Clinical Study Protocol

A Phase 1/2, Open-label, Single-arm Study to Assess the Safety, Tolerability, and Efficacy of ST-400 Autologous Hematopoietic Stem Cell Transplant for Treatment of Transfusion-dependent β-thalassemia (TDT)

Protocol Number:	ST-400-01
BB-IND:	BB-IND-17658
Sponsor:	Sangamo Therapeutics, Inc. 7000 Marina Boulevard Brisbane, CA 94005

Clinical Study Protocol

A Phase 1/2, Open-label, Single-arm Study to Assess the Safety, Tolerability, and Efficacy of ST-400 Autologous Hematopoietic Stem Cell Transplant for Treatment of Transfusion-dependent β-thalassemia (TDT)

Protocol Number:	ST-400-01
BB-IND:	BB-IND-17658
Sponsor:	Sangamo Therapeutics, Inc. 7000 Marina Boulevard Brisbane, CA 94005
Indication:	Transfusion-dependent β-thalassemia (TDT)
Development Phase:	Phase 1/2
Study Duration:	Up to 182 weeks (3.5 years)
Study Design:	Open-label, single-arm
Number of Subjects:	6
Medical Monitor:	Didier Rouy, MD, PhD Senior Medical Director, Clinical Sciences Sangamo Therapeutics, Inc. Phone: (628) 252-7504 Fax: (510) 970-6009 drouy@sangamo.com
Date of Original Version:	August 15, 2017
Amendment 1:	June 28, 2018
Amendment 2:	October 10, 2018
Amendment 3:	February 22, 2019
Amendment 4:	March 10, 2020
Amendment 5:	April 09, 2021

This study will be conducted according to the principles of Good Clinical Practice and in accordance with the U.S. Code of Federal Regulations and the International Conference on Harmonization Guidelines.

Clinical Approval Signature Page

Protocol Number: ST-400-01

Protocol Title: A Phase 1/2, Open-label, Single-arm Study to Assess the Safety, Tolerability, and Efficacy of ST-400 Autologous Hematopoietic Stem Cell Transplant for Treatment of Transfusion-dependent β-thalassemia (TDT)

Didier Rouy

09APR2021

Didier Rouy, M.D., Ph.D.
Medical Monitor
Sr. Medical Director, Clinical Sciences
Sangamo Therapeutics, Inc.

Date

Investigator Agreement Page

Protocol Number:	ST-400-01
BB-IND:	BB-IND-17658
Protocol Title:	A Phase 1/2, Open-label, Single-arm Study to Assess the Safety, Tolerability, and Efficacy of ST-400 Autologous Hematopoietic Stem Cell Transplant for Treatment of Transfusion-dependent β-thalassemia (TDT)

I have read all pages of this clinical study protocol for which Sangamo Therapeutics, Inc. is the Sponsor. I agree to conduct the study as outlined in the protocol, and to comply with all terms and conditions set out therein. I confirm that I will conduct the study in accordance with ICH guidelines and applicable local regulations. I will ensure that sub-Investigator(s) and other relevant members of my staff have access to copies of this protocol and the ICH guidelines to enable them to work in accordance with the provisions of these documents.

_____ _____

Investigator Signature Date

_____

Investigator Printed Name

_____

Site Name

_____

Site Address

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SYNOPSIS	
Title	A Phase 1/2, Open-label, Single-arm Study to Assess the Safety, Tolerability, and Efficacy of ST-400 Autologous Hematopoietic Stem Cell Transplant for Treatment of Transfusion-dependent β-thalassemia (TDT)
Sponsor	Sangamo Therapeutics, Inc.
Investigational Product	ST-400 is composed of autologous CD34+ hematopoietic stem/progenitor cells (HSPCs) that are genetically modified *ex vivo* at the erythroid-specific enhancer of the *BCL11A* gene by Zinc Finger Nuclease (ZFN) mRNAs SB-mRENH1 and SB-mRENH2 to boost endogenous production of fetal hemoglobin (HbF).
Objectives	**Primary Objective** The primary objective of this study is to evaluate the safety and tolerability of ST-400 in patients with TDT. **Secondary Objective** The secondary objective of this study is to assess the efficacy of ST-400 in patients with TDT. **Exploratory Objectives** The exploratory objectives of this study are to: • Evaluate the gene modification characteristics (% and durability) at the erythroid-specific enhancer of the BCL11A gene after ST-400 treatment. • Assess the impact of ST-400 on the biochemical, imaging, functional, and bone marrow evaluations related to β-thalassemia and HSCT.
Outcome Measures	**Primary Outcome Measures** Safety and tolerability will be assessed by incidence of adverse events (AEs) and serious AEs (SAEs). Additional safety evaluations will include: • Routine hematology and chemistry laboratory testing, vital signs, physical exam, electrocardiogram (ECG), echocardiogram (ECHO), pulmonary function tests (PFTs), bone marrow aspiration, and concomitant medications. • Kinetics and success of hematopoietic reconstitution. • Duration of hospitalization after conditioning. • Screening for potential development of hematological malignancies. **Secondary Outcome Measures** Change from baseline will be assessed by: • Clinical laboratory measurement of Hb fractions (A and F in g/dL) and percent HbF.

	• Calculation of annualized frequency and volume of packed red blood cell (PRBC) transfusions after ST-400 infusion as compared to baseline transfusion support in the 2 years prior to screening. **Exploratory Outcome Measures** To assess the following: • Percentage and durability of gene modification at the erythroid-specific enhancer (ESE) of the *BCL11A* genes • Change from baseline in o thalassemia-related disease biomarkers. o endocrine function by lab testing. o cardiac function by ECHO. o iron content by MRI (liver and heart). o bone mineral density by dual-energy X-ray absorptiometry (DXA). o quality-of-life by Short Form Health Status Survey (SF-36 Survey). o overall function by Karnofsky performance score. o Percentage of F-cells • Efficiency of apheresis procedure. • Difference between % indels in ST-400 product and indels detected in bone marrow and blood following ST-400 infusion.
Study Population	Male or female subjects with TDT who are 18 years to 40 years of age, inclusive, and who are willing and able to undergo autologous hematopoietic stem cell transplantation (HSCT).
Inclusion & Exclusion Criteria	**Inclusion Criteria** 1. Signed informed consent form. 2. Male or female between 18 and 40 years of age, inclusive. 3. Clinical diagnosis of TDT with ≥8 documented RBC transfusion events per year on an annualized basis in the 2-years prior to Screening. 4. Confirmed β–thalassemia diagnosis by molecular genetic testing. 5. Clinically stable and eligible to undergo HSCT. 6. Able and willing to use an effective method of contraception (Section 11.1) from the signing of the informed consent through the Primary Study Period, for male and female subjects with reproductive potential. **Exclusion Criteria** 1. Previous history of autologous or allogeneic HSCT or solid organ transplantation. 2. Pregnant or breastfeeding female. 3. γ-globin allelic variants associated with clinically significant altered oxygen affinity (see Appendix 2). 4. Medical contraindication to mobilization, apheresis or conditioning. 5. Absolute neutrophil count (ANC) ≤1,000/μL. 6. Abnormal liver function tests defined by: • direct bilirubin ≥3x ULN, or • AST or ALT ≥3x ULN

	7. Renal dysfunction as defined by serum creatinine ≥2.0 mg/dL. 8. Bridging fibrosis, liver cirrhosis, or active hepatitis based on liver biopsy obtained in previous 12 months or at Screening. 9. Massive splenomegaly (based on Investigator's judgement). 10. Treatment with prohibited medications in previous 30 days 11. Clinically significant, active bacterial, viral, fungal, or parasitic infection (based on Investigator's judgement). 12. Diagnosis of HIV or evidence of active HBV or HCV infection based on Screening laboratory testing. 13. Karnofsky performance scale ≤60. 14. Corrected DLCO ≤50% of predicted or clinically-significant restrictive lung disease based on Screening pulmonary function tests (PFTs). 15. Congestive heart failure (NYHA Class III or IV), unstable angina, uncontrolled arrhythmia, or left ventricular ejection fraction (LVEF) <40%. 16. QTcF >500 msec based on Screening ECG. 17. Cardiac T2* MRI <10 msec based on Screening MRI. 18. History of significant bleeding disorder. 19. Current diagnosis of uncontrolled seizures. 20. History of active malignancy in past 5 years (non-melanoma skin cancer or cervical cancer in situ permitted), any history of hematologic malignancy, or family history of cancer predisposition syndrome without negative testing result in the study candidate. 21. History of or active alcohol or substance abuse that in the opinion of the Investigator may interfere with study compliance. 22. History of therapeutic non-adherence. 23. Currently participating in another clinical trial using an investigational study medication, or participation in such a trial within 90 days or less than 5 half-lives of the investigational product prior to Screening visit. 24. Previous treatment with gene therapy. 25. Allergy or hypersensitivity to busulfan or study drug excipients (human serum albumin, DMSO, and Dextran 40). 26. Any other reason that, in the opinion of the Investigator or Medical Monitor, would render the subject unsuitable for participation in the study.
Study Design	This is a Phase 1/2, open-label, multi-center, single-arm study conducted in six (6) subjects with a diagnosis of TDT. Eligible subjects will be enrolled and undergo apheresis to collect autologous CD34+ HSPCs. The CD34+ HSPCs will be treated *ex vivo* by transfection with ZFN mRNAs SB-mRENH1 and SB-mRENH2 to manufacture the study drug, ST-400. Subjects will receive conditioning therapy with intravenous (IV) busulfan before being infused with ST-400. Clinical and laboratory data will be collected for a total of 156 weeks post-infusion of ST-400.

Treatment Plan	CD34+ HSPCs will be mobilized in each subject using treatment with G-CSF and plerixafor. Mobilized CD34+ HSPCs will be collected from each subject on Days 5 and 6 (+Day 7 if needed to secure the rescue treatment) of mobilization by apheresis. The collected cells of each subject will be split into 2 portions, one portion for ST-400 drug manufacturing and the other portion set aside in the event a rescue treatment is indicated. The rescue treatment portion must comprise a minimum of 2.5 x 10^6 CD34+ HSPCs/kg. The rescue treatment portion will be cryopreserved unmodified and stored at the study site for availability in the event of delayed hematopoietic reconstitution or graft failure with aplasia. For subjects in whom the first apheresis cycle does not mobilize the minimum number of CD34+ HSPCs required for ST-400 drug manufacturing and for rescue treatment, the mobilization procedure may be repeated at the discretion of the Investigator based on the subject's clinical status. The remainder of a subject's mobilized and harvested cells will be purified for CD34+ HSPCs and undergo transfection with ZFN mRNAs SB-mRENH1 and SB-mRENH2 to disrupt the erythroid-specific enhancer of the *BCL11A* gene and yield ST-400. ST-400 will be cryopreserved and stored until subsequent steps in the clinical protocol are completed and the subject is ready for ST-400 infusion. A subject will not proceed to conditioning with IV busulfan until a sufficient quantity of rescue treatment is obtained and ST-400 is manufactured, passes quality control and release testing, and is confirmed as received at the clinical site. After release of ST-400 for clinical use, subjects will be admitted to the hospital to begin IV busulfan in a dedicated transplant unit. Subjects will receive a myeloablative dose of busulfan (3.2 mg/kg/day; administered IV via central venous catheter either once daily or every 6 hours) for 4 days (total dose of 12.8 mg/kg). After the first dose, the IV busulfan dose will be adjusted based on pharmacokinetic sampling to target a total area under the curve (AUC) of 16,000-20,000 µmol∗min. No sooner than 72 hours after the final dose of IV busulfan in the conditioning phase, subjects will be infused on Day 0 with ST-400. The minimum cell dose of ST-400 to be administered is 3.0 x 10^6 cells/kg. Subjects may receive more than one lot of ST-400 if multiple apheresis cycles were performed to achieve the minimum cell dose. The first 3 subjects will be considered sentinel subjects and will be enrolled sequentially as follows: The first subject will be enrolled and receive ST-400 infusion. The second subject may proceed with CD34+ HSPC mobilization and drug manufacturing at any time but shall not proceed to conditioning or ST-400 administration until the first subject has achieved successful hematopoietic reconstitution (defined as ANC ≥500 cells/µL for

	3 consecutive days and platelet count ≥20,000 cells/µL for 3 consecutive measurements spanning a minimum of 3 calendar days (in the absence of platelet transfusion in the preceding 7 days). Similarly, the third subject may not proceed to conditioning or ST-400 administration until the second subject has achieved successful hematopoietic reconstitution. If unexpected toxicities are reported, enrollment and dosing will be paused pending review. After the 3 sentinel subjects have achieved successful hematopoietic reconstitution, all safety data from the study will be reviewed by the Safety Monitoring Committee (SMC). See Section "12. Safety Monitoring" for additional details. If no study suspension or stopping rules are met and no significant safety concerns related to ST-400 are reported, the subsequent 3 subjects may receive ST-400 concurrently without waiting for other subjects to engraft. Should a subject experience delayed hematopoietic reconstitution or graft failure with aplasia (defined as persistent ANC <500 cells/µL through Day 42 post-infusion), the subject will receive the rescue treatment consisting of unmodified, autologous CD34+ HSPCs, and a SMC safety review will be performed. The rescue treatment may be administered at any time after ST-400 infusion if needed at the discretion of the Investigator based on clinical indications for subject safety.
Study Duration	Duration of study participation will be up to 182 weeks for each subject, divided into 4 study segments: • Screening (up to 6 weeks) • Mobilization, Apheresis, Conditioning (up to 20 weeks) • Primary Study Period (starting with ST-400 infusion on Day 0; 52 weeks) • Follow-up Study Period (104 weeks) Subjects will be hospitalized at the study center following IV busulfan conditioning and ST-400 infusion. In line with typical clinical practice, subjects may be discharged following primary hospitalization upon achieving (1) neutrophil recovery (defined per protocol as ANC ≥ 500 cells/µL on 3 consecutive days) and (2) all other institutional criteria required for discharge following standard myeloablative autologous hematopoietic stem cell transplantation (HSCT) (estimated 2 to 4 weeks; however, duration is variable depending on each subject's clinical course). Subjects will be evaluated at the study center weekly until Day 42, then at Days 56 and 90, and then every 13 weeks until the final Week 156 visit (see Appendix 1). Each subject will thus have a total of 3 years of monitoring following ST-400 infusion. To assess long-term safety, subjects will be asked to participate in a separate long-term follow-up (LTFU) study after they complete this study. The total combined (current and LTFU studies) follow up period will last 15 years.

Safety Monitoring	Safety and tolerability of ST-400 will be monitored throughout the study by assessing incidence of AEs/SAEs. Additional safety evaluations will include: • Routine hematology and chemistry laboratory testing, vital signs, physical exam, ECG, echocardiogram (ECHO), pulmonary function tests (PFTs), bone marrow aspiration, and concomitant medications. • Kinetics and success of hematopoietic reconstitution. • Duration of hospitalization after conditioning. • Screening for potential development of hematological malignancies. During the conditioning and immediate post-transplant period, subjects will be admitted to an inpatient transplant unit for treatment. Standard transplant medical care will be provided as per Investigator's judgement and study center practices. Additional safety precautions, assessments, and treatments conducted as part of standard post-transplant medical care outside of those specified in this study (e.g., clinical laboratory testing, immune function testing, CMV prophylaxis, G-CSF administration, etc.) will be undertaken as per Investigator's judgement and study center practice. All AEs, irrespective of whether they are related or not related to the apheresis, conditioning, or ST-400, will be assessed and reported. An external, independent SMC with appropriate medical and scientific expertise in TDT and HSCT will provide oversight of the study for patient safety. The SMC will be convened after the 3 sentinel subjects have achieved successful hematopoietic reconstitution based on the criteria defined, and subsequently approximately every 3-6 months during the study period to monitor the safety and tolerability of ST-400. The SMC may be convened earlier or at any time if deemed necessary by the Sponsor or SMC. Specifically, the SMC will be convened if a study suspension rule is met. When convened, the SMC will evaluate all available data to provide recommendations on changes to the study and whether enrollment or dosing should continue. The SMC will also provide recommendations on any ST-400-related AEs that should be characterized as dose-limiting toxicities of ST-400 treatment.
Study Suspension Rules	Study enrollment and dosing will be suspended pending SMC evaluation of clinical trial safety data if any of the following occurs: 1. A subject develops a Grade 3 or higher AE assessed as related to ST-400 by the Investigator or Sponsor. 2. A subject experiences delayed hematopoietic reconstitution or graft failure with aplasia requiring rescue treatment. 3. A subject develops a malignancy (excluding non-melanoma skin cancer and cervical cancer in situ). 4. A subject dies. 5. The SMC and/or Sponsor determine that an event or current data warrant further evaluation by the SMC. Subjects who have already been dosed with ST-400 will continue to be followed for safety monitoring as part of the clinical trial.

Study Stopping Rules	Study enrollment and dosing will be permanently discontinued if any of the following occurs: 1. Sponsor, in consultation with the SMC or Regulatory Agency, decides for any reason that subject safety may be compromised by continuing the study. 2. A subject develops a malignancy assessed as related to ST-400. 3. Any other risk of ST-400 deemed medically unacceptable by the Sponsor is identified. 4. Sponsor decides to discontinue the development of ST-400. Subjects who have already been dosed with ST-400 will continue to be followed for safety monitoring as part of the clinical trial.
Sample Size	This study will enroll and treat a total of 5 to 6 subjects. Subjects who do not receive ST-400 or are lost to follow-up or withdraw from the study before the Week 26 visit in the Primary Study Period (beginning with ST-400 infusion) will be replaced.
Statistical Analyses	Statistical analyses will primarily be descriptive, and no formal hypothesis testing will be conducted.

ABBREVIATIONS

AE	adverse event/experience
ALKP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)
ANC	absolute neutrophil count
AST	aspartate aminotransferase (SGOT)
AUC	area under the curve
BSC	BioSafety Committee
BUN	blood urea nitrogen
CBC	complete blood count
CCOA	Clinical Certificate of Analysis
CFU	colony forming units
CMV	cytomegalovirus
COA	Certificate of Analysis
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DLCO	diffusing capacity for carbon monoxide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPT	day post-transfection
DSB	double-strand break
DXA	dual-energy X-ray absorptiometry
ECG	electrocardiogram
ECHO	echocardiogram
ETV	early termination visit
FDA	Food and Drug Administration
G-CSF	granulocyte colony-stimulating factor
GVHD	graft-versus-host disease
HBV	hepatitis B virus
Hb	hemoglobin
HbF	fetal hemoglobin ($\alpha 2\gamma 2$)
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSCT	hematopoietic stem cell transplantation
HSPC	hematopoietic stem/progenitor cell
ICF	informed consent form
IEC	Independent Ethics Committee
Indels	insertions and deletions
IRB	institutional review board
ITT	intent-to-treat
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
IV	intravenous
IVIG	intravenous immunoglobulin
kg	kilogram

LDH	lactate dehydrogenase
LTFU	Long-Term Follow-Up
LVEF	left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter
MRI	magnetic resonance imaging
mRNA	messenger RNA
NYHA	New York Heart Association
NIH	National Institutes of Health
NSG	NOD/SCID/IL2rγ^{null}
PFT	pulmonary function test
PRBC	packed red blood cell
RBC	red blood cell
RNA	ribonucleic acid
SAR	serious adverse reaction
SAE	serious adverse event
SB-mRENH1	mRNA encoding the left optimized ZFN targeted to the *BCL11A* erythroid-specific enhancer
SB-mRENH2	mRNA encoding the right optimized ZFN targeted to the *BCL11A* erythroid-specific enhancer
SCD	sickle cell disease
SCID	severe combined immunodeficiency
SF-36	36-Item Short Form Health Status Survey
SMC	Safety Monitoring Committee
SMT	Safety Monitoring Team
SOE	Schedule of Events
SUSAR	suspected unexpected serious adverse reaction
TDT	transfusion-dependent β-thalassemia
ULN	upper limit of normal
UPLC	Ultra performance liquid chromatography
VOD	veno-occlusive disease
WBC	white blood cell
ZFN	zinc finger nuclease
ZFP	zinc finger protein

1. INTRODUCTION

1.1 β-thalassemia

β-thalassemia is an inherited anemia characterized by absent or defective β-globin chain synthesis (Higgs & Engel, 2012). The defect causes an imbalance in globin chain production, and a reduction in hemoglobin (Hb; which is made up of two α-globin and two β-globin chains). As a consequence of the globin chain imbalance, unstable α-globin chain tetramers form in red blood cells (RBCs) or RBC precursors, and intramedullary destruction, apoptosis, ineffective erythropoiesis, iron overload, and profound anemia occur (Origa, 2017).

