STATISTICAL ANALYSIS PLAN

A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease

Protocol HGB-206

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Protocol Version and Date:	Version 13.0, 17 Jan 2023
Name of Test Drug:	bb1111 (USAN/INN: lovotibeglogene autotemcel, also known as LentiGlobin BB305 Drug Product for Sickle Cell Disease)
Phase:	Phase 1/2
Methodology:	Open-label, Safety, and Efficacy
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Analysis Plan Date:	4 Mar 2024
Analysis Plan Version:	Version 3.0
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APPROVAL OF STATISTICAL ANALYSIS PLAN

SIGNATURE PAGE

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Sponsor: **bluebird bio, Inc.**

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
6MWT	6-minute walk test
AAPT	ACTTION - American pain society pain taxonomy
ACS	Acute chest syndrome
ACTH	Adrenocorticotropic hormone
ACTTION	Analgesic, anesthetic, and addiction clinical trial translations, innovations, opportunities, and networks
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AUC	Area under curve
AST	Aspartate aminotransferase
BMD	Bone mineral density
BNP	Brain natriuretic peptide
C	Conditioning
CBC	Complete blood count
CD	Cluster of differentiation
CKD-EPI	Chronic kidney disease epidemiology collaboration
CI	Confidence interval
CRF	Case report form
CRP	C-reactive protein
CR	Complete response
CS	Clinically significant
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
D1	Day 1 (date/time of drug product infusion)
DBP	Diastolic blood pressure
DCO	Data cut-off date
DLco	Carbon monoxide diffusing capacity
DLT	Dose limiting toxicities
DMC	Data monitoring committee
DOB	Date of birth
DP	Drug product
DXA	Dual x-ray absorptiometry
eGFR	Estimated glomerular filtration rate
FEV_1	Forced expiratory volume in the one second
FISH	Fluorescence in situ hybridization
Free T3	Free triiodothyronine
Free T4	Free thyroxine
FSH	Follicle-stimulating hormone
FVC	Forced vital capacity
GGT	Gamma-glutamyl transferase
GR	Globin response

Abbreviation	Definition
GRRs	Global reference ranges
GVHD	Graft-versus-host disease
Hb	Hemoglobin
HbA	Hemoglobin A
HbA_2	Hemoglobin A_2
HbA^{T87Q}	Hemoglobin containing $\beta^{A\text{-}T87Q}$-globin
HbF	Hemoglobin F
HbS	Hemoglobin S
HLGT	High level group term
HLT	High level term
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HU	Hydroxyurea
ICF	Informed consent form
ISA	Integration site analysis
IS	Integration Site(s)
ITT	Intent-to-treat
IV	Intravenous
LDH	Lactate dehydrogenase
LFTs	Liver function tests
LH	Luteinizing hormone
LVEF	Left ventricular ejection fraction
LVV	Lentiviral vector
MedDRA	Medical dictionary for regulatory activities
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
NCS	Not clinically significant
NGS	Next generation sequencing
NE	Neutrophil engraftment
NCI-CTCAE	National cancer institute-common terminology for adverse events
OS	Overall survival
PB	Peripheral blood
PBL	Peripheral blood leukocyte
PCS	Potentially clinically significant
PD	Pharmacodynamics
PFT	Pulmonary function tests
pRBC	Packed red blood cell(s)
PRO	Patient reported outcome
PROMIS	Patient reported outcomes measurement information system
PT	Preferred term
Q6H	Every 6 hours
QD	Once daily

Abbreviation	Definition
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell
RDW	Red cell distribution width
RCL	Replication competent lentivirus
Rel Day	Relative study day
RTF	Rich text files
RV	Respiratory volume
SAE	Serious adverse event
SAP	Statistical analysis plan
SCD	Sickle cell disease
SD	Standard deviation
SEP	Successful engraftment population
SI	System of Units
SMQs	Standardized MedDRA Queries
SOC	System organ class
SOE	Schedule of events
sVOE	Severe vaso-occlusive event
sVOE-75	At least a 75% reduction in annualized severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to informed consent
sVOE-CR	Complete resolution of severe VOE between 6 months and 18 months after drug product infusion
sVOE-CR24	Complete resolution of severe VOE between 6 months and 24 months after drug product infusion
SpO2	Peripheral capillary oxygen saturation
TB	Total bilirubin
TCD	Transcranial doppler
TLC	Total lung capacity
TP	Transplant population
TPVOE	Transplant population for VOE
TRJV	Tricuspid regurgitant jet velocity
TSH	Thyroid stimulating hormone
VC	Vital capacity
VCN	Vector copy number
VOC	Vaso-occlusive crisis
VOE-CR	Complete resolution of VOE between 6 months and 18 months after drug product infusion
VOE-CR24	Complete resolution of VOE between 6 months and 24 months after drug product infusion
VOE	Vaso-occlusive event
WBC	White blood cell
WHO	World health organization
WPAI-GH	Work productivity and activity impairment questionnaire-general health

1. INFORMATION FROM THE STUDY PROTOCOL

1.1. Introduction and Objectives

1.1.1. Introduction

This document is the statistical analysis plan (SAP) for Study HGB-206, A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease. Unless otherwise specified, the protocol indicated in this SAP is Protocol HGB-206 Version 13.0, dated 17 January 2023.

