1 TITLE PAGE

Clinical Study Protocol

A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34$^+$ Human Hematopoietic Stem and Progenitor Cells (CTX001) in Subjects With Severe Sickle Cell Disease

Study Number: CTX001-121

IND Number: 18143

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Summary of Changes to the Protocol

The previous version of this protocol (Version 6.9 [US], 22 September 2021) was amended to create the current version (Version 6.11 [US], 15 September 2022). The protocol history is below:

Protocol History	
Version and Date of Protocol	**Comments**
Version 1.0, 13 April 2018	Original version
Version 2.0, 26 June 2018	Global version - Added secondary endpoint of proportion of subjects with sustained HbF ≥20% for 6 months, in the absence of treatment with HU, starting 6 months after CTX001 infusion - Added G-CSF to list of prohibited medications - Added assessment of lymph nodes and the neurological system to the abbreviated physical examination
Version 2.1 (DE), 15 February 2019	Country-specific version (Germany)
Version 3.0, 10 December 2018	Global version (except Germany) - Added secondary endpoint of relative change from baseline in annualized rate of hospitalization for severe VOCs starting 6 months after CTX001 infusion - Added to inclusion criterion #4 that pretreatment severe VOC events will be adjudicated by the Endpoint Adjudication Committee - Updated statistical analysis to include efficacy boundaries for the expanded study - Updated schedule of PRO assessments - Removed PROMIS-Fatigue and PROMIS-Cognitive function PROs
Version 4.0, 19 September 2019	Global version (except Germany) - Added βS/β0 genotype - Updated number planned initial number subjects to up to 17 subjects - Added testing for HTLV-1 antibodies to exclusion criterion 19 - Specified that subjects must have at least a screening assay for syphilis performed - Updated exclusion criterion #7 so that subjects with ≥10 hospitalizations or emergency department visits in the year before screening that are consistent with chronic pain related to sickle cell disease are excluded - Updated that RBC exchange transfusion should be performed 3 (±1) days before mobilization/apheresis and busulfan conditioning - Specified goal of RBC transfusions is to maintain HbS level <30% of total Hb and total Hb concentration ≤11 g/dL - Modified the timing for initiating an additional mobilization cycle to remove the restriction that it cannot be more than 60 days after the end of the prior cycle from initiated at least 14 days after the first day of the prior mobilization cycle - Extended the allowed delay for mobilization and apheresis, and myeloablative conditioning from 2 to 3 months

	- Updated that plerixafor will be administered 2 to 3 hours before the start of apheresis - Updated timing of $CD34^+$ counts post plerixafor administration and prior to or after completion of apheresis - Specified that coagulation markers will be collected daily and not weekly until discharge - Allowed for platelet transfusions to maintain platelets >50,000/μL - Updated testing window for assessments during pre-conditioning to 30 to 7 days before busulfan conditioning - Added instructions to allow repeat testing of individual screening assessments in certain cases - Added that female subjects undergoing fertility preservation who are unable to use hormonal contraception must use true abstinence and added acceptable contraceptive methods for male partners of female subjects - Deleted requirement that deferasirox be stopped 30 days before starting busulfan conditioning. All iron chelation drugs must be discontinued at least 7 days before starting myeloablative conditioning with busulfan - Added section to specify that the proportion of alleles with intended genetic modification present in each batch of CTX001 will be assessed - Added that, for subjects who withdraw from this study, data collected in Study VX18-CTX001-131 up to 2 years after CTX001 infusion will be included in the analyses
Version 4.1 (DE), 04 October 2019	Country-specific version (Germany)
Version 4.2 (IT), 24 July 2020	Country-specific version (Italy)
Version 5.0, 04 February 2020	Global version (except Germany) - Added pediatric subjects (12 to <18 years) and provided therapeutic rationale and statement of risk for this population - Added that approximately 6 adult subjects will be dosed with CTX001 before conditioning and dosing of the first pediatric subject - Added HbS and Hb collection for mobilization cycles in the schedule of assessments - Added that Hb concentration be documented after transfusion - Added option for parents to sign assent for pediatric subjects - Added history of abnormal TCD for subjects 12 to <18 years as an exclusion criterion - Modified inclusion criterion for the upper limit of direct bilirubin from 2× ULN to 2.5× ULN - Added updates to inclusion criteria and contraception requirements to specify that they apply to male subjects of reproductive capacity and modified documentation requirements of male infertility - Updated enrollment suspension criteria and individual stopping rules to include failure to manufacture target cell dose of CTX001 - Added transcranial Doppler ultrasonography for subjects 12 to 16 years

	- Added that AE collection excludes AEs possibly related to fertility preservation and specified timing for Grade 3 or greater AEs that trigger a DMC - Added Lansky performance status scale, youth and proxy versions of EQ-5D, Wechsler Abbreviated Scale of Intelligence, PedsQL Sickle Cell Disease Module, Pediatric Quality of Life Inventory, option of gonadal tissue banking for pre-pubescent subjects - Added that for subjects <34 kg, institutional and/or regional practice characteristics may be used for busulfan dosing - Added statement that subgroup analysis such as descriptive summaries by age group will be provided as appropriate
Version 5.1 (DE), 07 February 2020	Country-specific version (Germany)
Version 5.2 (DE), 21 May 2020	Country-specific version (Germany)
Version 6.0 (EUR), 14 December 2020	Region-specific version (Europe, except Germany and France)
Version 6.1 (DE), 08 January 2021	Country-specific version (Germany)
Version 6.2 (FR), 05 March 2021	Country-specific version (France)
Version 6.3 (NA), 21 May 2021	Region-specific version (North America) withdrawn in US
Version 6.4 (EUR), 21 May 2021	Current region-specific version (Europe, except Germany and France)
Version 6.5 (FR), 02 June 2021	Current country-specific version (France)
Version 6.6 (DE), 15 June 2021	Country-specific version (Germany)
Version 6.7 (US), 02 July 2021	Country-specific version (US) • Clarified that subjects should receive an RBC exchange or simple transfusion within 3 days before the start of each mobilization/apheresis cycle • Added that use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor. • Added collection of signs/symptoms consistent with myocardial infarction or cerebral vascular accident to physical examinations • Clarified that subjects should receive an RBC exchange or simple transfusion up to 7 days before the start of busulfan conditioning • Added change in urine albumin to creatinine ratio (UACR) over time and removed change in hemolytic index as an exploratory endpoint • Added guidelines for busulfan starting dose for subjects weighing <34 kg • Added collection of NT-proBNP for the assessment of pulmonary hypertension • Added collection of signs/symptoms consistent with myocardial infarction or cerebral vascular accident, new or worsening osteonecrosis, and retinopathy to physical examinations • Added that Tanner staging will be performed for subjects 12 to <18 years old until they reach Tanner Stage 5 during physical examination at baseline (Screening) and 12 and 24 months after CTX001 infusion • Added UACR for the assessment of albuminuria

	• Added methemoglobin as a measure of Hb oxidation status and erythropoietin as a measure of tissue oxygen delivery • Added measurement of ferritin to assess iron status • Added collection of reticulocytes/erythrocytes to clinical laboratory assessments • Clarified that hematology assessments should be done immediately before starting apheresis on Days 1 through 3 • Removed pre-apheresis $CD34^+$ count 15 minutes before apheresis and added that $CD34^+$ stem cells must be counted within 30 minutes of completion of apheresis • Because an ECG is performed prior to eligibility confirmation and mobilization, removed extra ECG that occurred between these 2 assessments • Removed echocardiogram assessment at the 1-month visit • Added $\beta S/\beta^+$ genotype to the inclusion criteria • Specified non-imaging and imaging TCD values in the inclusion and exclusion criteria • Updated exclusion criteria to add that the subject must not have any risk factors that would increase the likelihood of busulfan-related toxicities and excluded subjects with positive hepatitis B surface antigen • Updated to include the option of additional mobilization cycles if the dose target for manufacturing is not met • Added that if available, the kinetics of $CD34^+$ cell release for the subject may be used by the investigator, in consultation with the medical monitor and apheresis specialist, to alter the timing of the start of apheresis relative to the time of plerixafor dosing for the purpose of maximizing the collection of $CD34^+$ stem cells. • Updated that if a subject is deemed not to be eligible for myeloablative conditioning, this procedure can be delayed until expiry date of subjects' CTX001 Product • Added references to the CTX001 Product Receipt, Storage, and Infusion Manual and Apheresis Collection Manual • Clarified that historical VOCs (during the 2 years prior to screening) will be adjudicated by the EAC • Added treatment discontinuation information for crizanlizumab (ADAKVEO) and voxelotor (OXBRYTA) prior to mobilization; added background information on approved therapies crizanlizumab and voxelotor • Updated timing for starting iron chelation until at least 3 months after CTX001 infusion • Modified stopping rules so that the same SAE at least possibly related to CTX001 but not related to busulfan in 3 subjects will trigger an ad hoc DMC review and not suspension of enrollment based on demonstrated benefit of CTX001 and number of subjects dosed to date. Removed occurrence of VOC associated with a Grade 4 treatment-related AE in 2 subjects, during or immediately after stem cell mobilization and collection as an enrollment suspension criterion based on treatment experience to date.

	• Updated stopping rules for individual subjects to include failure to adequately mobilize and/or collect adequate numbers of $CD34^+$ cells or manufacture the required dose of CTX001 • Removed reference to partial withdrawal of consent for subjects who do not enroll in the long-term follow-up study but consent to have their information collected through medical record review or telephone interviews • Updated timing of manufacturing and release of CTX001 to approximately 11 weeks • Added VOD diagnostic criteria • Specified that for subjects already on a transfusion regimen, the MMSE and WASI should be administered before the first mobilization/apheresis cycle • Clarified that NK cells can be identified as $CD16^+$ and/or $CD56^+$ cells • Clarified neutrophil and platelet engraftment definitions and added a platelet engraftment definition for subjects who are discharged from the hospital before platelet engraftment has occurred; added that platelet levels will be monitored daily in the hospital and every 2 to 3 days until platelet engraftment is achieved • Clarified that 42 days after CTX001 infusion is Study Day 43. • Specified that if engraftment is not achieved within 42 days after CTX001 infusion (after Study Day 43) the investigator will determine whether and when the subject will receive the backup CD34+ stem cells according to his/her judgement based on all available clinical information (e.g., the patient's clinical status, presence of myeloid precursors on a bone marrow aspirate, presence of monocytes and neutrophils in the peripheral blood, whether engraftment has occurred by approximately Day 60). The investigator is recommended to consult with the study steering committee chair (or designee).
Version 6.8 (DE), 18 August 2021	Current country-specific version (Germany)
Version 6.9 (US), 22 September 2021	Country-specific version (US) • Updated study to Phase 1/2/3 • Updated primary efficacy, key secondary efficacy, and secondary endpoints • Updated statistical and interim analyses
Version 6.10 (CA), 22 September 2021	Current country-specific version (CA)
Version 6.11 (US), 15 September 2022	Current country-specific version (US)

Key changes made in the current version of the protocol are summarized below.

Change and Rationale	**Affected Sections**
Added key secondary efficacy endpoint of VF9 as a clinical endpoint to provide an earlier estimate of efficacy	Section 2, Section 7.2, Section 12.3.3.2
Removed "and the key secondary" from the Interim Analysis section, which describes the use of the Primary Efficacy Set for analysis, because the analysis of the key secondary endpoint of VF9 will not be based on the Primary Efficacy Set	Section 12.3.8.1

Typographical and administrative changes were also made to improve the clarity of the document.

2 PROTOCOL SYNOPSIS

Title A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified CD34^{+} Human Hematopoietic Stem and Progenitor Cells (CTX001) in Subjects With Severe Sickle Cell Disease

Brief Title A Study to Evaluate the Safety and Efficacy of a Single Dose of CTX001 in Subjects With Severe Sickle Cell Disease

Clinical Phase and Clinical Study Type Phase 1/2/3 Safety and Efficacy Study

Objectives

Primary Objective

Evaluate the safety and efficacy of a single dose of autologous CRISPR-Cas9 modified CD34^{+} human hematopoietic stem and progenitor cells (hHSPCs) (CTX001) in subjects with severe sickle cell disease (SCD)

Secondary Objectives

- Assess the effects of infusion of CTX001 on disease-specific events and clinical status
- Quantify gene editing efficiency

Exploratory Objective

Assess the ability of biomarkers to characterize CTX001 effect and predict treatment outcomes

Endpoints

Primary Endpoints

Safety Endpoints

- Successful neutrophil engraftment within 42 days after CTX001 infusion (Study Day 43)
- Time to neutrophil engraftment
- Time to platelet engraftment
- Safety and tolerability assessments based on adverse events (AEs), clinical laboratory values, and vital signs
- Transplant-related mortality (TRM) within 100 days after CTX001 infusion
- TRM within 1 year after CTX001 infusion
- All-cause mortality

Primary Efficacy Endpoint

- Proportion of subjects who have not experienced any severe vaso-occlusive crisis (VOC) for at least 12 consecutive months (VF12) after CTX001 infusion. The evaluation of VF12 starts 60 days after last red blood cell (RBC) transfusion for post-transplant support or SCD disease management.

Key Secondary Efficacy Endpoints

- Proportion of subjects free from inpatient hospitalization for severe VOC sustained for at least 12 months (HF12) after CTX001 infusion. The evaluation of HF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Proportion of subjects who have not experienced any severe VOC for at least 9 consecutive months (VF9) any time after CTX001 infusion. The evaluation of VF9 starts 60 days after last RBC transfusion for post-transplant support or

SCD disease management for subjects who reach at least 12 months of follow-up.

Secondary Endpoints

- Proportion of subjects with reduction in annualized rate of severe VOCs at the time of analysis from baseline by at least 90%, 80%, 75%, 50% up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Relative change from baseline in annualized rate of severe VOCs up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Duration of severe VOC free in subjects who have achieved VF12
- Relative change from baseline in rate of inpatient hospitalizations for severe VOCs up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management
- Relative change from baseline in annualized duration of hospitalization for severe VOCs up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Proportion of subjects with sustained HbF ≥20% at the time of analysis for at least 3 months, 6 months, or 12 months. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Change in number of units of RBCs transfused for SCD-related indications over time
- HbF concentrations over time
- Hemoglobin (Hb) concentrations over time
- Change from baseline in reticulocyte count (percent reticulocytes and absolute reticulocyte count) over time
- Change from baseline in indirect bilirubin over time
- Change from baseline in haptoglobin over time
- Change from baseline in lactate dehydrogenase over time
- Proportion of alleles with intended genetic modification present in peripheral blood leukocytes over time
- Proportion of alleles with intended genetic modification present in CD34+ cells of the bone marrow over time
- Change in patient reported outcomes (PROs) over time in adults (≥18 years) using;
    - Pain-scale:11-point numerical rating scale (NRS)
    - Functional assessment of cancer therapy-bone marrow transplant (FACT-BMT)
    - Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me)
    - EuroQol Quality of Life Scale (EQ-5D-5L)
- Change in PROs over time in adolescents (12 to <18 years of age) using;
    - Pain-scale: 11-point NRS
    - Pediatric Quality of Life Inventory (PedsQL Teen self-report and parent proxy versions)
    - PedsQL SCD module (Teen self-report and parent proxy versions)

- EQ-5D-Youth (EQ-5D-Y self-report and parent proxy versions)

Exploratory Endpoints

- Change in tricuspid regurgitant jet velocity (TRV) over time
- Change in proportion of circulating erythrocytes expressing fetal hemoglobin (F-cells) over time
- Change in inflammatory and endothelial activation markers over time
- Change in urine albumin to creatinine ratio (UACR) over time

Number of Subjects

Up to 17 subjects with possible expansion to a total of up to approximately 45 subjects dosed

Study Population

Subjects 12 to 35 years of age (inclusive at time of informed consent) with documented βS/βS, βS/β0, or βS/β^+ genotype who have severe SCD. Severe SCD is defined by the occurrence of at least 2 of the following events each year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, HU if indicated)

- Acute pain event that requires a visit to a medical facility and administration of pain medications (opioids or intravenous [IV] non-steroidal anti-inflammatory drugs [NSAIDs]) or RBC transfusions
- Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever
- Priapism lasting >2 hours and requiring a visit to a medical facility
- Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

Investigational Drug

Drug product: CTX001 (also known as exagamglogene autotemcel [exa-cel])

Description: CTX001 is composed of autologous $CD34^+$ hHSPCs modified with CRISPR-Cas9 at the erythroid lineage-specific enhancer of the *BCL11A* gene.

Route of administration: IV infusion

Study Design

This is a single-arm, open-label, multi-site, single-dose, Phase 1/2/3 study in subjects with severe SCD. The study will evaluate the safety and efficacy of a single dose of autologous CRISPR-Cas9 modified hHSPCs (CTX001) and will include up to 17 subjects, 12 to 35 years of age, inclusive. The study may be expanded to include a total of up to approximately 45 subjects dosed.

The study will be conducted in 4 stages:

<u>Stage 1: Screening and Pre-mobilization Period</u>

During this period, subjects who meet the inclusion criteria have the option to undergo fertility preservation via cryopreservation of oocyte or sperm. For pre-pubescent subjects, gonadal tissue collection and cryopreservation may be performed per local practice and standard of care. After eligibility is confirmed, subjects will begin RBC exchange or simple transfusions for a minimum of 8 weeks before the planned start of mobilization and will continue receiving these transfusions until they begin busulfan conditioning. The goal of these RBC transfusions is to maintain hemoglobin S (HbS) level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL. Treatment with HU, crizanlizumab (ADAKVEO®) and voxelotor (OXBRYTA®) should be stopped at least 8 weeks before planned mobilization.

Subjects should receive an RBC exchange or simple transfusion within 3 days before the start of each mobilization/apheresis cycle.

Use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor.

Stage 2: Mobilization, Autologous $CD34^+$ Stem Cell Collection, CTX001 Manufacture and Disposition

Each subject will undergo stem cell mobilization with plerixafor only. Peripheral blood mononuclear cells (PBMC) will be collected by apheresis. Subjects should receive an RBC exchange or simple transfusion within 3 days before the start of each mobilization/apheresis cycle. Use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor.

On Day 1, subject will receive plerixafor 2 to 3 hours before the start of apheresis. Day 2 and Day 3 (if required) will be the same as Day 1. One of the collection days will be used to collect an additional 2×10^6 $CD34^+$ cells/kg as a back-up for rescue therapy in the event of non-engraftment with CTX001. The targeted $CD34^+$ cell collection is at least 15×10^6 $CD34^+$ cells/kg for manufacturing of CTX001 in order to achieve a minimum target dose of 3×10^6 $CD34^+$ cells/kg.

If the first mobilization and apheresis cycle does not yield enough cells for both the minimum CTX001 product and safety backup or if a subject cannot complete apheresis, up to 2 additional mobilization and apheresis cycles will be allowed to collect additional cells. If the minimum cell dose target for manufacturing and backup is not met after the first 3 cycles, additional mobilizations may be conducted based on discussion and agreement with the subject, the investigator, and the medical monitor, taking into account the safety, tolerability, and efficacy of previous mobilizations and collections, a reasonable likelihood that the additional mobilizations can complete a full manufactured cell dose, and the overall benefit/risk profile for the subject. Such a discussion would take place after each additional cycle. Mobilization cycles will be initiated at least 14 days after the first day of the prior mobilization cycle. Each mobilization cycle will be evaluated for the overall efficacy of mobilization and apheresis. Subjects may be discontinued at any cycle at the discretion of the investigator in discussion with the medical monitor, if further mobilization is deemed unproductive. Collection of backup $CD34^+$ stem cells must occur prior to proceeding to Stage 3.

Stage 3: Myeloablative Conditioning (Stage 3A) and Infusion of CTX001 (Day 1, Stage 3B)

Stage 3A – Myeloablative Conditioning: During CTX001 manufacturing and before the planned start of busulfan conditioning, subjects will continue to receive RBC exchange or simple transfusions with the goal of maintaining an HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL.

If the planned start of busulfan conditioning is >4 months after completion of mobilization, the investigator may stop the RBC transfusion regimen and restart HU, crizanlizumab, or voxelotor for those subjects who have been previously treated with these agents. If the RBC transfusion regimen is interrupted, subjects should begin RBC exchange or simple transfusions a minimum of 8 weeks before the planned start of busulfan conditioning with the goal of maintaining an HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL. Subjects should receive an RBC exchange or simple transfusion up to 7 days before the start of busulfan conditioning.

Use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor.

After the CTX001 product is received at the site and it has been confirmed that the backup $CD34^+$ stem cells remain available and in acceptable condition to be

administered if needed, the subject will receive busulfan. The starting dose of busulfan will be 3.2 mg/kg administered IV once daily for 4 consecutive days. However, busulfan may be administered as 0.8 mg/kg every 6 hours (q6h) for 4 consecutive days, per the site's standard practice. For subjects less than 34 kg, weight-based starting dose recommendations for busulfan are as follows[1]:

- For subjects weighing 16 to 23 kg, 1.1 mg/kg q6h or 4.4 mg/kg qd
- For subjects weighing >23 to 34 kg, 0.95 mg/kg q6h or 3.8 mg/kg qd

Target busulfan AUC or cumulative exposure for each dosing regimen will remain the same across all age groups

Stage 3B – CTX001 Infusion:

Infusion of CTX001 will occur at a minimum of 48 hours after the completion of the busulfan infusion and at a maximum of 7 days after the completion of the busulfan infusion.

On Day 1, the entire dose (all vial[s]) of CTX001 will be thawed and administered through a central venous catheter.

Stage 4: Follow-up Through Engraftment and Up To 2 Years After CTX001 infusion

Stage 4A – Post-infusion In-hospital Follow-up: Subjects will be monitored daily in the transplant unit and receive supportive care according to standard practices for subjects undergoing hematopoietic stem cell transplant (HSCT).

Stage 4B – Post-engraftment Follow-up: After discharge from the transplant unit subjects will be followed for approximately 2 years after the CTX001 infusion.

Study Duration

The duration of Stage 1 will be approximately 2 to 4 months; Stage 2 approximately 2 to 4 months; Stage 3 approximately 1 month; Stage 4 approximately 2 years.

Subjects will be followed on study for a total of approximately 2 years after CTX001 infusion.

Additionally, all subjects who receive CTX001 will be asked to enroll in the long-term follow-up study (Study VX18-CTX001-131), after completion or withdrawal/discontinuation from Study CTX001-121.

Assessments

Efficacy: HbF levels, VOCs, RBC transfusions, alleles with intended genetic modification, reticulocytes, indirect bilirubin, haptoglobin, lactate dehydrogenase, PROs

Safety: Engraftment, TRM, clinical laboratory assessments, vital signs, height, weight, ECGs, physical examinations (PEs)

Exploratory: TRV, N-terminal prohormone b-type natriuretic peptide (NT-proBNP) levels, inflammatory and endothelial activation markers, F-cells distribution, UACR

Statistical Analyses

The study will initially enroll up to 17 subjects to provide a preliminary evaluation of safety and efficacy of CTX001. The study may be expanded to include a total of up to approximately 45 subjects dosed. This expanded sample size will provide an overall power of at least 95% to rule out a response rate of 50% or less for the primary efficacy endpoint when the true response rate is 80% for the primary efficacy endpoint.

Assuming that the study will be expanded to a total of approximately 45 subjects, 3 interim analyses (IAs) may be performed following a group sequential testing

procedure in the expanded study to allow for early evaluation of efficacy. The actual alpha spending will be based on the information available at the IA.

The proportion of subjects who have not experienced any severe VOC for at least 12 months, with the 1-sided *P* value and the 2-sided exact Clopper-Pearson CI, will be provided.

Proportion of subjects free from inpatient hospitalization for severe VOCs after CTX001 infusion sustained for at least 12 months will be summarized.

DMC Reviews

A data monitoring committee (DMC) will be formed before the first subject is dosed. The DMC's objectives and operational details will be defined in a separate document (DMC charter). The DMC will be charged with ensuring the safety of the subjects, safeguarding their interests, and ensuring the quality and integrity of the trial. The DMC will conduct reviews of study data as outlined in the DMC charter. The DMC may recommend that the sponsor suspend enrollment, amend the study, or discontinue the study at any time.

The DMC will review available engraftment and safety data for the first subject before the second subject will undergo myeloablation. The DMC will also review data from the second subject if the second subject infused with CTX001 experiences Grade ≥3 AEs other than those typically associated with busulfan conditioning or autologous transplant procedure through engraftment or 30 days post infusion, whichever is longer, before the remaining subjects can undergo myeloablation and CTX001 infusion.

The DMC will review the available engraftment, safety and efficacy data from the first 2 adult subjects with at least 3 months of data following CTX001 infusion, before expanding enrollment to subjects 12 to <18years of age. Approximately 6 adult subjects will be dosed with CTX001 before the conditioning and dosing of the first pediatric subject.

Further, the DMC will review safety and efficacy data after at least 6 subjects have received CTX001 infusion in order to recommend whether or not to expand the study to include up to approximately 45 subjects dosed (expansion cohort).

The Sponsor or designee will be responsible for promptly alerting the DMC regarding any suspected, unexpected, serious adverse reaction (SUSAR).

An ad hoc DMC safety review will occur if the following criterion is met:

- The same serious adverse event (SAE) at least possibly related to CTX001, but not related to busulfan in 3 subjects, with the exception of:
    - Asymptomatic elevations in amylase or lipase
    - Grade 3 elevation in ALT, aspartate transaminase (AST), alkaline phosphatase, gamma-glutamyl transferase (GGT), or bilirubin that resolve to baseline or within normal range in 10 days
    - Grade 3 infusion reaction (including those reported as a hypersensitivity/allergic event and/or signs or symptoms of infusion reactions) that resolves in 2 days
    - Grade 3 or 4 fever associated with engraftment that resolves in 4 days

If there is any such event(s), all relevant safety data will be reviewed with the DMC at an ad hoc DMC meeting. After review, the Sponsor may decide to continue the study, amend the study, or stop enrollment and remove all subjects who have not received CTX001 from the study.

3 SCHEDULE OF ASSESSMENTS

Schedules of assessments are in Table 3-1, Table 3-2, Table 3-3, Table 3-4, and Table 3-5.