Thalassemia is considered one of the most frequent genetic diseases in the world. It is estimated that about 1.5% of the global population are carriers of a β-thalassemia mutation, with about 60,000 symptomatic individuals born each year (Galanello & Origa, 2010).

The clinical severity of β-thalassemia is determined by the amount of normal hemoglobin produced. It defines three clinical and hematological conditions, classically referred to as β-thalassemia minor, β-thalassemia intermedia, and β-thalassemia major. Patients with β-thalassemia minor have mild or no anemia, and are usually asymptomatic carriers. Patients with β-thalassemia intermedia have a moderately severe anemia, and may benefit from transfusions to improve their quality-of-life, but later in life often develop a transfusion-dependent phenotype. Patients with β-thalassemia major have a severe anemia and require frequent blood transfusions for life. Although there are many combinations of β-globin mutations and genetic disease modifiers associated with the transfusion-dependent phenotype, collectively the condition is referred to in this study as transfusion-dependent β-thalassemia (TDT) (Galanello & Origa, 2010).

Improvements in health outcomes for patients with TDT have occurred over the past 50 years as the benefits of a supportive care program were recognized. The program consists of regular RBC transfusions, starting as soon as the diagnosis is established and anemia develops. The RBC transfusions are accompanied by regular iron chelation therapy to reduce the iron overload in vital organs caused by the transfusions. The supportive care program significantly ameliorates the morbidity of TDT. However, the program is time-consuming and resource-intense: treatment of a single patient in 2011 for 50 years was estimated to cost $1,971,380 USD (Koren et al., 2014) and result in 20% of treated patients having a life expectancy of less than 40 years (Modell et al., 2008).

The only proven cure for TDT is allogeneic hematopoietic stem cell transplantation (HSCT). However, allogeneic HSCT carries substantial risk of chronic morbidity (e.g., graft-versus-host disease [GVHD]) as well as a 10-15% risk of death based on 5-year mortality (Locatelli et al., 2013; Baronciani et al., 2016). In addition, published reports show that the probability of identifying a well-matched unrelated allogeneic donor within public registries is influenced by the ethnicity of the recipient; for example, among individuals of African descent, the probability of finding a suitable donor is estimated to be only 19% (Gragert et al., 2014). Thus, many, if not most, recipients lack a human leukocyte antigen (HLA)-matched donor for allogeneic HSCT, making this potential curative treatment unavailable.

In conclusion, the clinical paradigm for treatment of TDT consists of life-long maintenance of a complex and often morbid supportive care regimen to prolong survival and improve quality-of-life, or, if available, allogeneic HSCT to cure TDT but with risk of chronic disease and life-threatening complications. These options underscore the urgent need for new therapies for TDT.

1.2 Fetal Hemoglobin

Fetal hemoglobin (HbF) is the major hemoglobin present during gestation until birth. HbF is generated by combining the protein product of one of two β-like globin genes, Gγ-globin and Aγ-globin, known collectively as γ-globin, with α-globin protein as tetramers (α2γ2). HbF levels decline progressively after birth as γ-globin protein production decreases, and around 6-12 months of age HbF is largely replaced by adult Hb, which consists of a tetramer of β-globin and α-globin proteins (α2β2). Concomitant with the decline in HbF levels, the symptoms of TDT frequently become clinically apparent in infants.

HbF normally only plays a minor role in adult physiology. However, published studies have demonstrated that congenital, acquired, and drug-induced increases in HbF are associated with reduced morbidity and improved clinical outcomes in patients with TDT. For example, large unbiased genetic studies identified associations between TDT disease severity and quantitative trait loci such as *BCL11A*, *Xmn1HBG2*, and *HBS1L-MYB* that are associated with increased levels of HbF (Thein et al., 2009), wherein the level of HbF in many cases was observed to be proportional to the degree of attenuation of TDT symptomology (Musallam et al., 2012). In addition, there are case reports of graft rejection after allogeneic HSCT for TDT in which elevated HbF levels persisted serendipitously after HSCT, and during which time the patients were transfusion-independent (Ferster et al., 1995; Paciaroni & Lucarelli, 2012).

1.3 BCL11A in Hemoglobin Regulation

BCL11A is a transcription factor that plays many roles in development and hematopoiesis. Genome-wide association and functional follow-up studies in cell and animal models have shown that *BCL11A* is an important silencer of HbF expression. In a seminal study, disruption of *BCL11A* by erythroid-specific conditional knockout in a transgenic humanized mouse model of sickle cell disease (SCD) lead to failure of hemoglobin switching, maintenance of high-levels of HbF, and improvements in hematologic and pathologic characteristics associated with SCD (Xu et al., 2011).

Inhibition of *BCL11A* therefore appears to be a potentially effective strategy for treating β-globin disorders such as TDT and SCD in humans. However, targeting the *BCL11A* gene for therapeutic approaches poses challenges due to the crucial role of *BCL11A* in development and hematopoiesis (Brendel et al., 2016). An alternative strategy targets an enhancer element that is located in the upstream noncoding region of the *BCL11A* locus. The enhancer is required for *BCL11A* expression in erythroid cells but not in other lineages. The enhancer was found to contain a common genetic variation associated with higher HbF levels (Bauer et al., 2013). It is therefore hypothesized that modification of this erythroid-specific enhancer of the *BCL11A* gene could boost endogenous HbF levels in erythroid cells without deleterious effects on global *BCL11A* function (see Figure 1).

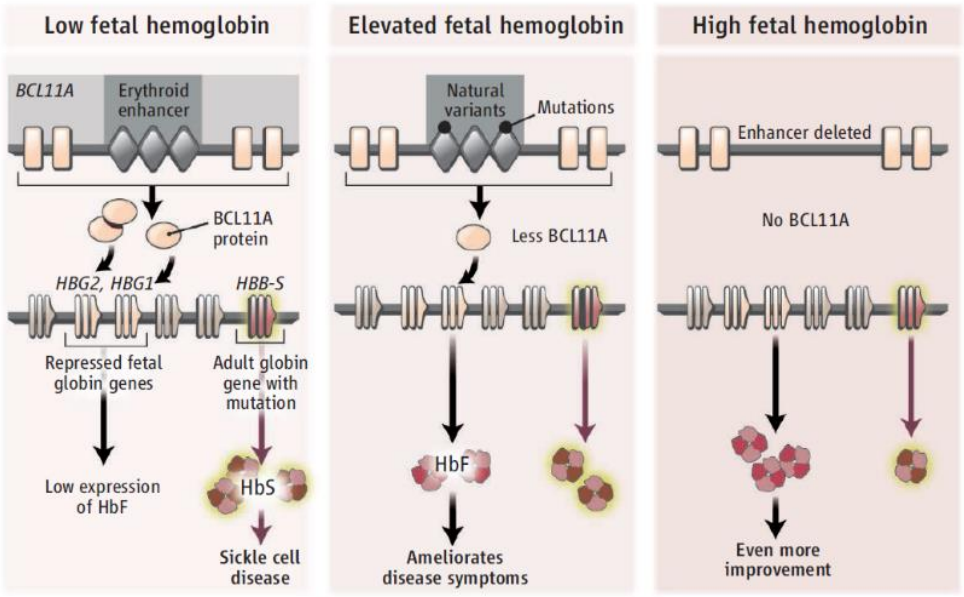

Figure 1: Regulation of hemoglobin switching by *BCL11A*

Adapted from Hardison & Blobel 2013.

1.4 Sangamo Zinc Finger Nucleases for Therapeutic Genome Editing

Zinc finger nucleases (ZFNs) are engineered proteins for site-specific genome editing. They combine the DNA recognition specificity of zinc finger proteins (ZFPs) with the nuclease domain of the type IIS restriction endonuclease *Fok*I to create double-strand breaks (DSBs) at precisely defined target sites in the genome. ZFPs contain arrays of Cys2-His2 zinc fingers, each recognizing approximately 3 base pairs of DNA. The *Fok*I nuclease domain has no sequence specificity, and must dimerize to cut DNA, which occurs efficiently only when 2 nuclease domain monomers are tethered next to each other in the proper orientation along a strand of DNA. Consequently, DNA cleavage activity is achieved only by the simultaneous binding of 2 independent ZFNs to adjacent sequences in the correct spatial orientation. Repair of the DSBs typically leads to the introduction of mutations, insertions, and deletions, termed “indels”, which can result in functional disruption of the target gene sequence.

Sangamo has engineered a ZFN pair consisting of a 6-finger ZFN (encoded by mRNA SB-mRENH1) and a 5-finger ZFN (encoded by mRNA SB-mRENH2) that binds to a 33 base pair (combined) target site in the erythroid-specific enhancer of the human *BCL11A* gene (with 7 base pairs between the ZFP recognition sites). Following the DSB created by the *Fok*I nuclease domain of the ZFNs, indels are created in the enhancer with functional disruption of a binding site for the erythroid transcription factor GATA1 (Vierstra et al., 2015; Canver et al., 2015) (see Figure 2). In a proof-of-concept study, targeted modification of the erythroid-specific enhancer of the *BCL11A* gene in hematopoietic stem/progenitor cells (HSPCs) using ZFNs resulted in robust engraftment with high levels of HbF in an immunodeficient mouse model (Chang et al., 2017).

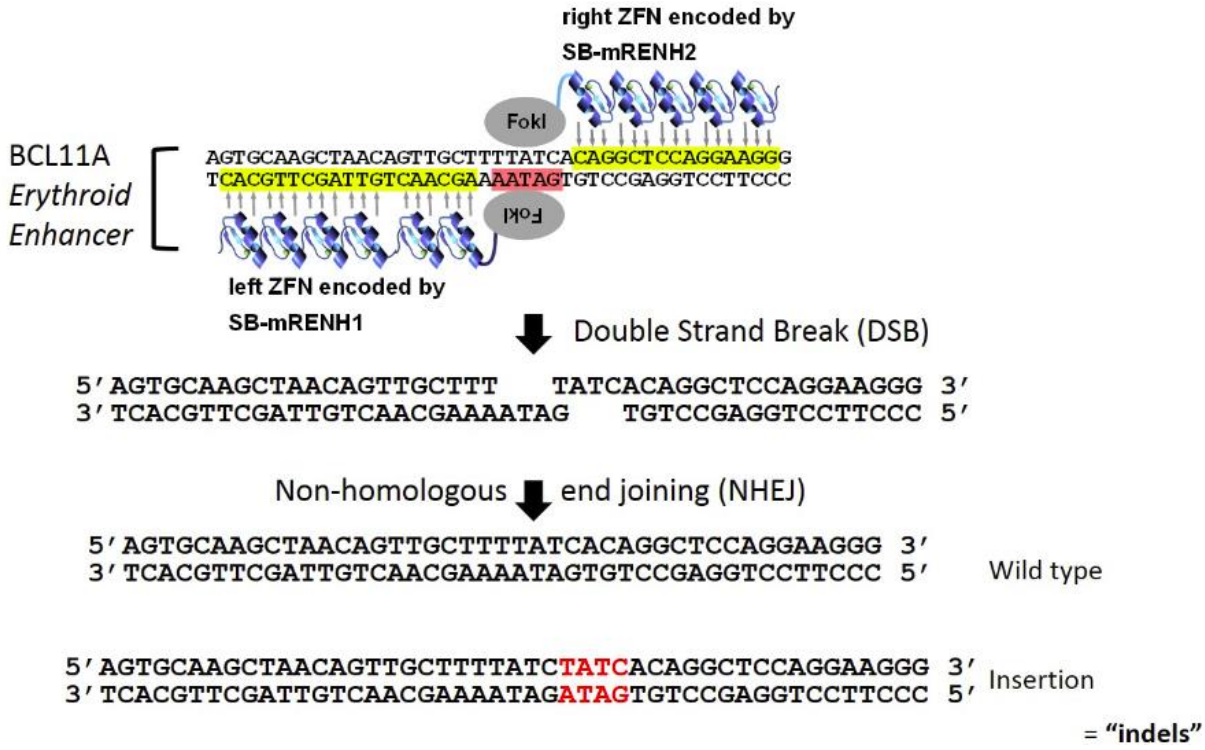

Figure 2: Gene Modification of BCL11A Erythroid Enhancer by Sangamo ZFNs

1.5 Clinical Rationale for ST-400 Mechanism of Action

Allogeneic HSCT for hereditary hematological conditions bears a risk of graft failure and GVHD. Autologous HSCT has fewer of these risks because the transplanted cells are derived from the patient. As a result, autologous HSCT with a patient's own genetically modified CD34+ HSPCs is expanding as a novel curative strategy.

Recent clinical trials in patients with SCD and TDT using autologous CD34+ HSPCs corrected *ex vivo* by a lentiviral vector with a functional β-globin transgene demonstrated improvement in hematological parameters and decreased transfusion requirements (Ribeil et al., 2017). Although promising, the use of a viral vector that randomly integrates in HSPCs after gene therapy is concerning as there is a theoretical risk of malignant transformation caused by random insertional mutagenesis and development of leukemia in a CD34+ HSPC sub-population (Stein et al., 2010).

ST-400 is a first-in-class *ex vivo* genome editing therapy that is being developed for the treatment of TDT. ST-400 is composed of autologous CD34+ HSPCs in which the erythroid-specific enhancer of the *BCL11A* gene is precisely modified without the use of integrating viral vectors (see Figure 3). The potential benefits of ST-400 include a) reduced risk of random insertional mutagenesis, and b) increased efficacy in TDT and SCD due to hemoglobin switching from defective Hb to HbF compared to approaches that rely only on the over-expression of β-globin.

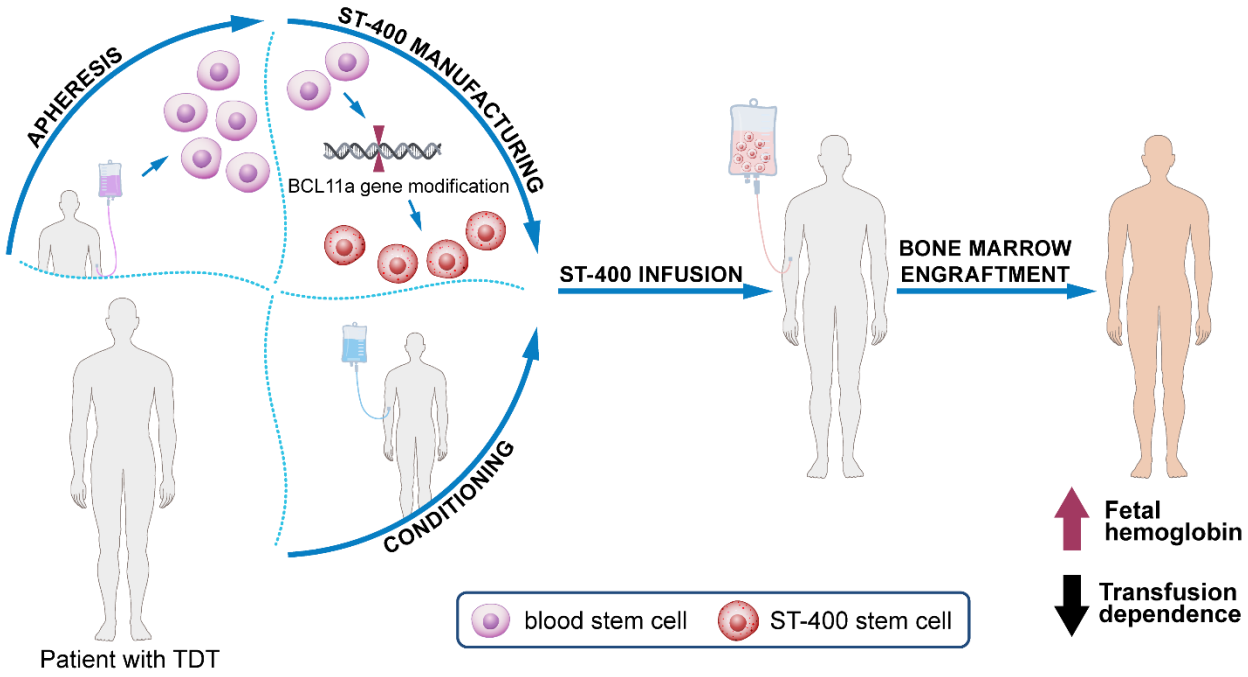

Figure 3: ST-400 Treatment

1.6 Preclinical Pharmacology Studies

Nonclinical studies were performed to support the use of ZFN mRNAs SB-mRENH1 and SB-mRENH2 in clinical studies. The studies characterized CD34+ HSPCs derived from healthy subjects and transfected with ZFN mRNAs SB-mRENH1 and SB-mRENH2 with regard to:

a) Level of disruption of the erythroid-specific enhancer of the *BCL11A* gene.

b) Potential of the transfected CD34+ HSPCs to differentiate into erythroid progeny (including assessment of cellular levels of γ-globin mRNA and protein in the erythroid progeny of the CD34+ HSPCs) *in vitro*.

c) Comparative activity and specificity of the original ZFN pair (ST-mRENH) and the optimized ST-400 ZFN pair (ST-mRENH1 and ST-mRENH2) in K562 cells and CD34+ HSPCs *in vitro*.

d) Potential of the transfected CD34+ HSPCs to engraft in NSG mice and differentiate into various hematopoietic lineages.

1.6.1 *In Vitro* Studies

For all of these studies, mobilized human CD34+ HSPCs were collected by apheresis from healthy subjects and purified. Purified CD34+ HSPCs were transfected with ZFN mRNAs SB-mRENH1 and SB-mRENH2. Untransfected CD34+ HSPCs from the same subjects served as controls. Forty-eight hours after transfection, the CD34+ HSPCs were harvested and frozen for use in three *in vitro* studies to determine erythroid differentiation potential of the transfected CD34+ HSPCs and to quantify on-target and off-target loci modification by the optimized ST-400 ZFN pair.

1.6.1.1 *In Vitro* Erythropoiesis

To analyze the effects of ZFN-mediated gene editing of the human erythroid-specific enhancer of the *BCL11A* gene, the transfected CD34+ HSPCs were placed in an *in vitro* erythropoiesis model known as "cRBC pooled differentiation" (Giarratana et al., 2011). Disruption of the erythroid-specific enhancer of the *BCL11A* gene was measured by MiSeq deep sequencing, and found to be ~75% indels, which is within the range expected during production of clinical material.

Reversed-phase UPLC of protein samples demonstrated that the ratio of γ-globin to β-globin and γ-globin to α-globin was increased approximately 3 to 4-fold in the erythroid progeny of the transfected CD34+ HSPCs compared to the untransfected CD34+ HSPCs. Thus, targeted gene modification by the optimized ST-400 ZFN pair resulted in a clinically-relevant effect, i.e., elevation of γ-globin protein. Additional details are provided in the Investigator's Brochure.

1.6.1.2 Colony-forming Unit (CFU) Potential

For assessment of the functional potential of human CD34+ HSPCs transfected with ZFN mRNAs SB-mRENH1 and SB-RENH2, the number and morphology of colonies formed by a fixed number of input cells in the CFU assay was used. The results showed that transfected CD34+ HSPCs differentiated into all hematopoietic lineages, including erythroid progenitors (CFU-E and BFU-E), granulocyte/macrophage progenitors (CFU-G/M/GM), and multi-potential progenitors (CFU-GEMM), without significant effects on differentiation potential compared to controls. Additional details are provided in the Investigator's Brochure.