The SAP is designed to outline the methods to be used in the analysis of study data in order to answer the study objectives. Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this study.

1.1.2. Study Objectives

The primary study objective is to:

- Evaluate the efficacy of treatment with bb1111 in subjects with severe sickle cell disease (SCD)

The secondary study objective is to:

- Evaluate the safety of treatment with bb1111 in subjects with severe SCD

1.2. Study Design

1.2.1. Synopsis of Study Design

This is a non-randomized, open label, multi-site, single dose, Phase 1/2 study in approximately 50 adults and adolescents with severe SCD. The study will evaluate the efficacy and safety of autologous hematopoietic stem cell transplantation (HSCT) using bb1111 (lovotibeglogene autotemcel, also known as LentiGlobin BB305 Drug Product for SCD), an autologous CD34+ cell-enriched population from patients with SCD that contains hematopoietic stem cells (HSCs) transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution.

The study has 4 distinct stages, as follows:

Stage 1 - Screening and eligibility assessment

Stage 2 - Stem cell harvest, drug product manufacture and disposition

Stage 3 - Myeloablative conditioning and drug product infusion

Stage 4 - Follow-up for approximately 24 months after drug product infusion

Subjects are placed into 1 of 3 non-randomly assigned Groups (Groups A, B, or C), depending on the date of informed consent and the method of stem cell collection for either rescue or drug

product manufacture. The detailed definition of the Groups can be found in study Protocol HGB-206 Section 5.3, and the main differences among these Groups are listed in Table 1.

Table 1. Study Design and Selected Details for Groups A, B and C

	Group A	Group B1	Group B2	Group C
Number of subjects consented and originally assigned to this group	9	1	3	51
Number of subjects received drug product infusion in this group	7	1	1	36
Number of subjects reassigned to Group C before drug product infusion	0	0	2	NA
Method of stem cell collection				
For drug product	BMH	BMH	BMH	Mobilization/ Apheresis
For back-up cells	BMH	BMH	Mobilization/ Apheresis	Mobilization/ Apheresis
Drug product manufacturing process	Process 1	Process 1 & Process 2	Process 2	Process 2a

BMH= bone marrow harvest; NA = not applicable

- Group A: Nine subjects who consented and had bone marrow harvested for stem cell collection were retrospectively assigned to Group A. Two of 9 withdrew from the study before initiating conditioning and did not receive drug product infusion. Seven of the 9 subjects received drug product infusion, and both drug product and back-up cells were derived from bone marrow harvest. All drug products were manufactured using Process 1.
- Group B1: One subject (PPI) who had consented under the protocol version 5.0 but had not yet been treated with drug product, was reconsented under the protocol version 6.0 and assigned to Group B1. For this subject, both drug product and back-up cells were derived from bone marrow harvest. However, this subject received two lots of drug product of which one was manufactured using Process 1 and the other was manufactured using Process 2.
- Group B2: Plerixafor mobilization and apheresis were used for collection of rescue cells and exploratory manufacturing development, and it was planned for subjects in Group B2 to then undergo bone marrow harvest for drug product manufacture. At least 3 subjects in Group B2 were originally planned for evaluation of dose limiting toxicities (DLT) at each dose of plerixafor. The Data Monitoring Committee (DMC) reviewed the adverse event (AE) profile during plerixafor treatment in mobilization Cycle 1 for the first subject in Group B2 before dosing plerixafor in the second subject and reviewed the AE profile of

the second subject before dosing plerixafor in the third subject. The DMC also reviewed aggregated safety data in Group B2 after all 3 subjects had been dosed with plerixafor and confirmed that there were no safety concerns precluding dosing of the subjects in Group C with plerixafor. Under protocol version 7.0, Group B2 subjects who tolerated plerixafor mobilization (for collection of rescue cells) were given the option to delay collection of cells for drug product manufacturing until Group C opened, and then they could undergo drug product manufacture from cells obtained by a subsequent cycle of plerixafor mobilization. Two of three subjects in Group B2 (PPI and PPI) were re-assigned to Group C, in which both drug product and back-up cells were derived from plerixafor mobilization and apheresis, and no new Subject ID was assigned to these 2 subjects. The single subject (PPI) remaining in Group B2 received 2 lots of drug product derived from bone marrow harvest, both of which were manufactured using Process 2.

- Group C: Opened after (1) confirmation of the safety and tolerability of plerixafor mobilization in Group B2, and (2) regulatory authority approval of the drug product manufacturing process with plerixafor-mobilized hematopoietic stem cells (HSCs). In Group C, both drug product and back-up cells are derived from plerixafor mobilization and apheresis, and drug product is manufactured using Process 2a.

Monitoring of adverse events will be conducted from the signing of informed consent.

- For Groups A and B: adverse events will be documented from the time Informed Consent is signed through the following time points:
  - ≥Grade 1 AEs: through 30 days after drug product infusion.
  - ≥Grade 2 AEs: through 12 months after drug product infusion.
  - All serious adverse events (SAEs), ≥Grade 3 AEs, and drug product related AEs: through the end of study.
- For Group C: all AEs for all subjects (excluding screen failures) will be recorded in the Case Report Form (CRF) starting from the time Informed Consent is signed through the end of study. All SAEs (including screen failures) will be reported from the signing of Informed Consent/Assent through end of study on the SAE report form. Group C subjects enrolled under Version 7.0 or earlier of Protocol HGB-206 will have AEs retrospectively documented to be in accordance with Version 8.0 or higher of Protocol HGB-206.