Table 3-1 Study CTX001-121: Screening (Stage 1)

Event/Assessment	Screening (Up to 8 Weeks)	Comments
Informed consent and where applicable, assent	X	Perform vital signs after consent even if collected prior to consent as part of standard of care
PRO assessment (≥18 years old): Pain scale (11-point NRS), ASCQ-Me, FACT-BMT, and EQ-5D-5L PRO assessment (<18 years old): Pain scale (11-point NRS), EQ-5D-Y, PedsQL, and PedsQL SCD module	X	Perform as the first assessment after signing informed consent (Section 11.12).
Demographics	X	Section 11.1
Medical history	X	Including medical records on SCD-related complications and associated treatment including medical facility visits, inpatient hospitalizations and transfusion records for at least 2 years before consent will be collected
Full physical examination[b]	X	Including assessment of spleen size, skin ulcers, and signs/symptoms consistent with myocardial infarction or cerebral vascular accident, new or worsening osteonecrosis, and retinopathy (Section 11.15.3)
Performance status	X	Section 11.15.3
Height and weight	X	Measure with shoes off (Section 11.15.4)
Vital signs	X	Includes blood pressure (systolic and diastolic), temperature, pulse rate, respiration rate, and pulse oximetry (Section 11.15.3)
SCD genotyping (central laboratory)	X	All subjects will be genotyped for SCD (alpha and *HBB* loci) to confirm βS/βS, βS/β0, or βS/β^+ genotype. Results will be required before busulfan conditioning begins. (Section 11.15.2)
Serum chemistry	X	Section 11.15.2
Coagulation	X	Section 11.15.2
Hematology	X	Section 11.15.2
Serum pregnancy test	X	For females of childbearing potential (Section 11.15.2)
Hemoglobin fractionation (central laboratory)	X	HbF for establishing eligibility; will also include HbS, HbA2, HbA (Section 11.7)
Infectious disease marker testing	X	Infectious disease marker testing will include HBV (HBcAb, HbsAg, and NAT); HCV (HCAb and NAT); HIV-1, HIV-2 (Antigen/Antibody immunoassay, if positive, then NAT); and syphilis[a] HTLV-1 Ab (if applicable) (Section 11.15.2)
Urinalysis	X	Section 11.15.2
12-lead ECG	X	Section 11.15.5
Brain MRI/MRA	X	To confirm absence of active Moyamoya disease. If a brain MRI/MRA has been performed within 3 months before date of consent, these results may be used, unless repetition of the assessment is clinically indicated (Section 11.4)
Transcranial Doppler (TCD) ultrasound	X	For subjects 12 to 16 years of age (Section 11.4)

Table 3-1 Study CTX001-121: Screening (Stage 1)

Event/Assessment	Screening (Up to 8 Weeks)	Comments
Echocardiogram	X	LVEF, TRV, and E/e' assessment (Section 11.4)
DLco [corrected]	X	Section 11.3
Adverse event assessment	X	Continuous from signing ICF and assent (where applicable)
Prior and concomitant medications	X	Continuous from signing ICF and assent (where applicable)

[a] Subjects must have at least a screening assay for syphilis performed (e.g., rapid plasma reagin [RPR] is preferred). If positive, a confirmatory test (e.g., FTA-ABS, TPPA) must be done to confirm the presence of active infection. Local testing algorithms for syphilis may be used, provided they contain these tests.

[b] Including Tanner Staging for subjects 12 to <18 years old who have not reached Tanner Stage 5.

Table 3-2 Study CTX001-121: Pre-mobilization Period and Autologous CD34+ Stem Cell Apheresis (Stage 1 and Stage 2)

			Mobilization and Apheresis[b]			
Event/Assessment	**After Eligibility Confirmation (Before Mobilization Start)** [a,b]	**Day -5 to Day -1**[a]	**Day 1**	**Day 2**	**Day 3 (if needed)**	**Comments**
Blood allelic editing (central laboratory)[c]	X					Peripheral blood sample; Collect before start of first RBC exchange or simple transfusion (Section 11.7)
Immunological testing [c]	X					Collect before start of first RBC exchange or simple transfusion (Section 11.15.2)
HbF distribution, F-cells (central laboratory) [c]	X					Collect before start of first RBC exchange or simple transfusion (Section 11.7)
Inflammatory and endothelial activation markers (central laboratory)[c]	X					Collect before start of first RBC exchange or simple transfusion (Section 11.7)
N-terminal prohormone b-type natriuretic peptide[c] (NT-proBNP; central laboratory)	X					Collect before start of first RBC exchange or simple transfusion (Section 11.7)
Bone marrow aspirate (central laboratory)	X					Collect before start of first cycle only. Baseline sample for genomic analysis, and exploratory biomarkers (Section 11.9)
Exploratory biomarker samples (central laboratory)[c]	X					Collect before start of first RBC exchange or simple transfusion (Section 11.7.1)
Urine sample collection (central laboratory)	X					For urine albumin to creatinine ratio Collect first morning void (Section 11.5)
Sperm or oocyte banking	X (optional)					If requested by subject or their legally authorized representative, if less than 18 years of age, prior to busulfan conditioning. Gonadal tissue banking may be done as appropriate per subject age and local site practice. (Section 11.10)
Infectious disease marker testing	X					Repeated within 28 days before the start of each planned apheresis cycle and results should be reported at least 5 business days before each planned cycle (Section 11.15.2)
Mini-Mental State Examination (subjects ≥18 years old) or Wechsler Abbreviated Scale of Intelligence (subjects <18 years old)	X					Age at time of assessment For subjects already on a transfusion regimen MMSE and WASI should be administered before the first mobilization/apheresis cycle Section 11.13 (MMSE), Section 11.14 (WASI)
Hemolysis markers[c]	X					Collect before start of first RBC exchange or simple transfusion. Hemolysis markers include haptoglobin and lactate dehydrogenase (Section 11.15.2)
Erythropoietin[c] (central laboratory)	X					Collect before start of first RBC exchange or simple transfusion (Section 11.15.2)

Table 3-2 Study CTX001-121: Pre-mobilization Period and Autologous CD34$^+$ Stem Cell Apheresis (Stage 1 and Stage 2)

			Mobilization and Apheresis[b]			
Event/Assessment	**After Eligibility Confirmation (Before Mobilization Start)** [a,b]	**Day -5 to Day -1**[a]	**Day 1**	**Day 2**	**Day 3 (if needed)**	**Comments**
Methemoglobin[c]	X					Collect before start of first RBC exchange or simple transfusion (Section 11.15.2)
Ferritin[c]	X					Collect before start of first RBC exchange or simple transfusion (Section 11.15.2)
Serum pregnancy test		X				Females of childbearing potential only. Must be performed within 3 days prior to starting **each** mobilization (Section 11.15.2)
Eligibility for mobilization/apheresis re-confirmed		X				Per site's standard guidelines. Subject must be hemodynamically stable and have no active infection per investigator judgment.
Abbreviated physical examination		X	X	X	X	Prior to each cycle and daily before mobilization (plerixafor) or before the start of apheresis (Section 11.15.3)
Performance status		X	X	X	X	Prior to each cycle and daily before mobilization (plerixafor) or before the start of apheresis (Section 11.15.3)
Vital signs		X	X	X	X	Prior to each cycle and daily before mobilization (plerixafor) or before the start of apheresis. Includes blood pressure (systolic and diastolic), temperature, pulse rate, respiration rate, and pulse oximetry (Section 11.15.3)
Weight		X				Measure with shoes off (Section 11.15.4)
12-lead ECG			X			Before mobilization (plerixafor) or before the start of apheresis on Day 1 of each cycle (Section 11.15.5)
Hematology		X	X	X	X	Prior to each cycle and immediately before starting apheresis on Day 1 through Day 3 (Section 11.15.2)
Serum chemistry		X	X	X	X	Prior to each cycle and daily before mobilization (plerixafor) or before the start of apheresis (Section 11.15.2)
Coagulation		X	X	X	X	Prior to each cycle and daily before mobilization (plerixafor) or before the start of apheresis (Section 11.15.2)
Placement of central venous catheter for apheresis	X (if needed)					
Pre-mobilization RBC exchange or simple transfusion regimen	X					Refer to Table 3-5
Plerixafor administration			X	X	X	Plerixafor will be administered 2 to 3 hours before the start of each day of apheresis. Plerixafor will be administered only if apheresis collection is planned for that day. Administration time can be adjusted based on CD34$^+$ kinetics and in conversation with the study medical monitor and the apheresis specialist.

Table 3-2 Study CTX001-121: Pre-mobilization Period and Autologous CD34$^+$ Stem Cell Apheresis (Stage 1 and Stage 2)

			Mobilization and Apheresis[b]			
Event/Assessment	**After Eligibility Confirmation (Before Mobilization Start)** [a,b]	**Day -5 to Day -1**[a]	**Day 1**	**Day 2**	**Day 3 (if needed)**	**Comments**
CD34$^+$ count			X	X	X	CD34$^+$ cells must be counted before plerixafor administration, 2 hours (± 15) minutes, 4 hours (± 15) minutes, and 6 hours (± 15) minutes post plerixafor administration. CD34$^+$ cells must be counted within 30 minutes after completion of apheresis Sites may perform additional peripheral CD34$^+$ counts per local standards.
Apheresis and shipment of CD34$^+$ stem cells to central manufacturer[c]			X	X		Target to process 22 liters (approximately 4 total blood volumes). For the mobilization/apheresis cycle, cells should be shipped to central manufacturer at the end of Day 1 and Day 2. After the first cycle, cells may be collected for manufacturing on Day 1 and Day 2 (as needed).
Apheresis for CD34$^+$ backup stem cells[d]					X	For the first mobilization/apheresis cycle, CD34$^+$ cells will be collected for backup on Day 3 only. After the first cycle, CD34$^+$ cells may be collected for backup on any day after sufficient CD34$^+$ cells are collected for manufacturing. Cells collected for backup will be stored at the study site. Backup CD34$^+$ stem cells may also be collected on manufacturing cell collection days ONLY after enough cells have been collected for manufacturing and after discussion with the medical monitor and apheresis specialist.
HbS and Hb levels			X	X[e]	X[e]	Daily before mobilization (plerixafor) or before the start of apheresis (Section 11.2)
Adverse event assessment	Continuous from signing ICF and assent (where applicable)					
Prior and concomitant medications	Continuous from signing ICF and assent (where applicable)					

[a] Assessments may be performed over multiple days or visits

[b] In addition to study-related visits, additional assessments or visits may be performed per institutional guidelines or as deemed appropriate by the investigator.

[c] For subjects on chronic transfusion, should be collected within 1 week of a subsequent RBC exchange or simple transfusion and before the first mobilization/apheresis cycle.

[d] For subsequent cycles (after the first mobilization/apheresis cycle) Days 2 and 3 of apheresis will be performed as needed.

[e] HbS collected on Day 2 or Day 3 only if a VOC or other serious adverse event has occurred.

Table 3-3 Study CTX001-121: Myeloablative Conditioning and Infusion of CTX001 (Stages 3A/3B)

Event/Assessment	Pre-conditioning[a,b]		Conditioning[b]	Infusion[b]	Comments
	30 to 7 Days Before Busulfan Initiation	3 to 7 Days Before Busulfan Initiation	Days -5 to -2	Day 1	
Serum pregnancy test		X			Females of childbearing potential only. Must be performed within 5 days of starting busulfan (Section 11.15.2)
Abbreviated physical examination		X	X	X	Section 11.15.3
Performance status		X	X	X	Section 11.15.3
Vital signs		X	X	X	Includes blood pressure (systolic and diastolic), temperature, pulse rate, respiration rate, and pulse oximetry Day 1: before starting infusion of the first vial of CTX001, at 30 (±5) minutes after start of CTX001 infusion, then every 30 (±5) minutes through 2 hours after the last vial of CTX001 has infused (Section 11.15.3)
Weight		X			Measured with shoes off (Section 11.15.4)
12-lead ECG		X		X	Day 1: ECGs before start of infusion (Section 11.15.5)
Hematology		X	X	X	Day 1: Collect laboratory assessments before start of infusion (Section 11.15.2)
Serum chemistry		X	X	X	Day 1: Collect laboratory assessments before start of infusion (Section 11.15.2)
Eligibility for myeloablation reconfirmed	X	X			30 to 7 days before busulfan initiation: If screening examination and start of pre-conditioning visit (30 days prior to busulfan) are ≥4 months apart, repeat pulmonary function tests and echocardiogram 3 to 7 days before busulfan initiation: Review most recent assessments (within 7 days prior to conditioning) as per site's standard guidelines for autologous stem cell transplant
Pre-conditioning transfusion regimen	X				Refer to Table 3-5. Treatment with HU, crizanlizumab (ADAKVEO®), and voxelotor (OXBRYTA®) should be stopped at least 8 weeks before planned mobilization.
Confirm rescue cells are cryopreserved in acceptable condition at the site		X			
Confirm central line is placed		X			
Confirm CTX001 has been received in acceptable condition at the site prior to initiating busulfan			X		

Table 3-3 Study CTX001-121: Myeloablative Conditioning and Infusion of CTX001 (Stages 3A/3B)

Event/Assessment	Pre-conditioning[a,b]		Conditioning[b]	Infusion[b]	Comments
	30 to 7 Days Before Busulfan Initiation	3 to 7 Days Before Busulfan Initiation	Days -5 to -2	Day 1	
Test dose of busulfan to determine optimal busulfan dose[c]	X (optional)				
Anti-seizure prophylaxis[e]			X		Initiate anti-seizure prophylaxis at least 12 hours before first dose of busulfan per hospital guidelines
IV busulfan administration[c]			X		Section 9.8.3
Busulfan PK	X[d]		X		Blood sample will be collected for PK analysis for busulfan on Day 1 and Day 3 of conditioning as per Appendix 15.1
IV infusion of CTX001				X	Subjects should be pre-medicated with an antihistamine (diphenhydramine hydrochloride) and an antipyretic (acetaminophen or paracetamol) per institutional guidelines (Section 10.1.4)
Adverse event assessment	Continuous from signing ICF and assent (where applicable)				
Prior and concomitant medications	Continuous from signing ICF and assent (where applicable)				

[a] Assessments may be performed over multiple days of visits.
[b] In addition to study-related visits, subjects will be followed per institutional guidelines or as deemed appropriate by the investigator.
[c] For subjects less than 34 kg, weight-based recommendation for busulfan dosing are according to Trame et al., 2001[1] provided that the target AUC is the same (Section 9.8.3).
[d] PK analysis will be performed if the test dose of busulfan is administered.
[e] Phenytoin is contraindicated.

Table 3-4 Study CTX001-121: Post-infusion Through Follow-up (Stage 4)

Event/ Assessment	Daily From Day 2 Until Discharge	Follow-up[a,b]													Comments
		D+30[c] M1 (±4d)	D+60 M2 (±7d)	D+90 M3 (±7d)	D+120 M4 (±7d)	D+150 M5 (±7d)	D+180 M6 (±14d)	D+270 M9 (±14d)	D+360 M12 (±14d)	D+450 M15 (±14d)	D+540 M18 (±14d)	D+630 M21 (±14d)	D+720 M24 (±14d)	ETF[cd]	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Includes blood pressure (systolic and diastolic), temperature, pulse rate, respiration rate, and pulse oximetry (Section 11.15.3)
Physical examination									X[e]				X[e]	X	Section 11.15.3
Abbreviated physical examination	X	X	X	X	X	X	X	X		X	X	X			Section 11.15.3
Performance status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.15.3
Post CTX001 infusion transfusion regimen							X								Refer to Table 3-5
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Platelet levels will be monitored daily while in the hospital and every 2 to 3 days after discharge until platelet engraftment is achieved (Section 11.15.2)
Serum chemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.15.2
Coagulation	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Section 11.15.2
Hemolysis markers		X		X			X	X	X	X	X	X	X	X	Hemolysis markers include haptoglobin and lactate dehydrogenase (Section 11.15.2)
Erythropoietin (central laboratory)				X			X	X	X		X		X	X	Section 11.15.2
Methemoglobin				X			X	X	X		X		X	X	Section 11.15.2
Ferritin									X				X	X	Section 11.15.2
Immunological testing				X			X		X				X	X	Section 11.15.2
Blood allelic editing (central laboratory)		X	X	X	X	X	X	X	X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.7)

Table 3-4 Study CTX001-121: Post-infusion Through Follow-up (Stage 4)

Event/ Assessment	Daily From Day 2 Until Discharge	Follow-up[a,b]													Comments
		D+30[c] M1 (±4d)	D+60 M2 (±7d)	D+90 M3 (±7d)	D+120 M4 (±7d)	D+150 M5 (±7d)	D+180 M6 (±14d)	D+270 M9 (±14d)	D+360 M12 (±14d)	D+450 M15 (±14d)	D+540 M18 (±14d)	D+630 M21 (±14d)	D+720 M24 (±14d)	ETF[cd]	
Hemoglobin fractionation (central laboratory)		X	X	X	X	X	X	X	X	X	X	X	X	X	Before scheduled transfusion (if applicable) (Section 11.7)
HbF distribution, F-cells (central laboratory)		X	X	X	X	X	X	X	X	X	X	X	X	X	Before scheduled transfusion (if applicable) (Section 11.7)
Inflammatory and endothelial activation markers (central laboratory)				X			X		X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.7)
NT-proBNP level (central laboratory)									X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.7)
Exploratory biomarker blood samples (central laboratory)				X			X		X		X		X	X	Before scheduled transfusion (if applicable) (Section 11.7.1)
Bone marrow aspirate (central laboratory)							X		X				X	X	For assessment of allelic editing, and exploratory biomarkers (Section 11.9)
Urine sample collection (central laboratory)									X				X	X	For urine albumin to creatinine ratio Collect first morning void (Section 11.5)
DLco [corrected]									X				X	X	Section 11.3
Echocardiogram									X		X		X	X	TRV, LVEF, and E/e' assessment (Section 11.4)
12-lead ECG									X				X	X	Section 11.15.5
PRO assessments For ≥18 years of age: EQ-5D-5L, FACT-BMT, and ASCQ-Me For <18 years of age: EQ-5D-Y, PedsQL, and PedsQL SCD module				X			X		X		X		X	X	Assessment should be performed as the first assessment at any visit, if possible (Section 11.12)

Table 3-4 Study CTX001-121: Post-infusion Through Follow-up (Stage 4)

Event/ Assessment	Daily From Day 2 Until Discharge	Follow-up[a,b]													Comments
		D+30[c] M1 (±4d)	D+60 M2 (±7d)	D+90 M3 (±7d)	D+120 M4 (±7d)	D+150 M5 (±7d)	D+180 M6 (±14d)	D+270 M9 (±14d)	D+360 M12 (±14d)	D+450 M15 (±14d)	D+540 M18 (±14d)	D+630 M21 (±14d)	D+720 M24 (±14d)	ETF[cd]	
PRO assessments (Pain-Scale [11-point NRS])				X	X	X	X	X	X	X	X	X	X	X	Assessment should be performed as the first assessment at any visit, if possible (Section 11.12)
Adverse event collection	Continuous from signing ICF and assent (where applicable)														
Prior and concomitant medication	Continuous from signing ICF and assent (where applicable)														

[a] Assessments may be performed over multiple days of visits.

[b] In addition to study-related visits, subjects will be followed per institutional guidelines or as deemed appropriate by the investigator.

[c] Month (M) is defined as 30 days. If Day 30/M1 occurs while the subject is still hospitalized, the daily assessments required before discharge should be done in addition to the Day 30/M1 assessments.

[d] Early Termination of Follow-Up (ETF). Assessments performed within 2 weeks of ETF Visit should not be repeated. Echocardiogram, DLco, and bone marrow aspirate should not be repeated if performed within 6 months of ETF Visit.

[e] Includes Tanner Staging for subjects 12 to <18 years old until they reach Tanner Stage 5.

Table 3-5 Study CTX001-121: Transfusion Regimen

Event/ Assessment	Pre-mobilization	Prior to Mobilization and Apheresis	Pre-conditioning		After Start of Busulfan to CTX001 Infusion	Post CTX001 Infusion	Notes
	Minimum 8 weeks	Within 3 days	Minimum 8 weeks	Up to 7 days			
RBC exchange or simple transfusion[a]	X[b]	X[c]	X[d]	X[c]	None	X[e]	[a] All transfusions of RBC products should be depleted of leukocytes and matched for at least the ABO blood group, the Kell (K) blood group, and Rh antigens C, D, and E [b] Initiate RBC exchange or simple transfusion a minimum of 8 weeks before planned start of mobilization with a goal of maintaining the target HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL; use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor. Treatment with HU, crizanlizumab (ADAKVEO), and voxelotor (OXBRYTA) should be stopped at least 8 weeks before planned mobilization. [c] Subjects should receive an RBC exchange or simple transfusion; use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor [d] Continue or reinitiate RBC exchange or simple transfusion a minimum of 8 weeks before start of busulfan conditioning with a goal of maintaining an HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL; use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor [e] As needed to maintain Hb ≥7 g/dL per institutional standards
Platelet transfusions						X	Target to maintain platelets >50,000/μL
HbS and Hb levels	X	X	X	X			Collect prior to and after each RBC exchange or simple transfusion, including the RBC exchange or simple transfusion required within 3 days before the start of each mobilization and apheresis cycle (Section 11.2)

4 TABLE OF CONTENTS

1 Title Page 1
Summary of Changes to the Protocol 2
2 Protocol Synopsis 8
3 Schedule of Assessments 14
4 Table of Contents 25
List of Tables 28
List of Figures 29
List of Abbreviations 30
Glossary of Terms 34
5 Introduction 35
5.1 Background 35
5.1.1 Hemoglobinopathies 35
5.1.2 Sickle Cell Disease 35
5.2 Therapeutic Rationale 36
5.2.1 CRISPR-Cas9 Gene Editing Approach 37
5.2.2 *BCL11A* Erythroid-lineage Specific Enhancer for Editing 39
5.2.3 CTX001 41
5.3 Nonclinical Experience 41
5.4 Clinical Experience 41
6 Study Objectives 42
6.1 Primary Objective 42
6.2 Secondary Objectives 42
6.3 Exploratory Objective 42
7 Study Endpoints 42
7.1 Primary Endpoints 42
7.1.1 Primary Efficacy Endpoint 42
7.1.2 Safety Endpoints 42
7.2 Key Secondary Efficacy Endpoints 42
7.3 Secondary Endpoints 43
7.4 Exploratory Endpoints 44
8 Study Population 44
8.1 Inclusion Criteria 44
8.2 Exclusion Criteria 45
9 Study Implementation 47
9.1 Study Design 47
9.1.1 Stage 1: Screening and Pre-mobilization Period 48
9.1.1.1 Rescreening and Repetition of Screening Assessment(s) 48
9.1.2 Stage 2: Mobilization, Autologous $CD34^+$ Stem Cell Collection, CTX001 Manufacture and Disposition 49
9.1.3 Stage 3: Myeloablative Conditioning (Stage 3A) and Infusion of CTX001 (Day 1, Stage 3B) 50
9.1.3.1 Stage 3A: Myeloablative Conditioning 50
9.1.3.2 Stage 3B: CTX001 Infusion (Day 1) 51

9.1.4 Stage 4: Follow-up Through Engraftment and Up To 2 Years After CTX001 Infusion 52
9.1.4.1 Stage 4A: Post-infusion In-hospital Follow-up (Engraftment) 52
9.1.4.2 Stage 4B: Post-engraftment Follow-up 52
9.2 Data Monitoring Committee 53
9.3 Steering Committee 54
9.4 Endpoint Adjudication Committee 54
9.5 Method of Assigning Subjects to Treatment Groups 54
9.6 Rationale for Study Elements 54
9.6.1 Study Design 54
9.6.1.1 Primary Efficacy Endpoint 55
9.6.2 Study Population 55
9.6.3 Dose 57
9.6.4 Study Duration 57
9.7 Prior and Concomitant Treatments and Procedures 57
9.7.1 Prior Medications 57
9.7.2 Venous Access 57
9.7.3 Prohibited Medications 57
9.8 Administration 58
9.8.1 RBC Transfusion 58
9.8.2 Mobilization and Apheresis 58
9.8.2.1 Mobilization 58
9.8.2.2 Apheresis Procedure 59
9.8.3 Conditioning: Busulfan Administration 60
9.8.4 CTX001 Infusion 61
9.8.5 Post-CTX001 Infusion Infection Prophylaxis and Surveillance 61
9.9 Enrollment Suspension Criteria and Stopping Rules 61
9.9.1 Enrollment Suspension Criteria 61
9.9.2 Stopping Rules for Individual Subjects 62
9.10 Removal of Subjects 63
9.11 Replacement of Subjects 63
10 Study Drug Information and Management 63
10.1 Preparation and Dispensing 63
10.1.1 Manufacture of CTX001 63
10.1.2 Shipment of CTX001 Product to Treatment Site 64
10.1.3 Storage of CTX001 Product 64
10.1.4 CTX001 Infusion Procedures, Dose, and Administration 64
10.2 Drug Accountability 65
10.3 Disposal of Unused Drug 65
10.4 Blinding and Unblinding 65
11 Assessments 65
11.1 Subject and Disease Characteristics 65
11.2 Transfusions 65
11.3 Diffusing Capacity of the Lungs for Carbon Monoxide 65
11.4 Imaging Assessments 66
11.5 Urine Albumin to Creatinine Ratio 66

11.6 Blood Collection for Hemoglobin Oxidation and Tissue Oxygen Delivery 66
11.7 Blood for Biomarker Assessments 66
11.7.1 Blood for Exploratory Research 66
11.8 Allelic Editing of CTX001 Drug Product 67
11.9 Bone Marrow Aspirate 67
11.10 Sperm and Oocyte Banking 67
11.11 Veno-occlusive Disease Diagnosis 67
11.12 Patient Reported Outcomes 67
11.12.1 Pain-Scale (11-point NRS) 67
11.12.2 EQ-5D-5L/EQ-5D-Y 68
11.12.3 FACT-BMT 68
11.12.4 ASCQ-Me 69
11.12.5 PedsQL and PedsQL Sickle Cell Disease Module 69
11.13 Mini-Mental State Examination 69
11.14 Wechsler Abbreviated Scale of Intelligence 70
11.15 Safety 70
11.15.1 Adverse Events 70
11.15.2 Clinical Laboratory Assessments 70
11.15.3 Physical Examinations and Vital Signs 72
11.15.4 Weight/Height 72
11.15.5 Electrocardiograms 73
11.15.6 Contraception and Pregnancy 73
11.15.6.1 Contraception 73
11.15.6.2 Pregnancy 75
12 Statistical and Analytical Plans 76
12.1 Sample Size and Power 76
12.2 Analysis Sets 76
12.3 Statistical Analysis 76
12.3.1 General Considerations 76
12.3.2 Background Characteristics 77
12.3.2.1 Subject Disposition 77
12.3.2.2 Demographics and Baseline Characteristics 77
12.3.2.3 Prior and Concomitant Medications 78
12.3.3 Efficacy Analysis 78
12.3.3.1 Analysis of Primary Variables 78
12.3.3.2 Analysis of Secondary Efficacy Endpoints 78
12.3.4 Safety Analysis 80
12.3.5 Engraftment 81
12.3.6 Adverse Events 81
12.3.6.1 Clinical Laboratory Assessments 81
12.3.6.2 Electrocardiogram 82
12.3.6.3 Vital Signs 82
12.3.6.4 Physical Examination 82
12.3.7 Exploratory Analysis 82
12.3.8 Interim and Independent Data Monitoring Committee Analyses 82
12.3.8.1 Interim Analysis 82

12.3.8.2 Independent Data Monitoring Committee Analysis 83
13 Procedural, Ethical, Regulatory, and Administrative Considerations 83
13.1 Adverse Event and Serious Adverse Event Documentation, Severity Grading, and Reporting 83
13.1.1 Adverse Events 83
13.1.1.1 Definition of an Adverse Event 83
13.1.1.2 Clinically Significant Assessments 83
13.1.1.3 Documentation of Adverse Events 84
13.1.1.4 Adverse Event Severity 85
13.1.1.5 Adverse Event Causality 85
13.1.1.6 Study Drug Action Taken 85
13.1.1.7 Adverse Event Outcome 86
13.1.1.8 Treatment Given 86
13.1.2 Serious Adverse Events 86
13.1.2.1 Definition of a Serious Adverse Event 86
13.1.2.2 Reporting and Documentation of Serious Adverse Events 87
13.1.2.3 Expedited Reporting and Investigator Safety Letters 88
13.2 Administrative Requirements 88
13.2.1 Ethical Considerations 88
13.2.2 Subject Information and Informed Consent 89
13.2.3 Investigator Compliance 89
13.2.4 Access to Records 89
13.2.5 Subject Privacy 90
13.2.6 Record Retention 90
13.2.7 Study Termination 90
13.2.8 End of Study 91
13.3 Data Quality Assurance 91
13.4 Monitoring 91
13.5 Electronic Data Capture 92
13.6 Publications and Clinical Study Report 92
13.6.1 Publication of Study Results 92
13.6.2 Clinical Study Report 93
References 94
14 Protocol Signature Pages 99
14.1 Sponsor Signature Page 99
14.2 Investigator Signature Page 100
15 Appendices 101
15.1 Busulfan PK Collection 101
15.2 European Society for Blood and Marrow Transplantation (EBMT) Criteria for VOD Diagnosis 102

