be notified and a work-up will be performed that may include the following:

- Physical exam

- CBC with differential and lymphocyte subsets

- Studies to rule out infectious cause

- Studies to rule out autoimmune disease

- Imaging studies, as appropriate

- Bone marrow analysis, flow, FISH and cytogenetic analysis and VCN and insertion site analysis of flow-sorted purified populations of lymphoid, myeloid cells and CD34+ cells

If there is no evidence of malignancy or myelodysplasia, subject will continue to be monitored as per the protocol-defined schedule of events, or more frequently at discretion of the investigator and sponsor.

If assessment of clonal dominance triggers clinical work-up for malignancy, the Principal Investigators, Sponsor and Project Manager will be notified. If a subject undergoes evaluation for malignancy based on laboratory or clinical indications, the DSMB, IRB and FDA will be informed for consideration of a temporary suspension of inclusion of new subjects in the study until diagnostic evaluation is complete.

2.5.5.2   Germline Transmission of Vector sequence

Hematopoietic cells are manipulated, and transduced ex vivo, and extensively washed prior to re-infusion. In addition, retroviral particles are inactivated by human complement in vivo and risk of shedding of vector particles has been quantified as minor.[147] Therefore the risk of gene transfer to other tissues, including gonads, is extremely small.

Hematopoietic progenitors are transduced ex vivo in a closed culture system. The vector does not contain replication competent viruses and will not be shed from transduced cells. The potential for transmission of vector sequences to other persons is therefore extremely small.

2.5.5.3   Quality Control of Cell Manipulation Process

Manipulation of cells ex vivo is potentially associated with microbial contamination. However, all steps of the manufacturing (CD34 selection, pre-stimulation, transduction, and final formulation) are performed in

an FDA-registered, Joint Commission (JC) and FACT accredited and CLIA-licensed GMP manufacturing facility (Connell and O'Reilly Cell Manipulation Core Facility) at the DFCI. The CMCF is classified as an ISO 7 cleanroom. Trained personnel following aseptic techniques and current GMP guidelines perform all procedures. Production and process controls are in place to minimize contamination as per GMP guidelines.

In addition, in process quality control samples are taken for microbial contamination testing prior to cryopreservation. Only products that pass all specifications will the thawed and infused.

2.5.5.4   Infusion of Transduced Cells

The released medicinal product comprising transduced CD34 cells are transported to the site frozen and thawed at the time of infusion at the bedside. The cryo tin containing the product is placed into a sterile thaw bag. The sealed bag is placed into a 37°C water bath until frost is gone from the outside of the tin. The exterior of the bag is sprayed or wiped with alcohol prior to placing the bag in the biological safety cabinet (if thawing is not at bedside). The tin is removed from the bag, opened, cell product removed, and placed into a new sterile thawing bag. The thawing bag with cells is placed into the 37 C water bath and gently rocked until the product is completely thawed. Product expiration is 4 hours post thaw start time. If thawing is not performed at bedside, these additional steps are needed: final infusion labels are attached to the thawed cell product. The product is transported to the bedside with ambient gel packs in a validated cooler (1-10°C).

Based on results from other gene transfer studies and our own experience, the infusion of cultured and gene altered autologous blood progenitors does not appear to be associated with any significant reactions. In this study, the medium used to culture the CD34+ cells is free of animal serum. However, infusion of any type of blood cell product can be associated with reactions resulting from clumping of these cells or other immediate reactions related to sticking of these cells to blood vessels in the lungs. Reactions are treated by stopping the infusion and providing oxygen, antihistamines, steroids and medications or fluids to increase blood pressure. Because infused cells in this study are autologous CD34+ HSCs, the possibility that this type of agglutination or vascular reaction will occur is very low. It is theoretically possible for the CD34+ cell cultures to become contaminated with microorganisms, but as described above, processes are in place to minimize contamination.

If we determine that the investigational drug presents an unreasonable and significant risk to subjects for any reason, we would discontinue immediately the investigation, notify the FDA, all institutional review boards and all investigators (21CFR 312.56(d)).

2.5.5.5   Testing for Replication Competent Lentivirus (RCL)

Generation of RCL is highly unlikely due to the multiply deleted nature of the vector and SIN configuration and has not been noted in any preclinical or clinical studies with similar vectors. Lentiviral supernatant and subject samples will however be tested for the presence of RCL by PCR methodology. Subject samples will be tested at time points 0 (pre-treatment), 3 months, 6 months, 12 months, and 24 months after treatment. After 24 months, RCL will continue to be checked yearly as part of the separate subsequent long-term follow up protocol as dictated by FDA guidance. In the unlikely event that post-treatment samples are positive, further analysis will be undertaken to characterize the RCL.

2.6   Supportive Care

Supportive care will be provided as per institutional guidelines for autologous transplantation except that red blood cell transfusions will be utilized to maintain Hb > 8gms/dl after infusion of

transduced cells. Disease modifying drugs are not allowed. Please contact study Chairs in the event the treating physician intends to prescribe disease modifying drugs while on study.

2.7   Treatment Failure/ Lack of Response

There are two potential failures in this therapeutic approach related to engraftment: 1) Failure as defined by absence of hematopoietic rescue (engraftment) as defined by absolute neutrophil count above 0.5×10^9 /L for three consecutive days, achieved within 7 weeks after the cell infusion; 2) Failure of engraftment or loss of genetically modified cells as determined by a VCN < 0.1 in peripheral blood MNC at 6 months. Either of these outcomes would be considered a treatment failure in terms of the primary endpoint.

Initiation of disease modifying agent(s) which includes hydroxyurea, L-glutamine, crizanlizumab, voxelotor for prevention or management of severe VOEs is considered treatment failure in terms of the primary endpoint.

Following treatment failure the subject will be commenced on the standard supportive treatment they were receiving prior to gene therapy.

In consultation with the applicable regulatory agencies a second administration of the gene therapy treatment could be considered 4 months after the initial infusion for a subject who fails to respond. Alternatively, the subject could be considered for an allogeneic transplant from an unrelated or haplo-identical donor. However, such subjects will already have contributed to the primary endpoint as "failures", and therefore, this second administration of gene therapy or allogeneic transplant will not affect the determination of the primary endpoint for these subjects.

2.8   Patient Withdrawal from Study and Off Study Criteria

2.8.1   Withdrawal of Individual Subjects, Off-study criteria, and follow-up of withdrawn subjects

Subjects who wish to discontinue from the study or the study treatment at any time are free to do so. The reasons for discontinuation should be documented by the investigator if possible.

Before administration of transduced CD34+ cells:

A subject can withdraw or be withdrawn from protocol treatment in the study at any time from enrollment until the transduced CD34+ cells have been administered. Subjects who are withdrawn prior to administration of transduced CD34+ cells will have no further data collected and will be replaced.

Before administration of CD34+ cells, a subject will be considered withdrawn/off study under the following circumstances:

a. Subjects from whom < 4 x 10^6 CD34+ cells/kg of final transduced drug product are estimated from combined collections will be approached to consider staying on study and undergoing another collection at least 2 weeks after the last. There will be a maximum of four total peripheral stem cell collections. The informed consent document has been written to inform subjects of this possibility. If the subject does not wish to stay on study, he or she will be withdrawn.

b. Subjects from whom transduced CD34+ cells do not pass release specifications (see **table 4-1**) will be approached to consider staying on study and undergoing another collection at least 2 weeks after the last. There will be a maximum of four total collections including the back-up collection. If the subject does not wish to stay on study, he or she will be withdrawn.

    c.   If prior to the administration of transduced CD34+ cells, the subject experiences a clinical event believed by the investigator to contraindicate the continuation of treatment, the subject will be withdrawn.

    d.   If the subjects/parent/guardian withdraws consent for study procedures and/or data collection the subject will be taken off study.

    e.   Death of subject

Subjects withdrawn from the study prior to administration of transduced CD34+ cells will resume normal clinical care. Efficacy and safety assessments will not be carried out from the point of withdrawal and data will not be recorded in the Case Report Forms (CRFs).

After administration of transduced CD34+ cells:

Once a subject has been administered the transduced CD34+ cells, study treatment is complete. Subjects taken off study after administration of CD34+ cells will continue data collection (unless the patient withdraws consent for data submission) and will NOT be replaced for the purposes of addressing the study objectives.

After administration of CD34+ cells, a subject will be considered withdrawn/off study under any of the following circumstances:

    a.   Failure of gene therapy as defined by absence of hematopoietic recovery (recovery defined as absolute neutrophil count above 0.5×10^9 /L for three consecutive days, achieved within 7 weeks after the procedure) will be taken off study.

    b.   Absence of vector-marked cells (defined as < 0.1% vector-positive peripheral blood mononuclear cells) at $\geq$ 6 months post-infusion on two consecutive determinations at least 1 month apart.

    c.   If the subjects/parent/guardian withdraws consent for study procedures and/or data collection the subject will be taken off study.

    d.   The subject lost to follow-up for 1 year will be taken off study.

    e.   Death of subject

Subjects that are withdrawn from the study due to any reason will be followed up according to protocol if gene marked cells >0.1% remain in peripheral blood. Further treatment and investigations will be carried out as necessary to ensure the safety and wellbeing of the subject.

Disposition of cells in case of withdrawal from study:

Storage of Stem Cells:

If back-up cells are not used, individual sites will store the cells in their freezers for 10 years. If after 10 years of storage the subject's physician determines that the products are no longer useful to him or her, the subject will be contacted (e.g. by registered mail) and offered the option to request transfer of the cellular products to another facility at the subject's cost. If we do not receive a request to transfer products, the products will be either discarded or de-identified and used for research, validation, or quality improvement projects. If after a time in storage, the product integrity or labeling no longer meets current standards for clinical use, the same notification process will be followed prior to product discard or de-identification and use by the lab.

Disposition of Gene-Modified Cells:

If some or all of the subject's gene-modified cells are not infused, the sponsor may decide if the cells will be discarded or used for research.

2.8.2   Premature termination of the study

Enrollment of the study will be suspended under the following circumstances:

a.   Expiration or exhaustion of vector stock may prevent further recruitment of patients.

b.   Any leukemia or cancer potentially related to provirus insertion or treatment-related death. Planned infusion of a gene-manipulated product in any subject already enrolled will be suspended; those who have already received a gene modified product will continue to be evaluated per protocol. The events will be reviewed as quickly as possible by the DSMB, IRB and FDA if one of these serious adverse events is observed.

c.   Overall stopping of trial would be made in consultation with the DSMB due to other serious and related adverse events occurring on trial. We will notify the FDA and each site Institutional Review Board if the study stopping rules are triggered.