1.6.1.3 Comparison of the Original and Optimized ZFN Pairs

Clinical development for the ST-400 β-thalassemia program yielded two related ZFN pairs, an original pair and a final optimized ST-400 ZFN pair that is much more precise. The comparative functional precision of cleavage of the original and optimized ST-400 ZFN pairs was evaluated. Candidate off-target sites were identified using an unbiased oligonucleotide duplex integration methodology, followed by analysis of off-target cleavage via MiSeq deep sequencing analysis. The results showed that the optimized ST-400 ZFN pair had a high level of both activity and specificity (>80% on-target modification in CD34+ HSPCs, and 0.15% aggregate off-target indels), with a more than 260-fold improvement compared to the original ZFN pair. Additional details are provided in the Investigator's Brochure.

1.6.2 *In Vivo* Studies

Human CD34+ HSPCs transfected with ZFN mRNAs SB-mRENH1 and SB-mRENH2 were assessed *in vivo* for their ability to engraft in NOD/SCID/IL2rγ^{null} (NSG) mice, maintain the disruption of the erythroid-specific enhancer of the *BCL11A* gene, and after engraftment in NSG mice differentiate into erythrocytes *in vitro*.

Female NSG mice were engrafted with 1 x 10^6 SB-mRENH1-/SB-mRENH2-transfected CD34+ HSPCs (from two healthy donors) or untransfected CD34+ HSPCs. Mice were followed for up to 19 weeks prior to scheduled necropsy.

All animals survived to their scheduled termination. The levels of modification at the erythroid-specific enhancer of the *BCL11A* gene were 79% and 75% in donors PB-MR-003 and PB-MR-004, respectively, at input after transfection, and dropped to stable levels of 42% and 50%, respectively, in peripheral blood cells at Week 19. Peripheral blood samples had almost identical modification levels as the corresponding bone marrow samples, and comparable modification was

observed in both BCL11A -dependent (CD19+ B cells; CD34+CD38^{high} primitive progenitors) and BCL11A independent (CD33+ myeloid cells) lineages. Overall, there was no difference between ZFN-treated and untreated CD34+ HSPCs in engraftment of the hematopoietic lineages.

Bone marrow was collected from the treated mice to assess for *in vitro* erythroid differentiation. The ratios of γ-globin to β-globin mRNA and γ-globin to α-globin mRNA were increased 1.5 to 3-fold, respectively, at Week 19. UPLC analysis to determine the ratios of γ-globin to α-globin protein and γ−globin to β-like globin protein showed similar increases.

To summarize, these studies demonstrated that CD34+ HSPCs transfected with the ST-400 optimized ZFN pair had a similar engraftment and differentiation potential as control untransfected CD34+ HSPCs. The transfected CD34+ HSPCs were durably maintained in the peripheral blood, the bone marrow, and in all lineages studied, with gene modification levels of 42% and 50% detectable at Week 19. Additional details are provided in the Investigator's Brochure.

1.7 Clinical Studies

This is a first-in human study for ST-400.

Several human clinical trials using *ex vivo* ZFN delivery for gene modification are ongoing or have been completed by the Sponsor (Tebas et al., 2014). These trials evaluated SB-728-T, an autologous enriched CD4+ T-cell product transfected *ex vivo* with ZFNs that target the CCR5 gene, in >80 HIV-infected subjects at doses ranging from 5 to 36 x 10^9 cells. The longest duration of follow-up has been over 5 years in the first subject infused. SB-728-T infusions were well tolerated with mostly mild and moderate reversible infusion related AEs (see Section 1.9.1.1 for additional details).

1.8 Targeted Patient Population

The targeted patient population of this study will be adult patients with TDT who require 8 or more RBC transfusion events per year and who are medically appropriate for the apheresis, mobilization, and conditioning regimen. Patients with various genotypes leading to severe beta thalassemia syndrome, therefore, will be considered for this study so long as they meet the clinical definition of TDT. Patients with sickling disorders, including Hemoglobin S/Beta-thalassemia, will not be considered for this study.

1.9 Risk Benefit Assessment

The risk-benefit of ST-400 treatment in patients with TDT is currently unknown.

1.9.1 Assessment of Risk

This is the first study in which ST-400 has been administered to humans, but the expectation of a favorable risk profile is supported by results obtained with previous studies in humans conducted using ZFNs in an autologous enriched T-cell product. Moreover, several *in vitro* and in vivo studies were conducted to assess the safety of ST-400.

Serious allergic reaction in association with ST-400 administration has been reported in one subject, characterized by symptoms of dysgeusia, cold sensation, chest pressure, dyspnea, hypotension, and bradycardia. The event was assessed as grade 3 and resolved within two hours of onset after treatment with IV fluids, diphenhydramine, hydrocortisone, and ranitidine. The reaction was assessed as likely related to the DMSO in the investigational product.

1.9.1.1 Previous Experience with ZFNs in Humans

SB-728-T, an autologous T-cell product consisting of CD4+ cells modified by ZFNs at the CCR5 gene locus, was administered to more than 80 human immunodeficiency virus (HIV)-infected subjects in 4 clinical trials. SB-728-T infusions were well tolerated with mostly mild and moderate reversible infusion-related AEs. One subject experienced a serious infusion related reaction with symptoms of fever, chills, joint pain, and back pain one day after SB-728-T infusion, which was assessed as related to the study treatment. There have been no reports of malignancy in these studies to date.

1.9.1.2 *In Vitro* Studies

The *in vitro* studies conducted included analysis of CD34+ HSPCs derived from healthy subjects and transfected with ZFN mRNAs SB-mRENH1 and SB-mRENH2 with regard to presence and type of mutations at the erythroid-specific enhancer of the *BCL11A* gene, karyotype, induced DSBs in DNA, molecular translocation, and evaluation of potential off-target transcriptional activity. An in vitro tumorigenesis study evaluated ZFN mRNAs SB-mRENH1 and SB-mRENH2 in a human fibroblast cell line.

Off-Target Genome Modifications by ST-400 ZFNs

The optimized ST-400 ZFN pair is highly specific for the erythroid-specific enhancer of the *BCL11A* gene and has but a minimal amount of detectable off-target activity. To further characterize candidate off-target loci and cleavage targets, indel analyses were conducted using MiSeq deep DNA sequencing and NextSeq ultra-deep DNA sequencing. MiSeq analysis showed very low levels of off-target cleavage of 0.15% or less. NextSeq analysis revealed extremely low levels of off-target cleavage of less than 0.01%. In comparison, indel levels at the targeted erythroid-specific enhancer of the *BCL11A* gene ranged from about 79 to 86%. These genome wide analyses indicate that indel levels at the BCL11A on-target locus exceed the levels of modification at all identified off-target sites combined by more than a factor of 300.

To determine the significance of the minimal off-target activity, a bioinformatics approach was employed in conjunction with a literature review of identified loci. For all identified off-target events, there was no evidence of modifications to coding regions of genes involved in critical hematopoietic functions. In addition, off-target events did not lead to modifications that are known to be associated with hematopoietic malignancies in humans. Additional details are provided in the Investigator's Brochure.

Karyotyping of ST-400 HSPCs

Karyotype analysis was performed to evaluate for potential abnormal chromosomal morphologies by the optimized ST-400 ZFN pair in CD34+ HSPCs derived from healthy subjects. The analysis showed that all cells were of human origin, and none had gross chromosomal abnormalities. These results demonstrated that there were no gross structural or numerical chromosomal abnormalities in CD34+ HSPCs transfected with the optimized ST-400 ZFN pair. Additional details are provided in the Investigator's Brochure.

Double Strand Breaks (DSBs) by ST-400 ZFN Pair

To characterize the profile of DSBs created by the optimized ST-400 ZFN pair, a p53-binding protein 1 (53BP1) assay was used. In this assay, the 53BP1 is recruited to sites of DSBs within 24 hours after they occur and used to visualize the DSBs by immunofluorescence microscopy (Schultz et al., 2000). The 53BP1 staining resulted in distinct bright foci marking the locations of DSBs. The results of this study demonstrated that the induction of 53BP1+ foci was transient, returning to background levels by 5 days post-transfection. Samples collected in parallel for MiSeq analysis showed that gene modification at the erythroid-specific enhancer of the *BCL11A* gene was ~79% indels at 2 days post-transfection. Thus, gene modification levels remained high as 53BP1 immunostaining levels decreased, demonstrating that the drop in 53BP1 signal was not due to loss of transfected cells over time. These findings are consistent with a transient (about 48 hour) period of ZFN expression and activity after transfection and rapid repair of the DSB. Additional details are provided in the Investigator's Brochure.

Colony Formation in Soft Agar by ST-400-Transfected Fibroblasts

For assessment of transformation/tumorigenic potential, anchorage-independent growth of human WI-38 fibroblasts transfected with ZFN mRNAs SB-mRENH1- and SB-mRENH2 was assessed in a soft agar transformation assay (Shin et al., 1975). Gene modification levels were measured at ~73% indels in the genetically modified WI-38 cells compared to ~0.3% in untransfected WI-38 cells. No anchorage-independent growth of the transfected and untransfected WI-38 cells was observed at any time point. The results show that transfection with ZFN mRNAs SB-mRENH1 and SB-mRENH2, and the resultant ZFN-mediated disruption at the erythroid-specific enhancer of the *BCL11A* gene in WI-38 cells, do not promote tumorigenicity. Additional details are provided in the Investigator's Brochure.

Molecular Translocation by ST-400 ZFN Pair

In depth characterization of rare translocation events occurring between the on-target locus (erythroid-specific enhancer of the *BCL11A* gene) and all known sites of off-target cleavage was conducted using a TaqMan-based assay developed by Sangamo that provides highly sensitive detection of translocation events at frequencies approaching one in 10^5 queried genomes. The assay was used to query for the presence and level of reciprocal translocations between the intended cleavage target in the erythroid-specific enhancer of the *BCL11A* gene and each previously identified off-target site. The assay revealed very low translocation levels involving just two of the off-target sites, with translocation frequencies ranging from 1 in 10^5 to 2 in 10^7 genomes. To determine the significance of these rare translocations, a bioinformatics approach was employed in conjunction with a literature review. None of the identified translocation events were known to be associated with hematopoietic malignancies in humans. Additional details are provided in the Investigator's Brochure.

Off-target Transcriptional Effects by the ST-400 ZFN Pair in Erythroid Progeny

To assess off-target transcriptional activity of the optimized ST-400 ZFN pair, the expression profile of 11 genes flanking the *BCLA11A* gene were analyzed using MiSeq deep sequencing. RNA was collected from the transfected CD34+ HSPCs on Day 14, at which time gene modification levels at the erythroid-specific enhancer of *BCL11A* gene were quantitated as >50% compared to controls. Levels of γ-globin mRNA in the transfected CD34+ HSPCs were increased about 2-fold (normalized to 18s RNA), reflecting decreased BCL11A expression resulting from

the on-target elimination of the GATA1 binding site in the erythroid-specific enhancer of the *BCL11A* gene. In contrast, the expression levels of the 11 genes flanking the *BCL11A* gene were similar to those of the 11 genes in the control cells. Expression levels of 4 other genes regulated by GATA1 (KLF1, SCL4A1, ZFPM1 and ALAS2) also were not affected. These results show that the activity of ZFN mRNAs SB-mRENH1 and SB-mRENH2 is restricted to repression of *BCL11A* gene transcription and its consequent downstream effects. Additional details are provided in the Investigator's Brochure.

1.9.1.3 *In Vivo* Studies

A previously conducted *in vivo* animal study found that a knockout of the mouse erythroid-specific enhancer of the *BCL11A* gene has no significant effects on erythropoiesis other than elevation of γ-globin levels (Smith et al., 2016).

To evaluate the potential tumorigenicity of ZFN-transfected HSPCs, an *in vivo* animal study was conducted in immune-deficient NSG mice. NSG mice are considered an optimal model for such studies because they accept transplantations of human HSPCs and establish long-lived grafts of the HSPCs and their progeny (Christianson et al., 1997; Shultz et al., 2005). The mice were engrafted with human CD34+ HSPCs derived from healthy subjects and transfected with ZFN mRNA SB-mRENH. SB-mRENH encodes the original two *BCL11A* enhancer-specific ZFNs, which are known to result in qualitatively equivalent gene modification at the erythroid-specific enhancer of the *BCL11A* gene as the optimized ZFN mRNAs SB-mRENH1 and SB-mRENH2. A total of 325 pre-conditioned female NSG mice were engrafted with 1 x 10^6 SB-mRENH-transfected HSPCs (n = 225) or untransfected HSPCs (negative control; n = 100), and maintained for up to 20 weeks prior to a scheduled necropsy. The engrafted mice were observed daily for evidence of AEs, underwent detailed clinical observation twice weekly, and were weighed weekly.

The cell dose of 1.0 x 10^6 CD34+ HSPC per mouse is approximately 5.0 x 10^7 cells/kg (assuming a 20 g mouse), which is 5-fold higher than the target clinical dose (1.0 x 10^7 cells/kg) for patients. As 225 mice received 1.0 x 10^6 ZFN-treated cells, a total of 2.25 x 10^8 cells were evaluated in the tumorigenicity study.

MiSeq deep sequencing confirmed that gene modification levels at the erythroid-specific enhancer of the BCL11A gene of 43% or greater throughout the study until completion of the experiment at Week 20. FACS analysis and multi-lineage differentiation of HSPCs showed successful engraftment, with hematopoietic progeny measured in blood and bone marrow of all animals during this study. Survival to study termination was ~95% (307/325), with 18/325 unscheduled deaths. Nine of the 18 early deaths were procedure- or husbandry-related. The remaining 9 deaths (2 in the untransfected control group, 7 in the ZFN-transfected group) were mice euthanized in poor clinical condition, mostly related to bone marrow hypoplasia caused by the irradiation procedure used for transplantation of human HSPCs.

In surviving animals, there were no significant differences in mean body weights or body weight gain between animals that received transfected or untransfected HSPCs over the course of the study. There were no treatment-related effects on peripheral blood and bone marrow morphology or lymphocyte counts. There were no pathologic findings related to administration of BCL11A enhancer-modified CD34+ HSPC or neoplasms shown to be of human origin.

In summary, this *in vivo* tumorigenicity study in engrafted NSG mice demonstrated no evidence of toxicity or neoplasia attributable to transfection with ZFN mRNA SBmRENH. Additional details are provided in the Investigator's Brochure.

1.9.2 Assessment of Benefit

TDT is associated with significant morbidity and mortality.

Over the past several decades, the incidence of early mortality due to TDT has been reduced with current supportive therapy, which consists of frequent and lifelong RBC transfusions and daily iron chelation therapy. However, this treatment is burdensome and expensive. Moreover, RBC transfusions cause iron overload that can be fatal when adherence to iron chelation therapy is poor.

β-thalassemia can be cured currently only by allogeneic HSCT. However, allogeneic HSCT is performed infrequently world-wide due to limited donor availability, toxicity, and cost. Furthermore, allogeneic HSCT bears risks of death and long-term complications (e.g., GVHD).

Thus, due to the limitations of existing therapies for TDT, there is an unmet medical need for which ST-400 could potentially provide life-long clinical benefit.

2. STUDY OBJECTIVES

It is hypothesized that treatment with ST-400 will induce a sustained high-level expression of γ-globin and production of HbF in patients with TDT, and ultimately reduce or eliminate the need for regular life-long RBC transfusions.

2.1 Primary Objective

The primary objective of this study is to evaluate the safety and tolerability of ST-400 in patients with TDT.

2.2 Secondary Objective

The secondary objective of this study is to assess the efficacy of ST-400 in patients with TDT.

2.3 Exploratory Objectives

The exploratory objectives of this study are to:

- Evaluate the gene modification characteristics (% and durability) at the erythroid-specific enhancer of the *BCL11A* gene after ST-400 treatment.
- Assess the impact of ST-400 on the biochemical, imaging, functional, and bone marrow evaluations related to β-thalassemia and HSCT.

3. STUDY OUTCOME MEASURES

3.1 Primary Outcome Measures

Safety and tolerability will be assessed by incidence of adverse events (AEs) and serious AEs (SAEs). Additional safety evaluations will include:

- Routine hematology and chemistry laboratory testing, vital signs, physical exam, electrocardiogram (ECG), echocardiogram (ECHO), pulmonary function tests (PFTs), bone marrow aspiration, and concomitant medications.
- Kinetics and success of hematopoietic reconstitution.
- Duration of hospitalization after conditioning.
- Screening for potential development of hematological malignancies.

3.2 Secondary Outcome Measures

Change from baseline will be assessed by:

- Clinical laboratory measurement of Hb fractions (A and F in g/dL) and percent HbF.
- Calculation of annualized frequency and volume of packed red blood cell (PRBC) transfusions after ST-400 infusion as compared to baseline transfusion support in the 2 years prior to screening.

3.3 Exploratory Outcome Measures

To assess the following:

- Percentage and durability of gene modification at the erythroid-specific enhancer (ESE) of the *BCL11A* genes.
- Change from baseline in
  - thalassemia-related disease biomarkers.
  - endocrine function by lab testing.
  - cardiac function by ECHO.
  - iron content by MRI (liver and heart).
  - bone mineral density by dual-energy X-ray absorptiometry (DXA).
  - quality-of-life by Short Form Health Status Survey (SF-36 Survey).
  - overall function by Karnofsky performance score.
  - Percentage of F-cells.
- Efficiency of apheresis procedure.
- Difference between % indels in ST-400 product and indels detected in bone marrow and blood following ST-400 infusion.

4. STUDY DESIGN

4.1 Overview

This is a Phase 1/2, open-label, multi-center, single-arm study.

A total of five (5) to six (6) subjects with a diagnosis of TDT will be enrolled and treated.

Eligible subjects will be enrolled and undergo apheresis to collect autologous CD34+ HSPCs. The CD34+ HSPCs will be treated *ex vivo* by transfection with ZFN mRNAs SB-mRENH1 and SB-mRENH2 to manufacture the study drug, ST-400. ST-400 will be cryopreserved until the subject is ready for conditioning.

Subjects will receive conditioning therapy with IV busulfan before being infused with ST-400.

Clinical and laboratory data will be collected for a total of 156 weeks post-infusion of ST-400.

4.2 Justification of Study Design

The primary objective of this first-in-human study is to assess the safety and tolerability of ST-400. Due to the invasive nature of the apheresis procedure and the potential toxicity of IV busulfan, no treatment control group is included in this study. A minimum of 2 years of retrospective clinical and hematological data will be collected for each subject, thus each subject will serve as their own control to provide reference data for evaluation of change from baseline of the study assessments, including transfusion requirements and circulating Hb and HbF levels.

4.3 Number of Subjects

Six (6) subjects who satisfy all eligibility criteria will be enrolled and treated in this study. Enrolled subjects who do not receive ST-400 or who are lost to follow-up or withdraw from the study before the Week 26 visit in the Primary Study Period (beginning with ST-400 infusion) will be replaced. The first 3 subjects will be considered sentinel subjects, and the second and third sentinel subjects will not begin conditioning with IV busulfan until the subject previously enrolled has achieved successful hematopoietic reconstitution.

4.4 Dose and Dose Justification

The minimum cell dose of ST-400 is 3.0×10^6 cells/kg, administered IV no sooner than 72 hours after the final dose of IV busulfan in the conditioning phase. To reduce the risk of graft failure, the minimal dose of ST-400 required in this study exceeds the generally recommended minimum cell dose of 2.0×10^6 CD34+ HSPCs/kg for autologous HSCT (Duong et al., 2014). To achieve the highest likelihood of efficacious ST-400 engraftment, the target dose of ST-400 is 10×10^6 cells/kg, and the maximum dose that may be given is 20×10^6 cells/kg.