For subjects in all 3 Groups who initiated any stem cell collection procedures (mobilization/apheresis or bone marrow harvest), all investigator vaso-occlusive events (VOEs) occurring on or after the signing of Informed Consent/Assent, regardless of the severity grade, will be documented from the time informed consent is signed through the last follow-up of the study. For subjects enrolled under Version 7.0 of Protocol HGB-206, the VOEs will be retrospectively documented to be in accordance with Version 8.0 or higher of Protocol HGB-206.

1.2.2. Randomization Methodology

Randomization was not performed as this is a single treatment, open-label study.

1.2.3. Unblinding

Unblinding is not applicable to this open-label study.

1.2.4. Stopping Rules

See the study Protocol Section 3.5 for stopping rules for this study.

1.2.5. Study Procedures

The schedule of events (SOE) to be performed is provided in the study Protocol Section 6.1.

1.2.6. Study Endpoints

1.2.6.1. Primary Efficacy Endpoint

VOE-CR, defined as complete resolution of vaso-occlusive events (VOEs), between 6 months and 18 months after drug product infusion.

1.2.6.2. Key Secondary Efficacy Endpoints

- sVOE-CR, defined as complete resolution of severe vaso-occlusive events (sVOEs), between 6 months and 18 months after drug product infusion.
- Globin Response, defined as meeting the following criteria for a continuous period of at least 6 months after drug product infusion:

  a. Weighted average HbA^{T87Q} percentage of non-transfused total Hb[1] $\geq$ 30%

  AND

  b. Weighted average non-transfused total Hb[1] increase of $\geq$ 3 g/dL compared to baseline total Hb[2] OR weighted average non-transfused total Hb[1] $\geq$ 10 g/dL

[1] Non-transfused total Hb is the total g/dL of HbS + HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-transfused total Hb" only for samples taken $\geq$ 60 days after last pRBC transfusion.
[2] Baseline total Hb is defined as follows:
The average of the 2 most recent Hb assessments made at or prior to the Screening evaluation, which meet the following criteria:
(i) Assessments must be separated by at least 1 month from each other.
(ii) Assessments must have been drawn no earlier than 24 months prior to informed consent and may include the Hb result from Screening.
(iii) The subject will not have received a pRBC transfusion within 3 months prior to each Hb assessment.
For subjects who are on chronic, recurrent transfusions, and do not have 2 Hb assessments which meet criteria (i), (ii), and (iii), the following criteria can be used: 2 Hb values which meet criteria (i) and (iii) that are found within 24 months prior to the start of a regular transfusion program.

1.2.6.3. Additional Secondary Efficacy Endpoints

Clinical and Disease Evaluation Endpoints:

- Change in the annualized number of VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent.
- Change in the annualized number of severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent.

- VOE-CR24, defined as complete resolution of VOEs between 6 months and 24 months after drug product infusion
- sVOE-CR24, defined as complete resolution of severe VOEs between 6 months and 24 months after drug product infusion
- sVOE-75, defined as at least a 75% reduction in annualized severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent

Characterization of Globin Response:

- Proportion of subjects who meet the definition of Globin Response at Month 24
- Duration of Globin Response

Hematologic Endpoints:

- Weighted average for the following at Month 6, 12, 18, and 24:
  - non-transfused total Hb[1]
  - HbS percentage of non-transfused total Hb[1]
  - HbS percentage of non-transfused total Hb[1] $\leq 70\%, \leq 60\%, \leq 50\%$
  - HbA^{T87Q} percentage of non-transfused total Hb[1]
  - non-HbS[3] percentage of non-transfused total Hb[1]
- Assessment of the following over time:
  - non-transfused total Hb[1]
  - HbS percentage of non-transfused total Hb[1]
  - HbA^{T87Q} percentage of non-transfused total Hb[1]
  - non-HbS[3] percentage of non-transfused total Hb[1]

[1] Non-transfused total Hb is the total g/dL of HbS + HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-transfused total Hb" only for samples taken $\geq$ 60 days after last pRBC transfusion.
[3] Non-HbS is the total g/dL of HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-HbS" only for samples taken $\geq$ 60 days after last pRBC transfusion.

- Change from baseline in hemolysis markers, including absolute reticulocyte count, % reticulocytes/erythrocytes, total bilirubin, haptoglobin, and lactate dehydrogenase
- Change from baseline in markers of iron stores including ferritin, liver iron content, and if assessed at baseline, cardiac iron content
- Change from baseline in annualized frequency and volume of packed red blood cell (pRBC) transfusions between 6 months and 24 months after drug product infusion
- Change from baseline in markers of stress erythropoiesis, including erythropoietin and serum transferrin receptor

SCD Burden and Chronic Complications Assessments:

- Change from baseline in renal function as measured by eGFR

- Change from baseline in cardiac-pulmonary function via echocardiogram (tricuspid regurgitant jet velocity [TRJV], LVEF) and pulmonary function tests
- Change from baseline in meters walked during 6-minute walk test

Hospitalizations and Quality of Life:

- Change from baseline in annualized VOE-related hospital admissions and days
- Change from baseline in patient-reported quality of life, as measured by Patient Reported Outcomes Measurement Information System (PROMIS)

1.2.6.4. Exploratory Efficacy Endpoints

- Change from baseline in cerebral vasculature and prior brain parenchymal injury evaluation at Month 12 and Month 24 (as measured by cerebral magnetic resonance angiography [MRA]/magnetic resonance imaging [MRI] in all subjects, and transcranial Doppler [TCD] for subjects ≤ 16 years old at Informed Consent)
- Change from baseline in bone mineral density (BMD) evaluation using dual x-ray absorptiometry (DXA) at Month 24
- Change from baseline in brain natriuretic peptide (BNP)
- Change from baseline in Patient Reported Outcome (PRO) measures including:
  - Overall health: EuroQol-5D (EQ-5D-3L or EQ-5D-Y)
  - Work productivity, as measured by the Work Productivity and Activity Impairment Questionnaire-General Health (WPAI-GH or Caregiver WPAI-GH)
  - Cognitive function as measured by PROMIS Short Form 6a
- Evaluation of chronic pain using AAPT
- Change from baseline in pain medication use
- Exploratory assays to assess change from baseline in sickle cell characteristics and bone marrow pathophysiology

1.2.6.5. Pharmacodynamic Endpoints

- Vector copy number (VCN) in peripheral blood over time
- Expression of $\beta^{A\text{-}T87Q}$-globin, β^{S}-globin, and other β-like globins in peripheral blood over time

Additional methods may be used to evaluate pharmacodynamics (PD).

1.2.6.6. Safety Endpoints

Safety will be evaluated by the following:

- Safety and tolerability of plerixafor for mobilization
- Success and kinetics of HSC engraftment
- Transplant-related mortality through 100 days post-drug product infusion and through 365 days post-drug product infusion

- Detection of vector-derived replication competent lentivirus (RCL) in any subject
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.)
- The number of subjects with persistent oligoclonality
- Frequency and severity of AEs/SAEs
- Laboratory parameters over time, including the following immunology tests: Screening for irregular antibodies, lymphocyte subpopulation evaluation (CD3, CD4, CD8, CD19, CD16/CD56)
- Incidence of acute and/or chronic graft-versus-host disease (GVHD)
- Other safety labs over time: chemistries, LFTs (AST, ALT, ALP, GGT, bilirubin [total and direct]), hematology, coagulation parameters
- Hormonal testing over time: estradiol (females only); total testosterone (males only) TSH, free T3 (triiodothyronine), free T4 (thyroxine), cortisol, ACTH, FSH, LH (all subjects)
- Change from baseline in methemoglobin concentration at Month 12 and Month 24
- Number of subjects with presence of a chromosomal abnormality or genetic mutation associated with hematologic malignancies over time

2. SUBJECT POPULATION

2.1. Population Definitions

The populations for evaluating efficacy, safety, or pharmacodynamic analyses are listed and defined in Table 2. Study endpoints and their corresponding analysis populations are listed in Table 3. Additional supportive or supplementary analysis might be performed in other analysis populations or groups.

Table 2. Population Definitions

Population	Definition
Intent-to-Treat (ITT) Population	All subjects who initiate any study procedures, beginning with stem cell collection procedures (mobilization/apheresis or bone marrow harvest)
Transplant Population (TP)	All subjects who receive drug product
Successful Engraftment Population (SEP)	A subset of TP subjects who, following busulfan myeloablation and drug product infusion, successfully engraft with drug product, defined as 3 consecutive absolute neutrophil count (ANC) $\geq 0.5\times10^9$/L laboratory values obtained on different days after the initial post-infusion nadir by Day 43
Transplant Population for VOE (TPVOE)	A subset of TP subjects who have at least 4 protocol VOEs in the 24 months prior to Informed Consent

Table 3. Endpoints and the Corresponding Analysis Population

Endpoints	Analysis Population[1]
Demographics and baseline characteristics	ITT
Efficacy endpoints	
Primary endpoint: VOE-CR	Group C subjects in TPVOE
Key secondary endpoint: sVOE-CR	Group C subjects in TPVOE
Key secondary endpoint: Globin Response	Group C subjects in TP
Other secondary endpoints:	
Globin Response at Month 24, Duration of Globin Response	Group C subjects who achieved Globin Response
sVOE-CR24, VOE-CR24, sVOE-75, Change in annualized number of sVOEs	Group C subjects in TPVOE
Change in annualized pRBC transfusions	Group C subjects in TP
Change in annualized VOE-related hospitalizations	TP
Change in annualized number of VOEs	TPVOE
Remaining other secondary endpoints	TP
Exploratory endpoints	TP
Pharmacodynamic endpoints	TP
Safety endpoints[2]	ITT or TP

Note: selected endpoints may be repeated in SEP as supportive, if different than TP.
[1] Additional supportive or supplementary analysis may be performed in other analysis population or groups.
[2] Selected safety endpoints, such as treatment-emergent adverse events, will be based on subjects in TP.

2.2. Protocol Deviations

All protocol deviations will be presented in a listing. The number and percentage of all major deviations in ITT population will be summarized by group. Protocol deviations due to COVID-19 will also be presented in a listing and summarized in a table.