CHAPTER 3

3   STUDY ENDPOINTS AND DEFINITIONS

3.1   Primary Endpoints

3.1.1   Occurrence of VOEs by Month 24 post-infusion

A complete absence of severe VOEs (defining VOE as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring parenteral opioids) in the period from Month 6 to Month 24 after gene therapy. For the purpose of this primary endpoint analysis, the first 6 months after infusion of the gene therapy product will be excluded from the VOE observation period. Patients with complete absence of severe VOEs will be classified as 'successes'. Patients with one or more severe VOEs from Month 6 to Month 24 after gene therapy, or who experience engraftment failure, or who initiate disease modifying agent(s) for prevention or management of severe VOEs, or who have less than 24 months of follow-up post-infusion, will be classified as 'failures'.

3.2   Secondary Endpoints

3.2.1   Hemoglobin Function

Total Hb of at least 10 g/dL or increase of > 2 g/dL over baseline; and total HbF > 20% with > 60% F cells. Each of these factors will be measured at Month 9, 12, 15, 18 and 24 post-infusion of gene modified cells. For each factor, the average value across the available time points (minimum of two required) will be utilized to determine if the endpoint criteria have been met.

3.2.2   Hemolysis

Hemolysis will be ascertained based on absolute reticulocyte count, lactate dehydrogenase and bilirubin at 18 months post-infusion.

3.2.3   Toxicities and Adverse Events

Adverse events (AEs) grade ≥2 according to CTCAE Version 5 that are related or possibly related to the study procedure will be tabulated through 24 months.

3.2.4   Percentage change in the annualized number of VOEs

For each evaluable patient, the percentage change in the annualized number of severe VOEs will be calculated as: (B - A) / A * 100%, where,

A=the annualized number of severe VOEs over the 24-month period prior to consent; and,

B=the annualized number of severe VOEs over the period from Month 6 to Month 24 after gene therapy.

For A, the annualized number of severe VOEs will be calculated as [(the number of severe VOEs) / 2 years]. For B, the annualized number of severe VOEs will be calculated as [(the number of severe VOEs) / (the number of years of observation during the period from Month 6 to Month 24 post-infusion)].

However, for evaluable patients who are lost to follow-up, die, or withdraw between Month 6 and Month 24, "B" will be imputed based on the severe VOE rate observed during the time period from Month 6 until

the time the patient is lost, dies or withdraws. The minimum length of the VOE observation period required for imputing the annualized VOE rate will be from Month 6 to Month 18 post-infusion. For example, if a patient experiences 2 severe VOEs from Month 6-18 (i.e., a rate of 0.167 severe VOEs per month) and is thereafter lost to follow-up, the imputed number of severe VOEs for the period from Month 6 to Month 24 is 3 severe VOEs. It follows that the annualized number, B, is equal to 2 severe VOEs.

As described in Background section 1.11, the post-infusion rate of severe VOEs has been observed to decrease over **time** [20]**.** Therefore, the above approach to deal with missing data provides a conservatively high estimate of the number of severe VOEs post-infusion, because it assumes that the initial post-infusion rate of severe VOEs will be maintained and not decrease over time.

3.2.5   Occurrence of VOEs by Month 18 post-infusion

A complete reduction in the number of severe VOEs (defining VOE as ACS or VOC requiring parenteral opioids) in a VOE observation period from Month 6 to Month 18 after gene therapy, as compared to the 24 months prior to consent. For the purpose of analysis, the initial 6 months after infusion will be excluded from the VOE observation period.

3.3   Exploratory Endpoints

3.3.1   Efficiency of BCL11A knockdown.

The amount of BCL11A protein on peripheral whole blood and sorted erythroid precursors at baseline, Month 6, 12, and 24 post infusion.

3.3.2   Transcranial Doppler Ultrasound

For subjects ≤ 14 years old at enrollment, TCD screening results from annual clinical TCD with data collected at Month 24 post-infusion of gene modified cells.

3.3.3   Organ Function

The following assessments at baseline, and Month 24 post infusion of gene modified cells (and additional timepoints as noted below and in Section 4.6):
1. Cerebrovascular (presence/absence of neurovascular disease per brain MRI/MRA)

2. Cerebral vascular events, including stroke

3. Renal (urine albumin creatinine ratio, serum creatinine, and cystatin C),

4. Cardiopulmonary (FVC, FEV1, FEV1/FVC, VC, TLC, RV, ERV, IC, FRC, DLCO, and oxygen saturation), Echocardiography for left ventricular shortening fraction or ejection fraction, and presence or absence of tricuspid regurgitation. If present, measure jet velocity as a measure of pulmonary hypertension

3.3.4   Household Material Hardship (HMH)

To describe the association between baseline socioeconomic status as measured by household material hardship (HMH) and clinical study end-points (reduction in VOE, Hb and HbF, changes in SCD-related organ assessments) and patient-reported outcomes. To describe the change in socioeconomic status from baseline to Month 24 post-infusion.

3.3.5   Patient Reported Outcomes

To describe the impact of the BCL11A shmiR gene therapy as measured by Health Related Quality of Life (HRQoL), using the Patient-Reported Outcomes Measurement Information System (PROMIS) domains for fatigue, pain interference, and sleep, from baseline to Month 24 after gene therapy.

3.3.6   Other Outcomes at Month 24 post-infusion of gene modified cells

At Month 24 post-infusion of gene modified cells, absence of all the following events:
1. Death

2. Lack of engraftment of gene-modified cells (requiring rescue with back-up CD34 cells)

3. Cerebral vascular events, including strokes

4. For subjects ≤ 14 years old, worsened annual TCD results leading to institution of transfusion therapy according to institutional standards

5. Lack or loss of engraftment of gene-modified cells as determined by VCN <0.1 in peripheral blood MNC at 6 months post-infusion

6. Development of MDS or leukemia that results secondary to vector-related insertional mutagenesis or to busulfan conditioning.

7. Presence of replication competent lentivirus

3.4   Biological Study Endpoints

3.4.1   RBC Survival

Exhaled alveolar end tidal carbon monoxide measurement (ETCO): a) upon entry to the trial; b) as part of the exchange or simple transfusion phase; and c) at Month 6, 12, 18 and 24 post-infusion. Measurements will be performed at regularly scheduled study visits.

3.4.2   RBC Oxygen Saturation

The number of RBCs with detectable polymer at venous pO2 (~35 mm Hg) compared to untreated baseline, using an assay of oxygen saturation at the single RBC level[99, 100].

3.4.3   Total Cerebral Blood Flow

The following measurements will be performed at baseline, Month 12, and Month 24 post-infusion for patients enrolled at BCH site only:

- Total cerebral blood flow with phase contrast MRI [101];

- Cerebrovascular reserve with breath hold functional MRI[102, 103]; and,

- Oxygen extraction fraction and cerebral blood flow by frequency-domain near-infrared and diffuse correlation spectroscopies (FD-NIRS-DCS) monitoring[104-106].

CHAPTER 4

4   PATIENT ENROLLMENT AND EVALUATION

4.1   Approaching Patients, Eligibility Screening and Obtaining Consent

Patients will be enrolled following diagnosis of SCD and referral to the study sites. Informed consent will be obtained from eligible patients and parents/guardians, and for older minors eligible for this study, assent or consent will be sought.

The Site Investigators will discuss the study at length with the parent/guardian of a potential new subject. A printed information leaflet will be supplied, and the parent/guardian given time to consider his/her decision. The parent/guardian will be encouraged to ask further questions about the study to the Investigator or the clinical nurse specialist. Should a parent/guardian decide that the patient will participate they will be invited to sign the study consent form. Children 12 years of age or over will be given an age specific patient information sheet and will be invited to sign an assent form.

4.2   Enrollment Procedures

Once protocol is finalized this section will have details on how to enroll a patient through the electronic case report system.

4.3   Initial Evaluation

After signing consent, subjects will undergo screening exam, labs, and evaluations to determine eligibility. Upon enrollment in the study, patients will be offered the opportunity for consultation regarding fertility preservation. Pre-transplant labs and evaluations are outlined in Section 4.6.

4.4   Hematopoietic stem cell procedures and processing timeline

Table 4-1. Hematopoietic stem cell procedures and processing

	Day – 54 or earlier	Days -53 to -50 or earlier	Days -49 to -7	Day -6	Days -5 to -2	Day 0
CD34+ collection (including back-up)	X[1]					
CD34 transduction and cryopreservation		X				
Release testing			X			
Subject admitted to transplant unit				X		
Conditioning (busulfan administration)					X	
Infusion of gene-modified cells						X

[1]Multiple collections of CD34+ cells may be required; each collection must be at least 4 weeks apart. A collection is defined as a cycle of peripheral blood mobilization (up to 4 days per cycle).

4.5   Study Monitoring

Pre-treatment and post-treatment investigations are summarized in Tabular format below (Table 4-2)

4.5.1   Pre-Treatment Investigations

Routine gene therapy pre-treatment work-up investigations will be carried out prior to gene therapy according to Table 4-2. Pre-Gene Transfer Evaluations. Work-up will include pre-treatment immune and hematologic evaluations, including a baseline diagnostic bone marrow aspirate and biopsy and any additional assessments determined by the investigator's clinical judgment. These assessments should be carried out within the pre-gene therapy treatment according to Section 4.6 Patient Evaluations. Please refer to Appendix D for recommended prioritization of lab tests.

4.5.2   Post-Treatment Investigations

From the day of infusion of the gene therapy product until hematopoietic recovery (engraftment, which is defined as absolute neutrophil count above 0.5×10^9 /L for three consecutive days), patients will be closely monitored clinically and with laboratory testing according to institutional standards of care. Typically, this would include daily physical exam, weight, and vital signs. Additionally, it would include at least three times weekly CBC and urinalysis, at least twice weekly chemistries, creatinine, bilirubin, alkaline phosphatase, AST, and ALT; and weekly viral surveillance cultures.

Subjects will then be monitored on protocol (with labs + visit, or labs only) at 1, 3, 6, 9, 12, 18, and 24 months after gene transfer. We have taken into consideration the volume of blood needed for protocol specified tests may not be feasible in children. Please refer to Appendix D for recommended prioritization of lab tests.

Monitoring of subjects will follow the schedule listed in 4.6 Patient Evaluations. Modifications to this protocol will be adopted as necessary to improve sample-processing capability, and a degree of flexibility regarding the actual dates of assessment will be maintained. Additional tests may be carried out in the event of a significant adverse effect to ensure optimal clinical care.

This protocol will collect data until 2 years after gene transfer. To comply with FDA requirements for follow-up of gene transfer subjects for 15 years after the procedure, each subject will be approached to enroll on a separate protocol for long-term monitoring between 2 and 15 years after gene transfer. Data collected on this study will consist of gene marking, archiving of samples for safety tests, collection of clinical follow-up data including recording the emergence of new malignancies or hematologic disorders.