List of Tables

Table 3-1 Study CTX001-121: Screening (Stage 1) 14
Table 3-2 Study CTX001-121: Pre-mobilization Period and Autologous $CD34^+$ Stem Cell Apheresis (Stage 1 and Stage 2) 16

Table 3-3 Study CTX001-121: Myeloablative Conditioning and Infusion of CTX001 (Stages 3A/3B) 19
Table 3-4 Study CTX001-121: Post-infusion Through Follow-up (Stage 4) 21
Table 3-5 Study CTX001-121: Transfusion Regimen 24
Table 9-1 Timing of the First Mobilization and Apheresis Cycle 59
Table 9-2 Timing of the Subsequent Mobilization and Apheresis Cycle (After the First Mobilization/Apheresis Cycle) 59
Table 11-1 Laboratory Test Panels 71
Table 12-1 Efficacy Boundaries and Probability of Crossing Efficacy Boundary Under Null Hypothesis for Primary Efficacy Endpoint at the Interim and Final Analyses 83
Table 13-1 AE and SAE Recording 84
Table 13-2 Grading of AE Severity 85
Table 13-3 Classifications for AE Causality 85
Table 13-4 Classifications for Study Drug Action Taken With Regard to an AE 86
Table 13-5 Classifications for Outcome of an AE 86
Table 15-1 EBMT Criteria for SOS/VOD Diagnosis in Adults 102

List of Figures

Figure 5-1 Polypeptide Chains in Adult Hemoglobin and Fetal Hemoglobin 35
Figure 5-2 Fetal Hemoglobin Protection of RBC Sickling 36
Figure 5-3 Schematic of the CRISPR-Cas9 Complex 38
Figure 5-4 CRISPR-Cas9-Mediated Genome Editing Strategies 39
Figure 5-5 Therapeutic Hypothesis for *BCL11A* Gene Editing 40
Figure 9-1 CTX001-121 Study Design 48
Figure 9-2 Mobilization and Apheresis Cycle 50

List of Abbreviations

Abbreviation	Definition
Ab	antibody
ACS	acute chest syndrome
AE	adverse event
Allo-HSCT	allogeneic hematopoietic stem cell transplant
ALP	alkaline phosphatase
ALT	alanine transaminase
ANC	absolute neutrophil count
ASCQ-Me	Adult Sickle Cell Quality of Life Measurement System
AST	aspartate transaminase
AUC	area under the concentration versus time curve
bpm	beats per minute
Cas9	CRISPR-associated 9 nuclease
CBC	complete blood count
CI	confidence interval
CRF	case report form
CRISPR	clustered regularly interspaced short palindromic repeats
crRNA	crisprRNA
CSSCD	Cooperative Study of Sickle Cell Disease
CTCAE	Common Terminology Criteria for Adverse Events
DLco	lung diffusing capacity for carbon monoxide
DMSO	dimethylsulfoxide
DMC	data monitoring committee
DNA	deoxyribonucleic acid
DSB	double-strand break
EAC	Endpoint Adjudication Committee
EBMT	European Society for Blood and Marrow Transplantation
EC	ethics committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EENT	eyes, ears, nose, and throat
EPO	erythropoietin
EQ-5D-5L	EuroQol Quality of Life Scale – 5 dimensions – 5 levels of severity
EQ-5D-Y	EuroQol Quality of Life Scale – 5 dimensions – youth
ETF	early termination of follow-up
exa-cel	exagamglogene autotemcel
FACT-BMT	functional assessment of cancer therapy-bone marrow transplant
FAS	Full Analysis Set
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor

Abbreviation	Definition
GGT	gamma-glutamyl transferase
GMP	Good Manufacturing Practice
GPS	Global Patient Safety
GvHD	graft-versus-host disease
GWAS	genome-wide association studies
Hb	hemoglobin
HBB	human B globin
HBcAb	hepatitis B core antibody
HbF	fetal hemoglobin
HbS	hemoglobin S
HbsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HbVar	Database of Human Hemoglobin Variants and Thalassemia Mutations
HCAb	hepatitis C positive antibody
HCV	hepatitis C virus
HDR	homology-directed repair
HF12	free from inpatient hospitalization for severe VOCs sustained for at least 12 months
hHSPCs	human hematopoietic stem and progenitor cells
HIV-1	human immunodeficiency virus-1
HIV-2	human immunodeficiency virus-2
HLA	human leukocyte antigen
HPFH	Hereditary Persistence of Fetal Hemoglobin
HRQoL	health-related quality of life
HSCT	hematopoietic stem cell transplant
HU	hydroxyurea
ICA	internal carotid artery
ICF	informed consent form
ICH	International Council for Harmonization
INR	international normalized ratio
IRB	institutional review board
IV	intravenous, intravenously
KCC	K-Cl cotransporter
LDH	lactate dehydrogenase
LT-HSC	long-term hematopoietic stem cells
LVEF	left ventricular ejection fraction
M	month
max	maximum value

Abbreviation	Definition
MCA	middle cerebral artery
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
min	minimum value
MMSE	Mini-mental State Examination
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging
mRNA	messenger RNA
MSH	Multicenter Study of Hydroxyurea
N	number of subjects
NAT	nucleic acid testing
NHEJ	non-homologous end-joining
NRS	numerical rating scale
NSAIDs	non-steroidal anti-inflammatory drugs
NSG	NOD SCID gamma
nt	nucleotide
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
PAM	protospacer adjacent motif
PBMC	peripheral blood mononuclear cells
PE	physical examination
PedsQL	Pediatric Quality of Life Inventory
PI	principal investigator
PK	pharmacokinetic(s)
PROs	patient-reported outcomes
PT	prothrombin time
PTT	partial thromboplastin time
q6h	every 6 hours
QC	quality control
QRS	the portion of an ECG comprising the Q, R, and S waves, together representing ventricular depolarization
QT	QT interval
QTcF	QT interval corrected by Fridericia's formula
RBC	red blood cell
RDW	red blood cell distribution width

Abbreviation	Definition
RNA	ribonucleic acid
RNP	ribonucleoprotein complex
RPR	rapid plasma reagin
SAE	serious adverse event
SAP	statistical analysis plan
SC	Steering Committee
SCA	sickle cell anemia
SCD	sickle cell disease
SD	standard deviation
sgRNA	single-guide RNA
SOP	standard operating procedure
SUSAR	suspected, unexpected, serious adverse reaction
TAMMV	time-averaged mean of the maximum velocity
TCD	transcranial Doppler
TDT	transfusion dependent β thalassemia
TE	treatment-emergent
tracrRNA	trans-activating RNA
TRM	transplant-related mortality
TRV	tricuspid regurgitant jet velocity
UACR	urine albumin to creatinine ratio
ULN	upper limit of normal
VAS	Visual Analogue Scale
VF9	have not experienced any severe VOC for at least 9 consecutive months
VF12	have not experienced any severe VOC for at least 12 consecutive months
VOC	vaso-occlusive crisis
WASI	Wechsler Abbreviated Scale of Intelligence
WBC	white blood cell
WHODD	World Health Organization Drug Dictionary

Glossary of Terms

Term	Definition
Engraftment	Engraftment is defined as the first day of 3 consecutive measurements of absolute neutrophil count (ANC) ≥500/μL on 3 different days achieved within 42 days after CTX001 infusion (Study Day 43) without use of unmodified $CD34^+$ cells after reaching the nadir, defined as ANC <500/μL
Enrollment	Subject has signed consent AND is confirmed to be eligible
Intended genetic modifications	Intended genetic modifications are defined as indels that modify the sequence of the erythrocyte-specific enhancer in intron 2 of *BCL11A*
Severe SCD	Severe sickle cell disease (SCD) is defined by the occurrence of at least 2 severe vaso-occlusive crises (VOCs) per year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, hydroxyurea)
Severe vaso-occlusive crises (VOCs)	Severe VOC is defined as any 1 of the following events: • Acute pain event that requires a visit to a medical facility and administration of pain medications (opioids or IV nonsteroidal anti-inflammatory drugs [NSAIDs]) or red blood cell (RBC) transfusions • Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever • Priapism lasting >2 hours and requiring a visit to a medical facility • Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

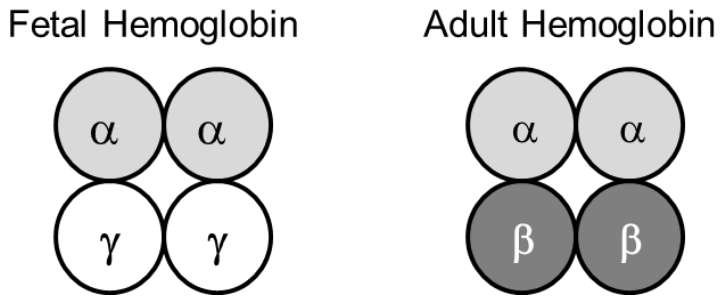
5 INTRODUCTION

5.1 Background

5.1.1 Hemoglobinopathies

Hemoglobin (Hb) is a tetramer formed of 4 globin peptides, each tightly associated with a heme group that contains an atom of iron. During gestation, the predominant form of hemoglobin is fetal hemoglobin (HbF), which is composed of 2 α-globin chains and 2 γ-globin chains. Shortly before birth, there is a switch from HbF to adult hemoglobin, which contains 2 α-globin and 2 β-globin polypeptide chains (Figure 5-1). The switch from HbF to adult hemoglobin is mediated by a transcriptional switch from γ-globin to β-globin within the β-globin gene cluster located on chromosome 11.

Figure 5-1 Polypeptide Chains in Adult Hemoglobin and Fetal Hemoglobin



Hemoglobinopathies are disorders caused by genetic defects that affect the production or function of hemoglobin molecules. Two of the most common of the hemoglobinopathies are sickle cell disease (SCD) and β-thalassemia.

5.1.2 Sickle Cell Disease

SCD is one of the most common monogenic disorders, affecting millions of people.[2] It is estimated to affect over 100,000 individuals in the US and about 42,000 individuals in Europe.[3] The most severe and prevalent form of SCD, referred to as sickle cell anemia (SCA), is an autosomal recessive disease due to homozygous mutations in which a valine replaces a glutamic acid at position 6 in the β-globin protein, which leads to polymerization of deoxygenated hemoglobin and red blood cell (RBC) sickling.

SCD is a chronic disease, characterized by recurrent acute vaso-occlusive crises (VOCs), which lead to acute pain, chronic hemolysis, anemia, progressive tissue injury, and organ dysfunction. The disease affects multiple organs causing acute and chronic complications such as acute chest syndrome (ACS), stroke, priapism, splenic sequestration, osteonecrosis, renal failure, pulmonary hypertension, liver disease, bone damage, limited growth, increased susceptibility to infections, fatigue, and progressive cognitive decline.[4, 5]

About 90% of children born with SCD in the US or EU will survive into adulthood, but their lifespan is shortened by 2 to 3 decades compared to the general population, with a median age of death of approximately 40 to 50 years.[6-10]

Approved therapies to prevent complications of SCD include hydroxyurea (HU) in the US and EU and L-glutamine oral powder, crizanlizumab, and voxelotor in the US.[11-15] These therapies

reduce complications of SCD; however, patients can still have breakthrough VOCs. However, HU is not effective in all patients, is not well tolerated, nor is it curative, and has carcinogenic and teratogenic risks. Allogeneic hematopoietic stem cell transplant (HSCT) is the only known cure for SCD, but HSCT is only available to about 20% of patients who have a matched donor[16], and graft-versus-host disease (GvHD) is a known risk.

Therefore, there is significant unmet medical need for the treatment of SCD.

CTX001 (also known as exagamglogene autotemcel [exa-cel]) is a cellular product that underwent CRISPR-Cas9 based gene editing intended to induce changes in the DNA sequence in the autologous $CD34^+$ hHSPC such that upon erythroid differentiation in the patient, the expression of γ-globin is increased, leading to an increase in HbF expression levels in adult erythroid cells. The increase in HbF is expected to ameliorate the clinical manifestations of SCD.

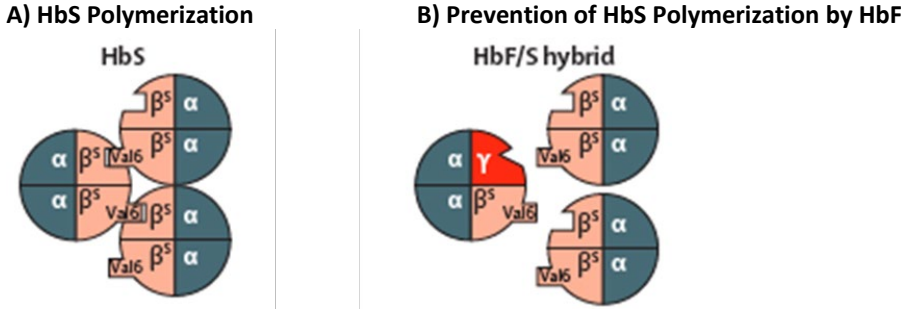
5.2 Therapeutic Rationale

The CRISPR-Cas9 editing therapeutic approach is to restore HbF production through editing of a non-coding region in the *BCL11A* gene. BCL11A is a transcriptional silencer of γ-globin gene expression and hence a negative modulator of HbF.

Several independent lines of evidence support the therapeutic hypothesis that an increase in HbF will ameliorate the clinical manifestations of SCD:

- Analyses of the molecular interactions show that HbF can inhibit the abnormal polymerization of hemoglobin S (HbS) that leads to RBC sickling (Figure 5-2).[17, 18]

Figure 5-2 Fetal Hemoglobin Protection of RBC Sickling



(A) HbS tetramers polymerize under deoxygenated conditions due to interactions between the mutated valine at position 6 and a hydrophobic patch of an adjacent HbS tetramer at positions 85 to 88.
(B) The anti-sickling effect of HbF is due to the substitution of a less hydrophobic amino acid at position 87 (glutamine rather than threonine), which disrupts the hydrophobic pocket and prevents the interaction of HbS tetramers to form polymers.

- Natural history and observational data support the protective role of HbF in the context of naturally occurring variations:
  - Infants with SCD are typically asymptomatic when their HbF levels are high, and only become symptomatic when HbF synthesis is significantly reduced, typically at approximately 6 to 9 months of age.[17]
  - Populations that have polymorphisms associated with higher concentrations of HbF (mostly in Saudi Arabia and Indian subcontinent) have milder forms of SCD.[19]

- Case reports and small studies show that people who are homozygous for the sickle variant but also have high expression of HbF through adulthood (hereditary persistence of fetal hemoglobin [HPFH]) have few or no SCD symptoms despite having HbS concentrations >60%.[20] In addition, people who are homozygous for deletional HPFH type 1 or 2 and harbor large deletions including the β-globin genes are clinically unaffected while they have 100% HbF.[20,21]

- HU, which is an approved pharmacologic therapy for the treatment of SCA and HbSC disease, increases production of HbF and ameliorates SCD symptoms.[22-25]
  - Post hoc analyses of a randomized, placebo-controlled study of HU in SCD patients (Multicenter Study of Hydroxyurea [MSH)] and an observational study of SCD patients (Cooperative Study of Sickle Cell Disease [CSSCD]) show a strong association between increased HbF and reduced pain rate.

5.2.1 CRISPR-Cas9 Gene Editing Approach

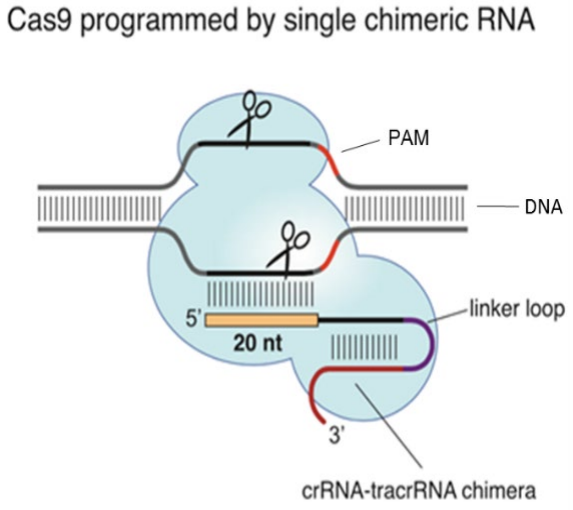
Gene editing using CRISPR-Cas9 can be used to create genetic modifications in $CD34^+$ human hematopoietic stem and progenitor cells (hHSPCs) with high specificity and frequency that will result in a similar phenotype to the naturally occurring HPFH-associated variants, and is expected to increase the production of HbF.

The CRISPR-Cas9 system is a naturally occurring defense mechanism in prokaryotes that has been repurposed as a RNA-guided DNA-targeting platform used for gene editing.[26] It relies on the DNA nuclease Cas9 and 2 noncoding RNAs, crisprRNA (crRNA) and trans-activating RNA (tracrRNA), to target the cleavage of DNA.

- crRNA drives sequence recognition and specificity of the CRISPR-Cas9 complex through Watson-Crick base pairing, typically with a 20-nucleotide (nt) sequence in the target DNA. Changing the sequence of the 5' 20nt in the crRNA allows targeting of the CRISPR-Cas9 complex to specific loci. The CRISPR-Cas9 complex only binds DNA sequences that contain a sequence match to the first 20nt of the single-guide RNA (sgRNA) if the target sequence is followed by a specific short DNA motif (with the sequence NGG) called a protospacer adjacent motif (PAM).
- TracrRNA hybridizes with the 3' end of crRNA to form an RNA-duplex structure that is bound by the Cas9 endonuclease to form the catalytically active CRISPR-Cas9 complex which can then cleave the target DNA.
- Once the CRISPR-Cas9 complex is bound to DNA at a target site, 2 independent nuclease domains within the Cas9 enzyme will each cleave one of the DNA strands 3 bases upstream of the PAM site, leaving a double-strand break (DSB) where both strands of the DNA terminate in a base pair (called a blunt end).

For the molecular reagents used in CTX001 production, the 2 RNA molecules (crRNA and tracrRNA) are joined by a 4nt linker loop to form a chimeric RNA called sgRNA named SPY101 (Figure 5-3). The CRISPR-Cas9 (sgRNA/Cas9) complex together forms a ribonucleoprotein complex (RNP) in situ.

Figure 5-3 Schematic of the CRISPR-Cas9 Complex

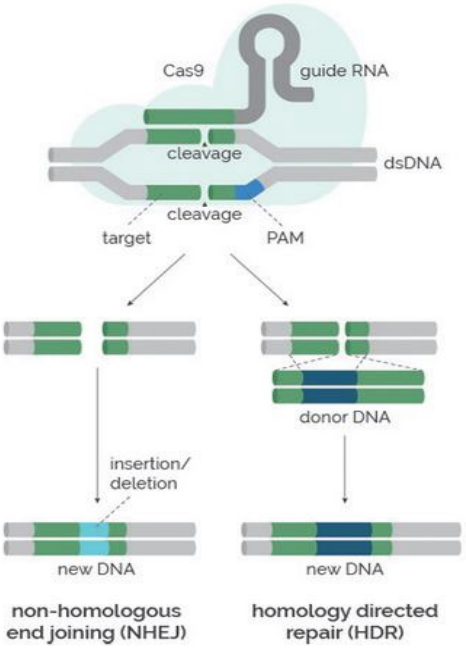


Complex containing a single gRNA wherein the crRNA and tracrRNA are joined by a linker loop (adapted from Jinek et al. 2012[26]).

After binding of CRISPR-Cas9 complex to DNA at a specific target site and formation of the site-specific DSB, the next key step is repair of the DSB. Cells use 2 main DNA repair pathways to repair the DSB: non-homologous end-joining (NHEJ) and homology-directed repair (HDR) (Figure 5-4).

NHEJ is a robust repair mechanism that appears highly active in the majority of cell types, including non-dividing cells. NHEJ is error-prone and can often result in the removal or addition of between 1 and several hundred nt at the site of the DSB, though such modifications are typically <20nt.[26,27] The resulting insertions and deletions (indels) can disrupt coding or noncoding regions of genes. Alternatively, HDR uses a long stretch of homologous donor DNA, provided endogenously or exogenously, to repair the DSB with high fidelity. HDR is active only in dividing cells and occurs at a relatively low frequency in most cell types. NHEJ is the preferable repair operant in our approach.

Figure 5-4 CRISPR-Cas9-Mediated Genome Editing Strategies



CRISPR-Cas9-mediated DNA cleavage creates a DSB at the target locus. The DSB can be repaired by NHEJ and HDR (Genome Editing Strategy Image, 2015).

5.2.2 *BCL11A* Erythroid-lineage Specific Enhancer for Editing

BCL11A is a transcriptional silencer of γ-globin gene expression and hence a negative modulator of HbF.[28,29,30] Multiple lines of evidence from published studies support that targeting of *BCL11A* will result in induction of HbF:

- In SCD transgenic mice, inactivation of *BCL11A* reverses γ-globin silencing and corrects disease pathophysiology.[31]
- Genome-wide association studies (GWAS) identified variants in *BCL11A* that lead to an increase in HbF and a reduction of the pain crisis rate in SCD patients.[29]
- Rare patients with microdeletions including the BCL11A locus (2p15-p16.1) show persistence of HbF and normal hematologic and immunologic function.[32]

Screening of guide RNA (gRNA) molecules that lead to a phenocopy of HPFH effects led to the identification of a guide (SPY101) that targets a critical erythroid-lineage specific transcription factor-binding site (GATA1). The transcription factor-binding site is located within the erythroid-lineage specific enhancer in the second intron of the *BCL11A* gene.

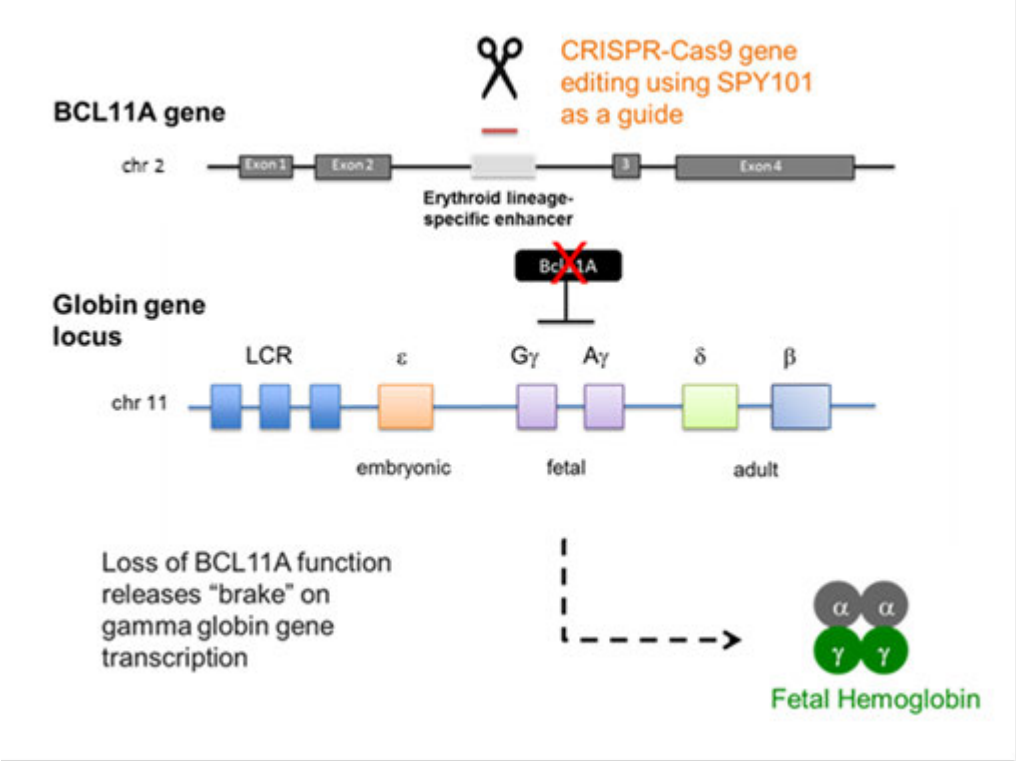
The erythroid specificity of this enhancer is supported by:

- In a transgenic mouse model carrying the human globin locus, *BCL11A* enhancer-deleted mice (BCL11A Δenh) induce human γ-globin expression in erythroid cells, but do not exhibit any other abnormalities including hematopoietic differentiation and cortical morphology.[33] In addition, while expression of *BCL11A* transcript was reduced >30-fold in erythroid cells of BCL11A Δenh mice, there was no reduction of *BCL11A* expression in hHSPCs, B-cells or cortical tissue.

- Engraftment studies in an immunocompromised mouse model using zinc finger nucleases to disrupt the erythroid lineage-specific enhancer of *BCL11A* in human CD34+ cells showed successful multilineage differentiation into lymphoid and myeloid cell lineages.[34]
- Data from 3 patients with microdeletions in a region that contains the *BCL11A* gene (2p15-p16.1) show that in humans, normal hematopoiesis can occur despite a significant reduction in the levels of BCL11A expression. These 3 patients, who had *BCL11A* haploinsufficiency and a large reduction in *BCL11A* expression across mononuclear and erythroid cell populations, also showed a large increase in HbF levels (16%, 24%, and 30%). However, a variety of hematologic parameters were normal, including blood counts, lymphocyte subtypes (assessed in 2 patients), and concentration of immunoglobulins (measured in 1 patient).
- Engraftment data from nonclinical studies show that disruption of the *BCL11A* erythroid lineage-specific enhancer using SPY101-RNP-edited human CD34+ cells did not impact multilineage differentiation into lymphoid and myeloid cell lineages.

Because the repair process uses NHEJ, the gene editing will generate indels within the non-coding *BCL11A* erythroid lineage-specific enhancer on chromosome 2, thus down-regulating *BCL11A* in erythroid precursors with no effect expected in other hematopoietic lineages. This noncoding change is expected to reactivate γ-globin gene transcription and elevate HbF protein in RBCs (Figure 5-5).

Figure 5-5 Therapeutic Hypothesis for *BCL11A* Gene Editing



5.2.3 CTX001

CTX001 is a cellular product consisting of autologous $CD34^+$ hHSPCs modified by CRISPR-Cas9-mediated gene editing. The target of the CRISPR-Cas9 gene editing is the erythroid lineage-specific enhancer region of the *BCL11A* gene located on intron 2 between exons 2 and 3 on chromosome 2. The edits created with the highly specific guide, SPY101, target a critical transcription factor binding site (GATA1) at the erythroid lineage-specific enhancer region (identified as DNase I hypersensitive site +58 [DHS+58]) of the *BCL11A* gene.[34-36] The gRNA-Cas9 RNP (SPY101-RNP) introduces DSBs in DNA in a sequence-dependent manner. Repair of the DSB by NHEJ results in DNA indels, intended to disrupt GATA1 binding, thereby lowering *BCL11A* transcription, with concomitant increases in γ-globin and HbF levels. Since SPY101-RNP precisely targets the non-coding erythroid lineage-specific enhancer region of the BCL11A gene and not the BCL11A coding sequence itself, SPY101-RNP is expected to modulate the levels of expression of the *BCL11A* gene and protein in cells solely of the erythroid lineage and not affect non-erythroid hematopoietic lineages.