Categorization of protocol deviations will be determined by a review of the protocol deviation data collected on the case report form (CRF). Determination of major/minor and categorization of each protocol deviation type will be made prior to database lock.

3. GENERAL STATISTICAL METHODS

3.1. Sample Size Justification

The sample sizes for Groups A and B are not determined by formal statistical methods.

Approximately 41 subjects will be enrolled in Group C with approximately 35 subjects meeting the severe VOE criteria as defined in Inclusion Criterion #3.1.

- For the primary endpoint, VOE-CR: assuming 80% of Group C subjects in the TPVOE will meet the primary efficacy endpoint, 35 subjects will provide more than 99% power to reject the null hypothesis of 40% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 60% (21 out of 35) of subjects meeting the primary efficacy endpoint, if exactly 35 subjects with at least 4 VOEs in the 24 months prior to ICF are enrolled and receive drug product in Group C.
- For the key secondary endpoint, sVOE-CR: assuming 85% of Group C subjects in the TPVOE will meet the primary efficacy endpoint under the alternative hypothesis, 35 subjects will provide more than 99% power to reject the null hypothesis of 50% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 69% (24 out of 35) of subjects meeting the key secondary endpoint, if exactly 35 subjects who have at least 4 VOEs in the 24 months prior to ICF are enrolled and received drug product in Group C.
- For the key secondary endpoint, Globin Response: assuming 70% of Group C subjects in the TP will meet the key secondary efficacy endpoint under the alternative hypothesis, 41 subjects will provide approximately 96% power to reject the null hypothesis of 40% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 59% (24 out of 41) of subjects meeting the key secondary efficacy endpoint, if exactly 41 subjects are enrolled and received drug product in Group C.

3.2. General Methods

All outputs will be incorporated into Adobe Acrobat portable document format (PDF) files or rich text files (RTF), sorted, and labelled according to the International Council for Harmonization (ICH) recommendations, and formatted to the appropriate page size(s).

Upon consultation with the Medical Monitor, screen-failed subjects may be allowed for re-screening for eligibility to enter the study with a new subject ID, and the re-screening eligible subjects will be presented by the new subject ID in the study. A listing will be provided for screening failure patients. For re-screened subjects, the listing will include original and new subject ID, original screening and re-screening date and result. The demographic information and the group enrolled (if it is available) will also be provided.

Subjects will be presented by study assignment group. No new Subject ID was assigned for two subjects (PPI and PPI) who were originally assigned to Group B2 and then were re-assigned to Group C in which both drug product and back-up cells were derived from plerixafor mobilization. These two subjects will be included in Group C for all the analyses.

If a subject in the TP develops a hematologic malignancy during the study, the following rules may apply:

- Tables and figures of efficacy endpoints, including but not limited to Hb fractions via high-performance liquid chromatography (HPLC), VOEs, sVOEs, and peripheral blood VCN, will be based on the data up to the date of diagnosis of the malignancy. For binomial proportion endpoints assessing periods of time [e.g., VOE-CR, sVOE-CR] the subject will be considered a non-responder if evaluable for the endpoint and the hematologic malignancy occurs during the endpoint period. For continuous endpoints (e.g., Hb fractions, PB VCN, annualized number of VOEs) data will be censored after the time of hematologic malignancy.
- All the efficacy endpoints will be included in the listings.
- Safety endpoints will be based on the data through the end of study.

For continuous variables, descriptive statistics such as the number of observations or subjects, mean, standard deviation (SD), median, 25% percentile (Q1) and 75% percentile (Q3) as needed, minimum and maximum values will be presented. Mean, median, Q1, Q3, and 95% confidence intervals (CI) will be rounded to 1 additional decimal place compared to the original data (maximum of 3 decimal places). The SD will be rounded to 2 additional decimal places compared to the original data. Minimum and maximum will be displayed with the same accuracy as the original data. The maximal decimals for SD will be 4.

For categorical variables, summary of the number and percentage within each category of the parameter will be presented. A shift table, for change from baseline, can be provided as appropriate. In the shift table, baseline, the worst value within each visit window post-drug product infusion (if there is more than 1 value), and the worst value during the entire follow-up post-drug product infusion will be presented as needed.

Descriptive summary statistics, as well as 2-sided 95% CI, will be presented on selected parameters, as described in the sections below. The exact CI for proportion will be calculated using Clopper-Pearson exact method.

Longitudinal data (collected serially over time on study) will be presented by appropriate time intervals, such as monthly, quarterly, and so forth, depending on the nature of the data.

For purposes of calculations, a month will be defined as 365.25/12 (30.4375) days and a year as 365.25 days. For reporting by month, calculations will be rounded to the nearest day (i.e., the calculated value at 18 months, 547.9, would be rounded to 548 days; the calculated value at 6 months, 182.6, would be rounded to 183 days).

By-subject listings of data for all completed and discontinued subjects, including screen failures (as appropriate), will be provided. All data listings that contain an evaluation date will contain a relative study day (Rel Day). Pre- and post- drug product infusion study days are numbered relative to the day of drug product infusion, which is designated as Day 1.

3.3. Computing Environment

All planned statistical analyses and data summarizations will be performed using SAS statistical software Version 9.4 or higher, unless otherwise noted.