4.6 Patient Evaluations

Table 4-2: REQUIRED ASSESSMENTS

	Pre-gene transfer			Admission to HSCT	Month 1 post-infusion	Month 3 post-infusion	Month 6 post-infusion	Month 9 post-infusion	Month 12 post-infusion	Month 18 post-infusion	Month 24 post-infusion
	Screening/ Eligibility	Baseline	Collection for back-up and GT product								
Informed Consent	X										
History and Physical	X	X		X	X	X	X	X	X	X	X
Growth (height and weight) and Vital Signs	X	X			X	X	X	X	X	X	X
Pre-HSCT Studies		X^E	X^E								
Performance Status	X										
PFTs	X										X
VOEs (history per definition)	X				X^H	X^H	X^H	X^H	X^H	X^H	X^H
ECHO	X										X
TCD if < 14 years old at timepoint (regularly scheduled yearly exams as per standard of care at institution)		X									X
Urine albumin, serum cystatin C		X									X
Brain MRI/MRA		X							X		X
CBC/differential + reticulocyte count+ iron studies ^{G}	X			X	X	X	X	X	X	X	X
Bone Marrow Aspirate/Biopsy ^{I}		X									
HPLC ("Hb electrophoresis") to measure HbF		X		X	X	X	X	X	X	X	X
Chem-10 panel, LDH, LFTs	X			X	X	X	X	X	X	X	X
PT/PTT	X										
T/B/NK cell subsets and IgG, IgM, IgA		X					X		X		X
B cell memory panel and gamma/delta T cell assay		X									X
Comprehensive globin sequencing		X									
Peripheral CD34 cell count and CRP		X	X^F								
Vector copy number (PB sorted lineages) ^{B}		X			X	X	X	X	X	X	X

	Pre-gene transfer			Admission to HSCT	Month 1 post-infusion	Month 3 post-infusion	Month 6 post-infusion	Month 9 post-infusion	Month 12 post-infusion	Month 18 post-infusion	Month 24 post-infusion
	Screening/ Eligibility	Baseline	Collection for back-up and GT product								
Flow cytometry of PB to measure F cells and exploratory testing		X			X	X	X	X	X	X	X
Record interval transfusions (ml/kg)	X				X	X	X	X	X	X	X
PROMIS surveys		X									X
HMH Survey		X									X
Record interval clinical events C	X				X	X	X	X	X	X	X
Integration site analysis D							X		X	X	X
RCL		X					X	X		X	X	X
Save serum, cells, and DNA		X					X	X		X	X	X
Adverse event and Concomitant Medication Monitoring	X	X	X	X	X	X	X	X	X	X	X

A The follow-up timing windows will be: Month 1 (+/- 3 days), Month 3 (+/- 10 days), Month 6 (+/- 30 days), Month 9 (+/- 30 days), Month 12 (+/- 30 days), Month 18 (+/- 30 days), and Month 24 (+/- 30 days).

B To comply with FDA guidance, testing for presence of vector sequences will be performed every 6 months for 2 years post-infusion, and then yearly, or until no vector sequences are detected in the sample on consecutive periodic tests. After this two-year study, subjects will be followed on a long-term follow-up study.

C Clinical events to be recorded include: VOC requiring ER visit or hospitalization, acute chest syndrome, stroke, and splenic sequestration.

D To comply with FDA guidance, integration site analysis will be performed every 6 months for 2 years post-infusion, then yearly. The amount of blood drawn will follow institutional guidelines of the treating hospital. After this two-year study, subjects will be followed on a long-term follow-up study. Further close monitoring will be performed as necessary.

E Refer to Table 4-1 for specific time limits

F Peripheral CD34 cell count and CRP will be assessed at baseline and at various times during pre- and post-plerixafor administration at the investigator's discretion.

G Iron studies will be assessed at baseline and only if clinically indicated or at the investigator discretion after gene transfer.

H Continuous assessment of VOEs after infusion.

I Marrow aspirate for morphology, cytogenetics, FISH studies and store cells.

Table 4-3: Pre-Transplant Investigations

The following is a list of studies typically performed prior to standard autologous transplant or for patients receiving transplant for sickle cell disease at Boston Children's Hospital. These are listed as a guideline for typical clinical care:

Study	Perform prior to cell collection	Perform prior to admission for conditioning
Serologic studies*	<30 days prior	<30 days prior
Electrocardiogram	<3 months prior	-
Echocardiogram	<3 months prior	<30 days prior
PFTs and pulmonary consult**		<60 days prior
Brain MRI/A		<60 days prior
Chest x-ray		On admission
Dental		<30 days prior
Ophthalmology		<30 days prior
Audiology		< 3 months prior
Glomerular filtration rate or creatinine clearance		<30 days prior
Baseline chemistries***		<30 days prior
G6PD testing	-	<3 months prior
Blood type	<30 days prior	<30 days prior

* includes cytomegalovirus IgG, CMV PCR, herpes simplex type I and II combined IgG, EBV capsid IgG, Varicella IgG, HIV DNA PCR, hepatitis A IgM, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody, hepatitis C antibody, toxoplasma IgG, RPR. Positivity for these tests may require further testing to rule out or treat active infection prior to enrollment.

** for patients too young to participate in PFTs, allowable screening includes normal CXR and oximetry >95% on room air

*** includes BUN, creatinine, AST, ALT, alkaline phosphatase, LDH, bilirubin total and direct

Prior to any interventions, all pre-transplant patients are offered a consultation regarding fertility preservation, including discussion of sperm/testicular tissue or ovarian preservation.

4.6.1　Assessment of Safety

A full physical examination (including chest auscultation, anthropometric measurement, body temperature, heart rate, respiratory rate and blood pressure measurement) will be carried out at each follow-up visit to evaluate the subject's condition.

Safety of the gene transfer procedure will be assessed through evaluation of adverse reactions after infusion and adverse reactions observed by the investigator or reported by the subject/parent/guardian during the study period.

Subject blood samples will be analyzed pre-gene transfer, at Month 3, 6, 12, 18 and 24 after gene therapy, for replication competent lentivirus (RCL), an essential safety test to detect the potentially pathogenic wildtype strains of the virus. After 24 months, RCL will continue to be checked yearly as part of the separate subsequent long-term follow up protocol as dictated by FDA guidance.

Safety of transduced cells to promote hematological recovery after conditioning will be assessed by ANC above 0.5×10^9 /L for three consecutive days, achieved before or at 7 weeks post-infusion by complete blood count and differential on subject blood samples.

As part of the overall safety evaluation, analysis of the lentiviral integration sites will be performed in different cell subpopulations and to investigate specific clonal expansions. This analysis is described in detail in Section 5.2.2. Quantification of transgene copy numbers is determined in peripheral blood mononuclear and sorted cell populations by real-time PCR methodology.

4.6.2   Assessment of Efficacy

All of the analyses will be carried out on pre- and post-treatment samples as described in the schedule of required assessments in Table 4-2. The results will be interpreted as a change in value through a longitudinal analysis, for each subject.

4.6.2.1   Presence of the Transgene in Peripheral Blood

Quantitative PCR will be performed to assess presence of the transgene (vector copy number) in peripheral blood cells. The test will be performed on low density mononuclear cells, and purified cells of the granulocyte, lymphoid, and erythroid lineage precursors when possible. Increase in fetal hemoglobin levels

HPLC will be used to measure HbF at the protein level on peripheral blood erythroid cells. Patients receiving chronic monthly transfusions for primary or secondary stroke prophylaxis will have the HPLC performed the day prior to scheduled transfusions.

4.6.2.2   Increase in hemoglobin level in the peripheral blood

Routine complete blood counts will be obtained.

4.6.2.3   Percent of F cells

Flow cytometry using fluorescent antibodies targeting HbF will be used to determine the percent of peripheral F cells.

4.6.2.4   Reduction in Hemolysis

Laboratory testing that will assess level of hemolysis includes reticulocyte count, LDH, and bilirubin levels.

4.6.2.5   Evaluation of clinical manifestations of SCD

Episodes of serious VOC (requiring ER visits or inpatient hospitalizations), acute chest syndrome, stroke, splenic sequestration, and any other sickle cell complications that require hospital admission, will be assessed by interval review of the patient's clinical history. These will be summated as "VOE" for the purpose of quantitative analysis of the primary endpoint. For the purpose of this evaluation, VOE will be

defined as at least one of the following: At least 4 VOEs within the 24 months prior to consent (VOE defined as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥24 - hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring parenteral opioids.

At each visit, interval red blood cell transfusions required will be recorded, measured in mL per kg of packed RBCs (number of units of pRBCs is acceptable for adult patients.)

4.6.2.6   Assessment of immune reconstitution within the lymphocytic lineage

Laboratory testing will include measurement of T, B, and NK cell subsets, B cell maturity (memory panel), and gamma/delta and alpha/beta T cells, all by flow cytometry. In addition, levels of immunoglobulins A, G, and M will be assessed by immunoturbimetric assay.

4.6.2.7   Assessment of Socioeconomic status and outcomes for children with SCD receiving gene therapy

Baseline socioeconomic status will be assessed by a brief, trial-embedded parent/participant-completed survey of HMH at trial entry(T1) and Month 24 post-infusion (T2) to allow for exploratory analysis of the association between SES and clinical, laboratory, and patient-reported outcomes (see Appendix B)

4.6.2.8   Patient Reported Outcomes

Health related quality of life (HRQoL), using the Patient-Reported Outcomes Measurement Information System (PROMIS) domains for fatigue, pain interference, and sleep (see Appendix B).

4.6.2.9   Neuromonitoring

Brain MRI/MRA

A routine brain MRI study will be performed at baseline, Month 6, 12 and 18 post-GT. The MRI study will include sagittal volumetric T1-weighted imaging, axial T2-weighted imaging, axial FLAIR imaging, axial diffusion tensor imaging (DTI), axial susceptibility-weighted imaging (SWI), pseudo-continuous arterial spin labeling PCASL, whole-brain 3D time of flight MRA and velocity encoded phase contrast (PC) MRI of the bilateral internal carotid arteries and the basilar artery. Images will be reviewed centrally for the presence of acute ischemic injury, prior hemorrhage, prior ischemic injury, evidence of narrowing or slow flow in the major intracranial arteries and regional decreases cerebral blood flow (CBF). In addition, PC MRI will be combined with brain volume determined with the volumetric T1-weighted images, to estimate whole-brain cerebral perfusion.