5.3 Nonclinical Experience

Details of the nonclinical efficacy and toxicology studies can be found in the Investigator's Brochure.

Briefly, in *in vitro* studies investigating CTX001, CRISPR-Cas9 gene editing at BCL11A gene erythroid enhancer of healthy donor $CD34^+$ cells led to a mean (SD) increase in γ/(γ+β)-globin mRNA ratios of 0.41 (0.15) and mean (SD) percentage of HbF/(HbF+HbA) protein levels of 29% (11%). Mean (SD) allele editing frequency was 80% (6%) and was uniform across subpopulations of $CD34^+$ cells, including long-term hematopoietic stem cells (LT-HSC). The majority of editing was bi-allelic.

In mice xenotransplantation studies, there was no difference in engraftment between NOD SCID gamma (NSG) mice infused with CTX001 and with control (mock or EGFP) edited $CD34^+$ hHSPCs at 16 weeks post-transplantation. The mean (SD) frequency of edited alleles present in the bone marrow samples at 16 weeks was 91% (15%). Furthermore, the engrafted cells were able to maintain their multi-lineage potential.

In the nonclinical safety assessment of CTX001, on-target and potential off-target editing was systematically evaluated using multiple complementary methods. CTX001 demonstrated a high rate of on-target indels (approximately 88%) and no off-target editing compared with unedited healthy donor cells. Karyotyping analysis did not identify any chromosomal translocations or other detectable abnormalities.

A tumorigenicity study did not reveal any neoplastic or myeloproliferative lesions in the mice receiving CTX001.

5.4 Clinical Experience

The current study is a Phase 1/2/3 study with CTX001 in subjects with SCD. CTX001 is also being evaluated in subjects with transfusion dependent β-thalassemia (TDT) in Study CTX001-111. At least 1 subject has been dosed with CTX001 in these studies and enrollment is ongoing.

6 STUDY OBJECTIVES

6.1 Primary Objective

- Evaluate the safety and efficacy of a single dose of autologous CRISPR-Cas9 modified $CD34^+$ hHSPCs (CTX001) in subjects with severe SCD

6.2 Secondary Objectives

- Assess the effects of infusion of CTX001 on disease-specific events and clinical status
- Quantify gene editing efficiency

6.3 Exploratory Objective

- Assess the ability of biomarkers to characterize CTX001 effect and predict treatment outcomes

7 STUDY ENDPOINTS

7.1 Primary Endpoints

7.1.1 Primary Efficacy Endpoint

- Proportion of subjects who have not experienced any severe VOC for at least 12 consecutive months (VF12) after CTX001 infusion. The evaluation of VF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.

7.1.2 Safety Endpoints

- Successful neutrophil engraftment within 42 days after CTX001 infusion (Study Day 43)
- Time to neutrophil engraftment
- Time to platelet engraftment
- Safety and tolerability assessments based on adverse events (AEs), clinical laboratory values, and vital signs
- Transplant-related mortality (TRM) within 100 days after CTX001 infusion
- TRM within 1 year after CTX001 infusion
- All-cause mortality

7.2 Key Secondary Efficacy Endpoints

- Proportion of subjects free from inpatient hospitalization for severe VOCs sustained for at least 12 months (HF12) after CTX001 infusion. The evaluation of HF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Proportion of subjects who have not experienced any severe VOC for at least 9 consecutive months (VF9) any time after CTX001 infusion. The evaluation of VF9 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management for subjects who reach at least 12 months of follow-up.

7.3 Secondary Endpoints

- Proportion of subjects with reduction in annualized rate of severe VOCs at the time of analysis from baseline by at least 90%, 80%, 75%, 50% up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Relative change from baseline in annualized rate of severe VOCs up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Duration of severe VOC free in subjects who have achieved VF12
- Relative change from baseline in rate of inpatient hospitalizations for severe VOCs up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Relative change from baseline in annualized duration of hospitalization for severe VOCs up to 24 months after CTX001 infusion. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Proportion of subjects with sustained HbF ≥20% at the time of analysis for at least 3 months, 6 months, or 12 months. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Change in number of units of RBCs transfused for SCD-related indications over time
- HbF concentrations over time
- Hb concentrations over time
- Change from baseline in reticulocyte count (percent reticulocytes and absolute reticulocyte count) over time
- Change from baseline in indirect bilirubin over time
- Change from baseline in haptoglobin over time
- Change from baseline in lactate dehydrogenase over time
- Proportion of alleles with intended genetic modification present in peripheral blood leukocytes over time
- Proportion of alleles with intended genetic modification present in $CD34^+$ cells of the bone marrow over time
- Change in patient reported outcomes (PROs) over time in adults (≥18 years) using;
    - Pain-scale:11-point numerical rating scale (NRS)
    - Functional assessment of cancer therapy-bone marrow transplant (FACT-BMT)
    - Adult Sickle Cell Quality of Life Measurement System (ASCQ-Me)
    - EuroQol Quality of Life Scale (EQ-5D-5L)
- Change in PROs over time in adolescents (12 to <18 years of age) using;

- Pain-scale: 11-point NRS
- Pediatric Quality of Life Inventory (PedsQL; Teen self-report and parent proxy versions)
- PedsQL SCD module (Teen self-report and parent proxy versions)
- EQ-5D-Youth (EQ-5D-Y self-report and parent proxy versions)

7.4 Exploratory Endpoints

- Change in tricuspid regurgitant jet velocity (TRV) over time
- Change in proportion of circulating erythrocytes expressing fetal hemoglobin (F-cells) over time
- Change in inflammatory and endothelial activation markers over time
- Change in urine albumin to creatinine ratio (UACR) over time

8 STUDY POPULATION

Eligibility will be reviewed and documented by an appropriately qualified member of the investigator's team before subjects are enrolled.

Subjects who meet all of the inclusion criteria and none of the exclusion criteria will be eligible.

8.1 Inclusion Criteria

Subjects must meet all the following inclusion criteria to be eligible for enrollment into the study:

1. Subject (or their legally authorized representative or guardian) will sign and date an informed consent form (ICF) and, where applicable, an assent form.
2. Subjects 12 to 35 years of age, inclusive, on the date of informed consent.
3. Documented βS/βS, βS/β0, or βS/β^+. Subjects can be enrolled based on historical genotype results, but confirmation of genotype is required before busulfan conditioning. The β0 genotypes are defined using the HbVar Database.
4. Subjects with severe SCD. Severe SCD is defined by the occurrence of at least 2 of the following events per year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, HU):
    - Acute pain event that requires a visit to a medical facility and administration of pain medications (opioids or intravenous [IV] non-steroidal anti-inflammatory drugs [NSAIDs]) or RBC transfusions
    - Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever
    - Priapism lasting >2 hours and requiring a visit to a medical facility
    - Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

    Historical severe VOCs will be adjudicated by the Endpoint Adjudication Committee (EAC).

5. Normal transcranial Doppler (TCD) velocity (time-averaged mean of the maximum velocity [TAMMV] <170 cm/sec for non-imaging TCD and <155 cm/sec for imaging TCD) in the middle cerebral artery (MCA) and the internal carotid artery (ICA) for subjects 12 to 16 years of age.
6. Karnofsky performance status of ≥80% for subjects ≥16 years of age or Lansky performance status of ≥80% for subjects <16 years of age.
7. Eligible for autologous stem cell transplant as per investigator's judgment.
8. Female subjects of childbearing potential (postmenarcheal, has an intact uterus and at least 1 ovary, and is less than 1 year postmenopausal) must agree to use acceptable method(s) of contraception from consent through at least 6 months after CTX001 infusion.
9. Male subjects of reproductive capacity must agree to use effective contraception from start of mobilization through at least 6 months after CTX001 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.
11. Willing to participate in the long-term follow-up study (Study VX18-CTX001-131), after completion of this study.

8.2 Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for enrollment:

1. An available 10/10 human leukocyte antigen (HLA)-matched related donor.
2. Prior HSCT.
3. Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the investigator.
4. White blood cell (WBC) count $<3 \times 10^9$/L or platelet count $<50 \times 10^9$/L, not related to hypersplenism per investigator judgment.
5. Treatment with regular RBC transfusions that, in the opinion of the investigator, cannot be interrupted after engraftment.
6. Subjects with history of alloimmunization to RBC antigens and for whom the investigator anticipates that there will be insufficient RBC units available for the duration of the study.
7. More than 10 unplanned hospitalizations or emergency department visits related to SCD in the 1 year before screening that, in the opinion of the investigator, are consistent with significant chronic pain rather than acute pain crises.
8. HbF level >15.0%, irrespective of concomitant treatment with HbF-inducing treatments such as HU.
9. History of abnormal TCD (TAMMV ≥200 cm/sec for non-imaging TCD and ≥185 cm/sec for imaging TCD) for subjects 12 to 18 years of age.
10. History of untreated Moyamoya disease or presence of Moyamoya disease at Screening that in the opinion of the investigator puts the subjects at the risk of bleeding.
11. History of a significant bleeding disorder.

12. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk to the subject. This may include, but is not limited to: history of relevant drug allergies; history of cardiovascular or central nervous system disease; history or presence of clinically significant pathology; history of mental disease; or history of familial cancer syndrome.
13. Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder.
14. Advanced liver disease, defined as
    a. Alanine transaminase (ALT) >3 × the upper limit of normal (ULN) or direct bilirubin value >2.5 × ULN or
    b. Baseline prothrombin time (PT) (international normalized ratio [INR]) >1.5 × ULN, or
    c. History of cirrhosis or any evidence of bridging fibrosis, or active hepatitis on liver biopsy
15. Baseline estimated glomerular filtration rate <60 mL/min/1.73 m^2
16. Lung diffusing capacity for carbon monoxide (DLco) <50% of predicted value (corrected for hemoglobin and/or alveolar volume).
17. Left ventricular ejection fraction (LVEF) <45% by echocardiogram.
18. Prior treatment with gene therapy/editing product.
19. Intolerance, contraindication, or known sensitivity to plerixafor or busulfan. Subject must not have any risk factors in the opinion of the investigator that would increase the likelihood of busulfan-related toxicities. Prior anaphylactic reaction with excipients of CTX001 product (dimethylsulfoxide [DMSO], dextran).
20. Positive for the presence of human immunodeficiency virus-1 (HIV-1) or human immunodeficiency virus-2 (HIV-2) (positive for both antigen/antibody AND nucleic acid tests [NAT]), hepatitis B virus (HBV) (positive for Hepatitis B core antibody [HBcAb] or positive hepatitis B surface antigen [HBsAg] AND NAT tests), syphilis (positive screening AND positive confirmatory tests), or hepatitis C virus (HCV; positive for both antibody [HCAb] AND for NAT tests). Additional infectious disease markers should be obtained and tested as required by the local authority for the collection and processing of cellular therapy products. These additional tests (e.g., HTLV-1, HTLV-2, malaria, tuberculosis, toxoplasmosis, *Trypanosoma cruzi*, or West Nile virus) will be evaluated to determine overall impact to the subject and manufacturing of CTX001.
21. Participation in another clinical study with an investigational drug/product within 30 days of screening or fewer than 5 half-lives of the investigational agent, whichever is longer from screening.
22. Subjects who are not able to comply with the study procedures outlined in the protocol as judged by the investigator.
23. Pregnancy or breastfeeding.

9 STUDY IMPLEMENTATION

9.1 Study Design

This is a single-arm, open-label, multi-site, single-dose, Phase 1/2/3 study in subjects who have severe SCD. The study will evaluate the safety and efficacy of a single dose of autologous CRISPR-Cas9 modified hHSPCs (CTX001) and will initially include up to 17 subjects. The subjects will be 12 to 35 years (inclusive) of age. The study may be expanded to include a total of up to approximately 45 subjects with βS/βS, βS/β0, or βS/β^+ genotypes.

As a safety measure during the initial stages of the study, the first 2 subjects will be treated with CTX001 in a staggered manner to ensure that there is successful engraftment of the first subject before treating the second subject. The second subject will not undergo myeloablation until the first subject achieves neutrophil engraftment (defined as the first day of 3 consecutive measurements of absolute neutrophil count [ANC] ≥500/μL on 3 different days), the available engraftment and safety data have been reviewed by the data monitoring committee (DMC), and at least 30 days after infusion of CTX001 to the first subject. Once the second subject has achieved neutrophil engraftment and has not had Grade ≥3 AEs other than those typically associated with busulfan conditioning or autologous transplant procedure, the remaining subjects can undergo conditioning and CTX001 infusion concurrently. If the second subject infused with CTX001 experiences Grade ≥3 AEs other than those typically associated with busulfan conditioning or autologous transplant procedure through engraftment or 30 days post infusion, whichever is longer, a DMC meeting will be convened and data will be reviewed before the remaining subjects can undergo conditioning and CTX001 infusion, and a substantial amendment to the clinical trial application (CTA) will be submitted before the remaining subjects can undergo conditioning and CTX001 infusion.

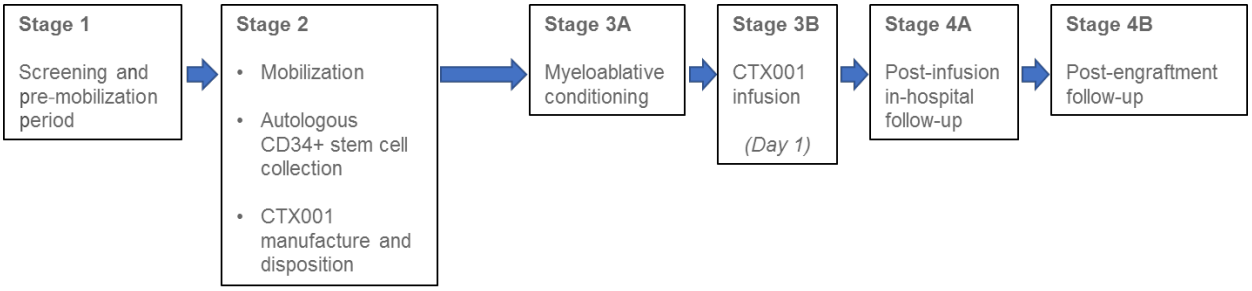
All steps that precede busulfan myeloablation such as consent, screening, and stem cell collection may proceed concurrently without staggering subjects.

The study will initially enroll adult subjects, 18 to 35 years of age. The decision to enroll adolescent subjects (12 to <18 years of age) will be based on a review of the engraftment, safety, and efficacy data from the first 2 adult subjects with at least 3 months of data following CTX001 infusion by the DMC. Approximately 6 adult subjects will be dosed with CTX001 before the conditioning and dosing of the first pediatric subject.

The decision to expand the study to include a total of up to approximately 45 subjects dosed will be based on an examination of the totality of the safety and efficacy data by the Sponsor in consultation with the DMC and the Steering Committee (SC).

For each subject, the study will be conducted in 4 stages (Figure 9-1), which are described below. All subjects who receive CTX001 will be asked to enroll into the long-term follow-up study (Study VX18-CTX001-131).

Figure 9-1 CTX001-121 Study Design



9.1.1 Stage 1: Screening and Pre-mobilization Period

Screening Visit assessments are listed in Table 3-1 and assessment during the pre-mobilization period are listed in Table 3-2.

The investigator (or an appropriate authorized designee at the study site) will obtain informed consent from each subject. After informed consent, subject eligibility will be determined.

Fertility preservation

Subjects who meet the inclusion criteria will have the option to undergo fertility preservation via cryopreservation of oocyte or sperm (this may occur at any time after eligibility is confirmed, before initiation of busulfan conditioning). For pre-pubescent subjects, gonadal tissue collection and cryopreservation may be performed per local practice and standard of care.

RBC transfusion

After eligibility is confirmed, subjects will begin RBC exchange or simple transfusions for a minimum of 8 weeks before the planned start of mobilization and will continue receiving these transfusions until they begin busulfan conditioning. The goal of these RBC transfusions is to maintain HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL. Use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor. Treatment with HU, crizanlizumab (ADAKVEO®) and voxelotor (OXBRYTA®) should be stopped at least 8 weeks before planned mobilization.

9.1.1.1 Rescreening and Repetition of Screening Assessment(s)

Individuals who do not meet the eligibility criteria for participation upon initial screening (screen failure) can be rescreened up to 2 times. All screening tests should be repeated to determine eligibility except for: genotyping (if performed during the initial screening); DLco and echocardiogram (if performed within 3 months), and infectious disease markers (if performed within 60 days) unless judged by the investigator to be necessary. Brain MRI/MRA should be repeated only if there are signs of neurological symptoms as judged by the investigator.

Repetition of individual screening assessment(s) that did not meet eligibility requirements is not permitted with the following exceptions:

- If there is clear evidence of a laboratory error (e.g., hemolyzed sample) or equipment malfunction, collection of a repeat sample for the appropriate laboratory test or assessment may be permitted with the approval of the medical monitor.

- Individual laboratory results that are related to a temporary, reversible condition in the opinion of the investigator may be retested once after the condition resolves or within 30 days, whichever is earlier.

If repeat values of the individual assessment(s) are within the eligibility criteria and completed within the screening window, then the subject is eligible for the study.

Rescreened participants will keep the same participant number assigned during the initial screening process.

9.1.2 Stage 2: Mobilization, Autologous CD34+ Stem Cell Collection, CTX001 Manufacture and Disposition

Assessments during mobilization and apheresis are listed in Table 3-2.

Before starting administration of plerixafor, subjects will be assessed by the study investigator to confirm whether they are eligible to proceed with apheresis (as per local guidelines).

Each subject will undergo stem cell mobilization with plerixafor only (see Section 9.8.2.1 for details). Peripheral blood mononuclear cells (PBMC) will be collected by apheresis.

Subjects should receive an RBC exchange or simple transfusion within 3 days before the start of each mobilization/ apheresis cycle. Use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor.

On Day 1, subjects will receive plerixafor 2 to 3 hours before the start of apheresis. Subjects will undergo apheresis for up to 3 consecutive days to collect $CD34^+$ hHSPCs (see Section 9.8.2.1 for details). Day 2 and Day 3 (if required) will be the same as Day 1. The targeted $CD34^+$ cell collection is at least 15×10^6 $CD34^+$ cells/kg for manufacturing of CTX001 in order to achieve a minimum target dose of 3×10^6 $CD34^+$ cells/kg. An additional 2×10^6 $CD34^+$ cells/kg will be collected as backup for rescue therapy in an event of non-engraftment with CTX001. Cell collection intended for manufacturing will only occur on Days 1 and 2 of apheresis. On these days collected cells intended for manufacturing will be shipped daily at 2°C to 8°C to the manufacturing facility. Day 3 of apheresis is reserved for collection of backup $CD34^+$ stem cells ONLY. These cells will not undergo the editing/manufacturing process and will be cryopreserved at the site (Figure 9-2).

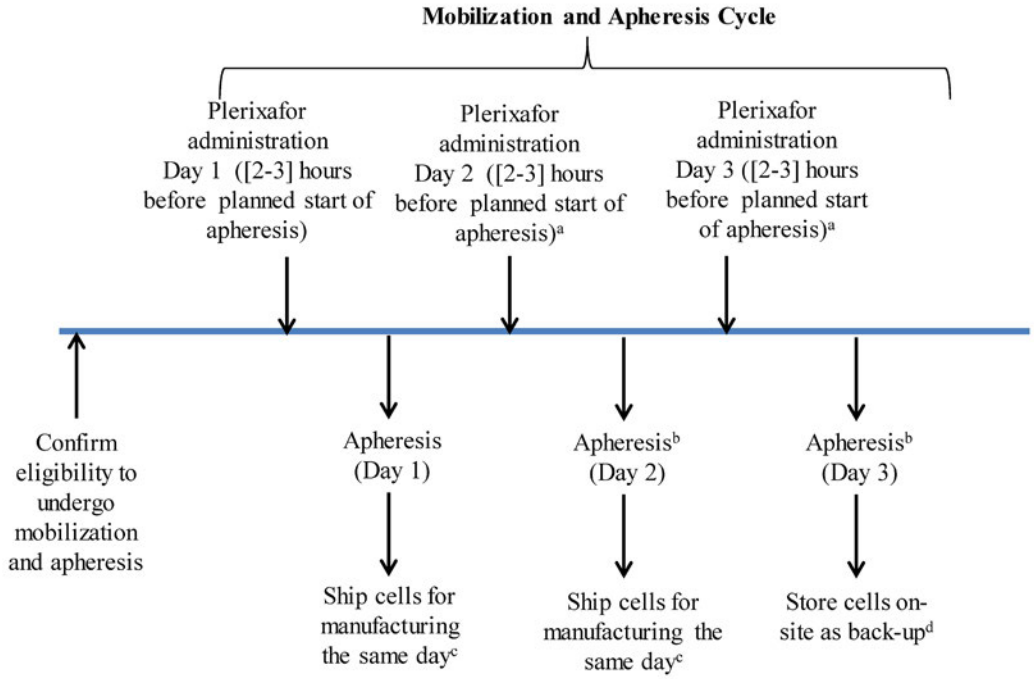
If the first mobilization and apheresis cycle does not yield enough cells for both the minimum CTX001 product and safety backup or if a subject cannot complete apheresis, up to 2 additional mobilization and apheresis cycles will be allowed to collect additional cells.

If the minimum cell dose target for manufacturing and backup is not met after the first 3 cycles, additional mobilizations may be conducted based on discussion and agreement with the subject, the investigator, and the medical monitor, taking into account the safety, tolerability, and efficacy of previous mobilizations and collections, a reasonable likelihood that the additional mobilizations can complete a full manufactured cell dose, and the overall benefit/risk profile for the subject. Such a discussion would take place after each additional cycle. Mobilization cycles will be initiated at least 14 days after the first day of the prior mobilization cycle. Each mobilization cycle will be evaluated for the overall efficacy of mobilization and apheresis. Subjects may be discontinued at any cycle at the discretion of the investigator in discussion with the medical monitor, if further mobilization is deemed unproductive.

Collection of backup $CD34^+$ stem cells must occur prior to proceeding to Stage 3.

If available, the kinetics of $CD34^+$ cell release for the subject may be used by the investigator, in consultation with the medical monitor and the apheresis specialist, to alter the timing of the start of apheresis relative to the time of plerixafor dosing for the purpose of maximizing the collection of $CD34^+$ stem cells.

Figure 9-2 Mobilization and Apheresis Cycle



a. Plerixafor will be administered only if apheresis collection is planned for that day.
b. For subsequent cycles (after the first mobilization/apheresis cycle), Days 2 and 3 of apheresis will be performed as needed.
c. For the first mobilization/apheresis cycle, cells should be shipped to central manufacturer at the end of the first and second collection day. For subsequent cycles (after the first mobilization/apheresis cycle), cells may be collected for manufacturing on Day 1 and Day 2 (as needed) if sufficient $CD34^+$ cells are not collected.
d. For the first mobilization/apheresis cycle, $CD34^+$ cells will be collected for backup on Day 3. For subsequent cycles (after the first mobilization/apheresis cycle), $CD34^+$ cells may be collected for backup on any day after sufficient $CD34^+$ cells are collected for manufacturing. Cells collected for backup dose will be stored at the study site.

9.1.3 Stage 3: Myeloablative Conditioning (Stage 3A) and Infusion of CTX001 (Day 1, Stage 3B)

Assessments during myeloablative conditioning and infusion of CTX001 are listed in Table 3-3.

9.1.3.1 Stage 3A: Myeloablative Conditioning

During CTX001 manufacturing and before the planned start of busulfan conditioning, subjects will continue to receive RBC exchange or simple transfusions with the goal of maintaining an HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL for at least 8 weeks (Table 3-5).

If the planned start of busulfan conditioning is >4 months after completion of mobilization, the investigator may stop the RBC transfusion regimen and restart HU, crizanlizumab, or voxelotor for those subjects who have been previously treated with these agents. If the RBC transfusion

regimen is interrupted, subjects should begin RBC exchange or simple transfusions a minimum of 8 weeks before the planned start of busulfan conditioning with the goal of maintaining an HbS level of <30% of total Hb while keeping total Hb concentration ≤11 g/dL.

Subjects should receive an RBC exchange or simple transfusion up to 7 days before the start of busulfan conditioning (Table 3-5). After the CTX001 product is received at the site and it has been confirmed that the backup $CD34^+$ stem cells remain available and in acceptable condition to be administered if needed, the subject will begin busulfan conditioning.

Use of simple transfusions instead of exchange transfusions should be made in consultation with the medical monitor.

Subjects will be assessed by the investigator to confirm their eligibility to proceed with myeloablative conditioning before administration of busulfan. If a subject is deemed not to be eligible for myeloablative conditioning, this procedure can be delayed until the expiry date of subject's CTX001 Product. Thereafter, the subject will be removed from the study and the Sponsor will be notified.

The starting dose of busulfan will be 3.2 mg/kg administered IV once daily for 4 consecutive days. However, busulfan may be administered as 0.8 mg/kg every 6 hours (q6h) for 4 consecutive days, per the site's standard practice. Clinical sites may use a test dose of busulfan 30 (± 2) days before beginning myeloablation to pre-determine the busulfan dose. For subjects less than 34 kg, weight-based starting dose recommendations for busulfan are as follows[1]:

- For subjects weighing 16 to 23 kg, 1.1 mg/kg q6h or 4.4 mg/kg qd
- For subjects weighing >23 to 34 kg, 0.95 mg/kg q6h or 3.8 mg/kg qd

Target busulfan AUC or cumulative exposure for each dosing regimen will remain the same across all age groups (Section 9.8.3).

Busulfan pharmacokinetics (PK) will be collected on Day 1 and Day 3 of conditioning. Additional details regarding busulfan PK are included in Appendix 15.1. The dose of busulfan may be adjusted as required based on the PK of the first busulfan dose to maintain appropriate levels for myeloablation. Additional details regarding busulfan administration are included in Section 9.8.3.

Infusion of busulfan should not take place if:

- CTX001 integrity has been compromised in any way and is no longer suitable for infusion (e.g., damage to the product container in transit to the clinical site).
- The subject has any clinical condition that, in the opinion of the investigator, would put the subject at unacceptable risk for transplantation.

If the busulfan infusion was given but the full dose was not administered, the investigator should immediately inform the medical monitor.

9.1.3.2 Stage 3B: CTX001 Infusion (Day 1)

The CTX001 infusion will occur at least 48 hours after completion of the busulfan infusion and no more than 7 days after completion of the busulfan infusion. Refer to the CTX001 Product Receipt, Storage, and Infusion Manual for detailed instructions related to the CTX001 product and administration.

On Day 1, the entire dose of CTX001 (3×10^6 to 20×10^6 $CD34^+$ cells/kg) will be infused through a central venous catheter. Each vial containing CTX001 should be infused within 20 minutes after thawing. Refer to the CTX001 Product Receipt, Storage, and Infusion Manual for detailed instructions related to the CTX001 product and administration.

If the investigator suspects that CTX001 integrity has been compromised in any way and is no longer suitable for infusion (e.g., damage to the product container in transit the clinical site), the investigator will not infuse CTX001 and will immediately contact the medical monitor.