3.4. Baseline Definitions

For annualized number of endpoints, such as annualized number of VOEs, sVOEs, hospitalizations and pRBC transfusions, baseline will be defined as the annualized number of the corresponding endpoint in the 24 months before Informed Consent.

The definition of baseline total Hb for Globin Response derivation can be found in Section 1.2.6.2. The baseline total Hb values are collected on the eCRF page of Baseline Hemoglobin. A subject will be considered as having no baseline Hb for Globin Response analysis if the subject has 1 or 0 qualifying Hb value from the Baseline Hemoglobin eCRF page.

For liver biopsy, liver MRI, Cardiac MRI, cerebral MRA/MRI, echocardiogram (including LVEF and TRJV), bone mineral density (BMD) and pulmonary function test (PFT), baseline will be defined as the first assessment during screening. If no records at screening, the last assessment prior to Informed Consent will be used.

Unless otherwise specified, for other efficacy, pharmacodynamic, and safety parameters, including shifts in key laboratory parameters, baseline will be defined as the first assessment during screening (which can be identified by the first record on or after date of Informed Consent Form [ICF], but before initiation of stem cell collection).

3.5. Methods of Pooling Data

Data will be presented by group. Efficacy analyses will focus on Group C. However, an overall column will be included as appropriate.

3.6. Adjustments for Covariates

No adjustment for covariates is planned for analysis of this study.

3.7. Multiple Comparisons/Multiplicity

Formal multiplicity adjustment will not be performed. It is expected that most of the secondary and exploratory efficacy endpoints will demonstrate a positive effect of drug product. There are multiple secondary endpoints, which will enable a more complete understanding of the clinical impact of therapy with drug product.

Further, the sample size for this study is modest, therefore consistency of effect in secondary endpoints will add credibility to the results of the primary efficacy analysis.

3.8. Subgroups

To assess the consistency in treatment effects across difference subgroup levels, subgroup analyses may be explored for efficacy endpoints, such as VOE-CR and sVOE-CR. Subgroup analyses will include, but may not be limited to, age group (adolescents [≥12 and <18 years old at Informed Consent] versus adults [≥18 years old at Informed Consent]), sex, race, alpha-globin genotype, and/or stroke history (Yes/No).

3.9. Withdrawals, Dropouts and Lost to Follow-Up

Subjects withdrawn from the study prior to drug product infusion may be replaced. Subjects who receive drug product but subsequently withdraw will not be replaced.

For subjects who withdraw for reasons other than withdrawal of consent, any SAEs open at the time of discontinuation should be followed up until resolution or are determined to be a stable or chronic condition.

If withdrawal is before drug product infusion, subjects should remain on study for at least 30 days after any invasive study procedure (e.g., mobilization, liver biopsy) before withdrawal. In the rare case a subject undergoes myeloablation and receives back-up cells instead of drug product, subject should remain on the study for at least 3 months post myeloablation.

If withdrawal is after drug product infusion, subjects will be asked to complete the same assessments as specified in the SOE for Month 24 (Early Termination Visit assessments) and will be asked to enroll in the long-term follow-up study.

3.10. Missing Data

The imputation rules for missing data can be found in Appendix 6.1, Table 11.

3.11. Analysis Visit Windows

All evaluation visits are expected to occur following scheduled evaluation per the protocol. The data used in summaries will be tabulated per the evaluation visit as recorded on the CRF. If the evaluation visit is missing due to early withdrawal or missing visits but there is data from unscheduled or additional visit that is inside an analysis visit window as defined in Appendix 6.2, Table 12, then the data from the unscheduled or additional visit will be used for the analysis visit window in data summaries.

For endpoints that are not Hb fractions, if subjects have multiple unscheduled or additional evaluations within an analysis visit window, the evaluation closest to the target visit date will be used in the summary tabulations. In case of evaluations equidistant to the target visit date within an analysis visit window, results of the earlier evaluation will be used. This applies to all assessments without designated visits.

For Hb fractions, if subjects have multiple unscheduled or additional evaluations within an analysis visit window, the mean of these evaluations will be used in the summary tabulations and the detailed rules can be found in Section 4.4.

Windows for time course distributions of AEs and concomitant medications are based on the following time periods:

- ICF until prior to either initiation of mobilization or bone marrow harvest (ICF to < MB)
- Initiation of mobilization (or bone marrow harvest) until prior to initiation of conditioning (MB to < C)
- Initiation of conditioning until prior to initiation of neutrophil engraftment (NE) (C to < NE)
- Initiation of NE through Month 24 (NE to M24)
- Day 1 (drug product infusion) until prior to initiation of NE (D1 to <NE)
- Day 1 (drug product infusion) through Month 24 (D1 to M24)
- Informed Consent Form through Month 24 (ICF to M24)

Note: initiation of NE is counted as the first date of the 3 consecutive ANC assessments that are $\geq 0.5\times10^9$/L obtained on different days after the initial post-transplant nadir by Day 43.

Windows for time course distributions of clinical laboratory shift tables (shift categories defined by CTCAE grade, Version 4.03) are based on the following time periods:

- Initiation of mobilization (or bone marrow harvest) until prior to initiation of conditioning (MB to < C)
- Initiation of conditioning until prior to initiation of NE (C to < NE)
- Initiation of NE through Month 12 (i.e., Day 365) (NE to M12)
- >Month 12 (i.e., Day 366) through Month 24 (>M12 to M24)
- Day 1 (drug product infusion) until prior to initiation of NE (D1 to <NE)
- Day 1 (drug product infusion) through Month 24 (D1 to M24)

3.12. Interim Analyses

Interim analyses are planned in support of regulatory submissions. The timing of these analyses and the number of subjects included in each analysis will take into account input from regulatory agencies and applicable regulatory guidance. The rationale for each analysis will be documented outside of this SAP.