In addition, at the BCH, a breath-hold functional MRI will be used to measure cerebrovascular reserve (CVR) with a hypercapnic challenge. Briefly, subjects will be instructed to breathe normally for 5 minutes. Then, subjects will be asked to take a normal inspiration and hold their breath without Valsalva maneuver for 16 seconds, followed by a 2-minute rest period of self-paced normal breathing. This procedure will be repeated four times. Acquired volumes will be motion-corrected and a general linear model fit to the acquired fMRI data on a voxelwise basis with regressors including a hemodynamic response function (HRF) specific to breath-holding tasks and a physiological regressor consisting of the average cerebellar signal intensity. A combined CVR measure will be calculated as the weighted average of relative counts of positive and negative HRF regression coefficients. These CVR measures will be acquired at BCH in combination with the routine MRI at baseline, Month 6, 12 and 18 post-GT.

Transcranial doppler ultrasound

Transcranial dopper ultrasounds (TCD) will be **performed to measure blood flow velocity. TCD will be performed as routine clinical study on an annual basis**. Timing of any additional TCD studies (ie to follow up a conditional TCD) would be investigator determined.

Frequency Domain-Near Infrared Spectroscopy-Diffuse Correlation Spectroscopy (FD-NIRS-DCS)

At the BCH site, FD-NIRS-DCS monitoring will be performed simultaneously with the MRI at baseline, 6, 12 and 18 months post-GT. A sensor will be secured to the left frontal area (Fp1 position based on the 10-20 system) to measure concentrations of cerebral tissue oxygenated and deoxygenated hemoglobin (HbO and HbR, respectively) and a quantitative index of CBF (CBFi). Cerebral tissue hemoglobin oxygen saturation (ctSO2) will be calculated as HbO/(HbO+HbR) and oxygen extraction fraction (OEF) as (SpO2-ctSO2)/SpO2, with arterial oxygen saturation (SpO2) recorded simultaneously from a pulse oximeter. An index of CMRO2 (CMRO2i) will be estimated by combining measured ctSO2 and CBFi, SpO2 and HgB from total blood count test (CMRO2=HgB*CBF*(SpO2-ctSO2)). For the breath-hold task, the breath holding index (BHI) will be determined from percentage increase in CBF from normocapnic baseline (CBF0) to the final CBF (CBFmax) at the end of the breath hold, normalized by the duration of the hold (BHI = (CBFmax-CBF0)*100/total apnea time).

At BCH, FD-NIRS-DCS will be monitored simultaneously with the TCD at baseline, 6, 12 and 18 months post-GT. The sensor will be placed as in the MRI study. Immediately following the TCD/FDNIRS-DCS study but with the FDNIRS-DCS sensor still in place, we will ask subjects to perform repeated stand-to-squat maneuvers (SSMs) to assess dynamic cerebral autoregulation (dCA) by FDNIRS-DCS. We will follow a standardized protocol where each subject will repeat 15 SSM of 10 seconds duration each for squats and stands, corresponding to a frequency of 0.05 Hz in the very low frequency band (VLF, 0.02–0.07 Hz) in which dCA is active. Pulsatile arterial blood pressure waveforms (ABP) will be recorded non-invasively using a Finapres device (Finapres Medical System, Amsterdam, Netherlands). Autoregulation will be evaluated quantitatively by transfer function analysis of the phase, gain, and coherence between ABP and CBF in the VLF band.

4.6.2.10 Echocardiogram

Echocardiography will be performed at baseline and 18 months after gene therapy to assess for left ventricular ejection fraction, left ventricular shortening fraction and presence or absence of tricuspid regurgitation. If tricuspid regurgitation is present, measure tricuspid regurgitant jet velocity as a proxy for pulmonary hypertension.

4.6.2.11 Pulmonary function tests and exercise capacity testing

Pulmonary function testing will be performed at baseline and 18 months post-GT. The complete pulmonary function test will include FVC, FEV1, FEV1/FVC, VC, TLC, RV, ERV, IC, FRC, DLCO, and oxygen saturation. 6 minute walk test will be performed at baseline, Month 12, and Month 24 post-GT.

4.6.2.12 Renal

Renal evaluation will occur at baseline and Month 18 post-GT. The assessments will include urine albumin creatinine ratio, and estimated GFR by the National Kidney Foundation Pediatric GFR Calculator (uses serum creatinine, serum cystatin C, height, and BUN.

4.7 Adverse Event Reporting

Adverse event (AE) reporting requirements are summarized below under section 4.7.4

4.7.1 Definitions

Adverse Event: Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) therefore is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the IMP/PTM medicinal (investigational) product.

An adverse event can be Anticipated or Unanticipated

- **Anticipated adverse events** are those that have been previously identified as resulting from the underlying disease, an autologous HCT, and not related to study drug. Investigator should refer to the anticipated event list to assess whether or not an event is anticipated. Anticipated events will be collected in the data capture system through calendar-driven forms (Toxicity, GVHD, etc.) and event-driven forms (Death, Infection, etc.). Events that meet serious criteria should be reported as detailed in Section 4.7.4.2.

- **Unanticipated adverse events** are those that vary in nature, intensity or frequency from information in the current adverse event list, the Investigator's Brochure, the package insert, or when it is not included in the informed consent document as a potential risk. Unanticipated events would also include those that have not been previously described as a result of the underlying disease requiring HCT, an autologous HCT.

The following are anticipated adverse events and do NOT require reporting as an AE/SAE:

- Graft failure will be reported through other study event-driven forms
- Medical or surgical procedures (the condition that leads to the procedure is the AE)
- Situations where an untoward medical occurrence has not taken place. For example:
  - Planned hospitalizations due to pre-existing conditions, which have not worsened (eg, HCT)
  - Hospitalizations that occur for procedures not due to an AE (eg, cosmetic surgery)
  - Hospitalizations for a diagnostic procedure where the hospital stay is less than 24 hours in duration or for normal management procedures (eg, liver biopsy)

Laboratory findings do NOT need to be reported as AEs in the following cases:

- Laboratory parameters already beyond the reference range at screening, unless a further increase / decrease can be considered an exacerbation of a pre-existing condition.

- Abnormal hematological laboratory parameters considered anticipated due to myeloablative conditioning regimen or other permitted chemotherapy treatments are not considered AEs.

- An abnormal laboratory value that cannot be confirmed after repeat analysis, preferably in the same laboratory (ie, the previous result could be marked as not valid and should not necessarily be reported as an AE).

Serious Adverse Event: A serious adverse event (SAE), as defined by per 21 CFR 312.32, is any adverse event that results in one of the following outcomes, regardless of causality and expectedness:

- **Results in death**
- **Is life-threatening.** Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- **Requires or prolongs inpatient hospitalization** (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- **Results in persistent or significant disability/incapacity.** Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- **Is a congenital anomaly or birth defect;** or
- **Is an important medical event** when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. 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.

Medical and scientific judgment should be exercised in deciding whether expected reporting is also appropriate in situations other than those listed above. For example, important medical events may not be immediately life threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed in the definition above (eg, suspected transmission of an infectious agent by a medicinal product is considered a Serious Adverse Event). Any event is considered a Serious Adverse Event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

4.7.2   Classification of Adverse Events by Severity

The severity refers to the intensity of the reported event. The Investigator must categorize the severity of each unexpected SAE according to the NCI CTCAE version 5.0. CTCAE guidelines can be referenced at the following website: http://ctep.cancer.gov/reporting/ctc.html. For any term that is not specifically listed in the CTCAE scale, intensity will be assigned a grade of one through five using the following CTCAE guidelines:

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

4.7.3   Classification of Adverse Events by Relationship to Investigational Product

The relationship of each reported event to the study therapy will be assessed by the Investigator; after careful consideration of all relevant factors such as (but not limited to) the underlying study indication,

coexisting disease, concomitant medication, relevant history, pattern of the SAE, temporal relationship to any study therapy interventions and dechallenge or rechallenge according to the following guidelines:

- **Possibly, Probably, or Definitely Related:** there is a reasonable possibility that the study therapy caused the event. A relationship of possibly, probably or definitely related to the investigational product is considered **related** for the purposes of regulatory authority reporting.

- **Unlikely, or Not Related:** There is no reasonable possibility that the investigational product caused the event. An unlikely or not related relationship to the investigational product is considered **not related** for the purposes of regulatory authority reporting. One or more of the following criteria apply:

4.7.4   Required Adverse Event Reporting

4.7.4.1   Non-serious Adverse Events

All non-serious adverse events will be reported from enrollment through the initiation of the infusion if determined by the investigator to be related to study procedures. All non-serious adverse events will be reported from the transduced product infusion through 30 days after completion of the infusion. From 31 days following completion of the infusion through the 24 month follow-up period, any clinically relevant grade 2 or higher non-serious adverse event requires reporting <u>if the event is determined by the investigator to be at least possible related to the study product</u>. At the end of the 24 month of study follow-up, participants will be offered enrollment in a long-term safety study that will include follow-up for up to 15 years following the transduced product infusion. Any non-serious event will be reported in a timely manner through the Adverse Event Form in the Advantage eClinical system (the EDC).

4.7.4.2   Serious Adverse Events (SAE)

Any SAE will be reported from enrollment through the initiation of the infusion if determined by the investigator to be related to study procedures. All SAEs will be reported from the transduced product infusion through 30 days after completion of the infusion. From 31 days following completion of the infusion through Month 24 post-infusion, any SAE requires reporting <u>if the event is determined by the investigator to be at least possible related to the study product</u>.

All unanticipated SAEs and all deaths from enrollment through completion of study defined follow-up will be reported following BMT CTN Administrative MOP Chapter 6. **Additionally, any grade 4 anticipated event not collected on the calendar-driven toxicity or specified event-driven form must also be reported through the expedited AE reporting system in Advantage eClinical. For this study, the reporting will be based on unanticipated in place of the MOP definition of unexpected.**

All reportable SAEs are to be reported through the expedited AE reporting forms in the EDC.

All the reported SAEs must be followed up until resolved, the Investigator judges to be no longer be clinically significant, or until they stabilize/become chronic to the extent that they can be fully characterized.

Serious adverse events (SAEs) will be reported through an expedited AE reporting system via electronic data capture. Life-threatening and fatal SAEs must be reported within 24 hours of knowledge of the event. All other SAEs must be reported within 1 business day of knowledge of the event. Events entered in the EDC will be reported using NCI's CTCAE Version 5.0.

Life-threatening or fatal events assessed to be unexpected and related to the gene modified autologous CD34+ cells are to be reported to the FDA by telephone or fax as soon as possible but no later than seven days after the IND's sponsor's receipt of the information. Reports of other serious, unexpected adverse events determined to be related to the gene modified autologous CD34+ cells are to be reported to the

FDA within 15 days after the sponsor's receipt of information. Other adverse events occurring on this study that do not meet the criteria for expedited reporting will be included in reports to the FDA annually.

The Data and Safety Monitoring Board will receive reports of all unanticipated/unexpected SAEs upon review by the BMT CTN Medical Monitor. Summary reports for all reported SAEs will be reviewed by the DSMB on a semi-annual basis.

Some anticipated serious events will be collected only on calendar-driven forms or event-driven forms (Graft failure and Death forms) and do not require reporting as an SAE.