9.1.4 Stage 4: Follow-up Through Engraftment and Up To 2 Years After CTX001 Infusion

9.1.4.1 Stage 4A: Post-infusion In-hospital Follow-up (Engraftment)

Subjects will be monitored in the transplant unit and receive supportive care according to standard practices for subjects undergoing HSCT. Subjects should receive RBC transfusions to maintain Hb ≥7 g/dL (Table 3-5) and platelet transfusions to maintain platelets >50,000/μL, or otherwise follow institutional practices. Subjects will be monitored for AEs and engraftment. Subjects will be discharged from the transplant unit upon neutrophil engraftment (ANC ≥500/μL for 3 consecutive measurements on 3 different days) and stabilization of major medical issues as per local hospital guidelines and investigator judgment. Details on post-infusion infection prophylaxis and surveillance are included in Section 9.8.5.

If engraftment is not achieved within 42 days after CTX001 infusion (after Study Day 43), the investigator will determine whether and when the subject will receive the backup $CD34^+$ stem cells according to his/her judgement based on all available clinical information (e.g., the patient's clinical status, presence of myeloid precursors on a bone marrow aspirate, presence of monocytes and neutrophils in the peripheral blood, whether engraftment has occurred by approximately Study Day 60). The investigator is recommended to consult with the study steering committee chair (or designee). In addition, the investigator may decide to administer the backup $CD34^+$ stem cells before Study Day 43 if clinically indicated.

9.1.4.2 Stage 4B: Post-engraftment Follow-up

Stage 4B starts after subjects have successfully engrafted, are clinically stable, and have been discharged from the transplant unit. Subjects will be followed for approximately 2 years after the CTX001 infusion with physical examinations (PEs), laboratory and imaging assessments, and AE evaluations.

Following engraftment, RBC transfusions should be avoided for subjects who have hemoglobin levels ≥7 g/dL, unless medically indicated (e.g., symptomatic anemia or as a requirement for surgery).

Following receipt of busulfan conditioning and CTX001, subjects should undergo at least yearly comprehensive PEs, and receive screening for malignancy based on appropriate country-specific cancer guidelines and subject medical history. Subjects should also undergo appropriate malignancy evaluation if they have unexplained symptoms, signs, or laboratory abnormalities that could be related to an underlying malignancy. Examples include unexplained weight loss or fever, lymphadenopathy, abnormal blood counts. The medical monitor should be notified if a subject is diagnosed with a malignancy.

All subjects who receive CTX001 product will be asked to enroll in the long-term follow-up study (Study VX18-CTX001-131), following completion or withdrawal/discontinuation from Study CTX001-121. Data collected in the long-term follow-up Study VX18-CTX001-131, up to 2 years after CTX001 infusion, will be included in the analyses.

9.2 Data Monitoring Committee

A DMC will be formed before the first subject is dosed. The DMC's objectives and operational details will be defined in a separate document (DMC charter). The DMC will be charged with ensuring the safety of the subjects, safeguarding their interests, and ensuring the quality and integrity of the trial. The DMC will conduct reviews of study data as outlined in the DMC charter. The DMC may recommend that the Sponsor suspend enrollment, amend the study, or discontinue the study at any time.

The DMC will review available engraftment and safety data for the first subject before the second subject can undergo myeloablation. The DMC will also review data from the second subject if the second subject infused with CTX001 experiences Grade ≥3 AEs other than those typically associated with busulfan conditioning or the autologous transplant procedure through engraftment or 30 days post infusion, whichever is longer, before the remaining subjects can undergo myeloablation and CTX001 infusion.

The DMC will review the available engraftment, safety, and efficacy data from the first 2 adult subjects with at least 3 months of data following CTX001 infusion, before expanding enrollment to subjects 12 to <18 years of age. Approximately 6 adult subjects will be dosed with CTX001 before the conditioning and dosing of the first pediatric subject.

Further, the DMC will review safety and efficacy data after at least 6 subjects have received CTX001 in order to recommend whether or not to expand the study to include up to approximately 45 subjects dosed (expansion cohort).

The Sponsor or designee will be responsible for promptly alerting the DMC regarding any suspected, unexpected, serious adverse reaction (SUSAR).

An ad hoc DMC safety review will occur if the following criterion is met:

- The same SAE at least possibly related to CTX001 but not related to busulfan in 3 subjects, with the exception of
    - Asymptomatic elevations in amylase or lipase
    - Grade 3 elevation in ALT, aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), or bilirubin that resolve to baseline or within normal range in 10 days
    - Grade 3 infusion reaction (including those reported as a hypersensitivity/allergic event and/or signs or symptoms of infusion reactions) that resolves in 2 days
    - Grade 3 or 4 fever associated with engraftment that resolves in 4 days

If there is any such event(s), all relevant safety data will be reviewed with the DMC at an ad hoc DMC meeting. After review, the Sponsor may decide to continue the study, amend the study, or stop enrollment and remove all subjects who have not received CTX001 from the study.

9.3 Steering Committee

The Steering Committee (SC) will provide expert guidance on the study execution strategy and help with increasing study awareness and enrollment. Details of the SC structure and function, frequency of meetings, and data planned for review will be included in the SC charter.

9.4 Endpoint Adjudication Committee

The EAC will be composed of an independent, external group of experts with appropriate clinical and scientific background to evaluate VOCs. The EAC will be adjudicating historical VOCs (during the 2 years prior to screening) and on-study VOCs to ensure that the events meet the study's definition of a severe VOC. Historical VOCs that occur within the 2-year period prior to screening, including those which may begin just prior to the 2-year window and end during the 2-year window, will contribute to the determination of eligibility. For subjects with one or more rescreening, all severe VOCs that occurred during the 2 years before the last rescreening date will be adjudicated. The EAC will adjudicate historical VOCs to ensure that the number of historical VOCs are sufficient for eligibility. Details of the EAC structure, function, and process will be included in the EAC charter.

9.5 Method of Assigning Subjects to Treatment Groups

This is an open-label study with a single treatment group.

9.6 Rationale for Study Elements

9.6.1 Study Design

The study is single-arm and open-label because of the need for stem cell transplant procedure to deliver CTX001.

The overall process is consistent with procedures used for autologous HSCT in patients with malignant diseases, with a few exceptions that are described below. Therefore, the risk associated with the procedures in this study is not expected to be significantly different from the standard risks of these procedures.

Prophylactic RBC exchange or simple transfusions will be administered for a minimum of 8 weeks before the planned start of mobilization through CTX001 infusion, to decrease the risk of VOCs during mobilization and busulfan conditioning. The rationale for this transfusion guidance stems from evidence demonstrating that transfusion of non-sickle RBCs by exchange techniques into patients with SCD can mitigate physiologic complications such as stroke[37], disease-related complications after surgical procedures[38], ACS[39], and VOCs.[40] Stem cell transplant and gene therapy protocols for patients with SCD often involve RBC exchange transfusion to decrease HbS to <30% prior to conditioning and mobilization.[41, 42]

Following mobilization with plerixafor alone, $CD34^+$ stem cells will be collected by apheresis. Collection of stem cells by apheresis rather than bone marrow harvest allows for easier isolation of $CD34^+$ cells as the process is less invasive for subjects and does not require general anesthesia. The decision to use plerixafor alone instead of plerixafor in combination with granulocyte colony-stimulating factor (G-CSF), which is the standard regimen used for collection, comes from the fact that administration of G-CSF can induce severe VOCs in subjects with SCD, which can be fatal.[43] Data from studies in healthy subjects and subjects with β-thalassemia show that plerixafor alone can effectively mobilize $CD34^+$ cells.[44,45, 46] These studies also show that $CD34^+$ cells collected using plerixafor alone in healthy subjects

successfully engraft when administered in the context of an allogeneic HSCT. Inability or anticipated inability to successfully manufacture the target dose of CTX001 is among the stopping criteria for this study.

Busulfan alone will be used for conditioning. For allogeneic stem cell transplantation, busulfan is typically combined with cyclophosphamide or fludarabine, since this combination provides both myeloablation and immunosuppression.[47] For autologous transplantation, as in the proposed clinical study with CTX001, immunosuppression to prevent GvHD is not necessary and single agent busulfan will provide predominantly a myeloablative effect.[48] Regimens using busulfan conditioning have been used in allogeneic transplantation of SCD and have resulted in successful engraftment, fewer treatment-related complications, and stable donor chimerism.[49] In addition, other gene therapy studies have successfully used conditioning with busulfan alone in disease areas such as β-thalassemia, SCD, and severe combined immunodeficiency due to adenosine deaminase deficiency.[50, 51]

The study will initially enroll only adult subjects, and enrollment will be expanded to adolescent subjects after data are available from adults (see Section 9.1). There is a strong rationale for including adolescents because of the similarities with adults in terms of etiology and pathophysiology of SCD and expected response to treatment. Overall, the study procedures and potential risks of HSCT with CTX001 are expected to be similar in adult and adolescent populations.[52-54]

9.6.1.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the proportion of subjects who have not experienced any severe VOC for at least 12 consecutive months (VF12) after CTX001 infusion. The evaluation of VF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management. The start of observation 60 days following the last RBC transfusion is consistent with sufficient time to allow for the known lifespan of transfused RBCs[55] as well as the resolution of transient increases in HbF associated with the transplant procedure.[56] A minimum of 12 months duration of absence of severe VOC is robust and considered to be highly unlikely to be due to chance, in patients who have 2 or more severe VOCs per year in the 2 years prior to screening.

9.6.2 Study Population

This study will include adults aged 18 to 35 years, and adolescent-aged children (12 to <18 years of age) with sickle cell disease.

Adolescent-aged children will be included once efficacy and safety have been shown in adults. The rationale for inclusion of adolescent-aged children is that the etiology and pathophysiology of SCD and the potential benefit for reducing and ameliorating disease activity are similar across age groups. Additionally, there are potential benefits in children compared with adults including preserving end-organ function and better tolerability of study procedures, including conditioning and transplantation.[52-54, 57]

Greater than 95% of all children with SCD in developed countries are expected to reach the age of 18; however, mortality rises steeply at the transition into adulthood as a result of chronic organ damage and dysfunction which begins in childhood without overt symptoms. Sickle cell disease in both children and adults is due to the same underlying pathophysiology of abnormal polymerization of HbS and loss of deformability of circulating erythrocytes, with subsequent vaso-occlusion, and chronic hemolysis.[53, 58] The predominant acute features of SCD in both age

groups are also similar, including painful crises, acute chest syndrome, priapism, and chronic hemolysis, eventually leading to progressive end-organ damage including silent and overt cerebral infarction, osteonecrosis, pulmonary hypertension and renal disease.[52] Importantly, with earlier intervention, progressive chronic organ damage may be prevented. Notably, younger patients without major organ dysfunction will often better tolerate myeloablative conditioning and the transplant procedure. This is consistently shown by the improved survival in patients who undergo allogeneic HSCT (allo-HSCT) from an HLA-identical sibling at a younger age.[54, 57, 59]

Therapies to prevent complications of SCD include HU (US and EU), crizanlizumab (ADAKVEO; US only), voxelotor (OXBRYTA; US only), and L-glutamine oral powder (US only).[13,12,11] These therapies reduce complications of SCD; however, patients can still have breakthrough VOCs. HU is not effective in all patients and its long-term benefit on organ dysfunction progression is not known. ADAKVEO, recently approved in the US, is indicated for the reduction of VOC frequency in adults and pediatric patients aged 16 years and older with sickle cell disease. Pivotal studies showed a reduction in overall VOC events with treatment compared with placebo. Voxelotor (OXBRYTA), which is conditionally approved in the US, is indicated for the treatment of SCD in adults and pediatric patients 12 years of age and older. Pivotal studies showed an overall improvement in total hemoglobin levels in treated patients compared with placebo. Importantly, while these therapies may show reductions in some disease manifestations, HLA-matched sibling allo-HSCT is the only known cure for SCD but <20% of patients have a matched related donor and GvHD remains a significant risk. Therefore, there is significant unmet medical need for the treatment of patients with severe SCD.

This study will enroll subjects with severe SCD. Severe SCD is defined by the occurrence of at least 2 of the following events each year during the 2-year period before screening, while receiving appropriate supportive care (e.g., pain management plan, HU if indicated):

- Acute pain event that requires a visit to a medical facility and administration of pain medications (opioids or IV NSAIDs) or RBC transfusions
- Acute chest syndrome, as indicated by the presence of a new pulmonary infiltrate associated with pneumonia-like symptoms, pain, or fever
- Priapism lasting >2 hours and requiring a visit to a medical facility
- Splenic sequestration, as defined by an enlarged spleen, left upper quadrant pain, and an acute decrease in hemoglobin concentration of ≥2 g/dL.

Recurrent VOCs will serve as a clinical endpoint to allow assessment of the clinical benefits by assessing the change in severe pain crises and other VOC manifestations.

Subjects with baseline HbF >15% will not be eligible for enrollment. Since very few patients with SCD who have high HbF levels have recurrent severe VOCs, an upper limit for HbF was included to support the fact that the events collected from the medical records are acute severe VOCs related to SCD.

Subjects who have a history of requiring regular RBC transfusions to prevent SCD complications and who, in the judgment of the investigator, cannot stop RBC transfusions after CTX001 infusion will not be eligible for enrollment because in order to accurately assess sustained HbF ≥20%, RBC transfusion will have to be stopped after CTX001 infusion as specified per study protocol.

9.6.3 Dose

Autologous transplantation for various indications typically uses at least 2×10^6 to 2.5×10^6 $CD34^+$ cells/kg to support engraftment.[60-62] To ensure engraftment in all subjects in the SCD study, the Sponsor selected a conservative minimum dose of 3×10^6 $CD34^+$ cells/kg, which is 20% to 50% higher than the typical minimum dose for autologous transplantation. In principle, infusion of a higher number of $CD34^+$ stem cells after myeloablation is associated with more rapid engraftment, durability, and efficacy of the treatment.[63]

Based on current manufacturing capabilities and projected cell yields, the Sponsor proposes to set a maximum cell dose limit of 20×10^6 $CD34^+$ cells/kg. The Sponsor, along with the DMC, will monitor for any potential dose related toxicities.

9.6.4 Study Duration

The total duration of follow-up for subjects who receive CTX001 will be 15 years following infusion.

Subjects will be followed on Study CTX001-121 for 2 years after CTX001 infusion. Because the study will enroll subjects with at least 2 severe VOCs per year during the 2-year period before screening, a 2-year follow-up will allow meaningful assessment of the decrease in the annualized incidence of VOCs.

Additionally, all subjects who receive CTX001 will be asked to enroll in the long-term follow-up study (Study VX18-CTX001-131), after completion or withdrawal/discontinuation from Study CTX001-121. The planned duration of Study VX18-CTX001-131 is up to 15 years' follow-up after CTX001 infusion. This is to ensure that any potential long-term AEs related to CTX001 are captured and to evaluate long-term treatment outcomes as well as to provide a longer follow-up for efficacy. The impact of the treatment on SCD burden and chronic complications will also be monitored.

9.7 Prior and Concomitant Treatments and Procedures

9.7.1 Prior Medications

All medication taken within 30 days before signing of the ICF and assent (where applicable) will be recorded.

Retrospective information on RBC transfusions will be recorded from 24 months prior to date of consent and assent (where applicable).

9.7.2 Venous Access

A central venous catheter will be used for administration of the conditioning regimen and infusion of CTX001. A central venous catheter may also be used for apheresis, for RBC exchange or simple transfusions, and for clinical care of the subject following CTX001 infusion as per investigator judgment.

9.7.3 Prohibited Medications

HU: Treatment with HU should be discontinued at least 8 weeks before planned start of mobilization. Subjects may be restarted on HU after mobilization and apheresis if deemed necessary by the investigator and if >4 months are planned between completion of mobilization

and start of busulfan conditioning. If HU is restarted, treatment with HU should be discontinued once RBC transfusions are restarted before busulfan conditioning.

After CTX001 infusion, once engraftment is achieved, HU may be restarted if the subject experiences VOCs or other complications that are judged by the investigator to be related to SCD and warrant re-initiation of HU. If HU is restarted, it is recommended that HU be progressively tapered and discontinued, if considered medically acceptable.

L-Glutamine: Treatment with L-Glutamine should be discontinued after CTX001 infusion.

Crizanlizumab (ADAKVEO): Treatment with ADAKVEO should be discontinued at least 8 weeks before planned start of mobilization. Subjects may be restarted on ADAKVEO after mobilization and apheresis if deemed necessary by the investigator. ADAKVEO should be discontinued at least 8 weeks before the start of busulfan conditioning.

Voxelotor (OXBRYTA): Treatment with OXBRYTA should be discontinued at least 8 weeks before planned start of mobilization. Subjects may be restarted on OXBRYTA after mobilization and apheresis if deemed necessary by the investigator. OXBRYTA should be discontinued at least 8 weeks before the start of busulfan conditioning.

HbF-inducing agent (other than HU): Treatment with any HbF-inducing treatment should be discontinued after CTX001 infusion.

G-CSF: G-CSF should not be administered after CTX001 infusion. If engraftment does not occur by Day 21 post-CTX001 infusion, G-CSF (e.g., filgrastim) may be administered. The use of G-CSF post-CTX001 infusion should be discussed with the study medical monitor prior to initiation.

Iron Chelators: Chelation must be discontinued at least 7 days before the start of myeloablative conditioning with busulfan.

Iron chelation with deferasirox, deferoxamine, or deferiprone should not be started until at least 3 months after CTX001 infusion to allow for stable hematopoietic recovery and avoid potential myelosuppressive effects.

9.8 Administration

9.8.1 RBC Transfusion

Refer to Table 3-5 for details on RBC transfusion.

9.8.2 Mobilization and Apheresis

9.8.2.1 Mobilization

The decision on whether a central line is needed for mobilization will be made by the apheresis-experienced nurse or physician. Mobilization will be with plerixafor only. G-CSF should NOT be administered.

Subjects will receive plerixafor at a dose of 0.24 mg/kg via subcutaneous injection 2 to 3 hours before the start of apheresis. Weight taken within 5 days before the first day of mobilization will be used for calculating the recommended dose. If multiple weights have been taken during the 5 days prior to first day of mobilization, then the weight recorded closest to time of mobilization should be used for calculation of the plerixafor dose. Refer to Table 9-1 and Table 9-2 for the full dosing schedule.

$CD34^+$ cell count in peripheral blood will be performed as listed in Table 3-2.

If available, the kinetics of $CD34^+$ cell release for the subject may be used by the investigator, in consultation with the medical monitor and the apheresis specialist, to alter the timing of the start of apheresis relative to the time of plerixafor dosing for the purpose of maximizing the collection of $CD34^+$ stem cells.

Refer to the plerixafor package insert for product details. Refer to the Apheresis Collection Manual for further details on mobilization, apheresis, and infusion procedures.

Table 9-1 Timing of the First Mobilization and Apheresis Cycle

	Mobilization and Apheresis		
Drug	**Day 1**	**Day 2**	**Day 3**
Plerixafor administration (2 to 3 hours before the start of apheresis)[a]	X	X	X
$CD34^+$ stem cell apheresis for CTX001 product and backup $CD34^+$ stem cells	X[b]	X[b]	X[c]

[a] Plerixafor will be administered only if apheresis collection is planned for that day.
[b] Cells should be shipped to central manufacturer at the end of the first and second collection day.
[c] The third day of apheresis is reserved ONLY for collection of backup $CD34^+$ stem cells. No collection of cells for manufacturing should occur on that day.

Table 9-2 Timing of the Subsequent Mobilization and Apheresis Cycle (After the First Mobilization/Apheresis Cycle)

	Mobilization and Apheresis		
Drug	**Day 1**	**Day 2**	**Day 3**
Plerixafor administration (2 to 3 hours before the start of apheresis)[a]	X	X[b]	X[b]
$CD34^+$ stem cell apheresis for CTX001 product and backup $CD34^+$ stem cells	X[c]	X[c]	X[d]

[a] Plerixafor will be administered only if apheresis collection is planned for that day.
[b] On Days 2 and 3 of apheresis will be performed as needed.
[c] Cells may be collected for manufacturing on Day 1 and Day 2 (as needed) if sufficient $CD34^+$ cells are not collected. $CD34^+$ cells may be collected for backup on any day after sufficient $CD34^+$ cells are collected for manufacturing.
[d] The third day of apheresis is reserved ONLY for collection of backup $CD34^+$ stem cells. No collection of cells for manufacturing should occur on that day.

9.8.2.2 Apheresis Procedure

PBMCs will be collected per clinical site standard operating procedures (SOPs) for up to 3 consecutive days. The targeted $CD34^+$ cell collection for manufacturing of CTX001 is at least 15×10^6 $CD34^+$ cells/kg. An additional 2×10^6 $CD34^+$ cells/kg will be collected as backup for rescue therapy in an event of non-engraftment with CTX001. Target to process 22 liters

(approximately 4 total blood volumes) as allowed per local regulations. Collection of backup $CD34^+$ stem cells must occur prior to proceeding to Stage 3 (busulfan conditioning).

If the first mobilization and apheresis cycle does not yield enough cells for both the minimum CTX001 product and safety backup, or if a subject cannot complete apheresis, 2 additional mobilization and apheresis cycles will be allowed to collect additional cells. The additional mobilization cycle should be initiated at least 14 days after the first day of the prior mobilization cycle. Based on the number of $CD34^+$ cells received at the manufacturing site and the manufactured CTX001 dose, the medical monitor will inform the clinical site if additional mobilization cycles will be necessary. If the minimum cell dose target for manufacturing and backup is not met after the first 3 cycles, additional mobilizations may be conducted based on discussion and agreement with the subject, the investigator, and the medical monitor, taking into account the safety, tolerability, and efficacy of previous mobilizations and collections, a reasonable likelihood that the additional mobilizations can complete a full manufactured cell dose, and the overall benefit/risk profile for the subject. Such a discussion would take place after each additional cycle. Each mobilization cycle will be evaluated for the overall efficacy of mobilization and apheresis. Subjects may be discontinued at any cycle at the discretion of the investigator in discussion with the medical monitor, if further mobilization is deemed unproductive.

Any AEs associated with the apheresis procedure should be managed as per the site's standard guidelines.

Cells should be shipped at the end of the first and the second collection day. Cells collected for a backup dose will be cryopreserved and stored at the study site. Additional details and instructions on shipment and receipt are included in the study Product Receipt, Storage, and Infusion Manual.

9.8.3 Conditioning: Busulfan Administration

Busulfan will be administered IV daily at a starting dose of 3.2 mg/kg/day for 4 consecutive days (based on weight collected within 7 days prior to the first day of busulfan administration). Once-daily dosing is the preferred schedule, but the busulfan dose regimen may be adjusted to be given q6h per site's standard practice. A test dose of busulfan may be performed 30 (± 2) days before beginning myeloablation to pre-determine the busulfan dose. For subjects less than 34 kg, weight-based starting dose recommendations for busulfan are as follows[1]:

- For subjects weighing 16 to 23 kg, 1.1 mg/kg q6h or 4.4 mg/kg qd
- For subjects weighing >23 to 34 kg, 0.95 mg/kg q6h or 3.8 mg/kg qd

Target busulfan AUC or cumulative exposure for each dosing regimen will remain the same across all age groups (see Section 9.1.3.1).

Busulfan PK will be collected on Day 1 and Day 3 of conditioning. Additional details regarding busulfan PK are included in Appendix 15.1. The dose of busulfan may be adjusted based on the PK of the first busulfan dose to maintain appropriate levels for myeloablation. The target AUC for subjects at a starting dose of 3.2 mg/kg/day for 4 days is 5000 μM·min (range: 4500 to 5500). The AUC for subjects receiving busulfan q6h for 4 days is 1125 μM·min (range: 900 to 1350). Refer to Appendix 15.1 for the busulfan PK collection schedule.

During busulfan conditioning, anti-seizure prophylaxis and other supportive measures should be instituted as per hospital guidelines.

9.8.4 CTX001 Infusion

CTX001 will be supplied in infusion vial(s). All vial(s) containing CTX001 should be infused. Further description is provided in the study Product Receipt, Storage, and Infusion Manual. Refer to Section 10 for study drug information and management.

9.8.5 Post-CTX001 Infusion Infection Prophylaxis and Surveillance

Following CTX001 infusion, subjects will undergo infectious surveillance and prophylaxis (bacterial, viral, fungal) as per local guidelines for HSCT and investigator judgment.

If a subject develops sepsis or systemic bacteremia following CTX001 infusion, appropriate cultures and medical management will be initiated.

9.9 Enrollment Suspension Criteria and Stopping Rules

9.9.1 Enrollment Suspension Criteria

The investigator will immediately notify the medical monitor of any of the following:

- Fatal or life-threatening serious adverse events (SAEs)
- SAEs related to CTX001
- Engraftment failures
- Malignancies
- Grade 4 VOCs associated with mobilization
- Failure to collect the target number of $CD34^+$ cells

Any SAEs will also be reported to Vertex Global Patient Safety in accordance with Section 13.1.2.2.

Enrollment will be temporarily suspended for any of the following **safety reasons:**

- Death (non-accidental) on study after CTX001 infusion.
- Detection of leukemia, myelodysplastic disease, or myeloproliferative disorder in a subject who has received CTX001.
- Failure to achieve neutrophil engraftment after infusion of CTX001 in 2 subjects, as defined by lack of engraftment within 42 days after infusion (Study Day 43) or administration of backup $CD34^+$ stem cells.
- Failure to manufacture the target cell dose of CTX001 in 3 of the first 6 subjects who have undergone the maximum allowed mobilization and apheresis cycles.

Enrollment will be temporarily suspended for **lack of efficacy** if:

- HbF is <5% at 9 months in 3 of the first 12 subjects

- The proportion of alleles with intended genetic modification present in peripheral blood leukocytes is <10% in 3 of the first 12 subjects at 2 consecutive time points after neutrophil engraftment

If there is any such event(s), enrollment will be temporarily suspended, and all available data will be reviewed. After evaluation, and following a review of the data with the DMC, the Sponsor may decide to restart enrollment in the study, amend the study, or permanently suspend enrollment and remove all subjects who have not received CTX001 from the study. A substantial amendment will be submitted before resuming enrollment.

Additionally, the Sponsor may convene an ad hoc DMC review of safety as outlined in Section 9.2 as well as temporarily suspend enrollment at any time if the benefit-risk assessment is suspected to be no longer acceptable. In this case, all available data will be reviewed by the DMC. Based on the review of data and the recommendations of the DMC, the Sponsor may decide to restart enrollment, amend the study, or permanently suspend enrollment and remove all subjects who have not received CTX001 from the study.

In the event enrollment is permanently suspended, subjects who are already enrolled in the study will not undergo stem cell mobilization or myeloablation with busulfan, and will have an end of study visit. Subjects who have already been treated with CTX001 will remain in the study and be followed up or transitioned to the long-term safety follow-up study (Study VX18-CTX001-131).

In the unlikely event that enrollment is suspended after a subject (or subjects) has received busulfan and before they have received CTX001, the decision to infuse CTX001 or the backup unedited $CD34^+$ cells will be up to the investigator's discretion after discussion of the potential risks with the subject. The investigator should inform the medical monitor of the decision before infusion of cellular product.

If enrollment is permanently suspended, the Sponsor will immediately inform all appropriate parties including principal investigators (PIs), Ethics Committees (EC), IRB, and competent authorities.