The summary tables for interim analysis will be presented based on all data available as of the data cut-off, except for these efficacy endpoints specified below. Only evaluable subjects will be included in summary tables:

- **VOE-CR, sVOE-CR, and change in annualized number of VOEs or sVOEs in the 6 to 18 months post-drug product infusion:** only include Group C subjects in the TPVOE who have or would have at least 18 months follow up post-drug product infusion.
- **sVOE-CR24, VOE-CR24, sVOE-75 and change in annualized number of VOEs or sVOEs in the 24 months post-drug product infusion:** only include Group C subjects in TPVOE who have Month 24 visit or would have at least 24 months follow-up post-drug product infusion.
- **Globin Response (GR):** only include subjects in TP who achieve Globin Response, have or would have at least 18 months follow up post-drug product infusion.
- **Globin Response at Month 24:** only include subjects in TP who achieve Globin Response and either (1) have Month 24 HPLC visit assessment or (2) would have at least 24 months follow up post-drug product infusion.
- **pRBC transfusions, and hospitalizations endpoint**: only include Group C subjects in the TP who have or would have at least 18 months of follow-up post-drug product infusion.

For Group A and B, the analysis population for interim analysis will be based on the same criteria as the criteria of interim analysis for Group C.

3.13. Final Analysis

A final analysis will be performed when last subject has completed the Month 24 Visit as specified in the protocol or discontinued from the study.

3.14. Additional Data Review

Safety data are reviewed on an ongoing basis for signal detection, DMC meetings and supporting preparation of regulatory submission documents. Analyses of study data may also be performed for the purposes of internal data review, regulatory agency interactions, and updating the scientific community.

4. STATISTICAL ANALYSIS METHODS

4.1. Subject Disposition and Analysis Populations

An overall disposition for all subjects who sign the main Informed Consent will be summarized by investigational sites and overall. The number of subjects who are screen-failures and the reasons for screen failures will be listed. Subjects are considered as screen failures if they sign the main Informed Consent but cannot finish screening or are ineligible based on those assessments. For subjects who prematurely discontinue from the study, the premature discontinuation reasons will be summarized. For subjects who are in the ITT population, a summary table will be provided by group and overall that includes the number of subjects who reach each of the following milestones: initiated mobilization or BMH, initiated myeloablative conditioning, infused with drug product, achieved NE, achieved PE, prematurely discontinued from the study, and completed the Month 24 Visit.

Analysis populations, such as ITT, TP, SEP, and TPVOE, will be summarized in a table by group and overall. A listing of analysis populations will also be provided, and the listing will include subject ID, investigational site, the Group assigned and whether the subject is in these analysis populations (Yes/No).

4.2. Demographics and Baseline Characteristics

For subjects in the ITT population, demographic and baseline characteristics will be summarized by group and overall using descriptive statistics. Details will be presented in by-subject listings. Baseline information, such as annualized number of VOEs, sVOEs, frequency and volume of pRBC transfusions in the 24 months prior to Informed Consent will also be summarized for the applicable analysis population in the corresponding efficacy endpoints.

4.2.1. Demographics

The following demographics categories will be summarized:

- Age (in years) at the time of Informed Consent or Assent (if relevant), and age groups (≥12 to <18 versus ≥18 to ≤50)
- Country of birth, sex, race, and ethnicity
- Body mass index (BMI, kg/m^2) and BMI groups (< 18.5, >=18.5 and < 25.0, >=25.0 and < 30.0 and >=30.0)

Demographics tables will be repeated for the TP and TPVOE.

4.2.2. SCD-specific medical history:

The following SCD-specific medical history categories will be summarized:

- age (in years) at SCD diagnosis
- previous hydroxyurea (HU) use, including history of HU intolerance or failure
- previous L-glutamine use (Yes/No)
- previous iron chelator use (Yes/No)

- baseline VOEs and sVOEs, defined as annualized number of VOEs and sVOEs occurring in the 24 months prior to Informed Consent
- age (in years) at the time of starting regular transfusion if it is applicable
- only for Group C subjects: baseline pRBC transfusions, defined as annualized frequency and volume (in mL/kg) of pRBC transfusions received in the 24 months prior to Informed Consent
- baseline total Hb, as defined in Section 1.2.6.2

- SCD genotype (include alpha-globin and beta-globin genotypes as available)
- TRJV status at baseline (< 2.5 m/sec versus ≥ 2.5 m/sec)

4.2.3. Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities ([MedDRA], Version 23.0 or higher). Medical history will be presented in a listing and summarized by Primary System Organ Class (SOC) and Preferred Term (PT).

4.2.4. Prior and Concomitant Medication and Procedures

Prior and Concomitant Medications and Procedures will be coded using the WHODrug (March 2016 version or higher). Medications will be provided in tables and listings, according to the intervals as mentioned in Section 3.11, with an additional category, "< ICF".