4.7.4.3   Vaso-Occlusive Event Reporting

Any vaso-occlusive event (VOE), defined in section 2.1 should be reported on an event-driven VOE CRF with a single VOE event per form. Any VOE must be reported on this form from enrollment through Month 24 after gene transfer infusion. All VOEs will be reviewed and confirmed by 2 independent hematologists with expertise in sickle cell disease. The review will occur within 4 weeks of notification of the event.

4.7.4.4   Toxicity Reporting

Toxicities are a subset of anticipated adverse events determined to be from the underlying disease process, an autologous HCT, concomitant therapy and generally are <u>not</u> considered to be related to the study product.

Once the participant has reached 31 days from last dose of study drug, toxicities listed in should be reported at the specified time points on the Toxicity CRF. **If an event listed is determined to be serious and related to the study product, it should be reported via the expedited reporting process as outlined in section 4.4.4.2.**

4.7.5   Pregnancy and Breastfeeding

Any occurrence of pregnancy in a female participant or female partner of a male participant must be reported through the expedited AE reporting system in the EDC.

4.8   Center of International Blood and Marrow Transplant Research (CIBMTR) Data Reporting

Centers participating in this BMT CTN trial should register pre- and post-infusion outcomes on patients enrolled on this trial with CIBMTR on the autologous transplant track. Reporting will include sickle cell disease Forms (pre- and post-infusion) The CIBMTR will assume responsibility for follow up of patients beyond the trial period. Enrollment on BMT CTN #2001 must be indicated on the pre-transplant registration form. CIBMTR pre- and post-transplant Report Forms must be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

CHAPTER 5

5   STATISTICAL CONSIDERATIONS

5.1   Study Design & Primary Endpoint

This is an open-label, non-randomized, multi-center, phase 2, single arm study involving a single infusion of autologous CD34+ HSC cells transduced with the lentiviral vector containing a shRNA targeting BCL11a.

Using an exact one-stage rule, we will test to determine if there is a sufficiently high proportion of patients who are classified as a 'success' according to the definition of the primary endpoint for complete absence of severe VOEs.   As described in section 3.1.1:

VOE is defined as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring parenteral opioids. The primary endpoint, as determined within each patient, is a complete absence of severe VOEs in the period from Month 6 to 24 after gene therapy. This is a binary endpoint, whereby each patient will be classified as to whether a complete absence has occurred (Yes= 'success' / No= 'failure'). Patients with no severe VOEs from Month 6 to Month 24 after gene therapy will be classified as 'successes.' Patients with one or more severe VOEs from Month 6 to Month 24 after gene therapy will be classified as 'failures.' Patients with less than 24 months of follow-up post-infusion will be classified as 'failures.' Patients who experience engraftment failure will be classified as 'failures': 1) Primary engraftment failure requiring a back-up infusion; or, 2) Failure of engraftment or loss of gene modified cells as determined by a PB MNC <0.1 at 6 months post-infusion. Patients who initiate disease modifying agent(s) which includes hydroxyurea, L-glutamine, crizanlizumab, voxelotor for prevention or management of severe VOEs will be classified as failures.

There will be no inferential statistical futility monitoring of the primary endpoint. Statistical monitoring rules will be used to monitor for primary engraftment failure and the occurrence of unacceptable events. Analyses of exploratory and biological objectives will be descriptive only.

5.1.1   Accrual and study duration

The estimated accrual rate of SCD patients meeting the eligibility criteria of this study is 20-25 patients per year. Taking into account the potential for 2-5 patients to be withdrawn prior to infusion (inevaluable), it will take ~2 years to enroll 27-30 patients to achieve the required 25 evaluable patients. With a minimum of 24 months follow-up post-infusion on the last patient, the total study duration will be about 4 years.

5.1.2   Evaluability

Evaluability for the Primary Objective

To be evaluable for the analysis of the primary efficacy objective, a patient must receive an infusion of transduced CD34+ cells. Patients who are lost to follow-up, die, or withdraw from the study after infusion and before Month 24 post-infusion will be evaluable and classified as 'failures.' Any subject withdrawn from protocol treatment prior to administration of transduced CD34+ cells will be considered inevaluable and replaced in the study.

Evaluability for Secondary Objective 3.2.1

To be evaluable for the analysis of Secondary Objective 3.2.1, a patient must receive an infusion of transduced CD34+ cells. Patients who are lost to follow-up or withdraw from the study prior to having assessments at least two of the time points (Month 9, 12, or 18 post-infusion), or fail to have known data for all three of the secondary endpoint criteria (Hb, HbF, F cells) at least two of the time points (Month 9, 12, or 18 post-infusion) for any reason, will be evaluable and classified as 'failures.'

Evaluability for Secondary Objective 3.2.4

To be evaluable for calculation of the % change in the annualized number of severe VOEs, a patient must receive an infusion of transduced CD34+ cells and have a minimum VOE observation period from Month 6 to Month 18 post-infusion. In other words, follow-up to Month 18 will be required to be evaluable for imputation of the annualized severe VOE rate.

Evaluability for Secondary Objective 3.2.5

To be evaluable, a patient must receive an infusion of transduced CD34+ cells. Patients who are lost to follow-up, die, or withdraw from the study after infusion and before Month 18 post-infusion will be evaluable and classified as 'failures.'

All patients enrolled on the study will be evaluable for Secondary Objective 3.2.2, exploratory and biologic objectives, and analyses of safety.

5.1.3   Endpoint windows

The secondary endpoints are designed to be measured at a specific time point, but a clinical assessment at that exact time may not always be possible. For practical purposes of collecting data, 2-month non-overlapping windows will be placed around each of the time points, such that the endpoint can be measured at any time within a given window. For example, the Month 12 post-infusion assessments can be measured anytime within a window 11-13 months post-infusion, and the Month 18 post-infusion assessments can be measured anytime within a window 17-19 months post-infusion.

Endpoint windows are not applicable to the primary endpoint, Secondary Objective 3.2.4 (percentage change in the annualized number of severe VOEs), or Secondary Objective 3.2.5. Patients will be assessed in an ongoing fashion for the occurrence of VOEs after infusion.

5.2   Sample Size and Power Considerations

5.2.1   Sample Size Justification

The sample size of 25 evaluable patients will be sufficient to provide at least 90% power for a one-stage rule, with a one-sided type 1 error of 0.022, to evaluate the primary endpoint.

5.2.2   Guidelines for Safety Monitoring

Rule A - Safety Stopping Rule for primary engraftment failure:

There are two different types of engraftment failure that contribute to this stopping rule: 1) Primary engraftment failure requiring a back-up infusion; and, 2) Failure of engraftment or loss of gene modified cells as determined by a PB MNC <0.1 at 6 months post-infusion. A single occurrence of either type of these engraftment failures within the first 3 patients will require a DSMB review. During the accrual of up to 25 evaluable patients, if at any time there are 3 or more engraftment failures (of either type), then the study will be closed to further accrual pending full DSMB review. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures.   This rule has the following operating

characteristics: The null hypothesis is that the proportion of patients with engraftment failure is ≤4%, and the alternative is that the proportion of patients with engraftment failure is ≥20%. This rule has 90.2% power and a Type I error of 7.6%.

<u>Rule B - Safety Stopping Rule for unacceptable events:</u>

We will count the number of patients with an "unacceptable event". Unacceptable events are defined as:

    i.   death;

    ii.   any leukemia potentially related to provirus insertion;

    iii.   abnormal clonal bone marrow cytogenetics; or,

    iv.   myelodysplasia

If at any time one or more subjects experience an unacceptable event, the rule will be triggered. If the rule is triggered, planned infusion of a gene-manipulated product in any subject already enrolled will be suspended; those who have already received a gene modified product will continue to be evaluated per protocol. The events will be reviewed as quickly as possible by the study committee, the DSMB, IRB and FDA for consideration of possible study modifications or stoppage.

This rule has the following operating characteristics: The null hypothesis is that the proportion of patients with unacceptable events is ≤0.4%, and the alternative is that the proportion of patients with unacceptable events is ≥9.3%. In a sample size of 25, this rule has 90.5% power and a Type I error of 9.2%.

<u>Rule C – Efficacy monitoring rule</u>

A one-stage design will be used to test for a sufficient number of patients who are a treatment 'success' per the primary endpoint criteria for VOE. After enrollment of 25 evaluable patients, if less than 18 patients are a 'success', then the trial will be terminated for lack of a sufficient efficacy signal. If 18 or more patients are a 'success', then this will be considered evidence of efficacy. If at any time there are 8 or more patients who are a failure (i.e., 18 successes will not be achieved), the trial will be referred to the DSMB for consideration of modification or closure.

This one-stage rule has the following operating characteristics: The null hypothesis is that the proportion who are a 'success' is ≤50%, and the alternative is that the proportion who are a 'success' is ≥80.5%. In a sample size of 25 evaluable patients, this rule has 90.3% power and a one-sided Type I error of 2.2%. The justification for the choice of 50% for the null hypothesis is described in detail in section 1.11.

5.3   Demographics and Baseline Characteristics

As part of the primary analysis, a descriptive summary of patient demographics and characteristics will be presented. The number and percentage of patients will be tabulated by sex, race, ethnicity, and genotype. The quartiles, minimum, and maximum of baseline values for age, WBC, Hb, and platelet count will be calculated.

5.4   Analysis of Primary Endpoint

To address this efficacy objective, we will apply the one-stage design described above in Rule C. The primary endpoint is binary: each evaluable patient will be classified as either a 'success' (complete absence of severe VOEs) or a 'failure'. The VOE observation period is from Month 6 to Month 24 post-infusion. Patients who have no severe VOEs from Month 6 to Month 24 post-infusion will be classified as "successes". Patients who report one or more severe VOEs from Month 6 to Month 24 post-infusion will

be classified as 'failures'. Patients who are lost to follow-up, die, or withdraw from the study after infusion and before Month 24 post-infusion will be classified as 'failures'. Patients who experience engraftment failure will also be classified as 'failures': 1) Primary engraftment failure requiring a back-up infusion; or, 2) Failure of engraftment or loss of gene modified cells as determined by a PB MNC <0.1 at 6 months post-infusion. Patients who initiate disease modifying agent(s) which includes hydroxyurea, L-glutamine, crizanlizumab, voxelotor for prevention or management of severe VOEs will be classified as failures.

In a descriptive fashion, we will track the number and proportion of evaluable patients who are lost to follow-up, die, or withdraw from the study.

5.5 Analysis of Secondary Endpoints

5.5.1 Hemoglobin Function

This endpoint has two components:

- Total Hb of at least 10 g/dL or increase of >2 g/dL over baseline; AND

- Total HbF >20% with >60% F cells post-infusion of gene modified cells.