To put the human cell dose in perspective to the number of treated cells evaluated in the 5-month NSG mouse tumorigenicity study, the mouse cell dose of 1.0×10^6 CD34+ HSPC is approximately 5.0×10^7 cells/kg, which is 5-fold higher than the target clinical dose (1.0×10^7 cells/kg) for patients. As 225 mice received 1.0×10^6 ZFN-treated cells, a total of 2.25×10^8 cells were evaluated in the tumorigenicity study. There was no evidence of treatment-related toxicity or neoplasia in the 5-month mouse study.

4.5 Study Duration

The duration of study participation will be up to 182 weeks for each subject, divided into 4 study segments:

- Screening (up to 6 weeks)
- Mobilization, Apheresis, Conditioning (up to 20 weeks)
- Primary Study Period (starting with ST-400 infusion on Day 0; 52 weeks)
- Follow-up Study Period (104 weeks)

To assess long-term safety, subjects will be asked to participate in a separate long-term follow-up (LTFU) study after they complete this study. The total combined (current and LTFU studies) follow up period will last 15 years.

4.6 Visit Schedule

Subjects will complete all screening visit procedures and will have eligibility confirmed before proceeding to mobilization & apheresis (see Figure 4). Subjects will be hospitalized at the study center following IV busulfan conditioning and ST-400 infusion. In line with typical clinical practice, subjects may be discharged following primary hospitalization upon achieving (1) neutrophil recovery (defined per protocol as ANC ≥ 500 cells/µL on 3 consecutive days) and (2) all other institutional criteria required for discharge following standard myeloablative autologous HSCT (estimated 2 to 4 weeks; however, duration is variable depending on each subject's clinical course). Subjects will be evaluated at the study center weekly until Day 42, then at Days 56 and 90, and then every 13 weeks until the final Week 156 visit. Each subject will thus have a total of 3 years of monitoring following ST-400 infusion. For a detailed schedule of visits and procedures see Section 7 and Appendix 1.

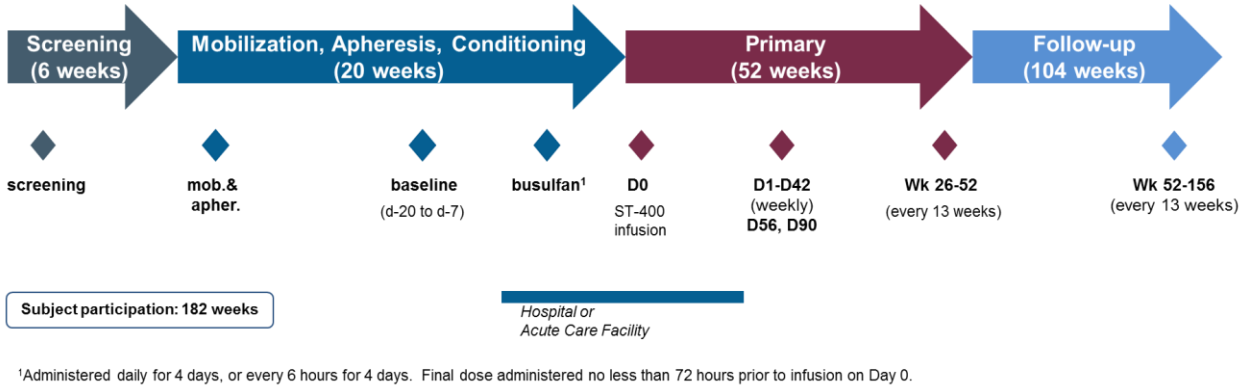

Figure 4: Schema of Study Visits

4.7 Study Procedures

Subjects who meet eligibility criteria and are enrolled will be scheduled for the Mobilization & Apheresis visit to prepare for and subsequently begin mobilization. Subsequently, CD34+ HSPCs will be mobilized in each subject following G-CSF administration on Days 1-6 of mobilization (plus Day 7 if rescue treatment is collected on Day 7) and plerixafor administration prior to each apheresis (Days 5 and 6, plus Day 7 if rescue treatment is collected on Day 7). Timing of plerixafor

administration with respect to the start of apheresis may be approximately 10 to 12 hours before apheresis, but is at the discretion of the Investigator. Timing of G-CSF administration may be 1-2 hours prior to the start of apheresis, but is at the discretion of the Investigator (see Figure 5). Mobilized CD34+ HSPCs will be collected from each subject by apheresis on Days 5 and 6 (+ Day 7 if needed to secure the rescue treatment) with a target of 25 x 10^6 CD34+ HSPCs/kg total to be submitted for manufacturing, although smaller yields are acceptable. Within this timeframe, apheresis and mobilization may be started earlier and/or discontinued earlier based on institutional practices, cell yields, and Investigator's judgement. Splenectomized subjects will receive a reduced dose of G-CSF, with further dose adjustment as necessary, to target a WBC below 100,000 cells/µL. G-CSF and plerixafor dosing, timing, or route of administration may be modified or discontinued at the discretion of the Investigator if the WBC exceeds 100,000 cells/µL or if the subject becomes symptomatic.

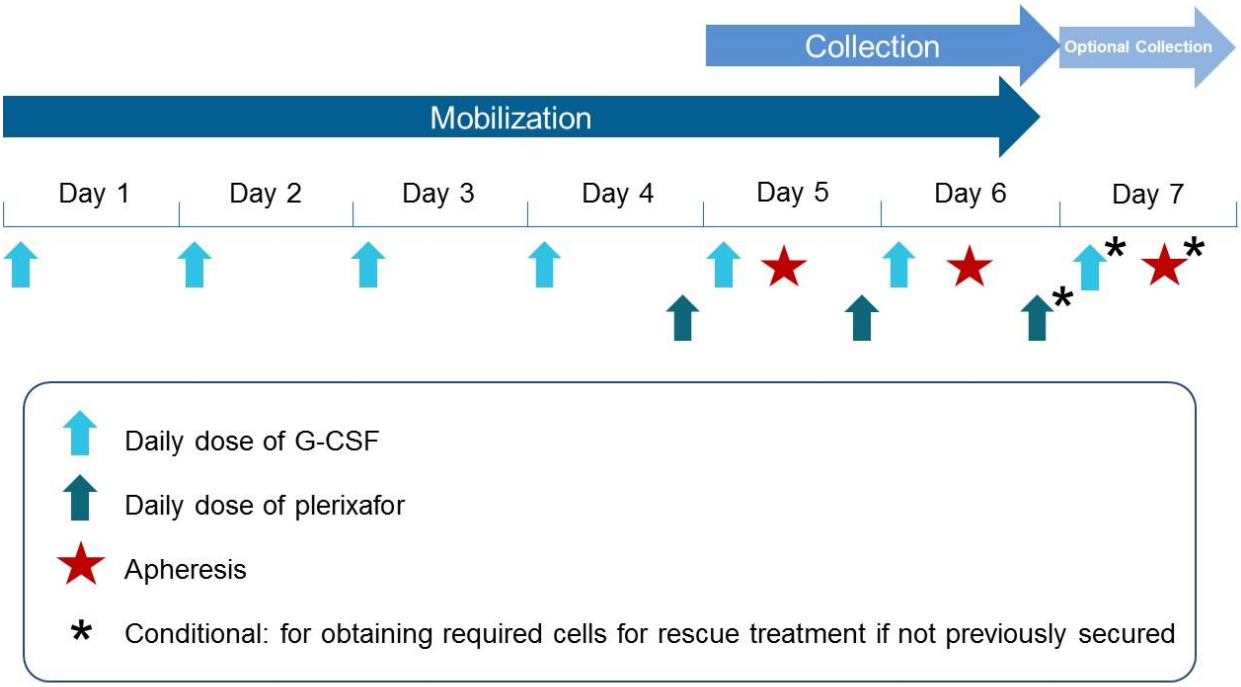

Figure 5: Schema of Mobilization and Apheresis

The collected cells of each subject will be split into 2 portions, one portion for ST-400 drug manufacturing and the other portion set aside in the event a rescue treatment is indicated.

The rescue treatment portion must comprise a minimum of 2.5 x 10^6 CD34+ HSPCs/kg. The rescue treatment portion will be cryopreserved unmodified and stored at the study site for availability in the event of delayed hematopoietic reconstitution or graft failure with aplasia. If the first apheresis cycle does not mobilize the minimum number of CD34+ HSPCs required for ST-400 drug manufacturing and for rescue treatment, 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, but may occur no sooner than 2 weeks after the initial apheresis.

Mobilized and harvested cells collected for ST-400 manufacturing will be sent by courier to the Good Manufacturing Practices (GMP) manufacturing facility. A CD34+ cell selection followed by transfection with ZFN mRNAs SB-mRENH1 and SB-mRENH2 to disrupt the erythroi-specific enhancer of the *BCL11A* gene will be performed to generate the ST-400 study drug. ST-400 will be cryopreserved and stored until all the clinical protocol segments up to and including the Baseline visit procedures are completed and the subject is ready for ST-400 infusion.

A subject will not proceed to conditioning with IV busulfan until a sufficient quantity of rescue treatment is obtained and ST-400 is manufactured, passes quality control and release testing, and is confirmed as received at the clinical site.

After receipt of ST-400 at the clinical site, subjects will be admitted to the hospital to begin IV busulfan in a dedicated transplant unit. Subjects will receive a myeloablative regimen of busulfan (3.2 mg/kg/day; administered IV via central venous catheter either once daily or every 6 hours) for 4 days (total dose of 12.8 mg/kg, which is considered standard-of-care for autologous transplantation). After the first dose, the IV busulfan dose will be adjusted based on pharmacokinetic sampling and study center practices to target an area under the curve (AUC) of 4,000-5,000 µmol∗min for daily dosing or an AUC of 1,000-1,250 µmol∗min for every 6 hour dosing for a total regimen target AUC of 16,000-20,000 µmol∗min.

IV busulfan pharmacokinetic targeting may be modified for subsequent subjects based on experience with previous subjects after discussion with the Safety Monitoring Committee (SMC). Therapeutic drug monitoring to determine clearance of busulfan after 4 days of dosing is complete is not required but may be performed at the discretion of the Investigator in accordance with study center practices.

No sooner than 72 hours after the final dose of IV busulfan in the conditioning phase, subjects will be infused on Day 0 with ST-400. The minimum cell dose of ST-400 to be administered is 3.0×10^6 cells/kg. Subjects may receive more than one lot of ST-400 if multiple apheresis cycles were performed to achieve the minimum cell dose.

The first 3 subjects will be considered sentinel subjects and will be enrolled sequentially as follows (see Figure 6): The first subject will be enrolled and receive ST-400 infusion. The second subject may proceed with CD34+ HSPC mobilization and drug manufacturing at any time but shall not proceed to conditioning or ST-400 administration until the first subject has achieved successful hematopoietic reconstitution as defined by absolute neutrophil count (ANC) ≥500 cells/µL for 3 consecutive days and platelet count ≥20,000 cells/µL for 3 consecutive measurements spanning a minimum of 3 calendar days (in the absence of platelet transfusion in the preceding 7 days). Similarly, the third subject shall not proceed to conditioning or ST-400 administration until the second subject has achieved successful hematopoietic reconstitution.

If unexpected toxicities are reported, enrollment and dosing will be paused pending SMC review. The SMC will also provide recommendations on any ST-400-related AEs that should be characterized as dose-limiting toxicities of ST-400 treatment.

After the 3 sentinel subjects have achieved successful hematopoietic reconstitution, all safety data from the study will be reviewed by the SMC. If no study suspension or stopping rules are met and no significant safety concerns related to ST-400 are reported, the subsequent 3 subjects may receive ST-400 concurrently without waiting for other subjects to engraft.

Should a subject experience delayed hematopoietic reconstitution or graft failure with aplasia, the subject will receive the rescue treatment consisting of unmodified, autologous CD34+ HSPCs, and a SMC safety review will be performed. Delayed hematopoietic reconstitution or graft failure with aplasia is defined as persistent ANC <500 cells/µL through Day 42 post-infusion. The rescue treatment may be administered at any time after ST-400 infusion if needed at the discretion of the Investigator based on clinical indications for patient safety.

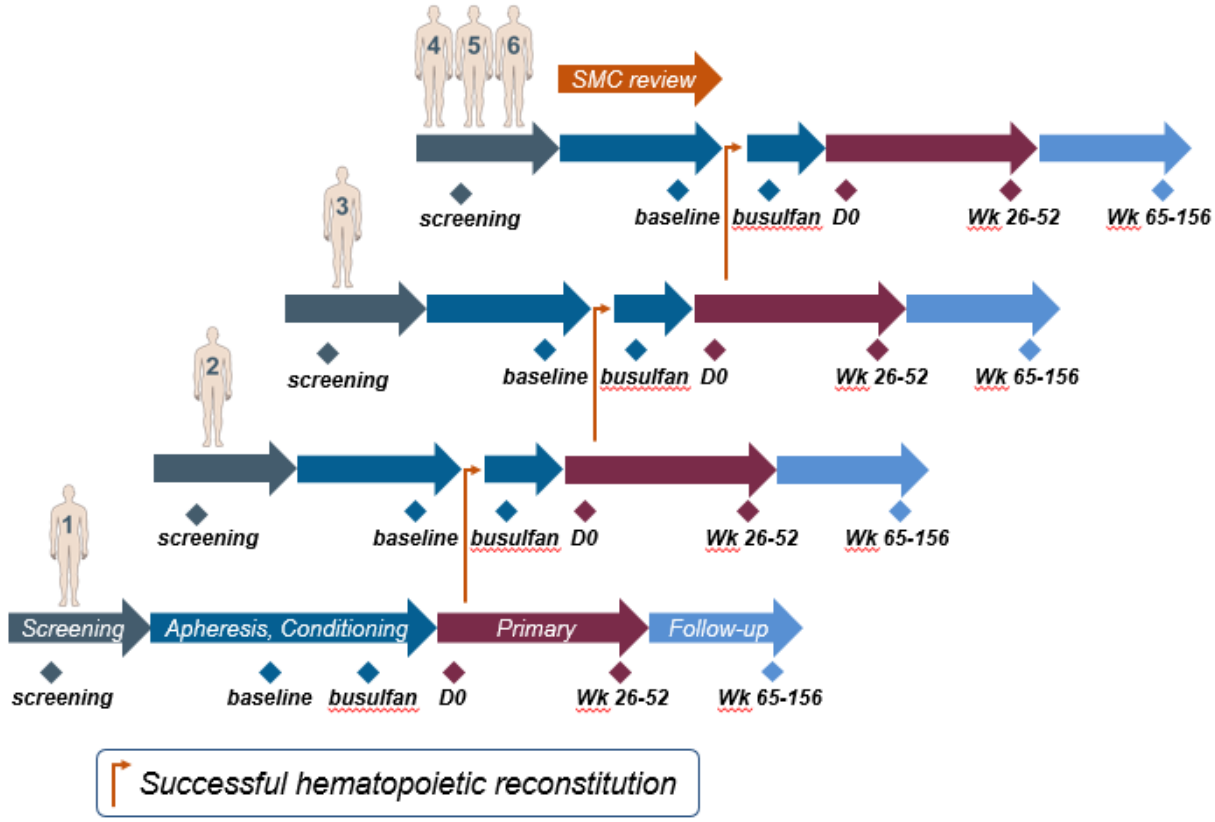

Figure 6: Schema of Subject Enrollment

4.8 Study Suspension Rules

Study enrollment and dosing will be suspended pending SMC evaluation of clinical trial safety data if any of the following occurs:

1. A subject develops a Grade 3 or higher AE assessed as related to ST-400 by the Investigator or Sponsor
2. A subject experiences delayed hematopoietic reconstitution or graft failure with aplasia requiring rescue treatment.
3. A subject develops a malignancy (excluding non-melanoma skin cancer and cervical cancer in situ).
4. A subject dies.
5. The SMC and/or Sponsor determine that an event or current data warrant further evaluation by the SMC.

Upon suspension, the SMC will be convened and will evaluate all data and provide recommendations on whether enrollment or dosing should continue, whether changes should be made to the study, and, in consultation with Sponsor, whether the study should be stopped.

Subjects who have already been dosed with ST-400 will continue to be followed for safety monitoring as part of the clinical trial.

4.9 Study Stopping Rules

Study enrollment and dosing will be permanently discontinued if any of the following occurs:

1. Sponsor, in consultation with the SMC or Regulatory Agency, decides for any reason that subject safety may be compromised by continuing the study.
2. A subject develops a malignancy assessed as related to ST-400.
3. Any other risk of ST-400 deemed medically unacceptable by the Sponsor is identified.
4. Sponsor decides to discontinue the development of ST-400.

Subjects who have already been dosed with ST-400 will continue to be followed for safety monitoring as part of the clinical trial.

5. SUBJECT SELECTION

5.1 Inclusion Criteria

1. Signed informed consent form (ICF).
2. Male or female between 18 and 40 years of age, inclusive.
3. Clinical diagnosis of TDT with ≥8 documented RBC transfusion events per year on an annualized basis in the 2-years prior to Screening.
4. Confirmed β–thalassemia diagnosis by molecular genetic testing.
5. Clinically stable and eligible to undergo HSCT.
6. Able and willing to use an effective method of contraception (see Section 11.1) from the signing of the informed consent through the Primary Study Period, for male and female subjects with reproductive potential.

5.2 Exclusion Criteria

1. Previous history of autologous or allogeneic HSCT or solid organ transplantation.
2. Pregnant or breastfeeding female.
3. γ-globin allelic variants associated with clinically significant altered oxygen affinity (see Appendix 2).
4. Medical contraindication to mobilization, apheresis or conditioning.
5. ANC ≤1,000/μL.
6. Abnormal liver function tests defined by:
   - direct bilirubin ≥3x ULN, or
   - AST or ALT ≥3x ULN
7. Renal dysfunction as defined by serum creatinine ≥2.0 mg/dL.
8. Bridging fibrosis, liver cirrhosis, or active hepatitis based on liver biopsy obtained in previous 12 months or at Screening.
9. Massive splenomegaly (based on Investigator's clinical judgement).
10. Treatment with prohibited medications in previous 30 days.
11. Clinically significant, active bacterial, viral, fungal, or parasitic infection (based on Investigator's judgement).
12. Diagnosis of HIV or evidence of active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection based on Screening laboratory testing.
13. Karnofsky performance scale ≤60.
14. Corrected DLCO ≤50% of predicted or clinically-significant restrictive lung disease based on Screening pulmonary function tests (PFTs).
15. Congestive heart failure (NYHA Class III or IV), unstable angina, uncontrolled arrhythmia, or left ventricular ejection fraction (LVEF) <40%.

16. QTcF >500 msec based on Screening ECG.
17. Cardiac T2* MRI <10 msec based on Screening MRI.
18. History of significant bleeding disorder.
19. Current diagnosis of uncontrolled seizures.
20. History of active malignancy in the past 5 years (non-melanoma skin cancer or cervical cancer in situ permitted), any history of hematologic malignancy, or family history of cancer predisposition syndrome without negative testing result in the study candidate.
21. History of or active alcohol or substance abuse that in the opinion of the Investigator may interfere with study compliance.
22. History of therapeutic non-adherence.
23. Currently participating in another clinical trial using an investigational study medication, or participation in such a trial within 90 days or less than 5 half-lives of the investigational product prior to Screening visit.
24. Previous treatment with gene therapy.
25. Allergy or hypersensitivity to busulfan or study drug excipients (human serum albumin, DMSO, and Dextran 40).
26. Any other reason that, in the opinion of the Investigator or Medical Monitor, would render the subject unsuitable for participation in the study.

6. INFORMED CONSENT

At the screening visit, the Investigator or designated personnel will explain to each subject the nature of the study, its purpose, the procedures, the expected duration, alternative therapies available, and the risks and potential benefits of participation. The subject will receive an ICF, with the opportunity to ask questions, and will be informed that participation is voluntary, and that he/she can withdraw from the study at any time without any impact upon his/her future clinical care. After this explanation and before any study-specific procedures are performed, the subject must voluntarily sign and date the ICF. The subject will receive a copy of the signed and dated written ICF and any other written information required for the study. Each subject will be re-consented at the time of any informed consent amendment, as applicable, and will be provided a copy of the revised consent form.