9.9.2 Stopping Rules for Individual Subjects

Stopping rules for individual subjects are as follows:

- Any medical condition that, in the opinion of the investigator, would put the subject at risk for continuing study-related procedures or follow-up
- If a subject becomes pregnant or begins breastfeeding before start of busulfan conditioning
- Inability or anticipated inability to successfully manufacture the target cell dose of CTX001.
- If a subject is found not to have met eligibility criteria or has an important protocol deviation before the start of busulfan conditioning that, in the opinion of the investigator, would put the subject at risk for continuing study-related procedures or follow-up
- Failure to adequately mobilize and/or collect adequate numbers of $CD34^+$ cells or manufacture the required dose of CTX001

9.10 Removal of Subjects

A subject (or legally authorized representative) may withdraw consent to participate in the study at any time. If a subject withdraws consent the date and reason for withdrawal should be documented. Subject data collected up to the date of consent withdrawal will be included in the analyses. If the subject withdraws consent for the study, no further evaluations will be performed, and no additional samples will be collected. The Sponsor may retain and continue to use any data and samples collected before such withdrawal of consent. Wherever possible, the tests and evaluations listed for the end of study visit will be carried out. The Sponsor will be notified of all study withdrawals.

If a subject withdraws consent to participate in the study after infusion of CTX001, the subject will be asked to enroll in the long-term follow-up study (Study VX18-CTX001-131). If the subject refuses to participate in the long-term follow-up study, the subject will be asked if they consent (and assent, where applicable) to have their information collected through review of medical records or quarterly telephone interviews. All subjects who withdraw after CTX001 infusion should be followed for safety whenever possible.

Other reasons for removal of a subject before start of busulfan conditioning include:

- Any medical condition that, in the opinion of the investigator, would put the subject at risk for continuing study-related procedures or follow-up
- If a subject is found not to have met eligibility criteria or has an important protocol deviation which compromises subject safety
- Subject becomes pregnant or begins breastfeeding
- Failure to successfully manufacture the minimum dose of CTX001 or deliver CTX001 to the site
- Administrative decision by Sponsor

If a subject chooses to withdraw from the study after the start of busulfan conditioning and before administration of CTX001, the subject's stored, unedited backup $CD34^+$ stem cells will be infused for safety reasons.

If a 10/10 HLA matched related donor becomes available after enrollment but before starting busulfan conditioning, then the subject will be withdrawn from the study.

9.11 Replacement of Subjects

Subjects who fail screening, withdraw consent prior to CTX001 infusion, or do not receive CTX001 infusion for any other reason may be replaced.

10 STUDY DRUG INFORMATION AND MANAGEMENT

10.1 Preparation and Dispensing

10.1.1 Manufacture of CTX001

Manufacturing and release of the cell product inclusive of quality release testing is expected to take approximately 11 weeks. Following arrival of the cells at the manufacturing facility, $CD34^+$ cells are isolated and incubated in culture media. The cells are then recovered, pooled, and

treated with CRISPR-Cas9 gene-editing reagents. The cells are again allowed to recover in culture medium before harvest, formulation, sampling for quality control (QC) testing, filling, and cryopreservation in the vapor phase of liquid nitrogen (≤-135°C). Product will then be shipped at ≤-135°C to the clinical site in 1 or more 20-mL vials. All manufacturing procedures are carried out in GMP cleanrooms.

10.1.2 Shipment of CTX001 Product to Treatment Site

CTX001 (packaged in vial[s]) will be shipped cryopreserved to the clinical sites in sealed dry nitrogen shippers (≤-135°C) validated for at least 10 days. On receipt, package integrity will be checked and the cellular product label(s) will be confirmed against the donor's unique identifier. Vial(s) will be labeled for "autologous use only" with donor identification, batch number, and expiry date in accordance with local regulatory requirements. Additional details and instructions are included in the study Product Receipt, Storage, and Infusion Manual.

10.1.3 Storage of CTX001 Product

CTX001 will be stored at the site in the frozen state at a temperature of ≤-135°C until just prior to the scheduled infusion. Additional details and instructions are included in the study Product Receipt, Storage, and Infusion Manual.

10.1.4 CTX001 Infusion Procedures, Dose, and Administration

CTX001 will be formulated in CryoStor CS5 medium which contains 5% DMSO and Dextran-40. Histamine release associated with DMSO can result in adverse effects including nausea, vomiting, diarrhea, flushing, fevers, chills, headache, dyspnea, rashes, bronchospasm, anaphylaxis, vasodilation and hypotension, and mental status changes. Given the risk of these AEs, subjects will be pre-medicated with an antihistamine (diphenhydramine hydrochloride) and an antipyretic (acetaminophen or paracetamol) before dosing with CTX001. These medications may be repeated every 3 to 4 hours as needed as per institutional guidelines and investigator judgment.

Emergency medical equipment should be available during the infusion in the event a subject has an allergic response, severe hypotensive crisis, or any other infusion-related reaction.

The single dose of CTX001 will be given at least 48 hours and within 7 days after the last busulfan dose. CTX001 vial(s) should be thawed just prior to the scheduled infusion as per local site SOPs. The entire dose of CTX001 should be infused within 20 minutes of thaw. Detailed instructions for the thaw and infusion of cells are in the study Product Receipt, Storage, and Infusion Manual. Hospital guidelines will be used to maintain chain of custody for CTX001 from the stem cell laboratory to the subject. Before infusion, local procedures should be followed regarding the verification of subject identity and product details to ensure a match as well as integrity of the product.

Subjects will receive CTX001 on Day 1 via infusion through a central venous catheter. All vial(s) containing CTX001 should be infused.

All procedures involving CTX001 must be performed using aseptic techniques by trained personnel according to the SOP at the clinical site.

In the unlikely event that CTX001 infusion does not occur within 7 days after the last dose of busulfan, subjects should receive the backup $CD34^+$ stem cells.

10.2 Drug Accountability

The investigator and Sponsor are responsible for accountability and traceability of CTX001.

The investigator will ensure that CTX001 is used in accordance with this protocol. Detailed accountability records indicating CTX001 inventory at each clinical site, use by each subject, and disposal will be maintained by the clinical sites. These records will document that subjects were provided the CTX001 dose as specified in the protocol and should reconcile CTX001 received by the subject. The Sponsor or its designee will review CTX001 accountability at the clinical sites on an ongoing basis during monitoring visits.

10.3 Disposal of Unused Drug

All excess material containing CTX001 will be considered hazardous waste and disposed of in compliance with applicable regulations and standard hospital procedures. Destruction will be adequately documented.

10.4 Blinding and Unblinding

This is an open-label study.

11 ASSESSMENTS

The schedule of assessments is shown in Table 3-1, Table 3-2, Table 3-3, Table 3-4, and Table 3-5.

In addition to study-related visits, subjects will be followed per institutional guidelines or as deemed appropriate by the investigator.

11.1 Subject and Disease Characteristics

Demographics (e.g., sex, age, race, ethnicity), 2 years of transfusion history (simple or RBC exchange transfusions), and 2 years of SCD-related complications and associated treatment (including inpatient hospitalization and medical facility visits) will be recorded. SCD history such as genotype and age of diagnosis will also be recorded. Past and present medical history, including targeted medical history and additional medical history considered by the investigator to be significant, will also be recorded.

11.2 Transfusions

Any transfusion received and reason for transfusion (mL and number of transfused units) will be documented from the time of consent through the end of the study. Additionally, Hb concentration before and after transfusion will be documented. HbS will be collected prior to and after all transfusions, and on Day 1 of mobilization/apheresis cycle. HbS will be collected on Day 2 or Day 3 of mobilization/apheresis cycle only if a VOC or serious adverse event has occurred.

11.3 Diffusing Capacity of the Lungs for Carbon Monoxide

DLco (corrected) will be assessed. Pulmonary function tests will be performed with the subject in a seated position and should be performed per site's SOPs for pre-transplant workup and using a calibrated spirometer.

11.4 Imaging Assessments

A brain MRI/MRA will be performed (for assessment of Moyamoya disease) at screening. If a brain MRI/MRA has been performed within 3 months before date of consent, those results may be used, unless repetition of the assessment is clinically indicated.

Non-imaging or imaging TCD will be performed following the technique used in the Stroke Prevention Trial in Sickle Cell Anemia study,[64] in children 12 to 16 years old to determine eligibility.

A transthoracic cardiac echocardiogram (for assessments that should include, but not limited to, LVEF, TRV, and E/e') will be performed and read by trained medical personnel. Subjects who develop signs/symptoms of congestive heart failure at any point during the study will undergo LVEF measurement by echocardiogram.

11.5 Urine Albumin to Creatinine Ratio

First morning void urine samples will be collected for UACR to evaluate for albuminuria.

11.6 Blood Collection for Hemoglobin Oxidation and Tissue Oxygen Delivery

Methemoglobin concentration will be measured in the blood to assess Hb oxidation status. Blood erythropoietin (EPO) level will be measured to assess the status of tissue oxygen delivery (or normal tissue oxygenation).

11.7 Blood for Biomarker Assessments

Blood samples will be collected for evaluation of biomarkers and their ability to characterize the effect of CTX001 and predict treatment outcomes.

Blood samples will be collected to evaluate:

- Protein based biomarkers, including but not limited to (1) hemoglobin fractionation and quantitation in peripheral blood to assess bulk HbF levels, (2) total hemoglobin concentration, (3) the proportion of circulating erythrocytes expressing fetal hemoglobin (F-cells), (4) inflammatory (CRP, IL6, IL8) and endothelial activation markers (E-Selectin, P-Selectin)[2], and (5) N-terminal prohormone b-type natriuretic peptide (NT-proBNP) level (for the assessment of pulmonary hypertension).
- Proportion of alleles with intended genetic modification present in peripheral blood leukocyte DNA.

Detailed procedures for the collection of blood samples and additional procedures for processing and handling samples will be provided in a separate document.

11.7.1 Blood for Exploratory Research

Blood samples will be collected for potential exploratory research to better understand CTX001 and its impact on hemoglobinopathies.

Samples may be used for exploratory research to identify molecular (genomic, metabolic and/or proteomic) biomarkers that may be indicative of clinical response, resistance, safety, pharmacodynamic activity (e.g., anti-Cas9 antibodies in cases of significant [>50%] HbF decrease), and/or the mechanism of action of treatment. Samples of apheresed stem cells and

CTX001 will be collected during manufacturing for potential future genome analysis in case of development of malignancy thought to be related to CTX001 and for exploratory research to better understand CTX001 and its impact on SCD. Samples will not impact a subject's minimum final CTX001 dose.

Detailed procedures for the collection of blood samples and additional procedures for processing and handling samples will be provided in a separate document.

11.8 Allelic Editing of CTX001 Drug Product

The proportion of alleles with intended genetic modification present in each batch of CTX001 will be assessed.

11.9 Bone Marrow Aspirate

Bone marrow aspirate samples will be collected for allelic editing testing to assess the proportion of alleles with the intended genetic modification present in bone marrow DNA.

Samples may also be used for potential future genome analysis in case of development of malignancy thought to be related to CTX001. Additionally, samples may be used for exploratory research to identify molecular (genomic, metabolic and/or proteomic) biomarkers that may be indicative of clinical response, resistance, safety, pharmacodynamic activity, and/or the mechanism of action of treatment.

Detailed procedures for processing and handling bone marrow aspirate samples will be provided in a separate document.

11.10 Sperm and Oocyte Banking

Subjects will be offered the opportunity to undergo sperm banking or oocyte preservation according to local SOPs and collection protocols. The procedure is optional and subjects who choose to undergo the procedures (or if less than 18 years of age, their legally authorized representative who chooses to have them undergo the procedures) will do so after eligibility is confirmed and prior to busulfan conditioning (Stage 3A). For pre-pubescent subjects, gonadal tissue collection and cryopreservation may be performed per local practice and standard of care.

11.11 Veno-occlusive Disease Diagnosis

Recommendation is that VOD diagnosis (after other causes have been ruled out) is based on EBMT VOD criteria (Appendix 15.2).[65] VOD severity grading will be according to standard guidelines (Table 13-2).

11.12 Patient Reported Outcomes

PRO assessments should be performed as the first assessment after obtaining informed consent (and assent, where applicable), as well as be completed by subjects at the beginning of a study visit prior to any assessments.

11.12.1 Pain-Scale (11-point NRS)

Numerical rating scale is a 1-dimensional measure of reporting intensity of pain in adults and adolescents. The 11-point NRS is a segmented visual analogue scale (VAS) including numbers from 0 to 10, '0' representing no pain to '10' representing worst possible pain. Each respondent will select a whole number on the scale that reflects their pain intensity.

11.12.2 EQ-5D-5L/EQ-5D-Y

The EuroQol Questionnaire – 5 dimensions – 5 levels of severity (EQ-5D-5L) assesses an adult subject's health status in a standardized way and consists of 2 parts: the EQ-5D descriptive system and the EQ VAS.

The EQ-5D-5L descriptive system comprises the same 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The respondent is asked to indicate his/her health state by ticking (or placing a cross) in the box against the most appropriate statement in each of the 5 dimensions. This decision results in a 1-digit number expressing the level selected for that dimension. The digits for 5 dimensions can be combined in a 5-digit number describing the subject's health state.

The EQ VAS records the subject's self-rated health on a 100-point VAS with endpoints labeled 'the best health you can imagine' and 'the worst health you can imagine.' This information can be used as a quantitative measure of health as judged by the individual respondents.

For subjects under the age of 18, child-friendly versions of EQ-5D-L are available for self (EQ-5D-Youth version; EQ-5D-Y) and parent proxy completion (EQ-5D-Y proxy version). In this study, the EQ-5D-Y will be administered for self-completion to subjects 12 to <18 years of age and the parent proxy version for 12 to <18 years of age subjects who are unable to fill in the questionnaire themselves. Subjects who are <18 at Screening should continue to complete the EQ-5D-Y or parent proxy version for the entire study; they will not change to the adult version (EQ-5D-5L) when they reach 18 years of age.

The EQ-5D-Y consists of 2 pages, the EQ-5D-Y descriptive system and the EQ VAS. The descriptive system comprises the same 5 dimensions as the EQ-5D-5L but using a child-friendly wording. Unlike EQ-5D-5L which utilizes 5 levels, each dimension in the Youth version has 3 levels: no problems, some problems, a lot of problems. The respondent is asked to indicate his/her health state by ticking (or placing a cross) in the box against the most appropriate statement in each of the 5 dimensions. The EQ VAS used in the Youth version is identical to the EQ VAS used in EQ-5D-5L. Similar to EQ-5D-5L, responses can be used to determine a quantitative measure of health outcome (i.e., health utilities).

11.12.3 FACT-BMT

The Functional Assessment of Cancer Therapy – for adult subjects undergoing bone marrow transplantation (FACT-BMT) questionnaire is a validated self-report questionnaire that includes physical, social, family, emotional, and functional well-being. The FACT-BMT consists of the FACT-General (constitutes the core of all subscales) and treatment-specific concerns of bone marrow transplantation.

Each statement in the FACT-BMT has a 5-point Likert-type response scale ranging from 0 to 4 (0 = "not at all"; 1 = "a little bit"; 2 = "somewhat"; 3 = "quite a bit"; and 4 = "very much"). The subject is asked to circle or mark 1 number per line to indicate his/her response to the statement as it applies to the past 7 days. Questionnaires are then scored, and the higher the score, the better the QOL.

This information can be used to provide a holistic assessment that identifies subject's needs, which may not be revealed by a standard clinical consultation.

11.12.4 ASCQ-Me

ASCQ-Me is a disease-specific health-related quality of life (HRQoL) questionnaire that measures physical, mental and social health along with information on severity of disease in adult SCD patients. It includes the following domains: emotional impact, pain impact, pain episodes, sleep impact, social functioning impact, stiffness impact, and SCD medical history checklist. Most dimensions have 5 levels: never, rarely, sometimes, often, and always or not at all, little bit, somewhat, quite a bit, and very much. Questions on the SCD medical history checklist are indicated by yes or no options, and pain episode frequency and severity are indicated by frequency of events. ASCQ-Me domains are scored using a T-score metric with a mean of 50 for reference population and an SD of 10. Scores can be interpreted by considering the direction of scoring and the difference between the reported score and the mean of 50 in reference population (SD 10).

11.12.5 PedsQL and PedsQL Sickle Cell Disease Module

As a pediatric version of FACT-BMT does not exist, the Pediatric Quality of Life Inventory questionnaire (PedsQL - Teen version) will be administered to subjects 12 to <18 years of age for self-completion. A parent proxy version is available for subjects who are unable to complete the questionnaire themselves. Subjects who are <18 years of age at Screening should continue to complete the PedsQL or parent proxy version for the entire study and will not complete the FACT-BMT when they reach 18 years of age.

The PedsQL is a brief, standardized, generic instrument that systematically assesses subjects' and parents' perceptions of HRQoL in pediatric subjects with chronic health conditions using pediatric cancer as an exemplary model.[66] The teen version of PedsQL (self-report and parent proxy versions) comprises of 23 items across 4 domains including Physical Functioning, Emotional Functioning, Social Functioning, and School Functioning.[67]

The PedsQL Sickle Cell Disease Module (PedsQL SCD; Teen version) is a disease-specific module of PedsQL that will be administered to subjects 12 to <18 years of age for self-completion in place of ASCQ-Me (validated only in adults). Subjects should continue to complete the PedsQL SCD module or parent proxy version for the entire study and will not complete the ASCQ-ME when they reach 18 years of age. The tool measures self-reported HRQoL in SCD subjects across 9 domains (43 items in total): Pain and Hurt, Pain Impact, Pain Management and Control, Worry I, Worry II, Emotions, Treatment, Communication I, and Communication II. A parent proxy version is also available for subjects who are unable to complete the questionnaire themselves.[68]

When completing the PedsQL and PedsQL SCD Module questionnaires, respondents are asked to report the degree to which each item has been a problem over the past month by selecting the most appropriate response on a 5-point scale ranging from 0 (never a problem) to 4 (almost always a problem). Responses are transformed to a 0 to 100 score, with higher scores reflecting better HRQoL.

11.13 Mini-Mental State Examination

Adult subjects will be evaluated for cognitive function using the Mini-Mental State Examination (MMSE, Version 2) after eligibility is confirmed and before start of first RBC exchange or simple transfusion. For subjects already on a transfusion regimen, MMSE should be administered before the first mobilization/apheresis cycle.

11.14 Wechsler Abbreviated Scale of Intelligence

Subjects (12 to <18 years of age) will be evaluated for cognitive ability using the 2-subtest form of the second edition Wechsler Abbreviated Scale of Intelligence (WASI) after eligibility is confirmed and before the start of first RBC exchange or simple transfusion. For subjects already on a transfusion regimen, WASI should be administered before the first mobilization/apheresis cycle.

11.15 Safety

Safety evaluations will include engraftment, TRM, AEs, clinical laboratory assessments, clinical evaluation of vital signs, ECGs, and PEs.

11.15.1 Adverse Events

All AEs will be assessed, documented, and reported in accordance with ICH GCP Guidelines. Section 13.1 outlines the definitions, collection periods, criteria, and procedures for documenting, grading, and reporting AEs.

11.15.2 Clinical Laboratory Assessments

Blood and urine samples will be collected according to Table 3-1 through Table 3-4. Additional clinical laboratory tests may be obtained at any time as clinically necessary for safety purposes at the investigator's discretion.

In certain circumstances, additional testing may be required depending on the subject's history and local requirements for cell processing (e.g., malaria, *Trypanosoma cruzi*).

Females of childbearing potential (as defined in Section 11.15.6) must have a negative pregnancy test result at screening, within 3 days prior to starting mobilization, and within 5 days before the initiation of busulfan conditioning.

The laboratory test panels are shown in Table 11-1.

Table 11-1 Laboratory Test Panels

Hematology	Serum Chemistry	Urinalysis	Other Tests[a]
CBC with differential including: Hemoglobin WBC ANC Platelet count Reticulocyte count Reticulocytes/erythrocytes Nucleated RBCs MCV (Mean corpuscular volume) MCHC (Mean corpuscular hemoglobin concentration) MCH (Mean corpuscular hemoglobin) RDW (Red blood cell distribution width)[a]	ALT AST Bilirubin (total, indirect, and direct) Albumin Alkaline phosphatase Bicarbonate BUN Calcium Chloride Creatinine Glucose Magnesium Phosphorous Potassium Sodium Total protein	Protein Blood Nitrite Leukocyte esterase	At screening only: Genotyping of *HBB* and alpha loci (central laboratory) Hemoglobin fractionation (central laboratory) Allelic editing (blood; central laboratory) Allelic editing (bone marrow aspirate; central laboratory) HbF distribution, F-cells (central laboratory) $CD34^+$ cell count HbS Inflammatory and endothelial activation markers (central laboratory) NT-proBNP (central laboratory) UACR (urine albumin to creatinine ratio; central laboratory) Erythropoietin (central laboratory) Methemoglobin Ferritin If applicable: Serum pregnancy test

Table 11-1 Laboratory Test Panels

Infectious Disease Marker Testing[b]	Immunological Testing	Coagulation	Hemolysis Markers
HBV (HbcAb, HbsAg, and NAT) HCV (HCAb and NAT) HIV-1, HIV-2 [c] (Antigen/Antibody immunoassay, if positive, then NAT) Syphilis[d] HTLV-1 Ab[e]	CD4 CD8 CD19 CD16 and/or CD56[f] IgG IgD IgE IgM IgA	PT (Prothrombin time) aPTT (Activated partial thromboplastin time) INR	Haptoglobin Lactate dehydrogenase

[a] While a subject is on a regular transfusion regimen, samples for central labs should be collected immediately before transfusion, and must be collected within 7 days before the next scheduled transfusion. After dosing with CTX001, if the subject is no longer receiving regular transfusions, samples should be collected immediately before a transfusion and at least 2 weeks after a previous transfusion.
[b] Additional infectious disease markers testing should be done if required by local practice (e.g., HTLV-1, HTLV-2, malaria, tuberculosis, toxoplasmosis, *Trypanosoma cruzi*, or West Nile virus).
[c] In line with standard practice for donor screening, reflexive HIV testing is permitted.
[d] Subjects must have at least a screening assay for syphilis performed (e.g., rapid plasma reagin [RPR] is preferred). If positive, a confirmatory test (e.g., FTA-ABS, TPPA) must be done to confirm the presence of active infection. Local testing algorithms for syphilis may be used, provided they contain these tests.
[e] Testing for HTLV-1 Ab may be required depending on subject's history and characteristics.
[f] For identification of NK cells. NK cells can be defined as $CD16^+$, $CD56^+$ or $CD16^+/CD56^+$.

11.15.3 Physical Examinations and Vital Signs

A PE includes a review of the following systems: head, neck, and thyroid; eyes, ears, nose, and throat (EENT); respiratory; cardiovascular; lymph nodes; abdomen (including spleen); skin (including leg ulcers); musculoskeletal; neurological systems, signs/symptoms consistent with myocardial infarction or cerebral vascular accident; new or worsening osteonecrosis, retinopathy and performance status. Breast, anorectal, and genital examinations will be performed when medically indicated. Performance status will be assessed using the Karnofsky Performance Scale for subjects ≥16 years old and the Lansky Performance Scale for subjects <16 years old. After screening, any clinically significant abnormal findings in PEs will be reported as AEs. Tanner staging will be performed for subjects 12 to <18 years old until they reach Tanner Stage 5 according to Table 3-1 and Table 3-4.

The abbreviated PE will include an assessment of the following body systems: head, neck, and thyroid; EENT; cardiovascular system; respiratory system; skin (including leg ulcers); abdomen (including spleen), lymph nodes; and neurological systems. Additionally, assessment of signs/symptoms consistent with myocardial infarction or cerebral vascular accident, new or worsening osteonecrosis, and retinopathy will be assessed (M1 through M6, M9, M15, and M18 only). Vital signs include blood pressure (systolic and diastolic), temperature, pulse rate, pulse oximetry, and respiration rate. The subject will be instructed to rest for at least 5 minutes before vital signs are assessed.

11.15.4 Weight/Height

Subject weight (kg) and height (cm) will be measured with shoes off.

11.15.5 Electrocardiograms

Standard 12-lead ECGs will be performed using a machine with printout. Additional standard 12-lead ECGs will be performed at any other time if clinically indicated. The performance of all ECGs should adhere to the following guidelines:

- The subject should be instructed to rest for at least 5 minutes before having an ECG.
- The test should be performed in the supine position.

A printout of the ECG traces will be made for safety review by the investigator or qualified designee and maintained with source documentation. Clinically significant ECG abnormalities occurring during the study through the Safety Follow-up Visit will be recorded as AEs.

Interpretation of the tracing should be made by a qualified physician and documented in the medical record. Clinically significant abnormalities present at screening should be reported as part of the subject's medical history.

11.15.6 Contraception and Pregnancy

11.15.6.1 Contraception

Study participation requires a commitment to use 2 highly effective methods of birth control.

Female subjects of childbearing potential must agree to use acceptable, highly effective methods of contraception to avoid pregnancy from consent through at least 6 months after CTX001 infusion.

Non-sterile male subjects of reproductive capacity who are or may become sexually active with female partners of childbearing potential must agree to use acceptable, highly effective methods of contraception to avoid fathering a child from start of mobilization through at least 6 months after CTX001 infusion.

Acceptable and highly effective methods of contraception for subjects and their partners are listed below. If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.

Contraception for the couple is waived for the following:

- True abstinence from heterosexual intercourse for the subject, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- If the male is infertile (e.g., after bilateral orchiectomy) or pre-pubescent. Infertility may be documented through examination of a semen specimen before mobilization.
- If the female is of non-childbearing potential, as described below.

Female Subjects

Acceptable, highly effective methods of contraception to avoid pregnancy must be used from consent through at least 6 months after CTX001 infusion.

- Female bilateral tubal ligation performed at least 6 months previously.

- Female continuous use of an intrauterine device (non-hormone releasing or hormone releasing) for at least 90 days before mobilization.
- Female combined (estrogen and progestogen-containing) or progestogen-only hormonal contraception associated with inhibition of ovulation if successfully used for at least 60 days before mobilization or with a second form of approved contraception for at least 60 days after beginning hormonal contraception.
- Female subjects undergoing fertility preservation as described in Section 9.1.1 and who are unable to use hormonal contraception during this time must use true abstinence during the period of fertility preservation unless meeting another criterion listed as waivers of contraception for the couple below.

Acceptable contraceptive methods for male partners of female subjects of childbearing potential:

- Condom with spermicide (either as a single product if commercially available and/or allowed according to local regulations; otherwise condom and spermicide as separate products). Local regulations may require use of an additional acceptable method of contraception.
- Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of mobilization, and 1 barrier method of contraception.

Male Subjects

Acceptable contraceptive methods must be used from start of mobilization through at least 6 months after CTX001 infusion and include the following:

- Condom with spermicide (either as a single product if commercially available and/or allowed according to local regulations; otherwise condom and spermicide as separate products). Local regulations may require use of an additional acceptable method of contraception.
- Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of mobilization, and 1 barrier method of contraception.

Acceptable contraceptive methods for female partners of male subjects:

- Bilateral tubal ligation performed at least 6 months previously.
- Continuous use of an intrauterine device for at least 90 days before start of mobilization.
- Hormonal contraceptives, if successfully used for at least 60 days before start of mobilization.

Additional notes:

- Female condom cannot be used with male condom (as a double method of contraception) due to risk of tearing.
- The use of birth control methods does not apply if the female partner has had a bilateral oophorectomy, hysterectomy, or is postmenopausal (as defined below).
- Male subjects who are not sexually active at the time of screening must agree to follow the contraceptive requirements of this study if they become sexually active with a partner of the opposite sex.

- If over the course of the study the subject's status changes and the subject does not meet the criteria for waiving the contraception requirements, the subject must begin following the contraceptive methods listed above.
- If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.
- Male subjects must not donate sperm after start of mobilization, throughout the study, and for 6 months following CTX001 infusion.
- Unique situations that may not fall within the above specifications may be discussed with the Sponsor's medical monitor or designee on an individual basis.