This is a binary endpoint, whereby each patient will be classified as to whether all of the criteria in 1 AND 2 are met (Yes= 'success' / No='failure'). For each factor, the average value across the available time points will be utilized to determine if the endpoint criteria have been met, with a requirement for assessments at a minimum of two time points.

5.5.2 Hemolysis

For each evaluable patient, the percent change in a) the absolute reticulocyte count; b) lactate dehydrogenase; and, c) bilirubin, from the time of consent to 18-months after gene therapy will be calculated. In a descriptive analysis, the mean and standard deviation for each of these parameters will be calculated for baseline and Month 18 values, as well as for the percent change.

5.5.3 Safety

Safety stopping Rules A and B will be applied. We will conclude that lentiviral gene therapy has been safely administered if the conditions of Rule A (at least 23 subjects out of 25 evaluable subjects engraft without requiring a back-up infusion), and Rule B (none of the enrolled patients have an unacceptable event) are satisfied.

The number of treatment-related deaths will be tabulated, as well as the number of patients who have a) secondary malignancy; b) abnormal bone marrow cytogenetics; and c) myelodysplasia. The number and proportion of patients with toxicities and adverse events (AEs) grade ≥2 and related or possibly related to medicinal project will be listed by type, grade, and attribution, and expectedness, though Month 24. For each patient, only the highest grade of a given type of event will be counted. The number and proportion of patients with AEs will be calculated, by type of event and grade. Similarly, serious adverse events will be listed and tabulated.

The serial measurements of RCL for individual subjects will be plotted over time.

5.5.4 Percentage change in the annualized number of VOEs

Methods to calculate the % change in the annualized number of severe VOEs are presented in section 3.2.4. The median, inner-quartile range, minimum, and maximum of the percentage change in the annualized

number of severe VOEs will be calculated. Additionally, for descriptive purposes, the proportion of patients who achieve a ≥75% reduction in the annualized number of severe VOEs will be calculated, and a 95% two-sided exact binomial confidence interval will be placed on this proportion. VOEs reported less than 6 months after infusion will be descriptively summarized but will not play a role in addressing this objective.

5.5.5   Occurrence of VOEs by Month 18 post-infusion

Each evaluable patient will be classified as either a 'success' (complete reduction of severe VOEs) or a 'failure'. For this objective, the VOE observation period is from Month 6 to Month 18 post-infusion. Patients who have no severe VOEs from Month 6 to Month 18 post-infusion will be classified as "successes". Patients who report one or more severe VOEs from Month 6 to Month 18 post-infusion will be classified as 'failures'. Patients who experience engraftment failure, or are lost to follow-up, die, or withdraw from the study after infusion and before Month 18 post-infusion, will be classified as 'failures'. The proportion of evaluable patients who are successes will be calculated as (# of successes)/(number of evaluable patients), and a 95% two-sided exact binomial confidence interval will be placed on this proportion.

5.6   Analysis of Exploratory Endpoints

5.6.1   Efficiency of BCL11A knockdown

Western blots of the amount of BCL11A protein on peripheral whole blood and sorted erythroid precursors will be presented at baseline, Months 6, 12, and 24 post infusion. If numeric values are available, spaghetti plots will be generated over time.

5.6.2   Transcranial Doppler Ultrasound

Within the cohort of evaluable patients ≤14 years old: The proportion of patients with stable TCD screening results on routine annual clinical assessment with data collected at Month 24 post-infusion of gene modified cells will be calculated. A 95% two-sided exact binomial confidence interval will be placed on this proportion.

5.6.3   Organ Function

A descriptive analysis, as appropriate to the continuous or categorical nature of a given endpoint, will be performed of the following endpoints, at baseline, and Month 18 post-GT:

- Cerebrovascular: presence/absence of neurovascular disease per brain MRI/MRA;
- Renal: urine albumin creatinine ratio, serum creatinine, and cystatin C;
- Cardiopulmonary function: PFTs (FVC, FEV1, FEV1/FVC, VC, TLC, RV, ERV, IC, FRC, DLCO, and oxygen saturation), Echocardiography for left ventricular shortening fraction or ejection fraction, and presence or absence of tricuspid regurgitation. If present, measure jet velocity as a measure of pulmonary hypertension.

5.6.4   Household Material Hardship (HMH) Survey

The HMH Study (see Appendix B) will explore the association between HMH at study entry and trial-evaluated clinical and patient-reported endpoints (including Hb and HbF at Month 24 post-infusion, percentage reduction in severe VOEs, and changes in SCD-related organ assessments) and patient-reported outcomes. We will describe the proportion of study participants exposed to HMH at study entry scoring HMH as a categorical variable based on the number of domains of unmet need (food, housing, utilities,

transportation) from 0-4. We will describe the proportion of participants experiencing each clinical endpoint by HMH exposure with 95% exact confidence intervals. We will describe the change in HMH from study entry to Month 24 post-infusion. We will describe the associations between HMH at study entry (T1) and Month 24 (T2) and patient reported outcomes as evaluated by PROMIS measures of fatigue, pain interference and sleep.

5.6.5 Patient Reported Outcomes

PROMIS® measures of fatigue, pain interference and sleep will be scored in accordance with their scoring manuals (see Appendix B)[108-111]. For each test result, the change from baseline in the score will be calculated, and a 95% two-sided exact confidence interval will be placed on the score change. We will also report the mean ± standard deviation of the score at each timepoint, and the mean ± standard deviation of the score change, across all the subjects. All evaluable patients will be asked to complete these questionnaires.

5.6.6 Other Outcomes at Month 24 post-infusion of gene modified cells

Each evaluable patient will be classified as to whether none of the following criteria are met, i.e., a binary endpoint: "event-free at Month 24 post-infusion" or "not event-free at Month 24 post-infusion". Events are:

1. Death

2. Cerebral vascular events, including stroke

3. For subjects ≤ 14 years old, worsened annual TCD results leading to institution of transfusion therapy per institutional guidelines

4. Lack of engraftment of gene-modified cells (requiring rescue with back-up CD34 cells)

5. Lack or loss of engraftment of gene-modified cells as determined by VCN <0.1 in peripheral blood MNC at Month 6 post-infusion

6. Development of MDS or leukemia that results secondary to vector-related insertional mutagenesis or to busulfan conditioning.

7. Presence of replication competent lentivirus

A conservative approach will be taken regarding missing data. If the patient's status at Month 24 post-infusion is unknown for any of these events, the patient will be considered to be "not event-free at Month 24 post-infusion". The proportion of patients classified as "event-free at Month 24 post-infusion" will be calculated, and a 95% exact binomial two-sided confidence interval will be placed on the proportion. [Note: this is not a Kaplan-Meier estimate of 24-month event-free survival.]

In addition, for each event separately, the proportion of patients who are "event-free at Month 24 post-infusion" will be calculated.

5.7 Analysis of Biologic Endpoints

5.7.1 RBC survival

The mean (± standard deviation) of exhaled alveolar end tidal carbon monoxide measurement (ETCO) will be calculated: a) upon entry to the trial; b) as part of the exchange or simple transfusion phase; and c)post-infusion. Spaghetti plots will be generated over time.

5.7.2 RBC Oxygen Saturation

For each evaluable patient, the change from baseline to Month 18 post-infusion in the fraction of RBCs with detectable hemoglobin polymer at venous PO_2 (~35 mmHg) will be calculated. Across all evaluable patients, the mean (± standard deviation) of the change will be calculated. A paired t-test will be performed on the paired baseline and Month 18 values of RBC fraction. In a descriptive analysis, the median, inner-quartile range, minimum, and maximum will be calculated for baseline and at venous PO_2.

5.7.3 Total Cerebral Blood Flow

For each evaluable patient, the change from baseline to Month 24 post-infusion of total cerebral blood flow and cerebrovascular reserve (as measured by MRI) will be calculated, as quantified by the following measures:

- total cerebral blood flow with phase contrast MRI);

- cerebrovascular reserve (with breath hold functional MRI);

- oxygen extraction fraction by frequency-domain near-infrared and diffuse correlation spectroscopies (FD-NIRS-DCS) monitoring;

- cerebral blood flow by frequency-domain near-infrared and diffuse correlation spectroscopies (FD-NIRS-DCS) monitoring; and,

- percent of negatively reactive brain voxels.

At each time point (baseline, Month 12, Month 24) and for the change from baseline, we will report the mean (± standard deviation), median, inner-quartile range, minimum, and maximum of each of the measures. Spaghetti plots will be generated over time.

APPENDIX A

6   APPENDIX A - HUMAN SUBJECTS

6.1   Subject Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. All prospective patients will have the study explained by the PI of the research team. The nature of the tests and procedures to be done will also be explained along with the potential hazards, possible adverse reactions and financial costs. The patient and/or parent/guardian will be encouraged to ask further questions about the study to the Investigator or designee. Should a patient or parent/guardian decide that the patient will participate they will be invited to sign the study consent form.

Prior to the initiation of the study, defined as initiating any procedure for purposes of evaluating patient eligibility not otherwise a part of routine patient care, acknowledgement of the receipt of this information and the subject's freely tendered offer to participate will be obtained in writing from each subject in the study. Those patients under the age of consent will voluntarily assent to the study under the same circumstances as their legal guardian and will sign the assent form.

This protocol, informed consent, assent form, and any amendments to the protocol will be reviewed by the IRB prior to initiation. The study will not be initiated without the approval of the IRB, whose operations must be in compliance with CFR 56; Title 21.

Written notice that the protocol and informed consent/assent forms have been reviewed and approved by the IRB will be submitted to the Investigator and the Sponsor prior to study initiation

6.2   Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relaying the patient's identity with the ID code will be kept separately at the center. The ID code will be generated by and kept on file at the BMT CTN Data and Coordinating Center upon enrollment.

6.3   Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files will be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

6.4   Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects (found at http://www.wma.net/e/). The DSMB and local IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The protocol, Investigator's Brochure(s), informed consent, written information given to the patients, safety updates,

annual progress reports, and any revisions to these documents will be provided to the FDA by the Study Sponsor.

APPENDIX B

7   APPENDIX B - PATIENT REPORTED OUTCOMES AND HOUSEHOLD MATERIAL HARDSHIP SURVEY

7.1   Household Material Hardship (HMH) Survey

Assessment of Socioeconomic status and outcomes for children with SCD receiving gene therapy

Baseline socioeconomic status will be assessed by a brief, trial-embedded parent/participant-completed survey of HMH at trial entry (T1) to allow for exploratory analysis of the association between baseline SES and clinical and patient-reported outcomes. Follow-up socioeconomic data will be evaluated by HMH survey at 24-months (T2) to evaluate change over time and associations with PROs.