7. STUDY METHODOLOGY

Study procedures are described in the following section and summarized in the Schedule of Events (see Appendix 1). Additional detailed instructions will be provided in the Study Reference Manual, Imaging Manual, Laboratory Manual, and Study Cell Therapy Manual.

7.1 Screening Visits

The objective of the screening visits is to identify subjects who meet the stated inclusion and exclusion criteria and who are willing and able to participate in the study. Screening will take approximately 6 weeks (+/- 4 weeks), and may be performed across several visits.

The following assessments and procedures will be performed (see Section 9 for details on Study Assessments):

- Obtain signed and dated ICF and authorization document to use and disclose medical information (prior to performing any study-specific procedures)
- Obtain complete medical history, including concomitant medications; if the subject is not normally seen at the study center it may be necessary to obtain medical records to confirm study eligibility
- Collect demographic information
- Review inclusion and exclusion criteria
- Assign subject number
- Transfusion history
- Assess AEs
- Physical examination
- Vital signs
- Height
- Weight
- Karnofsky performance scale
- Pregnancy test (for females with reproductive potential only)
- PFTs
- Chest x-ray
- Infectious disease marker panel
- Clinical laboratory tests
- ECG
- Liver biopsy (unless performed within previous 12 months with proper medical documentation)
- Comprehensive hemoglobin analysis

- HbF quantification
- Thalassemia-related disease biomarkers
- Endocrine lab testing
- ECHO
- Liver MRI
- Cardiac MRI
- SF-36 Survey

7.2 Subject Enrollment

Before enrollment, study site personnel must verify that the subject fulfills all eligibility criteria. Date of enrollment will be captured in the casebook as the date eligibility is confirmed by the Investigator.

7.3 Mobilization, Apheresis, and Conditioning

After meeting eligibility criteria and enrolling in the study, each subject will proceed to mobilization, apheresis, and conditioning, which will span approximately 20 weeks (+/- 8 weeks) and will be performed across several visits as detailed below.

7.3.1 CD34+ HSPC Apheresis

Subjects who have signed the ICF and meet all the eligibility criteria will be scheduled for CD34+ HSPC mobilization. Sangamo should be contacted by the study center prior to scheduling CD34+ HSPC mobilization to ensure availability of the ST-400 manufacturing facility.

7.3.1.1 G-CSF and Plerixafor Mobilization and Apheresis

CD34+ HSPCs will be mobilized in each subject following G-CSF administration on Days 1-6 of mobilization (plus Day 7 if rescue treatment is collected on Day 7) and plerixafor administration prior to apheresis on Days 5 and 6 (plus Day 7 if rescue treatment is collected on Day 7). Timing of plerixafor administration with respect to the start of apheresis may be approximately 10 to 12 hours before apheresis; timing of both G-CSF and plerixafor is at the discretion of the Investigator. Within this timeframe, apheresis and mobilization may be started earlier and/or discontinued earlier based on institutional practices, cell yields, and Investigator's judgement. Splenectomized subjects will receive a reduced dose of G-CSF, with further dose adjustment as necessary to target a WBC below 100,000 cells/μL. G-CSF and plerixafor dosing, timing, or route of administration may be modified or discontinued at the discretion of the Investigator if the WBC exceeds 100,000 cells/μL or if the subject becomes symptomatic. Detailed guidelines for administering G-CSF and plerixafor are provided in the Study Cell Therapy Manual.

It is strongly recommended that a vascular access consultation be obtained before the mobilization begins, and a temporary central catheter is inserted before the apheresis procedure. Subjects should be hospitalized until the temporary central catheter has been removed at the conclusion of the apheresis procedures, or per study center standard of care. Although peripheral access during apheresis might be an option in some cases, this practice is discouraged due to diminished yields of CD34+ HSPCs.

The mobilized cells will be collected from each subject by apheresis on Days 5 and 6 (+ Day 7 if needed to secure the rescue treatment) with a target of >25 x 10^6 CD34+ HSPCs/kg; however, lower yields are acceptable. The rescue treatment portion must comprise a minimum of 2.5 x 10^6 CD34+ HSPCs/kg.

If the first apheresis cycle does not mobilize the minimum number of CD34+ HSPCs required for ST-400 drug manufacturing and for rescue treatment, 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, but may occur no sooner than 2 weeks after the initial apheresis.

The following assessments and procedures will be performed within one week prior to administration of the first mobilizing agent (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs (performed throughout the visit)
- Physical examination
- Vital signs
- Weight
- Clinical laboratory tests
- Pregnancy test (for females with reproductive potential only)
- Transfusion log (updated throughout study participation)

Prior to mobilization, it is recommended that subjects have a Hb level of ≥9.0 g/dL. Subjects may undergo a hypertransfusion procedure to achieve higher Hb levels at the discretion of the Investigator.

7.4 Gamete and Embryo Cryopreservation

Busulfan treatment is associated with a high incidence of gonadal toxicity and infertility. All patients enrolled on this study will be counseled regarding the risk of gonadal toxicity with busulfan, and the strategy of gamete cryopreservation. Each study subject will be able to choose whether he/she would like to pursue gamete cryopreservation. If gamete cryopreservation is chosen, it will be performed outside of the study. In some circumstances and at the recommendation of their treating physician, female participants may elect to undergo in vitro fertilization (IVF) using freshly obtained oocyte gametes. The associated costs borne by the Sponsor will be limited to the actual, reasonable cost of the gamete cryopreservation procedures (and for some female subjects, IVF), and storage of banked tissues and/or embryos for the duration of the subject's participation in this study. Scheduling of associated procedures should be initiated as soon as possible after enrollment, and any invasive surgical procedures related to gamete and/or embryo cryopreservation should be completed by approximately 30 days prior to ST-400 infusion at the Investigator's discretion to allow for adequate healing.

7.5 Baseline Assessments

The following assessments and procedures will be performed within 2 weeks prior to conditioning with IV busulfan (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs
- Physical examination
- Vital signs
- Weight
- Karnofsky performance scale
- Pregnancy test (for females with reproductive potential only, within one week prior to first busulfan infusion)
- Clinical laboratory tests
- ECG
- *BCL11A* gene modification assay
- HbF quantification
- Immunological assay
- Transfusion log
- Thalassemia-related disease biomarkers
- Endocrine lab testing
- Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy
- F-cell percentage
- DXA
- SF-36 Survey
- Bone marrow aspiration

If more than 30 weeks will elapse between the start of the Screening visit and the Baseline visit, the Investigator will confirm that the subject still meets all inclusion/exclusion criteria. At the Investigator's discretion, repeat testing may be performed as appropriate. At a minimum, the following tests will be repeated at Baseline:

- Pulmonary function tests (PFTs)
- Chest X-ray
- Echocardiogram (ECHO)

Results should be recorded in the eCRF, and the Investigator should assess results against the appropriate study inclusion and exclusion criteria to confirm the subject continues to meet study

eligibility before proceeding to Conditioning. This assessment should be documented in source. Subjects who no longer meet eligibility criteria will be discontinued from study participation.

7.6 Conditioning

A subject will not proceed to conditioning with IV busulfan until a sufficient quantity of rescue treatment is obtained and ST-400 is manufactured, passes quality control and release testing, and is confirmed as received at the clinical site.

After release of ST-400 for clinical use, subjects will be admitted to the hospital for central venous catheter insertion and to begin IV busulfan in a dedicated transplant unit. Subjects will be hospitalized in accordance with study center standard procedures for recipients of HSCT. The estimated duration of the hospitalization is approximately 2 to 4 weeks, but the actual duration shall be determined by the Investigator depending upon a subject's clinical course.

Seizure prophylaxis during IV busulfan is strongly recommended. Iron-chelating agents should be discontinued 2 weeks prior to IV busulfan, and may be restarted after successful hematopoietic reconstitution or at the discretion of the Investigator. Subjects will receive a myeloablative dose of busulfan (3.2 mg/kg/day; administered IV via central venous catheter either once daily or every 6 hours) for 4 days (total dose of 12.8 mg/kg). For detailed instructions for administering IV busulfan refer to the Study Cell Therapy Manual.

After the first dose, the IV busulfan dose will be adjusted based upon pharmacokinetic sampling and study center practices to target an AUC of 4,000-5,000 µmol∗min for daily dosing or an AUC of 1,000-1,250 µmol∗min for every 6 hour dosing for a total regimen target AUC of 16,000-20,000 µmol∗min. IV busulfan pharmacokinetic targeting may be modified for subsequent subjects based on experience with previous subjects after discussion with the SMC. Therapeutic drug monitoring to determine clearance of busulfan after 4 days of dosing is complete is not required but may be performed at the discretion of the Investigator in accordance with study center practices. Please refer to Section 9.3 for additional information on concomitant medications.

The following assessments and procedures will be performed daily during conditioning (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs
- Physical examination
- Vital signs
- Transfusion log
- Samples for central laboratory analysis ("Clinical laboratory tests") should be collected at least once during conditioning, with timing at the discretion of the Investigator

Additional safety precautions, assessments, and treatments conducted as part of standard transplant medical care outside of those specified in this study (e.g., clinical laboratory testing, immune function testing, cytomegalovirus (CMV) prophylaxis, etc.) will be undertaken as per Investigator's judgement and study center practice. These include, but are not limited to, antimicrobial prophylaxis, RBC transfusion to maintain hemoglobin at levels deemed appropriate for the subject, and platelet transfusions as needed.

7.7 Day 0 (ST-400 Infusion)

The ST-400 drug product will be infused no sooner than 72 hours after the final dose of IV busulfan in the conditioning phase. For detailed instructions for thawing and administering ST-400 see the Study Cell Therapy Manual.

The target cell dose of ST-400 to be administered is 10 x 10^6 cells/kg body weight and the minimum cell dose required is 3.0 x 10^6 cells/kg body weight (cell dose is determined by the yield during apheresis). Subjects may receive more than one lot of ST-400 if multiple apheresis cycles were required to ensure the minimum cell dose. No dose modifications of ST-400 are possible within an individual subject since this is a single infusion study. G-CSF may be administered post-transplant as per the Investigator's judgement.

The following assessments and procedures will be performed (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs
- Physical examination
- Serial vital signs as detailed in the Study Reference Manual
- Clinical laboratory tests
- ECG
- HbF quantification
- Transfusion log
- Thalassemia-related disease biomarkers

Vital signs (temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation) will be monitored before, during, and after the infusion as detailed in the Study Reference Manual, and as needed to maintain good clinical care in accordance with study center practice.

Additional safety precautions, assessments, and treatments conducted as part of standard post-transplant medical care outside of those specified in this study (e.g., clinical laboratory testing, immune function testing, cytomegalovirus (CMV) prophylaxis, G-CSF administration, etc.) will be undertaken as per Investigator's judgement and study center practice. This includes, but is not limited to, antimicrobial prophylaxis, G-CSF administration, RBC transfusion to maintain hemoglobin at levels deemed appropriate for the subject, and platelet transfusions as needed.

7.8 Days 7 (+/-1 day), 14 (+/-1 day), 21 (+/-1 day), 28 (+/-1 day), 35 (+/- 2 days), 42 (+/-2 days), 56 (+/-5 days), and 90 (+/-7 days); and Weeks 26, 39, and 52 (+/- 14 days) (Primary Study Period)

The following assessments and procedures will be performed at each visit unless stated otherwise (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs

- Physical examination
- Vital signs
- Karnofsky performance scale (Weeks 26 and 52)
- Pregnancy test (for females with reproductive potential only; Days 28, 56 and 90, and Weeks 26, 39, and 52)
- PFTs (Week 52)
- Clinical laboratory tests
- ECG (Day 28; Weeks 26 and 52)
- Screening for Malignancy (Days 28, 35, 42, 56, and 90; Weeks 26, 39, and 52)
- *BCL11A* gene modification assay (Days 14, 28, 42, 56, and 90; Weeks 26, 39, and 52)
- HbF quantification (Days 14, 28, 42, 56, and 90; Weeks 26, 39, and 52)
- Immunological assays (Day 90; Week 52)
- Transfusion log
- Thalassemia-related disease biomarkers (Day 90; Weeks 26, 39, and 52)
- Endocrine lab testing (Week 52)
- Subset analysis of gene modification (Week 39)
- Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy (Day 90; Weeks 26, 39, and 52)
- F-cell Percentage (Days 28, 56, and 90; Weeks 26 and 52)
- ECHO (Week 52)
- Liver MRI (Week 52)
- Cardiac MRI (Week 52)
- DXA (Week 52)
- SF-36 Survey (Weeks 26 and 52)
- Bone marrow aspiration:
    - Day 90: can be waived at the discretion of Investigator;
    - Bone marrow aspirate may be performed at *either* the Week 26 *or* the Week 39 visit; it should not be performed at both. Both of these procedures (at both Week 26 and Week 39) may be waived at the Investigator's discretion.
    - Week 52

7.9 Weeks 65, 78, 91, 104, 117, 130, 143, and 156 (+/- 14 days) (Follow-up Study Period)

The following assessments and procedures will be performed at each visit unless stated otherwise (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs
- Physical examination
- Vital signs
- Karnofsky performance scale (Weeks 104 and 156)
- Clinical laboratory tests
- ECG (Weeks 104 and 156)
- Screening for Malignancy
- *BCL11A* gene modification assay (Weeks 78, 104, 130, and 156)
- HbF quantification
- Immunological assays (Weeks 65 and 117)
- Transfusion log
- Thalassemia-related disease biomarkers (Weeks 78, 104, 130, and 156)
- Endocrine lab testing (Weeks 65 and 117)
- Subset analysis of gene modification (Weeks 91 and 143)
- Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy (Weeks 78, 104, 130, and 156)
- ECHO (Weeks 104 and 156)
- Liver MRI (Weeks 104 and 156)
- Cardiac MRI (Weeks 104 and 156)
- DXA (Weeks 104 and 156)
- SF-36 Survey (Weeks 104 and 156)
- Bone marrow aspiration (Week 104)

7.10 Early Termination Visit

Subjects who discontinue from the study prematurely will be asked to return to the study site for an early termination visit (ETV). It is at the discretion of the Investigator, in consultation with the Medical Monitor, to waive any procedure if the procedure has been performed within the standard interval of scheduled study visits per protocol or if the procedure would place undue burden on the subject.

The following assessments and procedures will be performed at the ETV (see Section 9 for details on Study Assessments):

- Review and record concomitant medications
- Assess AEs
- Physical examination
- Vital signs
- Karnofsky performance scale
- Pregnancy test (for females of reproductive potential only)
- Clinical laboratory tests
- ECG
- Screening for Malignancy
- *BCL11A* gene modification assay
- HbF quantification
- Immunological assays
- Transfusion log
- Thalassemia-related disease biomarkers
- Endocrine lab testing
- Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy
- ECHO
- Liver MRI
- Cardiac MRI
- SF-36 Survey
- Bone marrow aspiration

7.11 Rescue Treatment

Should a subject experience delayed hematopoietic reconstitution or graft failure with aplasia, the subject will receive the rescue treatment consisting of unmodified, autologous CD34+ HSPCs, and an SMC safety review will be performed under the study suspension rules. Delayed hematopoietic reconstitution or graft failure with aplasia is defined as persistent ANC <500 cells/µL through Day 42 post-infusion. The rescue treatment may be administered at any time after ST-400 infusion if needed at the discretion of the Investigator based on clinical indications for subject safety.

7.12 Unscheduled Visits

Unscheduled visits may be performed at any time during the study whenever necessary to assess or to follow-up on AEs, or as deemed necessary by the Investigator. Evaluations and procedures to be performed at unscheduled visits will be at the Investigator's discretion in consultation with the Sponsor, and may be based on those listed in the Schedule of Events (see Appendix 1).

7.13 Subject Withdrawal and Discontinuation from Study

Subjects may withdraw or should be discontinued from the study for any of the following reasons:

- Request by the subject to withdraw.
- Request of Sponsor or Investigator if he or she thinks the study is no longer in the best interest of the subject.
- Pregnancy of subject prior to IV busulfan infusion.
- Subject judged by the Investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.
- At the discretion of the institutional review board (IRB), Office for Human Research, regulatory authority (e.g., FDA), Investigator, or Sponsor.

Subjects will be strongly encouraged to continue and comply with follow-up safety evaluations. If a subject withdraws consent or discontinues from the study post-study treatment, a conference between the Investigator and Medical Monitor will take place to ensure and document that the subject understands the importance of the study follow-up and that the study treatment cannot be revised even if a subject drops out of the study follow-up. If the subject agrees, a reduced follow-up testing schedule may be arranged including telephone call and safety labs to assess treatment-related AEs and disease status.

7.14 Long-term Follow-up Protocol for Adverse Effects of Gene Therapy

All subjects will be followed for a total of 3 years after ST-400 dosing under this protocol.

To assess long-term safety, subjects will be asked to participate in a separate long-term follow-up (LTFU) study after they complete this study. The total combined (current and LTFU studies) follow up period will last 15 years. The LTFU study will be conducted by Bioverativ Therapeutics Inc. (Sanofi) as both companies are developing equivalent gene-edited cell products, ST-400 for the treatment of transfusion-dependent β-thalassemia and Sanofi BIVV003 for the treatment of sickle cell disease.

A separate informed consent will be obtained for subjects to be followed in a LTFU study.

8. STUDY PRODUCT AND OTHER MEDICATIONS

8.1 ST-400

ST-400 is composed of autologous CD34+ HSPCs that are genetically modified *ex vivo* at the erythroid-specific enhancer of the *BCL11A* gene by the ZFN mRNAs SB-mRENH1 and SB-mRENH2 to boost endogenous production of HbF.

Component	Unit Formula
BCL11A erythroid-specific enhancer modified CD34+ HSPCs	10 million cells/mL
Cryopreservation Buffer (CryoStor® CS10) containing 10% Dimethyl Sulfoxide (DMSO)	1 mL

ST-400 is cryopreserved in 50 mL CryoMACS® freezing bags (fill volume of approximately 10 to 20 mL; total cell count of approximately 1.0 x 10^8 to 2.0 x 10^8 cells) using a controlled rate freezer. Multiple freezing bags are used if cell yield exceeds the capacity of a single bag. Infusion bags are stored in vapor phase liquid nitrogen (at ≤ -150°C) at the manufacturing facility until they are ready to be shipped to the clinical study center.

ST-400 will be shipped to clinical study sites in a liquid nitrogen (LN2) Dry Shipper with a temperature monitoring device. A Certificate of Analysis (COA) and Clinical Certificate of Analysis (CCOA) will accompany each shipment. Each freezing bag will carry a label that identifies the product name, sponsor/manufacturer, protocol number, date of manufacture, subject ID number, subject initials, lot number, bag number, "FOR AUTOLOGOUS USE ONLY", and standard mandated language for products for investigational use. Additional details on the ST-400 investigational product and infusion protocol are provided in the Study Cell Therapy Manual.

8.1.1 Inventory, Storage, and Handling

Upon receipt and prior to administration, the infusion bag(s) are stored in the LN2 Dry Shipper or freezer capable of maintaining ≤ -150°C (with temperature monitoring).

No individuals other than those who are authorized by the Investigator to administer the study drug shall have access to the study drug. The Investigator agrees not to supply study drug to any person other than study personnel and subjects in this study.

The study center is required to maintain complete records of all study drug received during this study, as well as of any study drug that is dispensed or disposed of. At the conclusion or termination of this study, destruction of all drug supplies must be pre-approved and coordinated with Sangamo (see the Study Cell Therapy Manual for additional details).

8.1.2 Administration

ST-400 shall be infused no sooner than 72 hours after the final dose of IV busulfan in the conditioning phase.

Following thaw, ST-400 will be infused per detailed instructions set forth in the Study Cell Therapy Manual.

At the time of ST-400 receipt, 2 individuals will independently confirm that the information from the study drug is correctly matched to the subject.