Female Subjects of Non-childbearing Potential:

Female subjects of non-childbearing potential will not be required to use contraception. To be considered of non-childbearing potential, female subjects must meet at least 1 of the following criteria:

- Postmenopausal: Female subjects who have been amenorrheic for at least 2 years and have a serum follicle-stimulating hormone (FSH) level within the laboratory's reference range for postmenopausal females
- Documented hysterectomy and/or bilateral oophorectomy
- Subject is premenarchal.

Note: All other female subjects (including subjects with tubal ligations and subjects who do not have a documented hysterectomy) will be considered to be of childbearing potential.

11.15.6.2 Pregnancy

Subjects will be counseled to inform the investigator of any pregnancy that occurs during the study. If a subject becomes pregnant during the study, study interventions that may put the fetus at risk will be stopped immediately.

Pregnancies (both those of female subjects and female partners of male subjects) must be reported to the medical monitor and Vertex Global Patient Safety (GPS) within 24 hours of the site's knowledge using the Pregnancy Information Collection Form.

All pregnancies in subjects who have entered Stage 2 of the study (Figure 9-1) will be followed through to outcome, and the infant will be followed for 1 year after the birth, provided informed consent is obtained. A separate ICF will be provided to explain these follow-up activities. Birth outcomes must be reported to the Vertex GPS using the Pregnancy Information Collection Form, even if the subject was discontinued from the study.

Pregnancies themselves are not considered AEs or SAEs. However, any AEs or SAEs occurring during pregnancy are to be reported following AE and SAE reporting guidelines.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

12 STATISTICAL AND ANALYTICAL PLANS

This section presents a summary of the planned analyses for this protocol. Statistical analysis details will be provided in the statistical analysis plan (SAP).

12.1 Sample Size and Power

Assuming that the study will be expanded to include up to approximately 45 subjects dosed, 3 interim analyses (IAs) may be performed following a group sequential testing procedure in the expanded study to allow for early evaluation of efficacy. The actual alpha spending will be based on the information available at the IA. Details of the IAs are described in Section 12.3.8.1. This sample size will provide an overall power of at least 95% to rule out a response rate of 50% or less for the primary efficacy endpoint when the true response rate is 80% for the primary efficacy endpoint.

12.2 Analysis Sets

- Enrolled Set: All enrolled subjects who signed informed consent and meet the inclusion criteria.
- Full Analysis Set (FAS): subset of the enrolled set that includes subjects who receive CTX001 infusion.
- Primary Efficacy Set: subset of FAS that includes all subjects who have been followed for at least 16 months post CTX001 infusion at the time of analysis.
- Safety Analysis Set: subset of the enrolled set that includes subjects who start the mobilization regimen.

12.3 Statistical Analysis

Methodological and related details (e.g., missing data) will be in the SAP.

12.3.1 General Considerations

Continuous variables will be summarized using the following descriptive summary statistics: the number of subjects (n), mean, SD, median, minimum value (min), and maximum value (max).

Categorical variables will be summarized using counts and percentages. Percentages will be presented to 1 decimal place.

Uncertainty of estimates will be assessed by CIs. All subject data will be presented in the subject data listings; listings will display all subjects in the enrolled population, regardless of whether or not they received study drug. Longitudinal data will be presented by appropriate time intervals, such as monthly or quarterly depending on the nature of the data.

Baseline value, unless specified otherwise, will be defined as the most recent non-missing measurement (scheduled or unscheduled) collected during screening and before start of mobilization.

For number of severe VOCs: All pre- and post-CTX001 infusion severe VOCs will be adjudicated by an EAC to determine whether they meet the protocol criteria for severe VOCs. Pre-treatment baseline is defined as the average annual severe VOCs during the 2 years prior to

enrollment. Baseline will be calculated using records of all severe VOCs adjudicated by the EAC as meeting the protocol definition of severe VOC, during the 2 years prior to enrollment.

All VOCs that occur post-CTX001 up to 24 months and are adjudicated by the EAC as severe will be captured as post-CTX001 severe VOCs.

Baseline number of inpatient hospitalizations for severe VOCs will be calculated based on annualized number of hospitalizations for severe VOCs during the 2 years prior to most recent screening.

Change (absolute change) from baseline will be calculated as Post-baseline value − Baseline value.

Relative change from baseline will be calculated and expressed in percentage as 100% × (Post-baseline value − Baseline value)/Baseline value.

Treatment-emergent (TE) Period will include the time from CTX001 infusion to last study visit.

Incomplete/missing data will not be imputed, unless specified otherwise. All data will be evaluated as observed. For subjects who are lost to follow-up or die, safety and efficacy analyses will be based on their available data, before death or loss to follow-up. For subjects who withdraw from this study but enroll in the long-term follow-up study (Study VX18-CTX001-131), efficacy and safety will be based on data from both the parent and the long-term follow-up studies up to 2 years after CTX001 infusion, unless otherwise specified.

A listing of subjects who withdraw will be generated with the reason for withdrawal including death, AE, withdrawal of consent, and lost to follow-up.

Subgroup analyses, such as descriptive summaries of the key efficacy / safety endpoints by age groups as well as by genotypes, will be provided as appropriate. Details will be described in the SAP.

12.3.2 Background Characteristics

12.3.2.1 Subject Disposition

The number and percentage of subjects in the following categories will be summarized as appropriate based on the Enrolled Set:

- Subjects who are mobilized
- Subjects who are dosed with CTX001
- Subjects who are dosed with CTX001 with at least 1 year of follow-up post-CTX001 infusion
- Completed study (24-month post-CTX001 follow-up)
- Prematurely discontinued the study and the reasons for discontinuation

12.3.2.2 Demographics and Baseline Characteristics

The following characteristics will be summarized:

- Age, sex, race, ethnicity, weight, height, genotype
- Targeted medical history

- Results of screening imaging (cardiac) and selected laboratory tests
- Number and annualized rate of severe VOCs for past 2 years

12.3.2.3 Prior and Concomitant Medications

Medications used will be coded using the World Health Organization Drug Dictionary (WHODD):

Prior medication: any medication that started before CTX001 infusion.

Concomitant medication: medication continued or newly received on or after CTX001 infusion.

A given medication may be classified as prior, concomitant, or both prior and concomitant.

Prior medications and concomitant medications will be summarized descriptively using frequency tables by Preferred Name.

12.3.3 Efficacy Analysis

12.3.3.1 Analysis of Primary Variables

Details of the analyses will be provided in the SAP.

Analysis of Primary Efficacy Endpoint

The response status of the primary efficacy endpoint for a subject is evaluated starting 60 days after last RBC transfusion for post-transplant support or SCD disease management after CTX001 infusion. A subject will be considered to have met the primary efficacy endpoint if the subject has not experienced severe VOCs for a period of at least 12 consecutive months (VF12) after CTX001 infusion. The evaluation of VF12 starts 60 days after last RBC transfusion for post-transplant support or SCD disease management. The primary efficacy analysis will be performed on the Primary Efficacy Set. Response status, for subjects who are lost to follow-up or die, will be based on their available VOC data before death or loss to follow-up. Response status, for subjects who withdraw from the study but enroll in the long-term follow-up study (Study VX18-CTX001-131), will be based on their VOC data from both the parent and the long-term follow-up studies up to 2 years after CTX001 infusion.

The proportion of subjects who meet the primary efficacy endpoint will be provided, with the 1-sided *P* value and the 2-sided exact Clopper-Pearson CI.

All severe VOC events will be listed from 2 years prior to enrollment to 2 years post CTX001 infusion.

12.3.3.2 Analysis of Secondary Efficacy Endpoints

VOCs occurring from the time of screening to CTX001 infusion will not be included in any of the analyses of VOCs.

Key Secondary Efficacy Endpoints

HF12: All inpatient hospitalizations for severe VOCs will be listed, from 2 years prior to enrollment to 2 years after CTX001 infusion. Proportion of subjects free from inpatient hospitalization for severe VOC sustained for at least 12 months (HF12) after CTX001 infusion will be summarized.

A subject will be considered to have met this key secondary efficacy endpoint if he/she has no inpatient hospitalizations for severe VOCs for at least 12 consecutive months. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management. The analysis of HF12 will be performed on the Primary Efficacy Set.

VF9: The proportion of subjects who have not experienced any severe VOC for at least 9 consecutive months (VF9) any time after CTX001 infusion will be summarized.

A subject will be considered to have met this key secondary efficacy endpoint if he/she has no severe VOCs for at least 9 consecutive months. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management. The analysis of VF9 will be performed on the set of subjects with at least 12 months follow-up after CTX001 infusion.

Secondary Efficacy Endpoints

- Proportion of subjects with reduction in annualized rate of severe VOCs at the time of analysis from baseline by at least 90%, 80%, 75%, 50% up to 24 months after CTX001 infusion will be calculated with the corresponding 2-sided 95% exact Clopper-Pearson CI. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Relative change from baseline in annualized rate of severe VOCs up to 24 months after CTX001 infusion will be summarized. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Duration of severe VOC free in subjects who have achieved VF12 will be summarized descriptively. Duration of severe VOC free will be calculated since the last severe VOC after CTX001 infusion before achieving VF12, or CTX001 infusion (for subjects with no severe VOC post-CTX001).
- Relative change from baseline in rate of inpatient hospitalizations for severe VOCs up to 24 months after CTX001 infusion will be summarized. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Relative change from baseline in annualized duration of hospitalization for severe VOCs up to 24 months after CTX001 infusion will be summarized. The evaluation starts 60 days after last RBC transfusion for post-transplant support or SCD disease management.
- Proportion of subjects with sustained HbF ≥20% at the time of analysis for at least 3 months, 6 months, or 12 months, starting 60 days after last RBC transfusion for post-transplant support or SCD disease management will be calculated with the corresponding 2-sided 95% exact Clopper-Pearson CI.
- Relative change in annualized number of units of RBCs transfused for SCD-related indications will be summarized.
- HbF and Hb concentrations will be summarized as a continuous variable over time.
- Change from baseline in reticulocyte count (percent reticulocytes and absolute reticulocyte count) will be summarized as a continuous variable over time.
- Change from baseline in indirect bilirubin will be summarized as a continuous variable over time.
- Change from baseline in haptoglobin will be summarized as a continuous variable over time.

- Change from baseline in lactate dehydrogenase will be summarized as a continuous variable over time.
- Change in PROs will be summarized as a continuous variable over time.
- Proportion of alleles with intended genetic modification present in peripheral blood leukocytes will be summarized as a continuous variable over time.
- Proportion of alleles with intended genetic modification present in $CD34^+$ cells of the bone marrow will be summarized as a continuous variable over time.

12.3.4 Safety Analysis

Analysis based on the Safety Analysis Set will include:

- Proportion of subjects with neutrophil engraftment within 42 days after CTX001 infusion (Study Day 43)
- Time to neutrophil engraftment
- Time to platelet engraftment
- AEs, laboratory values, and vital signs from signing of informed consent through the Month 24 visit.
- Incidence of TRM within 100 days and 1 year post CTX001 infusion. TRM defined as death possibly related to the transplantation procedure as assessed by the investigator.
- All-cause mortality

The number and percentage of subjects with AEs and SAEs will be summarized by severity and seriousness in a tabular fashion according to the time periods defined in Table 13-1. The percentage of subjects with AEs and SAEs within each time period will be based on the subset of the Safety Set being followed beyond the start of that time period.

Time to neutrophil engraftment, defined as the first of 3 measurements on 3 consecutive days with ANC ≥500/μL from transplantation, will be analyzed by the Kaplan-Meier method. Engraftment failure is defined as not achieving neutrophil engraftment by 42 days post-CTX001 infusion (Study Day 43) or receipt of backup $CD34^+$ stem cells. The number and percentage of subjects with engraftment failure will be summarized.

Time to platelet engraftment, defined as first of 3 consecutive measurements on 3 separate days with platelet $\geq 50 \times 10^9$/L without a platelet transfusion for 7 consecutive days, will be assessed by the Kaplan-Meier method.

Laboratory abnormalities (values outside of normal ranges, and by Common Terminology Criteria for Adverse Events [CTCAE] grade), will also be tabulated.

The number and percentage of subjects with TRM within 100 days and 1 year post CTX001 infusion will be summarized, where TRM is defined as death at least possibly related to the transplantation procedure as assessed by the investigator. Relatedness between SAEs leading to death and transplantation will be as assessed by the investigators. If an SAE is assessed as being at least possibly related to the transplantation procedure, the death will be classified as transplant-related.

12.3.5 Engraftment

Neutrophil engraftment is defined as the first day of 3 consecutive measurements of ANC ≥500/μL on 3 different days, achieved within 42 days post CTX001 infusion (Study Day 43), without use of the unmodified $CD34^+$ cells after reaching the nadir, defined as ANC <500/μL.

Platelet engraftment is defined as the first day of 3 consecutive measurements of unsupported (no platelet transfusions for the last 7 days) platelet ≥50,000/μL on 3 different days after CTX001 infusion. For subjects discharged early day 7 after the last platelet transfusion will be the day of platelet engraftment, as long as 3 subsequent and consecutive unsupported measurements on 3 different days are >50,000/μL. For subjects who have been discharged prior to platelet engraftment, it is recommended to collect blood every 2 to 3 days to obtain an accurate assessment of platelet engraftment.

The number and percentage of subjects achieving successful engraftment, together with the 95% CI will be provided.

Time to neutrophil engraftment will be analyzed by the Kaplan-Meier method. Engraftment failure is defined as not achieving neutrophil engraftment by 42 days after CTX001 infusion (Study Day 43), or receipt of backup $CD34^+$ stem cells. The number and percentage of subjects with neutrophil engraftment failure will be summarized.

Time to platelet engraftment will be analyzed by the Kaplan-Meier method.

12.3.6 Adverse Events

AEs will be coded according to MedDRA. AE and SAE summaries will be presented by MedDRA System Organ Class and Preferred Term using frequency counts and percentages (i.e., number and percentage of subjects with an event). When summarizing the number and percentage of subjects with an event, subjects with multiple occurrences of the same AE (SAE) or a continuing AE (SAE) will be counted once. Only the maximum severity level will be presented in the severity summaries, and the strongest relationship level will be presented in the relationship summaries.

AEs summarized by different period include the following:

- AEs by strongest relationship
- AEs by maximum severity
- SAEs
- AEs leading to death

Details for imputing missing or partial start dates of AEs will be specified in the SAP.

12.3.6.1 Clinical Laboratory Assessments

For laboratory measurements, the raw values and change from baseline values of the continuous hematology and chemistry results, including coagulation studies will be summarized in SI units by visit.

In addition, a listing containing individual subject hematology, chemistry, and coagulation values outside the normal reference ranges will be provided. This listing will include data from both scheduled and unscheduled visits.

12.3.6.2 Electrocardiogram

For post-baseline ECG measurements, a summary of raw values and change from baseline values will be provided by visit for the following standard 12-lead ECG measurements: RR (msec), HR (beats per minute [bpm]), PR (msec), QRS duration (msec), QT (msec), and QT corrected for HR intervals (QTcF [msec]).

12.3.6.3 Vital Signs

For vital signs measurements, the raw values and change from baseline values will be summarized by visit: systolic and diastolic blood pressure (mm Hg), body temperature (°C), heart rate (bpm), and respiratory rate (breaths per minute).

12.3.6.4 Physical Examination

PE findings will be presented in individual subject data listings only.

12.3.7 Exploratory Analysis

Descriptive statistics will be used to describe the following exploratory endpoints (unless stated otherwise):

- Change in TRV and NT-proBNP level will be summarized as a continuous variable over time.
- Change in inflammatory markers will be summarized as a continuous variable over time.
- Change in endothelial activation markers will be summarized as a continuous variable over time.
- Change in proportion of circulating erythrocytes expressing fetal hemoglobin (F-cells) (pre-transfusion) over time
- Change in UACR will be summarized as a continuous variable over time.
- Correlations of response markers (transfusions, total and fetal hemoglobin) with pre-treatment variables (e.g., subject genotype), cell dose and percent edited cells in final product.

These data will be used for internal exploratory purposes and may or may not be included in the clinical study report.

12.3.8 Interim and Independent Data Monitoring Committee Analyses

12.3.8.1 Interim Analysis

Assuming that the study will be expanded to a total of approximately 45 subjects dosed, 3 IAs may be performed following a group sequential testing procedure in the expanded study to allow for early evaluation of efficacy. The actual alpha spending will be based on the information available at the IA. For the IAs, the primary analysis for the primary efficacy endpoint will be based on the Primary Efficacy Set.

Hypothesis testing for primary efficacy endpoint will be conducted per Table 12-1, where the overall type I error is controlled at 1-sided 2.5% based on the exact binomial distribution.

Table 12-1 Efficacy Boundaries and Probability of Crossing Efficacy Boundary Under Null Hypothesis for Primary Efficacy Endpoint at the Interim and Final Analyses

Analysis Time Point	Efficacy Boundary	Probability of Crossing (≥) Efficacy Boundary Under Null Hypothesis (Assuming a Response Rate of 50% or less)
IA 1: N = 10	9/10	1.074%
IA 2: N = 17	14/17	0.636%
IA 3: N = 30	22/30	0.806%
Final: N = 45	31/45	0.805%
Overall α Spending		2.420%

12.3.8.2 Independent Data Monitoring Committee Analysis

The DMC review will include review of safety and efficacy data. Details will be included in the DMC charter and DMC SAP.

13 PROCEDURAL, ETHICAL, REGULATORY, AND ADMINISTRATIVE CONSIDERATIONS

13.1 Adverse Event and Serious Adverse Event Documentation, Severity Grading, and Reporting

13.1.1 Adverse Events

13.1.1.1 Definition of an Adverse Event

An AE is defined as any untoward medical occurrence in a subject during the study; the event does not necessarily have a causal relationship with the treatment. This includes any newly occurring event or worsening of a pre-existing condition (e.g., increase in its severity or frequency) after the ICF is signed. A pre-existing condition that is diagnosed prior to subject signing ICF should be recorded in medical history.

An AE is considered serious if it meets the definition in Section 13.1.2.1.

13.1.1.2 Clinically Significant Assessments

Study assessments including laboratory tests, ECGs, PEs, and vital signs will be assessed and those deemed to have clinically significant worsening from baseline will be documented as an AE. When possible, a clinical diagnosis for the study assessment will be provided, rather than the abnormal test result alone (e.g., urinary tract infection, anemia). In the absence of a diagnosis, the abnormal study assessment itself will be listed as the AE (e.g., bacteria in urine or decreased hemoglobin).

An abnormal study assessment is considered clinically significant if the subject has 1 or more of the following:

- Concomitant signs or symptoms related to the abnormal study assessment
- Further diagnostic testing or medical/surgical intervention
- Discontinuation from the study

Repeat testing to determine whether the result is abnormal, in the absence of any of the above criteria, does not necessarily meet clinically significant criteria. The determination of whether the study assessment results are clinically significant will be made by the investigator.

A laboratory value that is Grade 4 will not automatically be an SAE. A Grade 4 laboratory value will be an SAE if the subject's clinical status indicates a life-threatening AE.

Busulfan will be administered with the intention and expectation of myeloablation. In this context, Grades 1 and 2 laboratory abnormalities reflective of and fully explained by busulfan myeloablation should not be considered clinically significant.

13.1.1.3 Documentation of Adverse Events

AEs will be monitored and collected as outlined in Table 13-1.

Table 13-1 AE and SAE Recording

Study Time Period	AEs	SAEs
Signing of the ICF to start of mobilization	All grades of study procedure-related AEs only (including AEs possibly related to transfusion regimen, and excluding AEs possibly related to fertility preservation procedures)	All SAEs
Start of first mobilization cycle to 2 weeks after end of last mobilization cycle	All AEs	All SAEs
Two weeks after end of last mobilization cycle to start of busulfan conditioning	All grades of study procedure-related AEs only (including AEs possibly related to transfusion regimen)	All SAEs
Start of busulfan conditioning to start of CTX001 infusion	All AEs	All SAEs
Start of CTX001 infusion to Month 24 Visit (end of study visit)	All AEs	All SAEs

All subjects will be queried, using nonleading questions, about the occurrence of AEs at each study visit. When possible, a constellation of signs and/or symptoms will be identified as 1 overall event or diagnosis. AEs for enrolled subjects will be recorded in the CRF and source document. AEs for subjects who are screened but not subsequently enrolled will be recorded only in the subject's source documents. The following data will be documented for each AE:

- Description of the event
- Classification of "serious" or "nonserious"
- Date of first occurrence and date of resolution (if applicable)
- Severity
- Causal relationship to study drug(s)
- Action taken

- Outcome
- Concomitant medication or other treatment given

13.1.1.4 Adverse Event Severity

The investigator will determine and record the severity of all serious and nonserious AEs. The guidance available at the following website will be consulted: CTCAE, Version 5.0, Cancer Therapy Evaluation Program, https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50 (accessed November 2018). The severity of an AE that does not appear in the CTCAE will be determined according to the definitions in Table 13-2.

Table 13-2 Grading of AE Severity

Classification	Definition
Mild (Grade 1)	Mild level of discomfort and does not interfere with regular activities
Moderate (Grade 2)	Moderate level of discomfort and significantly interferes with regular activities
Severe (Grade 3)	Significant level of discomfort and prevents regular activities
Life-threatening (Grade 4)	Any adverse drug event that places the subject, in the view of the investigator, at immediate risk of death
Death (Grade 5)	Any adverse event that results in the death of the subject

13.1.1.5 Adverse Event Causality

Every effort will be made by the investigator to assess the relationship of the AE, if any, to the study drug(s). Causality will be classified using the categories in Table 13-3.

Table 13-3 Classifications for AE Causality

Classification	Definition
Related	There is an association between the event and the administration of investigational study drug, a plausible mechanism for the event to be related to the investigational study drug and causes other than the investigational study drug have been ruled out, and/or the event reappeared on re-exposure to the investigational study drug.
Possibly related	There is an association between the event and the administration of the investigational study drug and there is a plausible mechanism for the event to be related to investigational study drug, but there may also be alternative etiology, such as characteristics of the subject's clinical status or underlying disease.
Unlikely related	The event is unlikely to be related to the investigational study drug and likely to be related to factors other than investigational study drug.
Not related	The event is related to an etiology other than the investigational study drug (the alternative etiology will be documented in the subject's medical record).

13.1.1.6 Study Drug Action Taken

The investigator will classify the study drug action taken with regard to the AE. The action taken will be classified according to the categories in Table 13-4.

Table 13-4 Classifications for Study Drug Action Taken With Regard to an AE

Classification	Definition
Dose not changed	Study drug dose not changed in response to an AE
Dose reduced	Study drug dose reduced in response to an AE
Drug interrupted	Study drug administration interrupted in response to an AE
Drug withdrawn	Study drug administration permanently discontinued in response to an AE
Not applicable	Action taken regarding study drug administration does not apply. "Not applicable" will be used in circumstances such as when the investigational treatment had been completed before the AE began and no opportunity to decide whether to continue, interrupt, or withdraw treatment is possible.

13.1.1.7 Adverse Event Outcome

An AE will be followed until the investigator has determined and provided the final outcome. The outcome will be classified according to the categories in Table 13-5.

Table 13-5 Classifications for Outcome of an AE

Classification	Definition
Recovered/resolved	Resolution of an AE with no residual signs or symptoms
Recovered/resolved with sequelae	Resolution of an AE with residual signs or symptoms
Not recovered/not resolved (continuing)	Either incomplete improvement or no improvement of an AE, such that it remains ongoing
Fatal	Outcome of an AE is death. "Fatal" will be used when death is at least possibly related to the AE.
Unknown	Outcome of an AE is not known (e.g., a subject lost to follow-up)

13.1.1.8 Treatment Given

The investigator ensures adequate medical care is provided to subjects for any AEs, including clinically significant laboratory values related to study drug. In addition, the investigator will describe whether any treatment was given for the AE. "Yes" is used if any treatment was given in response to an AE, and may include treatments such as other medications, surgery, or physical therapy. "No" indicates the absence of any kind of treatment for an AE.

13.1.2 Serious Adverse Events

13.1.2.1 Definition of a Serious Adverse Event

An SAE is any AE that meets any of the following outcomes:

- Fatal (death, regardless of cause, that occurs during participation in the study or occurs after participation and is suspected of being a delayed toxicity due to administration of the study drug)
- Life-threatening, such that the subject was at immediate risk of death from the reaction as it occurred

- Inpatient hospitalization or prolongation of hospitalization. Please note that hospital admissions during the conduct of the study that are per protocol (such as for mobilization/apheresis, central line placement, busulfan conditioning, CTX001 infusion) do not meet this criterion.
- Persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- Congenital anomaly or birth defect
- Important medical event that, based upon appropriate medical judgment, may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the outcomes listed above (e.g., an allergic bronchospasm requiring intensive treatment in an emergency room or at home)
- Engraftment failure: failure to achieve neutrophil engraftment by 42 days after CTX001 infusion (Study Day 43) or need to receive backup $CD34^+$ stem cells at any time during period of neutropenia
- Development of new malignancy

If a subject has a hospitalization or procedure (e.g., surgery) for an event or condition that occurred before the subject signed the ICF, and the hospitalization or procedure was planned before the subject signed the ICF, the hospitalization or procedure will not be considered to indicate an SAE, unless an AE caused the hospitalization or procedure to be rescheduled sooner or to be prolonged relative to what was planned. In addition, hospitalizations clearly not associated with an AE (e.g., social hospitalization for purposes of respite care) will not be considered to indicate an SAE.

Clarification will be made between the terms "serious" and "severe" because they are not synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event, as in mild, moderate, or severe myocardial infarction. The event itself, however, may be of relatively minor medical significance, such as a severe headache. This is not the same as "serious", which is based on subject/event outcome or action described above, and is usually associated with events that pose a threat to a subject's life or functioning. Seriousness, not severity, serves as a guide for defining expedited regulatory reporting obligations.

13.1.2.2 Reporting and Documentation of Serious Adverse Events

All SAEs that occur after obtaining informed consent and assent (where applicable) through the Month 24 Visit after infusion of CTX001, regardless of causality, will be reported by the investigator to Vertex GPS **within 24 hours of identification**. In addition, all SAEs that occur after the 24-Month Visit and are considered related to study drug(s) will be reported to Vertex GPS **within 24 hours of identification**.

For SAEs that occur after obtaining informed consent and assent (where applicable) through the 24-Month Visit, the SAE Form will be completed for new/initial events as well as to report follow-up information on previously reported events. Investigators are asked to report follow-up information as soon as it becomes available to ensure timely reporting to health authorities.

Please send completed SAE Forms to Vertex GPS via:

Email: [redacted] (preferred choice)

Fax: [redacted]

For questions, contact telephone: [redacted]

SAEs that occur after the 24-Month Visit and are considered related to study drug(s) will be recorded on the Vertex Organized Safety Information Collection Form (hereafter referred to as the "SAE Form") using a recognized medical term or diagnosis that accurately reflects the event. SAEs will be assessed by the investigator for relationship to the investigational study drug(s) and possible etiologies. On the SAE Form, relationship to study drug(s) will be assessed only as related (includes possibly related) or not related (includes unlikely related), and severity assessment will not be required. For the purposes of study analysis, if the event has not resolved at the end of the study reporting period, it will be documented as ongoing. For purposes of regulatory safety monitoring, the investigator is required to follow the event to resolution and report the outcome to Vertex using the SAE Form.