Social determinants of health, including poverty, contribute substantially to health outcomes in the United States across both adult and pediatric populations. Understanding whether novel curative approaches, such as gene therapy, achieve equitable outcomes (including survival, disease modifying end-points, healthcare utilization, and patient-reported symptom burden and quality-of-life) for those patients who successfully access them is essential in current era, as newer, innovative therapies have the potential to increase disparate outcomes by exacerbating issues of access to care. One in five U.S. children lives in poverty, and poverty is associated with inferior health-related quality of life and functional disability status in children with SCD[119, 120]. Among adults with SCD, over half of hospitalizations occur among patients from low-income areas, and two-thirds of hospitalizations for SCD are billed to Medicaid underscoring the persistent frequency of poverty in the adult SCD population[121]. Socioeconomic disparities in clinical trial participation have been extensively described in adult oncology[122, 123], and are posited to in part underly well-described survival disparities by socioeconomic status across numerous adult cancer. Equally as compelling, poverty-associated disparities in disease outcome and symptom burden have been described in the context of pediatric oncology multi-center clinical trials[124, 125]. Whether disparities in access and/or outcome based on socioeconomic status will extrapolate to gene therapy trials in SCD is not known. Comprehensively describing the sociodemographic characteristics of patients who successfully access gene therapy as a novel investigative therapy will allow for exploratory analysis of socioeconomic status and trial end-points, and will lay the groundwork for future efforts to ensure equitable therapeutic access as these therapies are scaled across the U.S. and world.

Patients or parent/guardians will complete a brief, survey at study entry (T1) and again at 24-months (T2). Socioeconomic status will be evaluated by household material hardship (HMH) (a concrete and remediable measure of poverty including unmet basic needs of food, housing, utilities and transportation)[126-128] to allow for an exploratory analysis of the association between baseline SES and clinical and patient-reported outcomes as collected on trial. We have extensive experience evaluating family-reported SES in pediatric stem-cell transplant and oncology populations with high willingness to participate and low participant burden using a brief survey instrument[129-131]

Survey administration

• Time-point: This is a survey administered at time of study entry (T1) and Month 24 post-infusion (T2).

- • Survey respondent: The HMH survey is intended to gather information about the socioeconomic status of the participant's primary household and can be completed by the patient or their corresponding identified primary caregiver. For participants <18 years of age,

parent/guardians of enrolled children are the survey respondents. For patients living in a two-parent/guardian household, it is acceptable for both parent/guardians to complete the survey, with one identifying as primary respondent for demographic questions. For patients with multiple households, one household should be identified as the primary household and the appropriate parent/guardian should complete the survey.

- Participants >=18 years of age living independently will complete the survey themselves.
- Participants >=18 years of age living with their parent/guardians (e.g. not living independently) may choose to complete the survey themselves or with input from their parent/guardian.

• Administration: The HMH survey takes approximately 3-minutes to complete, will preferentially be completed electronically (e.g. on a computer/iPad with electronic data capture). Surveys may be completed paper/pencil if electronic administration is not feasible. Surveys are available in English and Spanish. Surveys may be administered in ANY language with an appropriate interpreter. For those participants unable to complete the survey in-person, the HMH survey may be administered by study staff over the telephone or virtual/video platforms, or by a hard-copy provided to parent/patient to complete on their own and return to study team with mode of administration documented.

Survey Data Handling: HMH surveys administered via computer/iPad will have data automatically captured and stored in structured database. HMH surveys administered via paper copy should be reviewed by the site CRA for completeness.

7.2   Patient Reported Outcomes Background

FDA guidance for gene therapy recommends that sponsors should identify the specific aspects of the disease that are meaningful to the patient and might also be affected by the activity of the gene therapy product. (Human Gene Therapy for Rare Diseases: Guidance for Industry https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-gene-therapy-rare-diseases). Vaso-occlusive pain is the hallmark of sickle cell disease and is a major contributor to morbidity, impaired health related quality of life (HR-QoL), and healthcare utilization associated with this disease. In the PiSCES study, adult patients reported pain on more than half the diary days but unplanned health-care utilization only occurred on 3.5% of diary days[132]. Thus, the rate of visits to the ED or hospital is an incomplete measure of the burden of pain in SCD and its impact on the lives of patients living with the disease. A daily record maintained by patients is ultimately, the best way of capturing the multidimensional experience of pain and its impact on their lives. Since paper pain diaries are subject to errors and omissions, poor compliance, inflation in retrospective reports and inaccuracies because of high "faked" compliance due to backfilling of entries, electronic pain diary is used for real time direct capture of daily pain before and at various time points following gene therapy[133].

Patient experience data that includes data collected by patients, family members and caregivers provide information about the experiences with the disease, the impact of the disease on the patient's life, patient preferences with respect to treatment and the experience of the patient with a particular treatment (Reference: https://www.fda.gov/drugs/development-approval-process-drugs/cder-patient-focused-drug-development). Such data may provide important additional information about the clinical benefit of a gene therapy product. FDA encourages sponsors to collect patient experience data during product development, and to submit such data in the marketing application.

Similarly, understanding the patient perspective is key to advancing patient and provider decision making tools.. Syrjala et al. compared PROs of adult patients at 10 years post alloHCT for malignant disease to

suitably matched control patients who had not. This study, found that survivors and controls had similar rates of hospitalization and outpatient visits, but alloHCT survivors reported more medical problems, restrictions in social function, and denial of life and health insurance[137-140]. These data provide the rationale for more in-depth assessment of not only cost but also financial hardship after curative therapy for SCD from a patient perspective.

Collectively, these data speak to the need for a study in the unique population of patients with SCD who often have substantial comorbidities prior to allo or autoHCT that are likely to have a specific impact on allo or autoHCT outcomes both in the near- and long-term. It also provides a discrete metric for patients and providers to compare their outcomes and values of interest as they relate to each treatment strategy. We will assess HRQoL using the National Institutes of Health funded and developed the web-based Patient-Reported Outcomes Measurement Information System (PROMIS®) measures. PROMIS® contains a number of pediatric self-report forms that each assess domains of physical and emotional function over a seven-day time interval[141-144]. The PROMIS® measures have been used and validated in both newly diagnosed pediatric chronic diseases[145-147]. PROMIS® has provided tremendous insight into the HRQoL of pediatric illness and has demonstrated feasibility of its use to report PROs of pediatric sickle cell patients[145].

Specifically, the age appropriate PROMIS® v25 and PROMIS® v29 will be used. Each instrument contains 7 measures and consists of 8 questions with the exception of fatigue which has 10. Each response is based on "experience during the past 7 days". Responses utilize a 5-point Likert scale ranging from "never" to "always" or from "with no trouble" to "not able to do"[148, 142]

7.3   PROMIS

Patient reported outcomes including the health related quality of life (HRQoL), will be assessed using age appropriate measures at study entry and at Month 24 after gene transfer.

PROMIS® contains a number of self-report forms that assess domains of fatigue, pain interference and sleep over a seven-day time interval. To assess HRQoL domains of fatigue, pain interference and sleep disturbance,we will administer age appropriate PROMIS® short forms for pediatric subjects (8- 18 years old); and adult subjects (18 years and over).

Table 7-1. PRO Items and Completion Time

Instrument	Number of items	Estimated completion time	Time of Assessment
Fatigue Adults: PROMIS_SF_v1.0_Fatigue_4a_ Children:PROMIS_Pediatric_SF_v2.0_Fatigue_10a	4 questions 10 questions		T1, T2
Pain Interference Adults: PROMIS_SF_v1.1_-_Pain_Interference_4a Children:PROMIS_Pediatric_SF_v2.0_Pain_Interference_8a	4 questions 8 questions	Total 5 mins	
Sleep Adults: PROMIS_SF_v1.0_-_Sleep_Disturbance_4a	4 questions		
Children:PROMIS_Pediatric_Short_Form_v1.0Sleep_Disturbance_4a	4 questions		

7.3.1   Details and Timing for PRO assessments

Study participants will include patients or their corresponding identified primary caregiver. All PRO questionnaires will be administered electronically and can be completed during either an inpatient stay, an

outpatient visit, or via password-protected website. Participants age 8-17.9 years will complete the pediatric PROMIS short forms (**Table 7-1**). Participants over age 18 years will complete adult PROMIS short forms. Surveys will be completed at study entry (T1) and Month 24 post-infusion (T2). Prospectively collected HrQoL, PRO measures will be administered at T1 and T2 (**Table 7-1**)[149, 150, 131].

APPENDIX C

8   APPENDIX C – PRES GUIDELINES

GUIDELINES FOR PREVENTION AND MANAGEMENT OF PRES

Mission Statement

To describe the pathophysiology, clinical diagnosis, prevention and management of Posterior reversible encephalopathy syndrome (PRES). PRES is a disorder of reversible subcortical vasogenic brain edema with a constellation of acute neurological symptoms and brain imaging findings of vasogenic edema predominantly involving the bilateral parieto-occipital regions. Patients with sickle cell disease are particularly susceptible to PRES.[1,2]

Pathophysiology

PRES results from endothelial injury related to abrupt blood pressure changes or direct effects of cytokines on the endothelium, which leads to the breakdown of the blood–brain barrier and subsequent brain edema. With early diagnosis and appropriate management, PRES is reversible, both radiographically and clinically, and generally has a favorable prognosis.

Clinical Presentation

Clinical presentation of PRES include[3,4,5,6,7,8] seizure (60–75%), encephalopathy (50–80%), headache (50%), visual disturbances (33%), focal neurological deficit (10–15%), and Status epilepticus (5–15%). Symptoms typically occur in the setting of renal failure, blood pressure fluctuations, cytotoxic drugs, autoimmune disorders, and pre-eclampsia or eclampsia.[6] Calcineurin inhibitor (CNI)-induced PRES may occur with elevated blood pressure within days to weeks of CNI initiation and typically occurs without elevated medication levels.[9] Clinical and radiographic recovery occur in 75–90% of patients with a mean

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time to full clinical recovery range of 2–8 days, although some patients can take several weeks to achieve full recovery.[9,10,11,12] Concomitant GVHD with the use of steroids is an important risk factor for PRES.[13]

Imaging

Brain imaging is useful to confirm the diagnosis of PRES and to exclude alternative diagnoses.[6,7,12,14] Although vasogenic edema can be visualized using non-contrast CT in some patients, brain MRI (particularly T2-weighted sequences such as fluid-attenuated inversion recovery [FLAIR]) is much more sensitive.[12,14] Brain imaging usually reveals vasogenic edema in the parieto-occipital regions of both cerebral hemispheres. The subcortical white matter is always affected, and the cortex is also often involved. The edema is usually asymmetric, but almost always bilateral. Three primary descriptive variations exist in approximately 70% of patients: a dominant parieto-occipital pattern, holo-hemispheric watershed pattern, and superior frontal sulcus pattern. Neither the pattern nor the severity of brain edema, is associated with the type or severity of clinical presentation.[6,12]

Differential Diagnosis

The symptoms and signs are non-specific, thus necessitating brain imaging with the primary intent to exclude alternative diagnoses. However, the diagnosis of PRES is not solely radiological; the clinical context and the judgment of the clinician are crucial to making the correct diagnosis.