Immediately prior to infusion, 2 individuals will independently and in the presence of the subject confirm that the information from the study drug is correctly matched to the subject.

8.1.3 Precautions

Emergency medical equipment and appropriately-trained medical personnel for the infusion of cryopreserved HSPC grafts must be available during the infusion in case the subject has an allergic response, severe hypotensive crisis, or any other reaction to the infusion.

Vital signs (temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation) must be taken before, during, and after infusion as detailed in the Study Reference Manual and in accordance with study center practice (see Section 9).

If a subject develops febrile neutropenia, veno-occlusive disease (VOD), or other AEs related to the conditioning procedure, appropriate medical management should be undertaken as per the Investigator's clinical judgement. AEs should be assessed and reported. Additional information, guidance, and recommendations on post-transplant care are provided in the Study Cell Therapy Manual and Study Reference Manual.

If possible contamination of the ST-400 study drug is suspected, the study site will not proceed with infusion and will contact Sangamo.

8.2 Intravenous Busulfan

This protocol will exclusively use IV busulfan; oral administration will not be allowed.

Busulfan is a bifunctional alkylating agent that causes DNA-DNA crosslinks and triggers cell apoptosis. Because it specifically affects non-dividing stem cells in the bone marrow, busulfan is used either alone or in combination with other agents (e.g., cyclophosphamide) as the myeloablative conditioning agent of choice for allogenic and autologous HSCT.

8.2.1 Inventory, Storage, and Handling

Please refer to package insert.

8.2.2 Administration

Please refer to package insert.

For detailed guidance on IV busulfan therapeutic drug monitoring and dose adjustment based upon pharmacokinetic sampling, refer to the Study Cell Therapy Manual.

8.2.3 Precautions

Please refer to the busulfan package insert for complete safety information.

8.3 G-CSF

G-CSF is a 175 amino acid polypeptide that regulates the production of granulocytes within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and activation. In addition, G-CSF is a potent inducer of CD34+ HSPC mobilization from the bone marrow into the bloodstream. G-CSF may be administered by either subcutaneous or IV route. Pegylated G-CSF should not be used. G-CSF and filgrastim are used interchangeably in this protocol.

8.3.1 Inventory, Storage, and Handling

Please refer to package insert.

8.3.2 Administration

Please refer to package insert and Study Cell Therapy Manual.

8.3.3 Precautions

Please refer to the G-CSF package insert for complete safety information.

8.4 Plerixafor

Plerixafor reversibly inhibits binding of stromal cell-derived factor-1-alpha (SDF-1α), expressed on bone marrow stromal cells, to the CXC chemokine receptor 4 (CXCR4), resulting in mobilization of CD34+ HSPCs from bone marrow into peripheral blood. Plerixafor used in combination with G-CSF results in synergistic increase in CD34+ HSPCs mobilization.

8.4.1 Inventory, Storage, and Handling

Please refer to package insert.

8.4.2 Administration

Please refer to package insert and Study Cell Therapy Manual.

8.4.3 Precautions

Please refer to the plerixafor package insert for complete safety information.

8.5 Concomitant Medication and Supportive Care

It is recommended that subjects have completed their routine vaccinations (hepatitis A, HBV, pneumococcus, influenza, and tetanus diphtheria booster) at least 30 days prior to the conditioning procedure.

It is further recommended that subjects receive all supportive therapy and standard medical care deemed appropriate by the Investigator and per study center standard procedures. This includes, but is not limited to, antimicrobial prophylaxis, G-CSF administration, RBC transfusion to maintain hemoglobin at levels deemed appropriate for the subject, and platelet transfusions as needed. Seizure prophylaxis during IV busulfan is strongly recommended. Iron-chelating agents should be discontinued 2 weeks prior to busulfan infusion, and may be restarted after successful hematopoietic reconstitution or at the discretion of the Investigator.

Concomitant medications such as acetaminophen or metronidazole should be avoided in the peri-transplant period as they may reduce the clearance of busulfan and thus cause excessive busulfan toxicity. Additional guidance on busulfan drug interactions with commonly used concomitant medications is provided in the Study Cell Therapy Manual.

Potential side effects that are associated with DMSO following ST-400 infusion may include transient fever, chills, and/or nausea. Serious allergic reaction in association with ST-400 administration has been reported (refer to Section 1.9.1 for additional details). Pre-medication or post-infusion treatment with acetaminophen, diphenhydramine hydrochloride (Benadryl), and glucocorticosteroids (such as hydrocortisone), may be administered and repeated as needed per site practice and Investigator's judgment.

Following the conditioning procedure, subjects will be isolated in single rooms to reduce the risk of infections, and appropriate precautions will be taken while the ANC is <500 cells/μL per the site's standard practice, including anti-bacterial and anti-fungal prophylaxis. Standard-of-care treatment should be given to manage febrile neutropenia. Routine monitoring for CMV re-activation and pre-emptive therapy should be performed as per study center practices. Medications for the prevention and treatment of hepatic VOD/sinusoidal obstruction syndrome, including ursodeoxycholic acid and defibrotide, may be administered at the discretion of the Investigator.

The Investigator will record on the concomitant medications page in the subject's electronic case report form (eCRF) all concomitant medications, including over-the-counter medicinal products, dietary supplements, herbal medications, and medications given in treatment of AEs, taken by a subject from Screening throughout this study.

8.6 Prohibited Medications

The following medications are prohibited during the study as they may interfere with safety and or efficacy evaluation of the study drug, unless the Investigator deems they are medically necessary:

- Hydroxyurea
- Erythropoietin or analogue
- Systemic chemotherapeutic agents
- Chronic systemic immunosuppressive medications
- Experimental medications
- Any medication expected to alter HbF levels

Subjects who have been treated with a prohibited medication in the 30 days prior to the screening visit are not eligible to participate in this study. Iron-chelating agents should be discontinued 2 weeks prior to conditioning with IV busulfan and may be restarted after successful hematopoietic reconstitution or at the discretion of the Investigator.

9. STUDY ASSESSMENTS

9.1 Medical History

A complete medical history, including prior surgeries and procedures, will be recorded for each subject at the screening visit.

9.2 Demographics

Demographic data on each subject (e.g., age, gender, race, ethnicity, etc.) will be obtained at the screening visit.

9.3 Concomitant Medications

All history of medications taken prior to consent (prior medications) or at the time of or following consent (concomitant medications) for each subject will be recorded. Please refer to Section 8.6 for the list of prohibited medications.

9.4 Transfusion History

History of RBC transfusions (mL packed RBC/kg, volume, date, etc.) will be recorded for each subject at the screening visit based on medical documentation. A minimum of 2 years of transfusion history prior to Screening is required to be eligible to participate in this study; however up to 3 years of history will be recorded if available.

9.5 Physical Exam

A physical examination will be conducted on each subject at the specified visits and will include at a minimum: general appearance; head, eyes, ears, nose, and throat (HEENT); as well as cardiovascular, dermatologic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. Any clinically significant changes from previous visits or new findings should be documented in the source and reported as an adverse event.

9.6 Vital Signs

Vital signs, including systolic/diastolic blood pressure, heart rate, temperature, respiratory rate, and oxygen saturation, will be recorded at the specified study visits for each subject. Subject height will be measured at the Screening visit only. Subject weight will be recorded at the Screening, Mobilization & Apheresis, and the Baseline visits.

9.7 Karnofsky Performance Scale

The Karnofsky Performance Scale is a simple, widely-accepted tool for evaluating functional impairment in patients. Each subject will be evaluated and scored at the specified visit using the Karnofsky Performance Status Scale Definitions Rating Criteria. Subjects with a score on the Karnofsky Performance Scale ≤60 at the screening visit are not eligible to participate in this study.

9.8 Pregnancy Test

Pregnancy testing will be conducted on all female subjects with reproductive potential by serum or urine analysis at specified visits. A female subject is considered of reproductive or childbearing potential, i.e., fertile, following menarche and until becoming post-menopausal (defined as no menses for 12 months without an alternative medical cause), unless permanently sterile due to hysterectomy, bilateral salpingectomy or bilateral oophorectomy. Serum pregnancy testing should be conducted at the Screening, Mobilization & Apheresis (within one week prior to administration of the first mobilizing agent) and Baseline visits (within one week prior to first busulfan infusion).

Urine pregnancy testing should be conducted at specified post-infusion visits (refer to Appendix 1). A positive or equivocal urine pregnancy test result will be confirmed with a serum pregnancy test. If the result is positive, the Sponsor will be notified directly, and a Pregnancy and Partner Pregnancy Reporting Form submitted within 24 hours of discovery. Pregnant or breastfeeding subjects are not eligible to enroll into this study.

9.9 Pulmonary Function Tests (PFTs)

Pulmonary function testing is a common method to evaluate respiratory function and oxygen transfer prior to myeloablative conditioning. Each subject will undergo PFTs at the screening visit and Week 52. Subjects with corrected DLCO ≤50% of predicted or clinically-significant restrictive lung disease are not eligible to participate in this study. If more than 30 weeks will elapse between the start of the Screening visit and the Baseline visit, PFT will need to be repeated to confirm inclusion/exclusion criteria (Section 7.5).

9.10 Chest X-ray

Chest X-ray (also known as AP radiograph of the chest) will be obtained on each subject at the screening visit to evaluate the general health of the subject and eligibility for the study per the Investigator's clinical judgement. If more than 30 weeks will elapse between the start of the Screening visit and the Baseline visit, a chest X-ray will need to be repeated to confirm inclusion/exclusion criteria (Section 7.5).

9.11 Infectious Disease Panel

Clinical laboratory testing for HIV, HBV, and HCV will be conducted at the screening visit. Subjects with a diagnosis of HIV or evidence of active HBV or HCV infection are not eligible to participate in this study.

9.12 Clinical Labs

Clinical laboratory testing will be conducted at the specified visits as detailed in Appendix 1. Additional testing outside of what is specified in the protocol may be conducted as part of the apheresis procedure or post-transplant medical care as per Investigator's clinical judgement and study center practices. Refer to Table 1 for the list of individual clinical laboratory tests that will be obtained in this study.

Table 1: Clinical Laboratory Tests

Hematology CBC with differential Reticulocyte count Peripheral smear	**Serum Chemistry** Sodium (Na) Potassium (K) Chloride (Cl) Carbonate (CO_3^{2-}) Calcium (Ca) Phosphate (PO_4^{3-}) Magnesium (Mg^{2+}) BUN Creatinine Glucose LDH Uric acid
Liver Function Bilirubin (total and direct) ALKP ALT AST Albumin Total protein Ferritin	**Urine** (with microscopic examination) Glucose Protein Bilirubin Blood pH Specific gravity Leukocyte esterase
CBC = complete blood count; ANC = absolute neutrophil count; ALKP = alkaline phosphatase; ALT = alanine aminotransferase (SGPT); AST = aspartate aminotransferase (SGOT); LDH = lactate dehydrogenase; BUN = blood urea nitrogen.	

9.13 Electrocardiogram (ECG)

A 12-lead ECG will be obtained for each subject at specified visits as a safety assessment to provide a baseline and to monitor for potential AEs on cardiac function/conduction.

9.14 Liver Biopsy

Liver biopsy is a routine procedure to detect occult liver disease that may predispose a subject to excessive busulfan toxicity. Subjects with bridging fibrosis, liver cirrhosis, or active hepatitis based on liver biopsy are not eligible to participate in this study. Liver biopsy must be performed at Screening or in the 12 months prior to Screening with adequate medical documentation.

9.15 Comprehensive Hemoglobin Analysis

Comprehensive hemoglobin analysis will be conducted on all subjects at Screening to assess for HBA1/2 (α-globin) and HBB (β-globin) mutations and/or deletion/duplication analysis, and HBD (δ-globin) and HBG1/2 (γ-globin) mutations. This testing will provide molecular confirmation and characterization of the β-thalassemia genotype. Subjects who are found to have γ-globin allelic variants associated with clinically significant altered oxygen affinity are not eligible to participate in this study (refer to Appendix 2).

9.16 Screening for Potential Hematological Malignancies

Although ST-400 is designed to have highly precise gene modification without insertional mutagenesis, the risk for development of a hematological malignancy remains undefined. Therefore, subjects will be closely monitored throughout the study for the potential development

of hematopoietic malignancies using clinical history, physical exams, laboratory assessments, and bone marrow aspirations for:

- new or worsened unexplained lymphadenopathy;
- new or worsened unexplained hepatosplenomegaly;
- unexplained WBC >30,000/µL or ANC <500 cells/µL confirmed on repeat testing;
- unexplained platelet count <50,000/µL confirmed on repeat testing;
- clinical suspicion of malignancy, which may include unexplained and persistent symptoms of weight loss, anorexia, fatigue, fevers, headache, etc., based on Investigator's judgement; or
- clinically-significant abnormality on bone-marrow aspiration suggestive of malignant transformation.

If any of the above criteria are met at any time, the Sponsor will be notified and the subject will undergo a clinical work-up for the evaluation of malignancy by a medical oncologist, which may include physical exam, laboratory testing, imaging, and bone marrow examination. In addition, an expedited analysis of markers of gene modification may be performed as described in Section 9.17. If the diagnosis of hematological malignancy is confirmed, a study suspension rule will have been met, and all enrollment/dosing will be halted. An SMC will then convene and review all data to provide recommendations on the future of the study.

In addition to the clinical monitoring described above, for each subject the presence or absence of clonal populations will be assessed over time using high-throughput sequencing technology. If "clonal dominance" within the indel pattern is detected (See Section 9.17), then the subject will undergo a clinical work-up for the evaluation of malignancy as above.

9.17 BCL11A Gene Modification Assay

The proposed mechanism of action of ST-400 is to produce precise genetic modification of the erythroid-specific enhancer of the *BCL11A* gene through the use of engineered ZFNs. The percentage and durability of gene modification at the *BCL11A* locus by ST-400 will be monitored at the time of product manufacturing and throughout the study. Analysis of the percentage of indels among all alleles, as well as dominance and frequency of any given indel will be performed using high-throughput sequencing technology.

Hematopoietic "clonal dominance" based on indel analysis will trigger a clinical work-up for the evaluation of hematological malignancy (see Section 9.16). "Clonal dominance" is defined using the following algorithm based on the dominant indel detected:

- If the dominant indel is ≥90% of the whole at any time in any bone marrow aspirate, unseparated whole blood or lineage-sorted blood samples, then "clonal dominance" has occurred;
- If the dominant indel is ≥30%, then analysis should be repeated on peripheral blood (unsorted, T-lineage and B-lineage) two more times with samples taken at 3-month intervals (ad hoc sampling to be conducted if necessary).

- If on 1st or 2nd recheck the dominant indel is <30%, then "no clonal dominance" and return to monitoring per the routine schedule of events;
- If on 1st recheck the dominant indel is ≥30% and <90%, and 2nd recheck it is <50%, then "no clonal dominance" and return to monitoring per the routine schedule of events;
- If on 1st recheck the dominant indel is ≥30% and <90%, and 2nd recheck is ≥50%, then "clonal dominance" has occurred.

9.18 Subset Analysis of BCL11A Gene Modification

This assay is the same as the BCL11A Gene Modification Assay, but is performed on lineage-specific hematopoietic cells, which have previously been sorted from the clinical sample.

Hematopoietic "clonal dominance" based on indel analysis is defined using the same algorithm presented above (see Section 9.17).

9.19 Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy

ST-400 is designed to have highly-precise gene modification at the erythroid-specific enhancer of the *BCL11A* gene with minimal gene modification detectable elsewhere in the genome. Clinical samples will be banked at specified time points, and in the event of a hematological malignancy, these samples may undergo additional retrospective analysis to better characterize the malignancy. These additional tests could include, but are not limited to, molecular translocation assays and analysis of off-target gene modification.

9.20 HbF Quantification

Genetic modification of the erythroid-specific enhancer of the *BCL11A* gene by ST-400 is expected to boost endogenous levels of HbF by diminishing the inhibitory effect of *BCL11A* on transcription of the γ-globin genes. Peripheral blood samples will be collected in order to measure Hb fractions (A and F in g/dL), and to quantify percent HbF for all subjects at Screening, Baseline, and throughout this study.

9.21 Immunological Assays

Immunological assays (including, but not limited to lymphocyte subsets and quantitative immunoglobulins) will be conducted on peripheral blood samples collected from all subjects at Screening and post-conditioning to evaluate hematopoietic reconstitution and immune recovery/function.

9.22 Transfusion Log

RBC transfusions will be closely monitored in this study. At the specified visits, any history of the occurrence or absence of RBC transfusions since the last visit should be elicited and documented (mL PRBC/kg, volume, date, etc.). Therapeutic phlebotomy, if required, should also be documented on the transfusion log.

Additionally, other blood products including, but not limited to, platelets, intravenous immunoglobin (IVIG), plasma, and granulocytes that are administered during the study should be documented in the transfusion log.

9.23 Thalassemia-related Disease Biomarkers

Thalassemia-related disease biomarkers will be tested throughout this study to evaluate the effect of ST-400 on erythropoiesis and iron overload. These exploratory biomarker tests may include, but are not limited to, iron metabolism, erythropoietin, haptoglobin, and hepcidin levels.

9.24 Endocrine Lab Testing

Chronic iron overload in TDT leads to iron deposition in endocrine organs and subsequent endocrine dysfunction. Endocrine laboratory testing may include, but is not limited to, thyroid studies, IGF-1, morning cortisol, adrenocorticotropic hormone (ACTH), HbA1C, and vitamin D studies.

9.25 Echocardiogram (ECHO)

A standard 2-dimensional Doppler ECHO will be obtained at the specified visits to evaluate cardiac function, including left ventricular ejection fraction (LVEF), regional wall motion, and valvular morphology and function. Subjects with LVEF <40% based on the Screening ECHO are not eligible to participate in this study. If more than 30 weeks will elapse between the start of the Screening visit and the Baseline visit, an ECHO will need to be repeated to confirm inclusion/exclusion criteria (Section 7.5).

9.26 Liver MRI

Liver disease and hepatomegaly are common co-morbidities of TDT due to increased RBC destruction and extramedullary erythropoiesis. The accelerated rate of erythropoiesis enhances dietary iron absorption from the gut, resulting in a chronic state of iron overload analogous to that seen in hereditary hemochromatosis. MRI is the standard method for evaluating iron deposition in the hepatocytes and Kupffer cells, revealing such hepatic iron overload as reduced R2-MRI assessed by FerriScan®.

9.27 Cardiac MRI

Cardiac abnormalities, including heart failure and fatal arrhythmias, are major complications of TDT and frequent causes of death. Life-long transfusion therapy ameliorates cardiac pathology; however, TDT patients frequently develop cardiac hemosiderosis due to myocardial iron deposition. Cardiac MRI is the standard for evaluation of iron deposition in the myocardium, revealing such myocardial iron overload as reduced myocardial T2* (T2 star). Subjects with myocardial T2*<10 msec at Screening are not eligible to participate in the study.

9.28 DXA

Osteoporosis and fractures are a common complication of TDT (Vogiatzi et al., 2009). DXA is a convenient and reliable method to determine bone mineral density and to evaluate the presence of osteoporosis and fracture risk with low exposure to radiation.

9.29 SF-36 Survey

The SF-36 Survey is a quality-of-life questionnaire for assessing quality of life that is widely-accepted, validated, and easily administered. The SF-36 Survey relies upon patient self-reporting. It is commonly utilized in clinical drug development, and has been studied in patient populations with TDT (Sobota et al., 2011).

9.30 Bone Marrow Aspiration

Bone marrow aspiration is a routine clinical procedure to characterize hematopoiesis. TDT leads to profound erythroid hyperplasia with a high degree of immature cells and erythroid precursors of often bizarre morphologies. Bone marrow aspiration will be obtained to evaluate changes from baseline in erythropoiesis and gene modification by ST-400, including in unsorted and lineage sorted subpopulations, and other exploratory analyses. Bone marrow aspirates will be assessed at the clinical site for routine hematopathology review to screen for potential malignant transformation per the site's standard clinical practice following transplantation. Unscheduled bone marrow aspiration may also be conducted at any time if clinically indicated, such as to evaluate the potential development of hematological malignancy.