In parallel with reporting via the SAE Form described above, the investigator must also **immediately** notify the medical monitor of any of the following:

- Fatal or life-threatening SAEs
- SAEs related to CTX001
- Engraftment failures
- Malignancies
- Grade 4 VOCs associated with mobilization (see Section 9.9.1)

13.1.2.3 Expedited Reporting and Investigator Safety Letters

The sponsor is responsible for reporting suspected, unexpected, serious adverse reactions (SUSARs) involving the study drug(s) to all regulatory authorities, IECs, and participating investigators in accordance with ICH Guidelines and/or local regulatory requirements, as applicable. In addition, the sponsor, or authorized designee, will be responsible for the submission of safety letters to central IECs.

It is the responsibility of the investigator or designee to promptly notify the local IRB/IEC of all unexpected serious adverse drug reactions involving risk to human subjects. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the study. Each site is responsible for notifying its IRB or EC of these additional SAEs.

13.2 Administrative Requirements

13.2.1 Ethical Considerations

The study will be conducted in accordance with the current ICH GCP Guidelines, which are consistent with the ethical principles founded in the Declaration of Helsinki, and in accordance with local applicable laws and regulations. The IRB/IEC will review all appropriate study documentation to safeguard the rights, safety, and well-being of the subjects. The study will be conducted only at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, sample ICF, advertisements (if applicable), written information given to the subjects (including diary cards), safety updates, annual progress reports, and any revisions to these

documents will be provided to the IRB/IEC by the investigator or Sponsor, as allowable by local applicable laws and regulations.

Ethics Committee/Institutional Review Board

The PI must obtain EC or IRB approval for the investigation prior to any study procedures, including screening, are performed. Initial EC or IRB approval, and all materials approved by the EC or IRB for this study including the subject ICF and recruitment materials must be maintained by the investigator and made available for inspection.

Ethics Review

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an EC or IRB as appropriate. The investigator must submit written approval to the Sponsor before he or she can perform any study related procedures on subjects.

The PI is responsible for informing the EC or IRB of any amendment to the protocol in accordance with local requirements. In addition, the EC or IRB must approve all advertising used to recruit subjects for the study. The protocol must be re-approved by the EC or IRB upon receipt of amendments and annually, as local regulations require.

The PI is also responsible for providing the EC or IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. The sponsor will provide this information to the PI.

13.2.2 Subject Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from the subject or legal representative or guardian (if applicable), and assent will be obtained from the subject (where applicable), before study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH GCP and all applicable laws and regulations and will be subject to approval by the sponsor or its designee.

13.2.3 Investigator Compliance

No modifications to the protocol will be made without the approval of both the investigator and the Sponsor. Changes that significantly affect the safety of the subjects, the scope of the investigation, or the scientific quality of the study (i.e., efficacy assessments) will require IRB/IEC notification before implementation, except where the modification is necessary to eliminate an apparent immediate hazard to human subjects. The Sponsor will submit all protocol modifications to the required regulatory authorities.

When circumstances require an immediate departure from procedures set forth in the protocol, the investigator will contact the Sponsor to discuss the planned course of action. If possible, contact will be made before the implementation of any changes. Any departures from the protocol will be fully documented in the source documentation and in a protocol deviation log.

13.2.4 Access to Records

The investigator will make the office and/or hospital records of subjects enrolled in this study available for inspection by the Sponsor or its representative at the time of each monitoring visit and for audits. The records will also be available for direct inspection, verification, and copying, as required by applicable laws and regulations, by officials of the regulatory health authorities

(FDA and others). The investigator will comply with applicable privacy and security laws for use and disclosure of information related to the research set forth in this protocol.

13.2.5 Subject Privacy

To maintain subject confidentiality and to comply with applicable data protection and privacy laws and regulations, all data provided to the Sponsor, study reports, and communications relating to the study will identify subjects by assigned subject numbers, and access to subject names linked to such numbers will be limited to the site and the study physician and will not be disclosed to the Sponsor. As required by applicable laws and regulations in the countries in which the study is being conducted, the investigator will allow Sponsor and/or its representatives access to all pertinent medical records to allow for the verification of data gathered and the review of the data collection process. The FDA and regulatory authorities in other jurisdictions, including the IRB/IEC, may also request access to all study records, including source documentation, for inspection.

For sites participating in the US, and in accordance with the HIPAA and associated regulations, an executed HIPAA authorization will be obtained by the site from each subject (or the legal representative of the subject) before research activities may begin. Each HIPAA authorization will comply with all HIPAA requirements including authorization allowing the site access to and use of the subject's personally identifiable health information, authorization for the site to disclose such information to Sponsor, the FDA, and other parties requiring access under the protocol, and statements as to the purpose for which such information may be used and for how long.

13.2.6 Record Retention

The investigator will maintain all study records according to ICH GCP Guidelines and/or applicable local regulatory requirement(s), whichever is longest, as described in the Clinical Trial Agreement. If the investigator withdraws from the responsibility of keeping the study records, custody will be transferred to a person willing to accept the responsibility and the Sponsor will be notified.

13.2.7 Study Termination

At any time, the Sponsor may terminate this study in its entirety or may terminate this study at any particular site. In addition, for reasonable cause, either the investigators or their IRBs/IECs may terminate the study at their center.

Conditions that may lead to reasonable cause and warrant termination include, but are not limited to:

- Subject or investigator noncompliance
- Unsatisfactory subject enrollment
- Lack of adherence to protocol procedures
- Lack of evaluable and/or complete data
- Potentially unacceptable risk to study subjects
- Decision to modify drug development plan
- Decision by the FDA or other regulatory authority

Written notification that includes the reason for the clinical study termination is required.

This study may be discontinued at any time due to safety concerns, efficacy concerns, failure to meet expected enrollment goals, administrative reasons or at the discretion of the Sponsor. Should the study be terminated prematurely, the Sponsor will provide written notification to all investigators and regulatory authorities and will specify the reason(s) for early termination. The investigator must inform the EC or IRB promptly and provide the reason(s) for the termination.

All subjects who receive CTX001 will be asked to enroll in Study VX18-CTX001-131, a long-term follow-up study, to be followed for a total of up to 15 years after infusion.

13.2.8 End of Study

The end of study is defined as the last scheduled visit (or contact) of the last subject.

End of study will be defined as the date the last subject completes the 24-month follow-up period or date of early withdrawal from the study. The Sponsor will notify all applicable regulatory agencies in accordance with local requirements when the study has ended.

13.3 Data Quality Assurance

Sponsor or its designated representative will conduct a study site visit to verify the qualifications of each investigator, inspect clinical study site facilities, and inform the investigator of responsibilities and procedures for ensuring adequate and correct study documentation.

The investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each subject. Study data for each enrolled subject will be entered into a CRF by study site personnel using a secure, validated, web-based electronic data capture (EDC) application. The Sponsor will have read-only access to site-entered clinical data in the EDC application.

Instances of missing, discrepant, or uninterpretable data will be queried with the investigator for resolution. Any changes to study data will be made to the CRF and documented in an audit trail, which will be maintained within the clinical database.

13.4 Monitoring

Before an investigational site can enter a subject into the study, a representative of the Sponsor or designee will visit the investigational study site to:

- Determine the adequacy of the facilities
- Discuss with the investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of the Sponsor or designee. This will be documented in a Clinical Study Agreement between the Sponsor and the investigator.

Monitoring and auditing procedures developed or approved by the Sponsor will be followed to comply with GCP Guidelines. On-site checking of the CRFs/SAE Forms for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed.

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor or designee (study site monitor), who will review the CRFs/SAE Forms and source documents. The study site monitor will ensure that the investigation is conducted according to the protocol design and regulatory requirements.

Additionally, during the conduct of the study, a study site monitor will have regular contact with investigational site, for the following reasons:

- Provide information and support to the investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data is being accurately recorded in the CRFs, and that investigational product accountability checks are being performed

The monitor will be available between visits if the investigator(s) or other staff needs information or support.

13.5 Electronic Data Capture

The Sponsor will provide the study sites with secure access to and training on the EDC application sufficient to permit study site personnel to enter or correct information in the CRFs on the subjects for which they are responsible.

A CRF will be completed for each consented study subject. It is the investigator's responsibility to ensure the accuracy, completeness, clarity, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data will indicate the subject's participation in the study and will document the dates and details of study procedures, AEs, other observations, and subject status.

The investigator, or designated representative, will complete the CRF as soon as possible after information is collected.

The audit trail entry will show the user's identification information and the date and time of any correction. The investigator will provide formal approval of all the information in the CRFs, including any changes made to them, to endorse the final submitted data for the subjects for whom the investigator is responsible.

The Sponsor will retain the CRF data and corresponding audit trails. A copy of the final archival CRF in the form of a compact disc (CD) or other electronic media will be placed in the investigator's study file.

13.6 Publications and Clinical Study Report

13.6.1 Publication of Study Results

Any and all scientific, commercial, and technical information disclosed by the Sponsor in this protocol or elsewhere will be considered the confidential and proprietary property of the Sponsor. The investigator will hold such information in confidence and will not disclose the information to any third party except to such of the investigator's employees and staff as have been made aware that the information is confidential and who are bound to treat it as such and to whom disclosure is necessary to evaluate that information. The investigator will not use such information for any purpose other than determining mutual interest in performing the study and, if the parties decide to proceed with it, for the purpose of conducting the study.

The investigator understands that the information developed from this clinical study will be used by the Sponsor in connection with the development of the study drug and other drugs and diagnostics, and therefore may be disclosed as required to other clinical investigators, business

partners and associates, the FDA, and other government agencies. The investigator also understands that, to allow for the use of the information derived from the study, the investigator has the obligation to provide the Sponsor with complete test results and all data developed in the study.

No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between the Sponsor and the investigator and/or the investigator's institution.

13.6.2 Clinical Study Report

A clinical study report, written in accordance with the ICH E3 Guideline, will be submitted in accordance with local regulations.

REFERENCES

1 Trame MN, Bergstrand M, Karlsson MO, Boos J, Hempel G. Population pharmacokinetics of busulfan in children: Increased evidence for body surface area and allometric body weight dosing of busulfan in children. Clin Cancer Res. 2011;17(21):6867-77.

2 Rees DC, Gibson JS. Biomarkers in sickle cell disease. Br J Haematol. 2012;156(4):433-45.

3 Hassell KL. Population estimates of sickle cell disease in the U.S. Am J Prev Med. 2010;38(4 Suppl):S512-21.

4 Azar S, Wong TE. Sickle cell disease. Medical Clinics of North America. 2017;101(2):375-93.

5 Dietz AC, Duncan CN, Alter BP, Bresters D, Cowan MJ, Notarangelo L, et al. The second pediatric blood and marrow transplant consortium international consensus conference on late effects after pediatric hematopoietic cell transplantation: defining the unique late effects of children undergoing hematopoietic cell transplantation for immune deficiencies, inherited marrow failure disorders, and hemoglobinopathies. Biol Blood Marrow Transplant. 2017;23(1):24-9.

6 Quinn CT, Rogers ZR, McCavit TL, Buchanan GR. Improved survival of children and adolescents with sickle cell disease. Blood. 2010;115(17):3447-52.

7 Telfer P, Coen P, Chakravorty S, Wilkey O, Evans J, Newell H, et al. Clinical outcomes in children with sickle cell disease living in England: a neonatal cohort in East London. Haematologica. 2007;92(7):905-12.

8 Powars DR, Chan LS, Hiti A, Ramicone E, Johnson C. Outcome of sickle cell anemia. Medicine. 2005;84(6):363-76.

9 Platt OS, Brambilla DJ, Rosse WF, Milner PF, Castro O, Steinberg MH, et al. Mortality in sickle cell disease -- life expectancy and risk factors for early death. N Engl J Med. 1994;330(23):1639-44.

10 Artz N, Whelan C, Feehan S. Caring for the adult with sickle cell disease: results of a multidisciplinary pilot program. J Natl Med Assoc. 2010;102(11):1009-16.

11 Madigan C, Malik P. Pathophysiology and therapy for haemoglobinopathies. Part I: sickle cell disease. Expert Rev Mol Med. 2006;8(9):1-23.

12 Platt OS. Hydroxyurea for the treatment of sickle cell anemia. N Engl J Med. 2008;358(13):1362-9.

13 Wilmore DW. Food and drug administration approval of glutamine for sickle cell disease: success and precautions in glutamine research. JPEN J Parenter Enteral Nutr. 2017;41(6):912-17.

14 Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. N Engl J Med. 2019;381(6):509-19.

15 Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, et al. Crizanlizumab for the prevention of pain crises in sickle cell disease. N Engl J Med. 2017;376(5):429-39.

16 Pereira AZ, Hellman R, Hamerschlak N, Kondo A, de Souza PM, Pedreira WL, Jr., et al. A child as a donor for hematopoietic stem cell transplantation: bioethical justification-a case study on sickle cell disease. Case Rep Hematol. 2017;2017(8394732):1-5.

17 Lettre G, Bauer DE. Fetal haemoglobin in sickle-cell disease: from genetic epidemiology to new therapeutic strategies. Lancet. 2016;387(10037):2554-64.

18 Sunshine HR, Hofrichter J, Eaton WA. Requirement for therapeutic inhibition of sickle haemoglobin gelation. Nature. 1978;275(5677):238-40.
19 Perrine RP, Pembrey ME, John P, Perrine S, Shoup F. Natural history of sickle cell anemia in Saudi Arabs. A study of 270 subjects. Ann Intern Med. 1978;88(1):1-6.
20 Ngo DA, Aygun B, Akinsheye I, Hankins JS, Bhan I, Luo HY, et al. Fetal haemoglobin levels and haematological characteristics of compound heterozygotes for haemoglobin S and deletional hereditary persistence of fetal haemoglobin. Br J Haematol. 2011;156(2):259-64.
21 Huisman TH, Schroeder WA, Charache S, Bethlenfalvay NC, Bouver N, Shelton JR, et al. Hereditary persistence of fetal hemoglobin. Heterogeneity of fetal hemoglobin in homozygotes and in conjunction with beta-thalassemia. N Engl J Med. 1971;285(13):711-6.
22 Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, Eckert SV, et al. Effect of hydroxyurea on the frequency of painful crises in sickle cell anemia. N Eng J Med. 1995;332(20):1317-22.
23 Luchtman-Jones L, Pressel S, Hilliard L, Brown RC, Smith MG, Thompson AA, et al. Effects of hydroxyurea treatment for patients with hemoglobin SC disease. Am J Hematol. 2016;91(2):238-42.
24 Yates AM, Dedeken L, Smeltzer MP, Lebensburger JD, Wang WC, Robitaille N. Hydroxyurea treatment of children with hemoglobin SC disease. Pediatr Blood Cancer. 2013;60:323-5.
25 Lebensburger JD, Patel RJ, Palabindela P, Bemrich-Stolz CJ, Howard TH, Hilliard LM. Hydroxyurea decreases hospitalizations in pediatric patients with Hb SC and Hb SB+ thalassemia. J Blood Medicine. 2015;6:285-90.
26 Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-21.
27 Cradick TJ, Fine EJ, Antico CJ, Bao G. CRISPR/Cas9 systems targeting beta-globin and CCR5 genes have substantial off-target activity. Nucleic Acids Res. 2013;41(20):9584-92.
28 Menzel S, Garner C, Gut I, Matsuda F, Yamaguchi M, Heath S, et al. A QTL influencing F cell production maps to a gene encoding a zinc-finger protein on chromosome 2p15. Nature Genetics. 2007;39(10):1197-9.
29 Lettre G, Sankaran VG, Bezerra MAC, Araujo AS, Uda M, Sanna S, et al. DNA polymorphisms at the BCL11A, HBS1L-MYB, and beta-globin loci associate with fetal hemoglobin levels and pain crises in sickle cell disease. Proceedings of the National Academy of Sciences. 2008;105(33):11869-74.
30 Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, et al. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. Proceedings of the National Academy of Sciences. 2008;105(5):1620-5.
31 Xu J, Peng C, Sankaran VG, Shao Z, Esrick EB, Chong BG, et al. Correction of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. Science. 2011;334(6058):993-6.

32 Basak A, Hancarova M, Ulirsch JC, Balci TB, Trkova M, Pelisek M, et al. BCL11A deletions result in fetal hemoglobin persistence and neurodevelopmental alterations. J Clin Invest. 2015;125(6):2363-8.

33 Smith EC, Luc S, Croney DM, Woodworth MB, Greig LC, Fujiwara Y, et al. Strict in vivo specificity of the Bcl11a erythroid enhancer. Blood. 2016;128(19):2338-42.

34 Canver MC, Smith EC, Sher F, Pinello L, Sanjana NE, Shalem O, et al. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. Nature. 2015;527(7577):192-7.

35 Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, et al. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science. 2013;342(6155):253-7.

36 Vierstra J, Reik A, Chang KH, Stehling-Sun S, Zhou Y, Hinkley SJ, et al. Functional footprinting of regulatory DNA. Nat Methods. 2015;12(10):927-30.

37 Adams RJ, McKie VC, Hsu L, Files B, Vichinsky E, Pegelow C, et al. Prevention of a first stroke by transfusions in children with sickle cell anemia and abnormal results on transcranial Doppler ultrasonography. N Engl J Med. 1998;339(1):5-11.

38 Howard J, Malfroy M, Llewelyn C, Choo L, Hodge R, Johnson T, et al. The Transfusion Alternatives Preoperatively in Sickle Cell Disease (TAPS) study: a randomised, controlled, multicentre clinical trial. Lancet. 2013;381(9870):930-8.

39 Miller ST, Wright E, Abboud M, Berman B, Files B, Scher CD, et al. Impact of chronic transfusion on incidence of pain and acute chest syndrome during the Stroke Prevention Trial (STOP) in sickle-cell anemia. J Pediatr. 2001;139(6):785-9.

40 Kalff A, Dowsing C, Grigg A. The impact of a regular erythrocytapheresis programme on the acute and chronic complications of sickle cell disease in adults. Br J Haematol. 2010;149(5):768-74.

41 Stenger E, Shenoy S, Krishnamurti L. How I treat hematopoietic cell transplantation for sickle cell disease. Blood. 2019;134(25):2249-60.

42 Uchida N, Leonard A, Stroncek D, Panch SR, West K, Molloy E, et al. Safe and efficient peripheral blood stem cell collection in patients with sicke cell disease using plerixafor. Haematologica. 2020;105:

43 Fitzhugh CD, Hsieh MM, Bolan CD, Saenz C, Tisdale JF. Granulocyte colony-stimulating factor (G-CSF) administration in individuals with sickle cell disease: time for a moratorium? Cytotherapy. 2009;11(4):464-71.

44 Schroeder MA, Rettig MP, Lopez S, Christ S, Fiala M, Eades W, et al. Mobilization of allogeneic peripheral blood stem cell donors with intravenous plerixafor mobilizes a unique graft. Blood. 2017;129(19):2680-92.

45 Devine SM, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. Blood. 2008;112(4):990-8.

46 Yannaki E, Karponi G, Zervou F, Constantinou V, Bouinta A, Tachynopoulou V, et al. Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of granulocyte-colony stimulating factor plus plerixafor in patients with beta-thalassemia major. Hum Gene Ther. 2013;24(10):852-60.

47 Ciurea SO, Andersson BS. Busulfan in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2009;15(5):523-36.

48 Deeg HJ, Schuler US, Shulman H, Ehrsam M, Renner U, Yu C, et al. Myeloablation by intravenous busulfan and hematopoietic reconstitution with autologous marrow in a canine model. Biol Blood Marrow Transplant. 1999;5(5):316-21.
49 Walters MC, Patience M, Leisenring W, Eckman JR, Scott JP, Mentzer WC, et al. Bone marrow transplantation for sickle cell disease. N Engl J Med. 1996;335(6):369-76.
50 Ribeil JA, Hacein-Bey-Abina S, Payen E, Magnani A, Semeraro M, Magrin E, et al. Gene therapy in a patient with sickle cell disease. N Engl J Med. 2017;376(9):848-55.
51 Thompson A, Kwiatkowski J, Rasko J, Hongeng S, Schiller GJ, Anurathapan U, et al. Lentiglobin gene therapy for transfusion-dependent beta-thalassemia: update from the northstar Hgb-204 phase 1/2 clinical study. Blood. 2016;128(22):1175.
52 Kanter J, Kruse-Jarres R. Management of sickle cell disease from childhood through adulthood. Blood Rev. 2013;27(6):279-87.
53 Quinn CT. Sickle cell disease in childhood: from newborn screening through transition to adult medical care. Pediatr Clin North Am. 2013;60(6):1363-81.
54 Cappelli B, Volt F, Tozatto-Maio K, Scigliuolo GM, Ferster A, Dupont S, et al. Risk factors and outcomes according to age at transplantation with an HLA-identical sibling for sickle cell disease. Haematologica. 2019;104(12):e543-e46.
55 Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G. Recommendations for the transfusion of red blood cells. Blood Transfusion. 7:49-64.
56 Meletis J, Dalekou M, Samarkos M, Paravasiliou E, Meletis C, Konstantopoulos K, et al. Fetal erythropoiesis after allogeneic bone marrow transplantation estimated by the peripheral blood erythrocytes containing hemoglobin F (F-cells). Hematology. 2001;5(6):447-53.
57 Gluckman E, Cappelli B, Bernaudin F, Labopin M, Volt F, Carreras J, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood. 2017;129(11):1548-56.
58 Elmariah H, Garrett ME, De Castro LM, Jonassaint JC, Ataga KI, Eckman JR, et al. Factors associated with survival in a contemporary adult sickle cell disease cohort. Am J Hematol. 2014;89(5):530-5.
59 Saraf SL, Rondelli D. Allogeneic hematopoietic stem cell transplantation for adults with sickle cell disease. J Clin Med. 2019;8(10):1565.
60 Haynes A, Hunter A, McQuaker G, Anderson S, Bienz N, Russell NH. Engraftment characteristics of peripheral blood stem cells mobilised with cyclophosphamide and the delayed addition of G-CSF. Bone Marrow Transplant. 1995;16(3):359-63.
61 Papadopoulos KP, Ayello J, Tugulea S, Heitjan DF, Williams C, Reiss RF, et al. Harvest quality and factors affecting collection and engraftment of CD34+ cells in patients with breast cancer scheduled for high-dose chemotherapy and peripheral blood progenitor cell support. J Hematother. 1997;6(1):61-8.
62 Tricot G, Jagannath S, Vesole D, Nelson J, Tindle S, Miller L, et al. Peripheral blood stem cell transplants for multiple myeloma: identification of favorable variables for rapid engraftment in 225 patients. Blood. 1995;85(2):588-96.
63 Shpall EJ, Champlin R, Glaspy JA. Effect of CD34+ peripheral blood progenitor cell dose on hematopoietic recovery. Biol Blood Marrow Transplant. 1998;4(2):84-92.
64 Adams RJ, McKie VC, Brambilla D, Carl E, Gallagher D, Nichols FT, et al. Stroke prevention trial in sickle cell anemia. Control Clin Trials. 1998;19(1):110-29.

65 Mohty M, Malard F, Abecassis M, Aerts E, Alaskar AS, Aljurf M, et al. Revised diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in adult patients: A new classification from the European Society for Blood and Marrow Transplantation. Bone Marrow Transplant. 2016;51(7):906-12.

66 Varni JW, Seid M, Knight TS, Uzark K, Szer IS. The PedsQL™ 4.0 Generic Core Scales: Sensitivity, Responsiveness, and Impact on Clinical Descision-Making. J Behav Med. 2002;25(2):175-93.

67 Varni JW [Internet]. Scaling and Scoring of the Pediatric Quality of Life Inventory™ PedsQL™ [cited 15 December 2019]. Available from: https://www.pedsql.org/PedsQL-Scoring.pdf.

68 Panepinto JA, Torres S, Bendo CB, McCavit TL, Dinu B, Sherman-Bien S, et al. PedsQL sickle cell disease module: feasibility, reliability, and validity. Pediatr Blood Cancer. 2013;60(8):1338-44.

14 PROTOCOL SIGNATURE PAGES

14.1 Sponsor Signature Page

Protocol #: CTX001-121	Version #: 6.11 (US)	Version Date: 15 September 2022
Study Title: A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified $CD34^+$ Human Hematopoietic Stem and Progenitor Cells (CTX001) in Subjects With Severe Sickle Cell Disease		

This clinical study protocol has been reviewed and approved by the sponsor.

Printed Name	Title
Signature	Date

14.2 Investigator Signature Page

Protocol #: CTX001-121	Version #: 6.11 (US)	Version Date: 15 September 2022
Study Title: A Phase 1/2/3 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified $CD34^+$ Human Hematopoietic Stem and Progenitor Cells (CTX001) in Subjects With Severe Sickle Cell Disease		

I have read Protocol CTX001-121, Version 6.11 (US), and agree to conduct the study according to its terms. I understand that all information concerning CTX001 and this protocol supplied to me is confidential.

Printed Name

Signature Date

15 APPENDICES

15.1 Busulfan PK Collection

Samples for PK analysis of busulfan will be drawn on the 1st and 3rd day of dosing. Recommended guidelines are below.

Recommendations for once daily (qd) dosing:

End of infusion, (± 5 minutes)
End of infusion + 15 minutes (± 5 minutes)
End of infusion + 30 minutes (± 5 minutes)
Start of infusion + 4 hours (± 15 minutes)
Start of infusion + 5 hours (± 15 minutes)
Start of infusion + 6 hours (± 15 minutes)
Start of infusion + 8 hours (± 15 minutes)

Recommendations for every 6 hours (q6h) dosing:

End of infusion (± 5 minutes)
End of infusion + 15 minutes (± 5 minutes)
End of infusion + 30 minutes (± 5 minutes)
Start of infusion + 3 hours (± 15 minutes)
Start of infusion + 4 hours (± 15 minutes)
Start of infusion + 5 hours (± 15 minutes)
Start of infusion + 6 hours (± 15 minutes)

Note: Samples should not be drawn from the lumen used to infuse busulfan. In both dosing schemes, the actual time of PK collection must be recorded, not just the nominal times provided above.

Clinical sites must either measure busulfan PK on the first and third days of administration, or alternatively, use a busulfan test dose prior to myeloablation to pre-determine the dose, with confirmatory PK measurements on the first day of dosing. Based on the available busulfan PK results, busulfan dose adjustments should be made for subsequent dosing. As part of this protocol, sites are permitted to perform daily busulfan monitoring, with subsequent dose adjustments, if that is routine practice. Specific details of busulfan dosing, PK, and dose adjustments will be included in the electronic case report form (eCRF).

15.2 European Society for Blood and Marrow Transplantation (EBMT) Criteria for VOD Diagnosis

Table 15-1 EBMT Criteria for SOS/VOD Diagnosis in Adults

Classical SOS/VOD **In the first 21 days after HSCT**	**Late onset SOS/VOD** **>21 days after HSCT**
Bilirubin ≥2 mg/mL and 2 of the following criteria must be present: • Painful hepatomegaly • Weight gain >5% • Ascites	Classical VOD/SOS beyond Day 21 OR Histologically proven SOS/VOD OR 2 or more of the following criteria must be present: • Bilirubin ≥2 mg/mL (or 34 μmol/L) • Painful hepatomegaly • Weight gain >5% • Ascites AND Hemodynamical or/and ultrasound evidence of SOS/VOD

Source: Mohty et al. 2016[65]
EBMT: European Society for Blood and Marrow Transplantation; HSCT: hematopoietic stem cell transplantation; SOS: sinusoidal obstruction syndrome; VOD: veno-occlusive disease