Differential diagnoses to be considered include infectious encephalitis, vasculitis, post-transplant lymphoproliferative disorder and progressive multifocal leucoencephalopathy.

Risk factors in HCT predisposing to PRES

Incidence of PRES in allo-HCT is 7-9% with greater risks with myeloablative than with non-myeloablative regimens (16% vs 3%). PRES commonly occurs in the first month following HCT and is associated with increased HLA mismatch and with acute GVHD. Low –level neurotoxicity such as tremors, anxiety, and psychiatric dysfunction has been observed in 10%-40% of patients receive CNIs. Cyclosporine can induce endothelial injury/dysfunction leading to enhanced vasoconstrictive effects, increased sympathetic activation, and coagulation effects. Blood levels of Cyclosporine do not appear to correlate with severe neurotoxicity or PRES. Immune challenge from the transplant such as rejection and GVHD, effects of chemotherapy, and sepsis may all contribute to the risk of PRES. Discontinuation or switch of CNIs usually results in clinical improvement.

Unique Risk for PRES in patients with SCD undergoing HCT

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Patients with SCD have impaired dynamic cerebrovascular autoregulation with decreased ability to buffer the transfer of blood pressure surges to cerebral tissue[15] as well as reduced cerebrovascular reserve capacity or vasodilatory capacity.[16] This may place them at unique risk for developing PRES. PRES has been reported in patients with SCD following severe acute chest syndrome, blood transfusion, hyper-transfusion with rapid increase in hemoglobin, recent use of corticosteroids, hypomagnesaemia, and in the absence of any precipitating factors.[17,18,19,20,21] The prevalence of seizures in children with SCD is 10 times that of the general population.[22,23,24] The observation of neurological complications in 30% of patients including intracranial hemorrhages in 38% of those with a previous history of stroke in an early series of patients with SCD undergoing HCT led to the universal adoption of measures for the prevention of PRES.[1] Measures for prevention of PRES include extended duration of anticonvulsant prophylaxis, intensified antihypertensive management and aggressive platelet support.[1]

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TABLE I

Medians and 90th Percentiles of Systolic and Diastolic Blood Pressure for Sickle Cell Anemia Subjects in the Cooperative Study of Sickle Cell Disease (CSSCD)

Age (Years)		2–3	4–5	6–7	8–9	10–11	12–13	14–15	16–17	18–24	25–34	35–44
Females N		257	97	72	68	57	71	89	81	227	199	66
SYS	Median	90	95	96	96	104	106	110	110	110	110	110
BP	90th	100	110	110	110	110	118	120	122	122	125	130
DIA	Median	52	60	60	60	60	62	70	70	64	68	70
BP	90th	62	70	70	70	74	74	80	78	80	80	84
Males N		276	111	78	66	75	61	75	53	179	166	41
SYS	Median	90	95	100	100	100	110	108	112	112	114	110
BP	90th	104	110	108	116	112	120	120	128	130	130	132
DIA	Median	54	60	60	60	60	64	64	70	68	70	70
BP	90th	66	68	68	70	70	72	78	80	80	80	84

SYS BP = systolic blood pressure; DIA BP = diastolic blood pressure.

Pegelow, C et al Am J Med 102 p171-177) 1997

Prevention of PRES in HCT for SCD

Prevention of PRES requires careful attention to the following measures (Table 2):

a. Control of Blood pressure. Blood pressure in patients with SCD has been reported

 to be lower than published standards for age, sex, and race-matched controls.[25,26,27] Decreased survival has been observed for patients with SCD whose systolic or diastolic pressures were above the 90th percentile for HbSS subjects.[27] Pressures above the 90th percentile for HbSS may overlap levels considered normal in non-SCD patients. Blood pressures may be elevated with fluid infusions or use of medications such as corticosteroids or CNIs. Supportive care orders must indicate the importance of keeping BP within 10% above the median for age for HbSS patients as described by Pegelow et al (Table I) or the baseline BP for the patient, whichever is lower. Mean arterial pressure (MAP) should be maintained at <70mmHg. Close monitoring and aggressive management with anti-hypertensive agents will be required to prevent PRES.

b. Maintenance of adequate platelet count. Thrombocytopenia and coagulopathy may be associated with

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increased risk of PRES-related ICH.[1],[28] It is therefore recommended to keep platelet count> 50, 000/μL.[1]
c. Maintenance of euvolemic state.

Large fluid shifts should be avoided.[28] Close attention must be paid to fluid balance since fluid overload with weight gain associated with increased blood pressure and consequently, increased risk of PRES.

Table 2. Measures for Prevention of PRES	
Measure	**Action**
Control of Blood pressure	Physician to be notified and PRN anti-hypertensives to be administered if systolic or diastolic BP exceeds 10% above median for age in HbSS patients or > 10% baseline for patient, whichever is lower.
Maintenance of adequate platelet count.	Transfuse to keep Platelets> 50, 000/μL
Maintenance of euvolemic state	Avoid rapid fluid shifts. Maintain weight as close to baseline as possible.
Maintenance of adequate magnesium level	Maintain serum magnesium level ≥ 1.8mg/dL (0.75mmol/L) when lab normal range (1.7–2.4 mg/dL or 0.7–1 mmol/L)
Prevention of seizures	Institute anticonvulsant therapy before Busulfan and continue through the duration of administration of any calcineurin inhibitor.

d. Maintenance of adequate level of serum Magnesium. Severe hypomagnesemia can present with clinical and radiological features similar to PRES.[29],[30] Magnesium sulphate is considered the drug of choice in the treatment of PRES associated with Eclampsia.[31] It is therefore recommended that patients receive magnesium supplementation in order maintain patient Maintain serum magnesium level ≥ 1.5mg/dL (0.75mmol/L) or ≥ 1.8 mg/dL when lab normal range (1.7–2.4 mg/dL or 0.7–1 mmol/L). Maintenance of mild hypermagnesemia with serum Magnesium 2-3mg/dL may be advisable, but maybe difficult to achieve

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because of side effects of P.O Magnesium. Bioavailability of magnesium in the aspartate, citrate, lactate, diglycinate and chloride forms is higher than magnesium oxide or sulfate.[32,33,34,35,36,37]

Treatment and management of PRES

The management of PRES consists of eliminating the precipitating cause, control of blood pressure and the institution of comprehensive supportive measures (Table 3). If PRES is caused by a specific medication, this medication should be discontinued. Failure to do so can perpetuate the syndrome. Alternative immunosuppression might be considered, but corticosteroids should be avoided. If steroids are added, careful consideration must be given to aggressive management of blood pressure. While it is important to treat the hypertension, the initial goal in treating patients with severe hypertension is to reduce blood pressure by 25% within the first few hours.**Error! Bookmark not defined.** Pronounced fluctuations of blood pressure should be avoided, and continuous infusions of intravenous drugs might be required. Excessive or rapid blood pressure reduction could provoke cerebral ischemia. Seizures are treated with antiepileptic medications. Other underlying disorders, such as sepsis, and flare-ups of autoimmune disorders, should be treated.

Table 3. Measures for Treatment and Management of PRES	
Measure	**Action**
Supportive care	Immediately admit to ICU and initiate management as below while evaluating cause for neurological manifestations. High index of suspicion and early diagnosis are key. Obtain MRI with FLAIR. Monitor neurological status closely.
Removal of drug precipitating PRES	Caution is advised in adding steroids because of the risk of hypertension and progression of PRES.
Table 3. Measures for Treatment and Management of PRES	
Measure	**Action**
Control of Blood pressure	Institute intravenous medications to control Blood pressure. Labetalol is drug of choice. Decrease BP by 25% over 6 hours. Gradual reduction of BP thereafter.
Maintenance of adequate platelet count.	Transfuse to keep Platelets> 50, 000/μL
Maintenance of euvolemic state	Avoid rapid fluid shifts. Maintain weight as close to baseline as possible. Close attention to intake and output. Avoid hypertransfusion. Keep Hb<12gm/dL.

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Maintenance of adequate magnesium level	Correct hypomagnesemia. Maintain serum magnesium level ≥ 1.8mg/dL (0.75mmol/L) when lab normal range (1.7–2.4 mg/dL or 0.7–1 mmol/L)
Treatment of seizures	Institute anticonvulsant therapy immediately.
Management of concurrent illness	Treat sepsis, or fluid overload.
Rehabilitation	Consider early introduction of physical and occupational therapy as appropriate

Prognosis

Despite its name, PRES is not always fully reversible. Early diagnosis and prompt management is crucial since the most severe forms of the PRES result in death, or chronic neurological sequelae.[38] Severe neurological injury and death can be attributed to intracranial hemorrhage, posterior fossa edema with brainstem compression, acute hydrocephalus[39], or marked diffuse cerebral edema and increased global intracranial pressure. Persistent neurological sequelae are reported in 10–20% of patients with PRES.[2,3,4,5,6,7,8]

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APPENDIX D

9   APPENDIX D - LABORATORY

9.1   Priority Lab Testing

Priority lab testing

1. Clinical care labs
2. CBC/diff/retic
3. HPLC for Hb electrophoresis
4. Chem panel
5. Flow for F cell cells
6. VCN
7. ISA
8. T/B cells
9. RCL
10. B cell memory panel

Labs for Higgins

1. Sample requirements: 0.3ml (or more) whole blood in EDTA (purple-top tube)
2. Helpful lab results (but not required)
    a. CBC/diff/retic
    b. HPLC for Hb electrophoresis
    c. Flow for F cell cells
    d. If above are not available, most recent CBC and Hb fractions

Labs for Manis

1. Flow for progenitor cell phenotyping (CD34) to be done in Manis lab (0.5ml, purple- can be obtained from sample 3 below)
2. Flow for red cell age (CD47 and RBC markers)
3. CD34 enumeration in flow lab

APPENDIX E

10 APPENDIX E - REFERENCES

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APPENDIX F

11 APPENDIX F - INVESTIGATOR SIGNATURE

INVESTIGATOR SIGNATURE

Title:	A Multi-Center, Phase 2 Gene Transfer Study Inducing Fetal Hemoglobin in Sickle Cell Disease
Protocol Number:	BMT CTN 2001

I confirm that I have read this protocol. I will comply with the protocol and the principles of Good Clinical Practice (GCP), as described in the United States Code of Federal Regulation (CFR) 21 Parts 11, 50, 54, 56, and 312 and the appropriate International Conference on Harmonization guidance documents.

_____

[Signature]

_____   _____

[Insert name and title.]   Date