9.31 Percentage of F-cells

F-cells are red blood cells that contain measurable amounts of HbF. Blood samples will be collected for analysis of percentage of F-cells.

10. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

AEs and SAEs will be monitored continuously during the study from the time that the subject has provided a signed ICF through the subject's last day of study participation. Subjects will be queried and events will be assessed at each clinic visit. Subjects will be reminded to immediately report any AE to the Investigator. All AEs will be recorded in the subject's CRF. The detailed description of the event will include appropriately graded severity of the AE and its relationship to the study drug.

10.1 Definitions of an Adverse Event

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related. An AE can include any of the following events that develop or increase in severity during this study:

- Any sign, symptom, or physical examination finding that worsens in nature, severity, or frequency compared to baseline status (i.e., prior to screening), whether thought to be related or unrelated to the condition under study.
- Any clinically significant laboratory abnormality or laboratory abnormality that requires medication or hospitalization. Abnormal laboratory results will be graded based on Common Terminology Criteria for Adverse Events (CTCAE) 5.0 criteria. An asymptomatic Grade 1 or 2 clinical laboratory abnormality should be reported as an AE only if it is considered clinically significant by the Investigator. A Grade 3 and 4 clinical laboratory abnormality that represents an increase in severity from baseline should be reported as an AE if it is not associated with a diagnosis already reported on the CRF.
- All events associated with the use of ST-400, including those occurring as a result of an overdose, abuse, withdrawal phenomena, sensitivity, or toxicity to ST-400.
- Concurrent illness.
- Injury or accident.

A pre-existing condition is one that is present prior to screening, and is to be reported as part of the subject's medical history. It should be reported as an AE only if the frequency, intensity, or the character of the condition worsens during study treatment.

10.2 Classification of an Adverse Event

The Investigator is responsible for determining

- if the AE meets the criteria for an SAE,
- the relationship of the AE to study treatment, and
- the severity of the AE.

10.2.1 Definition of a Serious Adverse Event

A SAE is any AE that in the view of either the Investigator or Sangamo results in any of the following outcomes:

- Death.

- Life-threatening threatening event (i.e., an event that places the subject at immediate risk of death); however, this does not include an event that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- Congenital anomaly/birth defect in the offspring of an exposed subject.
- A medically important event.

A medically important event is an AE that may not result in death, be life-threatening, or require hospitalization, but may be considered serious when, based upon appropriate medical judgment, the AE may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above. (Examples of such medical events include but are not limited to allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.)

10.2.2 Determination of Relationship of Adverse Event to Study Treatment

The Investigator is responsible for classifying an AE and SAE as either Related or Not Related to ST-400 treatment. Additional information will be collected on suspected relationship to study procedures (i.e., apheresis) and/or other protocol-required medications (i.e., plerixafor, G-CSF, and busulfan).

The following definitions should be considered when evaluating the relationship of AEs and SAEs to ST-400 treatment, study procedures, and/or other protocol-required medications.

- Not related

  An AE will be considered "not related" to the use of the investigational product or study procedures if there is not a reasonable possibility that the event has been caused by the product under investigation or the study procedures. Factors pointing toward this assessment include but are not limited to the lack of reasonable temporal relationship between administration of the product and the AE, the presence of a biologically implausible relationship between the product and the AE, or the presence of a more likely alternative explanation for the AE.

- Related

  An AE will be considered "related" to the use of the investigational product or study procedures if there is a reasonable possibility that the event may have been caused by the product under investigation or the study procedures. Factors that point toward this assessment include but are not limited to a positive rechallenge, a reasonable temporal sequence between administration of the product and the AE, a known response pattern of the suspected product, improvement following discontinuation or dose reduction, a biologically plausible relationship between the product and the AE, or a lack of an alternative explanation for the AE.

10.2.3 Determination of Severity of Adverse Event

Severity will be categorized by toxicity grade according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. AEs not listed in the CTCAE Version 5.0 will be evaluated by using the following criteria:

- 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.

In the event of death, the cause of death should be recorded and reported as an SAE. "Death" is not the AE; "death" is an outcome. The term "death" should be reported as an SAE only if the cause of death is not known and cannot be determined. If an autopsy is performed, a copy of the autopsy report should be obtained if possible. The Investigator should make every effort to obtain and send death certificates and autopsy reports to Sangamo.

10.2.4 Determination of Expectedness of Adverse Event

The expectedness of all AEs will be determined by the Sponsor according to the Investigator's Brochure.

10.3 Adverse Event Reporting

All AEs should be reported starting from the time of signing of the ICF through the subject's last study visit. The Investigator is responsible for evaluating all AEs, obtaining supporting documents, and determining that documentation of the event is adequate. The Investigator may delegate these duties to sub-Investigators but must assure that these sub-Investigators are qualified to perform these duties under the supervision of the Investigator. All AEs will be recorded in the subject's CRF.

10.4 Serious Adverse Event Reporting

The reporting period for all SAEs starts from the time of signing of the ICF through the subject's last study visit.

All SAEs, whether or not considered to be associated with the administration of ST-400, must be reported immediately to Sangamo or its designees through the electronic data capture (EDC) system within 24 hours of the study staff becoming aware of the event. A paper SAE Report Form may be used as a back-up option when the eCRF system is not functioning or is inaccessible. Please refer to the Study Reference Manual for additional SAE reporting guidelines.

Investigator must follow SAEs until resolution, stability, or return to baseline, even after the subject completes or discontinues the study (i.e., SAEs should not be left open).

The Medical Monitor will then advise the Investigator regarding the nature of any further information or documentation that is required. Follow-up reports must be submitted within 24 hours from the time that the additional information becomes available.

10.5 Specific Serious Adverse Events

In this study, the following AEs are considered medically important and must be reported as SAEs:

- Malignancy (excluding non-melanoma skin cancer and cervical cancer in situ)
- Grade ≥3 hypersensitivity reaction associated with administration of ST-400 according to CTCAE v5.0
- Delayed hematopoietic reconstitution or graft failure with aplasia requiring the administration of the rescue aliquot

10.6 Suspected Unexpected Serious Adverse Reactions

A suspected unexpected serious adverse reaction (SUSAR) is any SAE that is assessed as both unexpected and, in the view of the Principal Investigator and/or the Sponsor, as related to the study treatment.

The Sponsor or its designee will submit SUSAR reports to appropriate regulatory authorities, health authorities, and Investigators as per local laws and regulations.

The Investigator is responsible for promptly notifying the IRB in accordance with local regulations of all SAEs.

11. PROCEDURES FOR HANDLING SPECIAL SITUATIONS

11.1 Pregnancy and Contraception Requirements

Plerixafor and busulfan have been shown in animal studies to be teratogenic; busulfan has been shown to be genotoxic. The effects of administration of ST-400 on the pregnant female or the developing fetus are unknown. Thus, male subjects and female subjects with reproductive or childbearing potential must agree to use an effective method of contraception during the study.

Male and female subjects with reproductive potential must agree to use contraception from the signing the informed consent through the Primary Study Period.

Male subjects are considered fertile after puberty unless permanently sterile by bilateral orchidectomy. Female subjects are considered of reproductive or childbearing potential, i.e., fertile, following menarche and until becoming post-menopausal (defined as no menses for 12 months without an alternative medical cause), unless permanently sterile due to hysterectomy, bilateral salpingectomy or bilateral oophorectomy.

The following are considered effective methods of birth control:

- Combined (estrogen and progestogen containing) hormonal contraception associated with the inhibition of ovulation:
  - Oral, or
  - Intravaginal, or
  - Transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - Oral, or
  - Injectable, or
  - Implantable
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Bilateral tubal occlusion
- Vasectomized partner (provided that partner is the sole sexual partner)
- Sexual abstinence (refraining from heterosexual intercourse for the duration of the protocol-defined period)
- Male condom with spermicide AND cervical cap
- Male condom with spermicide AND diaphragm
- Male condom with spermicide AND cervical sponge

Females will be tested for pregnancy at specified visits (see Section 9.8).

- Pregnant females will not be enrolled in the study.
- Female subjects enrolled in the study who become pregnant prior to myeloablation/ST-400 administration will be discontinued from further study procedures to avoid potential risks to the developing fetus (see Section 7.13).

- Female subjects enrolled in the study who become pregnant following ST-400 administration will be allowed to continue in the study but will not undergo any scheduled procedures considered by the Investigator and Sponsor to have potential risk to the developing fetus.

Pregnancies or pregnancies of partners occurring in subjects during this study are to be reported on the Pregnancy Reporting Form. In general, it is expected that pregnancies will be reported in the same timeframe as SAEs. Regardless of whether the subject has discontinued participation in the study, the course of all pregnancies will be followed to partum at minimum.

Congenital abnormalities/birth defects in the offspring of subjects should be reported as an SAE if conception occurred during this study.

11.2 Medical Emergency

In a medical emergency requiring immediate attention, study site staff will apply appropriate medical intervention according to current standards of care. The Investigator (or designee) should contact the study's Medical Monitor or designee. Refer to Page 2 of this protocol for complete contact information for the Medical Monitor.

12. SAFETY MONITORING

An external, independent SMC with appropriate medical and scientific expertise in TDT and HSCT will provide oversight of the study for patient safety. A Chair will be appointed and minutes will be generated and retained for each of the SMC meetings.

The SMC will be convened after the 3 sentinel subjects have achieved successful hematopoietic reconstitution based on the criteria defined, and subsequently approximately every 3-6 months during the study period to monitor safety and tolerability of ST-400. The SMC may be convened earlier or at any time if deemed necessary by the Sponsor or SMC. Specifically, the SMC will be convened if a study suspension rule (see Section 4.8) is met. When convened, the SMC will evaluate all data available to provide recommendations on changes to the study and whether enrollment or dosing should continue. As a component of their ongoing review of safety data, the SMC will also provide recommendations on any ST 400-related AEs that should be characterized as dose-limiting toxicities of ST-400 treatment.

The SMC oversaw the safety of study participants from 29th March 2018 till 18th December 2020. Afterward, the safety oversight was transferred to a joint Safety Monitoring Team (SMT) composed of members of Sangamo and Sanofi considering both companies are developing equivalent gene-edited cell products (ST-400 and BIVV003) for 2 different indications (TDT and SCD), respectively.

The SMT is a cross-functional team composed of drug safety/pharmacovigilance, clinical development, clinical operations, and preclinical functions. The roles and responsibilities are detailed in the safety monitoring team charter.

13. STATISTICAL ANALYSIS

This is an open-label, single-arm study, and analyses will be primarily descriptive in nature. No formal statistical hypothesis testing is planned. In general, continuous variables will be summarized by means, standard deviations, medians, minimums and maximums. Categorical variables will be summarized with counts and percentages per category. Some summaries may be presented as graphs. Additional details regarding the analysis of study data will be provided in the Statistical Analysis Plan.

13.1 Analysis Populations

Two analysis populations will be considered in this study. The all-enrolled subjects population consists of all subjects enrolled into the study. The safety population consists of all subjects who receive any amount of ST-400 in the study.

13.2 Methods of Analysis

The study outcome measures are described in Section 3. Full details of the statistical methods will be described in the Statistical Analysis Plan.

13.3 Analysis of Primary Outcome Measures

The primary analysis of the primary outcome measure of this study is the incidence of AEs and SAEs in the safety population during the Primary Study Period, defined as the date of ST-400 infusion through the date of the Week 52 study visit (or date of ETV for subjects withdrawing prior to Week 52), inclusive.

All reported AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). AE incidence will be summarized for all subjects. In addition, separate summaries will be generated for serious adverse events, related adverse events and AEs by severity. Reported AEs starting prior to the Primary Study Period or starting after the Primary Study Period will be summarized or listed separately.

Other safety outcome measures (including results of laboratory tests [e.g., blood chemistry, hematology, and urinalysis] and assessments [e.g., vital signs, ECG, ECHO, PFTs, bone marrow aspiration], concomitant medications) will be summarized, and shifts from baseline will be evaluated where appropriate. Duration of hospitalization following ST-400 administration and time to hematopoietic reconstitution will be summarized.

13.4 Analysis of Secondary Outcome Measures

Baseline levels of Hb fractions (A and F in g/dL) and percent HbF will be determined based on the last assessment on or prior to the date of first administration of IV busulfan. HbF levels and change from baseline will be summarized by study visit.

Baseline annualized frequency and volume of PRBC transfusions will be based on the 2 year period prior to Screening. Frequency and volume of transfusion will be annualized by study period and overall, and compared descriptively to the baseline values.

13.5 Analysis of Exploratory Outcome Measures

Analysis details for the exploratory outcome measures will be provided in the Statistical Analysis Plan.

13.6 Other Study Data

Other study data that will be summarized include subject disposition (for the Primary Study Period and for the overall study), demographic and baseline characteristics, use of concomitant medications, study treatment administration details, and time on study.

13.7 Sample Size

The sample size for this single-arm study is not based on providing statistical power for hypothesis testing. However, for an AE with a true rate of 20%, a sample size of 6 subjects provides an approximately 74% likelihood that the event will be observed during the study.

13.8 Interim Analysis

Informal interim analyses may be performed at the Sponsor's discretion.

14. INVESTIGATOR OBLIGATIONS

The Investigator will ensure that the study is conducted in compliance with the protocol, Declaration of Helsinki, ICH Guidelines for Good Clinical Practice (E6), and all regulatory and study center requirements, including those for subject privacy, informed consent, Institutional Review Board or Ethics Committee approval, and record retention.

14.1 Informed Consent

An Investigator shall seek such consent only under circumstances that provide the prospective subject sufficient opportunity to consider whether or not to participate, and that minimize the possibility of coercion or undue influence. The information that is given to the subject or the representative shall be in language understandable to the subject or the representative. The Investigator must keep each subject's signed ICF on file for inspection by a regulatory authority at any time.

Sangamo will provide the Investigator with a template for the ICF. State and local laws and/or study center requirements may require the disclosure of additional information in the ICF. The proposed ICF must be submitted to Sangamo prior to submission to the IRB or Independent Ethics Committee (IEC) to ensure that it meets Sangamo standards for consent forms. The IRB or IEC must approve the ICF. A copy of the approved ICF must be submitted to Sangamo.

14.2 Future Use of Stored Specimens

With the subject's approval and as approved by local IRBs, de-identified biological samples (i.e., residual content of samples collected for protocol-defined analyses) may be stored for future research. These samples could be used for further research into the effects of ST 400 and/or the causes of TDT, its complications and other conditions for which individuals with TDT are at increased risk, to improve treatment, or for assay development to address TDT or related indications.

During the conduct of the study, an individual subject can choose to withdraw consent to have biological specimens stored for future research. However, data from samples that have already been tested will not be removed from the study.

14.3 Institutional Review Board and BioSafety Committee

This protocol, ICF, and relevant substantive data are to be submitted to the appropriate IRB and BioSafety Committee (BSC) for review and approval before the initiation of the study. Amendments to the protocol will also be submitted to the IRB and BSC (as appropriate) prior to implementation of the change. A letter documenting the IRB/BSC's approval must be received by Sangamo prior to initiation of the study.

14.4 Protocol Amendments

Any changes to this protocol will be initiated by Sangamo in writing as a protocol amendment. The amendment must be submitted to the IRB together with a revised ICF, if applicable. Written documentation of IRB approval must be received before the amendment can take effect.

14.5 Subject Privacy

Subject medical information obtained for the purposes of this trial is confidential, and disclosure to third parties other than those noted below is prohibited. Upon the subject's request and written permission, medical information may be given to his/her personal physician or other appropriate

medical personnel responsible for the subject's welfare. Data generated for this study must be available for inspection and/or provided on request to representatives of national or local health authorities, Sangamo, and the associated IRB/IEC.

Release of research results or data that reveal subject names or other identifiers, such as photographs, audio, or videotapes, must be carried out in accordance with appropriate privacy regulations. Written authorization must be obtained from the subject and IRB/IEC prior to release of such information. Identifiable subject data may not be used for purposes of promoting the study drug.

14.6 Other Reporting Obligations

The Investigator is also responsible for informing his/her IRB of the progress of the study and for obtaining annual IRB renewal. The IRB must be informed at the time of completion of the study. The Investigator should provide his/her IRB (if required by the institution) with a summary of the results of the study.

15. ADMINISTRATIVE CONSIDERATIONS

15.1 Study Documentation

The Investigator and study staff are responsible for maintaining a comprehensive and centralized filing system containing all study-related documentation. These files must be available for inspection at any time, shall be provided upon request, and should consist of the following elements:

- Subject files containing the completed medical records, supporting source documentation, electronic CRFs, and the IRB/IEC approved ICF signed by subjects
- Study files containing all versions of the IRB/IEC approved protocol with all amendments, IRB approved ICFs, copies of all pre-study documentation, required forms, and all correspondence to and from the IRB/IEC and Sangamo

The Investigator should maintain a list of appropriately qualified persons who are delegated to perform significant study-related activities. In addition, the Investigator should maintain a signature sheet to document signatures and initials of all persons authorized to make entries and/or corrections on the source documents and electronic CRFs.

15.2 Record Retention

The Investigator shall retain records required to be maintained under this part for a period of 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated. If no application is to be filed or if the application is not approved for such indication, the Investigator shall retain these records until 2 years after the investigation is discontinued. However, these documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with Sangamo. It is the responsibility of Sangamo to inform the Investigator as to when these documents no longer need to be retained.

15.3 Case Report Forms

The Investigator is responsible for the quality of the data recorded on the CRF. The data recorded should be a complete and accurate account of the subject's record collected during the study.

Clinical data will be recorded on CRFs provided by Sangamo. All forms must be legible and complete. The Investigator must review all entries for completeness and correctness. When changes or corrections are made on any CRF, an audit trail will be generated to record date and time when a change is made, who made the change, and reason for the change as needed. The original entry should not be obscured.

The Investigator agrees to complete and sign CRFs in a timely fashion, after completion of each subject visit, and make them available to the Study Monitor for full inspection. In addition, all data queries should be resolved promptly.

15.4 Termination of the Study

Sangamo retains the right to terminate the study and remove all the study materials from the study site at any time. Specific instances that may precipitate such termination are as follows:

- Completion of the study at an investigational site.
- Investigator withdrawal from participation in study.

15.5 Study Monitoring

Sangamo, as Sponsor of this study, is responsible to regulatory authorities for ensuring the proper conduct of the study with regard to protocol adherence and validity of the data recorded on the CRFs presented to the regulatory authorities. Sangamo has therefore assigned a Medical Monitor and site monitor to this study. Their duties are to aid the Investigator and, at the same time, Sangamo in the maintenance of complete, legible, well-organized, and easily retrievable data.

Individual study sites will be monitored by Sangamo or a representative at appropriate intervals to assure satisfactory consenting process, data recording, and protocol adherence. To perform their roles well, the monitors must be given direct access to primary subject data (source documents) that support data entered onto the CRFs. The Investigator and staff are expected to cooperate and provide all relevant study documentation in detail at each site visit on request for review. Each study center will also be routinely monitored by telephone and/or by email to keep abreast of subject status and to answer questions.

Regulatory authorities, the IRB/IEC, and/or Sangamo's Clinical Quality Assurance group may request access to all source documents, CRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

The Investigator or designated person should agree, as a minimum requirement, to record the following information in the subject notes:

- Protocol identification number, brief description, or title of study
- Date and statement that subject has signed ICF
- All study follow-up visit dates
- AE as described in Section 10 of this protocol

Entries in the subject notes must contain the signature or initials of the person making the entries.

15.6 Confidential Information and Publication

All information provided by Sangamo to the Investigator and any data or results generated in the performance of this clinical trial are considered confidential and remain the sole property of Sangamo. The Investigator shall maintain this information in confidence and use this information solely for in the conduct of the study unless otherwise expressly agreed to in writing by Sangamo.

The Investigator understands and agrees that Sangamo shall have the right to use the data or results generated in the performance of the study for any purpose, including in registration documents for regulatory authorities in the U.S. or abroad, or for public dissemination in the form of papers, abstracts, posters, or other informational materials to be presented at scientific meetings, or published in professional journals, or as a part of an academic thesis. The Investigator further understands and agrees that Sangamo shall have the right to first publication of the data or results of the study, which is intended to be a joint, multi-center publication of the study results made by Sangamo in conjunction with the Investigators from all appropriate investigational sites contributing data, analysis, and comments. Authorship of publications resulting from this study will be based on customary standards for attribution of authorship taking into consideration factors such as significance of contribution to the design of the study, analysis and interpretation of the data, and critical review of the publication. Subsequent to the first publication of the study results

by Sangamo, the Investigator may publish the Investigator's site-specific data or results. If the Investigator wishes to publish the Investigator's site-specific data or results, a copy of such proposed publications, papers, abstracts, or other written materials, or an outline of any proposed oral presentations, shall be submitted to Sangamo for review at least 60 days prior to submission of such written materials for publication, or any proposed oral presentation. Sangamo shall have the right to review and comment on such written material or outline, and to confirm the accuracy of the data described therein by comparison with that collected during this study. In addition, Sponsor shall have the right to require the Investigator to, and Investigator shall, remove specifically identified confidential information of Sangamo (other than the data or results of the study) and/or delay the proposed publication for an additional 60 days to enable Sangamo to file patent applications.

15.7 Study Funding

The costs necessary to perform the study will be agreed to by the Investigator and/or the management of the study facility, and will be documented in a separate financial agreement. All financial agreements will be signed by the Investigator and Sangamo.

16. REFERENCES

Baronciani D, Angelucci E, Potschger U, Gaziev J, Yesilipek A, Zecca M, Orofino MG, Giardini C, Al-Ahmari A, Marktel S, de la Fuente J, Ghavamzadeh A, Hussein AA, Targhetta C, Pilo F, Locatelli F, Dini G, Bader P, Peters C. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000-2010. Bone Marrow Transplant. 2016 Apr;51(4):536-41.

Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, Shao Z, Canver MC, Smith EC, Pinello L, Sabo PJ, Vierstra J, Voit RA, Yuan GC, Porteus MH, Stamatoyannopoulos JA, Lettre G, Orkin SH. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science. 2013 Oct 11;342(6155):253-7.

Brendel C, Guda S, Renella R, Bauer DE, Canver MC, Kim YJ, Heeney MM, Klatt D, Fogel J, Milsom MD, Orkin SH, Gregory RI, Williams DA. Lineage-specific *BCL11A* knockdown circumvents toxicities and reverses sickle phenotype. J Clin Invest. 2016 Oct 3;126(10):3868-3878.

Canver MC, Smith EC, Sher F, Pinello L, Sanjana NE, Shalem O, Chen DD, Schupp PG, Vinjamur DS, Garcia SP, Luc S, Kurita R, Nakamura Y, Fujiwara Y, Maeda T, Yuan GC, Zhang F, Orkin SH, Bauer DE. *BCL11A* enhancer dissection by Cas9-mediated in situ saturating mutagenesis. Nature. 2015 Nov 12;527(7577):192-7.

Christianson SW, Greiner DL, Hesselton RA, Leif JH, Wager EJ, Schweitzer IB, Rajan TV, Gott B, Roopenian SC, Schultz LD. Enhanced human CD4+ T cell engraftment in beta2-microglobulin-deficient NOD-scid mice. J Immunol. 1997 Apr 15;158(8):3578-86.

Chang KH, Smith SE, Sullivan T, Chen K, Zhou Q, West JA, Liu M, Liu Y, Vieira BF, Sun C, Hong VP, Zhang M, Yang X, Reik A, Urnov FD, Rebar EJ, Holmes MC, Danos O, Jiang H, Tan S. Long-Term Reconstitution and Fetal Globin Induction upon *BCL11A* Gene Editing in Bone-Marrow-Derived CD34(+) Hematopoietic Stem and Progenitor Cells. Mol Ther Methods Clin Dev. 2017 Jan 11;4:137-148.

Duong HK, Savani BN, Copelan E, Devine S, Costa LJ, Wingard JR, Shaughnessy P,Majhail N, Perales MA, Cutler CS, Bensinger W, Litzow MR, Mohty M, Champlin RE,Leather H, Giralt S, Carpenter PA. Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2014 Sep;20(9):1262-73.

Ferster A, Corazza F, Vertongen F, Bujan W, Devalck C, Fondu P, Cochaux P, Lambermont M, Khaladji Z, Sariban E. Transplanted sickle-cell disease patients with autologous bone marrow recovery after graft failure develop increased levels of fetal haemoglobin which corrects disease severity. Br J Haematol. 1995 Aug;90(4):804-8.

Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010 May 21;5:11.

Giarratana MC, Rouard H, Dumont A, Kiger L, Safeukui I, Le Pennec PY, François S, Trugnan G, Peyrard T, Marie T, Jolly S, Hebert N, Mazurier C, Mario N, Harmand L, Lapillonne H, Devaux JY, Douay L. Proof of principle for transfusion of *in vitro*-generated red blood cells. Blood. 2011 Nov 10;118(19):5071-9.

Gragert L, Eapen M, Williams E, Freeman J, Spellman S, Baitty R, Hartzman R, Rizzo JD, Horowitz M, Confer D, Maiers M. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. N Engl J Med. 2014 Jul 24;371(4):339-48.

Hardison RC, Blobel GA. Genetics. GWAS to therapy by genome edits? Science. 2013 Oct 11;342(6155):206-7.

Higgs DR, Engel JD, Stamatoyannopoulos G. Thalassaemia. Lancet. 2012 Jan 28;379(9813):373-83.

Koren A, Profeta L, Zalman L, Palmor H, Levin C, Zamir RB, Shalev S, Blondheim O. Prevention of β Thalassemia in Northern Israel - a Cost-Benefit Analysis. Mediterr J Hematol Infect Dis. 2014 Feb 17;6(1):e2014012.

Locatelli F, Kabbara N, Ruggeri A, Ghavamzadeh A, Roberts I, Li CK, Bernaudin F, Vermylen C, Dalle JH, Stein J, Wynn R, Cordonnier C, Pinto F, Angelucci E, Socié G, Gluckman E, Walters MC, Rocha V; Eurocord and European Blood and Marrow Transplantation (EBMT) group. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood. 2013 Aug 8;122(6):1072-8.

Modell B, Khan M, Darlison M, Westwood MA, Ingram D, Pennell DJ. Improved survival of thalassaemia major in the UK and relation to T2* cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2008 Sep 25;10:42.

Musallam KM, Sankaran VG, Cappellini MD, Duca L, Nathan DG, Taher AT. Fetal hemoglobin levels and morbidity in untransfused patients with β-thalassemia intermedia. Blood. 2012 Jan 12;119(2):364-7.

Origa R. β-Thalassemia. Genet Med. 2017 Jun;19(6):609-619.

Paciaroni K, Lucarelli G. Hemopoietic stem cell transplantation failure followed by switch to stable production of fetal hemoglobin. Blood. 2012 Jan 26;119(4):1091-2.

Ribeil JA, Hacein-Bey-Abina S, Payen E, Magnani A, Semeraro M, Magrin E, Caccavelli L, Neven B, Bourget P, El Nemer W, Bartolucci P, Weber L, Puy H, Meritet JF, Grevent D, Beuzard Y, Chrétien S, Lefebvre T, Ross RW, Negre O, Veres G, Sandler L, Soni S, de Montalembert M, Blanche S, Leboulch P, Cavazzana M. Gene Therapy in a Patient with Sickle Cell Disease. N Engl J Med. 2017 Mar 2;376(9):848-855.

Schultz LB, Chehab NH, Malikzay A, Halazonetis TD. p53 binding protein 1(53BP1) is an early participant in the cellular response to DNA double-strand breaks. J Cell Biol. 2000 Dec 25;151(7):1381-90.

Shin, SI et al. Tumorigenicity of virus-transformed cells in nude mice is correlated specifically with anchorage independent growth in vitro. Proc Natl Acad Sci U S A. 72(11), 4435-9 (1975).

Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Kotb M, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol. 2005 May 15;174(10):6477-89.

Smith EC, Luc S, Croney DM, Woodworth MB, Greig LC, Fujiwara Y, Nguyen M, Sher F, Macklis JD, Bauer DE, Orkin SH. Strict in vivo specificity of the *BCL11A* erythroid enhancer. Blood. 2016 Oct 5; 128: 2338-42.

Sobota A, Yamashita R, Xu Y, Trachtenberg F, Kohlbry P, Kleinert DA, Giardina PJ, Kwiatkowski JL, Foote D, Thayalasuthan V, Porter JB, Thompson AA, Schilling L, Quinn CT, Neufeld EJ; Thalassemia Clinical Research Network. Quality of life in thalassemia: a comparison of SF-36 results from the thalassemia longitudinal cohort to reported literature and the US norms. Am J Hematol. 2011 Jan;86(1):92-5.

Stein S, Ott MG, Schultze-Strasser S, Jauch A, Burwinkel B, Kinner A, Schmidt M, Krämer A, Schwäble J, Glimm H, Koehl U, Preiss C, Ball C, Martin H, Göhring G, Schwarzwaelder K, Hofmann WK, Karakaya K, Tchatchou S, Yang R, Reinecke P, Kühlcke K, Schlegelberger B, Thrasher AJ, Hoelzer D, Seger R, von Kalle C, Grez M. Genomic instability and myelodysplasia with monosomy 7 consequent to EVI1 activation after gene therapy for chronic granulomatous disease. Nat Med. 2010 Feb;16(2):198-204.

Tebas P, Stein D, Tang WW, Frank I, Wang SQ, Lee G, Spratt SK, Surosky RT, Giedlin MA, Nichol G, Holmes MC, Gregory PD, Ando DG, Kalos M, Collman RG, Binder-Scholl G, Plesa G, Hwang WT, Levine BL, June CH. Gene editing of CCR5 in autologous CD4 T cells of persons infected with HIV. N Engl J Med. 2014 Mar 6;370(10):901-10.

Thein SL, Menzel S, Lathrop M, Garner C. Control of fetal hemoglobin: new insights emerging from genomics and clinical implications. Hum Mol Genet. 2009 Oct 15;18(R2):R216-23.

Vierstra J, Reik A, Chang KH, Stehling-Sun S, Zhou Y, Hinkley SJ, Paschon DE, Zhang L, Psatha N, Bendana YR, O'Neil CM, Song AH, Mich AK, Liu PQ, Lee G, Bauer DE, Holmes MC, Orkin SH, Papayannopoulou T, Stamatoyannopoulos G, Rebar EJ, Gregory PD, Urnov FD, Stamatoyannopoulos JA. Functional footprinting of regulatory DNA. Nat Methods. 2015 Oct;12(10):927-30.

Vogiatzi MG, Macklin EA, Fung EB, Cheung AM, Vichinsky E, Olivieri N, Kirby M, Kwiatkowski JL, Cunningham M, Holm IA, Lane J, Schneider R, Fleisher M, Grady RW, Peterson CC, Giardina PJ; Thalassemia Clinical Research Network. Bone disease in thalassemia: a frequent and still unresolved problem. J Bone Miner Res. 2009 Mar;24(3):543-57.

Xu J, Peng C, Sankaran VG, Shao Z, Esrick EB, Chong BG, Ippolito GC, Fujiwara Y, Ebert BL, Tucker PW, Orkin SH. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. Science. 2011 Nov 18;334(6058):993-6.

APPENDIX 1: SCHEDULE OF EVENTS

PRIMARY STUDY PERIOD	Screening (6 weeks +/- 4 weeks)	Mobilization & Apheresis (within 1 week prior to first mobilizing agent administration)	Baseline (within 2 weeks prior to conditioning)	Conditioning	ST-400 Infusion (Day 0)	Day 7 (+/- 1 d)	Day 14 (+/- 1 d)	Day 21 (+/- 1 d)	Day 28 (+/- 1 d)	Day 35 (+/- 2 d)	Day 42 (+/- 2 d)	Day 56 (+/- 5 d)	Day 90 (+/- 7 d)	Week 26 (+/- 14 d)	Week 39 (+/- 14 d)	Week 52 (+/- 14 d)	ETV (s)
Informed Consent	X																
Medical History	X																
Demographics	X																
Inclusion/Exclusion Criteria	X																
Subject Number	X																
Transfusion History (a)	X																
Concomitant Medications	X (m)	X (o)	X	X (p)	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse Events	X	X (o)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam (b)	X	X (o)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X (o)	X	X	X (r)	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																
Weight	X	X	X														
Karnofsky Performance Scale	X		X											X		X	X
Pregnancy Test (c)	X	X(o)	X						X			X	X	X	X	X	X
Pulmonary Function Tests (PFTs)	X (f)															X	
Chest X-ray	X (f)																
Infectious Disease Panel	X																
Clinical Labs	X	X (o)	X	X (q)	X	X	X	X	X	X	X	X	X	X	X	X	X
ECG	X		X		X				X					X		X	X
Liver Biopsy (d)	X																
Hemoglobin Analysis	X																
HbF Quantification	X		X		X		X		X		X	X	X	X	X	X	X
BCL11A Gene Modification Assay			X				X		X		X	X	X	X	X	X	X
Thalassemia-related Disease Biomarkers (e)	X		X		X								X	X	X	X	X
Endocrine Lab Testing	X		X													X	X
ECHO	X (f)															X	X
Liver MRI	X															X	X
Cardiac MRI	X															X	X
SF-36 Survey	X		X											X		X	X
Transfusion Log		X (o)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Immunological Assays (g)			X										X			X	X
Subset Analysis of BCL11A Gene Modification															X		
Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy			X										X	X	X	X	X
DXA			X													X	
Bone Marrow Aspiration (h)			X										X (h)	X (h)	X (h)	X	X
% F-cells			X						X			X	X	X		X	
Screening for Malignancy									X	X	X	X	X	X	X	X	X
Gamete or Embryo Cryopreservation		X (approx. 30 days prior to ST-400 infusion)															
G-CSF (n, i)		X															
Plerixafor (n, i)		X															
Collection of CD34+ HSPCs (j)		X															

	Week 65 (+/- 14 d)	Week 78 (+/- 14 d)	Week 91 (+/- 14 d)	Week 104 (+/- 14 d)	Week 117 (+/- 14 d)	Week 130 (+/- 14 d)	Week 143 (+/- 14 d)	Week 156 (+/- 14 d)
Concomitant Medications	X	X	X	X	X	X	X	X
Adverse Events	X	X	X	X	X	X	X	X
Physical Exam (b)	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X
Karnofsky Performance Scale				X				X
Clinical Labs	X	X	X	X	X	X	X	X
ECG				X				X
HbF Quantification	X	X	X	X	X	X	X	X
BCL11A Gene Modification Assay		X		X		X		X
Thalassemia-related Disease Biomarkers (e)		X		X		X		X
Endocrine Lab Testing	X				X			
ECHO (f)				X				X
Liver MRI				X				X
Cardiac MRI				X				X
SF-36 Survey				X				X
Transfusion Log	X	X	X	X	X	X	X	X
Immunological Assays (g)	X				X			
Subset Analysis of BCL11A Gene Modification			X				X	
Sampling for Potential Retrospective Analysis in the Event of Hematological Malignancy		X		X		X		X
DXA				X				X
Bone Marrow Aspiration (h)				X				
Screening for Malignancy	X	X	X	X	X	X	X	X

(a) A minimum of 2 years of transfusion history prior to Screening is required to be eligible to participate in this study. Up to 3 years of history will be recorded if available.
(b) A physical examination will be conducted on each subject at the specified visits, and will include at a minimum: general appearance; head, eyes, ears, nose, and throat (HEENT); as well as cardiovascular, dermatologic, respiratory, gastrointestinal, musculoskeletal, and neurologic systems. Any clinically significant changes from previous visits or new findings should be documented in the source and reported as an adverse event.
(c) For female subjects with reproductive potential only. Serum pregnancy testing will be conducted at the 3 pre-infusion timepoints, and urine testing will be conducted for the post-infusion timepoints with confirmation by serum testing if urine testing is positive or equivocal. Baseline pregnancy testing should be conducted within 1 week prior to first busulfan infusion.
(d) May have been performed in prior 12 months with adequate medical documentation.
(e) Thalassemia-related disease biomarkers may include, but are not limited to, iron metabolism, erythropoietin, haptoglobin, and hepcidin levels.
(f) If more than 30 weeks will elapse between the start of the Screening visit and the Baseline visit, the following tests will be repeated at Baseline: pulmonary Function Tests (PFTs), chest X-ray, and echocardiogram (ECHO). Additional tests may be repeated as appropriate to confirm eligibility at the discretion of the Investigator.
(g) Immunological assays include, but are not limited to, lymphocyte subsets and quantitative immunoglobulins.
(h) Bone marrow aspiration at the Day 90 visit may be waived at the Investigator's discretion. Bone marrow aspirate may be performed at *either* the Week 26 *or* the Week 39 visit; it should not be performed at both. Both of these procedures (at both Week 26 and Week 39) may be waived at the Investigator's discretion. Unscheduled bone marrow aspiration may also be conducted at any time if clinically indicated, such as to evaluate the potential development of hematological malignancy.
(i) G-CSF and plerixafor dosing, timing, or route of administration may be modified or discontinued at the discretion of the Investigator if the WBC exceeds 100,000 cells/μL or if the subject becomes symptomatic.

(j) If the first apheresis cycle does not mobilize the minimum number of CD34+ HSPCs required for ST-400 drug manufacturing and for rescue treatment, 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.
(k) Subjects will not proceed to conditioning procedure with IV busulfan until a sufficient quantity of rescue treatment is obtained and ST-400 is manufactured, passes quality control and release testing, and is confirmed as received at the clinical site.
(l) Dose of ST-400 will be administered IV as a single dose no sooner than 72 hours after the final dose of IV busulfan in the conditioning phase.
(m) Any scheduled vaccinations (e.g., influenza) must be completed at least 30 days prior to the conditioning phase.
(n) Prior to mobilization it is recommended that subjects have a Hb level of ≥9.0 g/dL.
(o) Conducted within one week prior to administration of the first mobilization agent. Adverse Events assessed on each day of the visit.
(p) Seizure prophylaxis during IV busulfan is strongly recommended. Iron-chelating agents should be discontinued 2 weeks prior to busulfan and may be restarted after successful hematopoietic reconstitution or at the discretion of the Investigator. Concomitant acetaminophen or metronidazole should be avoided. Additional guidance on busulfan drug interactions with commonly used concomitant medications is provided in the Study Cell Therapy Manual.
(q) Samples for central laboratory analysis ("Clinical laboratory tests") should be collected at least once during conditioning, with timing at the discretion of the Investigator.
(r) Vital signs (temperature, heart rate, respiratory rate, blood pressure, and oxygen saturation) will be monitored before, during, and after the infusion as detailed in the Study Reference Manual, and as needed to maintain good clinical care in accordance with study center practice.
(s) It is at the discretion of the Investigator, in consultation with the Medical Monitor, to waive any procedure if the procedure has been performed within the standard interval of scheduled study visits.

APPENDIX 2: γ-GLOBIN ALLELLIC VARIANTS ASSOCIATED WITH CLINICALLY SIGNIFICANTLY ALTERED OXYGEN AFFINITY

HbF structural variants are rare. Although some HbF variants have no clinical significance, there are HbF mutations that lead to unstable hemoglobin or hemoglobins with deleteriously altered oxygen affinity. All subjects will have sequencing of the γ-globin gene evaluated at Screening. Subjects with γ-globin mutations that have clinical significance are not eligible to participate in this study. Examples of known clinically-significant γ-globin variants are provided below (not inclusive list):

- Hb F-Poole
- Hb F-M Osaka
- Hb F-La Grange
- Hb F-Cincinnati
- Large deletions such as γβ-thalassemia or εγδβ-thalassemia