4.3. Mobilization/BMH, Conditioning and Drug Product Infusion

4.3.1. Mobilization/BMH

Mobilization/BMH details to be summarized by group or by type of stem cell collection procedure (plerixafor mobilization and apheresis, or bone marrow harvest, as appropriate):

- For subjects who underwent BMH procedure
  - Number of BMH procedures
  - Nucleated Cells Harvested (10^8/kg) per BMH procedure and total
  - Number of CD34+ cells collected (cells×10^6/kg) per BMH procedure and total
  - CD34+ cells cryopreserved for rescue (10^6/kg) per BMH procedure
  - Total nucleated cells cryopreserved for backup (10^8/kg) per BMH procedure
- For subjects who underwent mobilization:
  - Total number of mobilization cycles
  - Number of apheresis procedures per mobilization cycle
  - Blood volume (mL) processed per apheresis day, per mobilization cycle, and total
  - Plerixafor (mg/kg) used per apheresis day, per mobilization cycle, and total
  - Number of CD34+ cells collected (cells×10^6/kg) per apheresis day, per mobilization cycle, and total
  - Total number of CD34+ cells sent for transduction (cells ×10^6/kg)
  - Total number of CD34+ cells collected for rescue (cells ×10^6/kg)

Additional parameters such as amount of anticoagulant, volume of anticoagulant in bag at end of collection, and hematopoietic progenitor cells collected by apheresis (HPC-A) volume will be provided in listings.

4.3.2. Busulfan Conditioning

Busulfan conditioning details to be summarized by group include the following:

- The total dose of busulfan (mg) infused
- The average daily dose of busulfan (mg/kg/day)
- The daily estimated average area under curve ([AUC], μM×min), which is estimated as the average AUC including both collected and derived AUC, where derived AUC is calculated as the average of the collected AUCs from CRF per busulfan dose multiplied by busulfan dose when AUC is missing but the busulfan dose is collected. If busulfan is administered as every 6 hours (Q6H), the AUC on each conditioning day will be summed first before the daily AUC calculation.
- Time (in months) from informed consent to initiation of stem cell collection
- Time (in months) from informed consent to initiation of conditioning
- Time (in months) from initiating of stem cell collection to initiation of conditioning

4.3.3. Drug Product (DP) Infusion

Drug product infusion details and the corresponding events to be summarized by group include the following:

- Time (in months) from Informed Consent to drug product infusion
- Time (months) from initiation of stem cell collection to drug product infusion
- Duration of hospitalization for conditioning and drug product infusion: defined as relative days from initiation of conditioning to post-drug product infusion discharge
- Number of drug product (DP) lots infused
- Total number of infused CD34+ cells (10^6/kg): combined total number of cells if more than one drug product lot. If total number of infused CD34+ cells in any individual drug product lot is reported as anomalous, the total number of CD34+ cells for the subject will not be calculated.
- Weighted average DP VCN (c/dg): weighted average per subject if more than one drug product lot infused, with weight based on the fraction of CD34+ cells in the lot/total CD34+ cells for all lots for the subject. If total number of CD34+ cells is not available, a simple average of DP VCN will be used.
- Weighted average DP %LVV+ cells (percent lentiviral vector positive cells in DP): weighted average per subject if more than one drug product lot infused, with weight based on the fraction of CD34+ cells in the lot/total CD34+ cells of all lots for the subject. If total number of CD34+ cells is not available, a simple average of DP % LVV+ cells will be used.

- Weighted average of DP VCN/DP %LVV+ cells: weighted average per subject if more than one drug product lot infused, with weight based on the fraction of CD34+ cells in the lot/total CD34+ cells of all lots for the subject. If total number of CD34+ cells is not available, a simple average of DP VCN/DP %LVV+ cells will be used.

4.4. Efficacy Analysis

The analysis populations for the efficacy endpoints identified below can be found in Table 3. Listings will be provided for all efficacy endpoints. Supportive figures will be provided as needed.

The definitions of investigator VOEs, protocol VOEs, adjudicated VOEs, protocol sVOEs, and adjudicated sVOEs are listed in Appendix 6.4, Table 14. All sVOE or VOE-related efficacy endpoints will be based on adjudicated sVOEs or adjudicated VOEs among Group C subjects in the TPVOE, with a 95% CI estimated for applicable binary endpoints; Supplementary analyses may also be performed based on protocol sVOEs, protocol VOEs or investigator VOEs. The endpoints will also be presented for Groups A and B subjects in the TPVOE without 95% CI estimated for the binary outcomes.

For Hb fraction analysis, the globin sample (within the analysis visit window) and the hematology sample will be merged by date. If the dates match, this Hb will be selected, even if a transfusion occurs on the same date. If a globin sample exists but there is no corresponding hematology sample with the same date, then the globin sample will be merged with the closest Hb result with no transfusion between the globin and hematology sample. If there are multiple Hb records on the same date, the one with the lowest value will be used. If the selected Hb is not within a ±7 day window of the globin sample, the fraction will be footnoted in the data listings. An analysis visit window will be applied for by visit summary tables and figures (see Appendix 6.2, Table 12 for analysis visit window). If there are multiple fractions for a subject within a given analysis visit window, an average of the fractions and Hb used to derive the factions will be calculated and included in summary tables and figures.

The estimands for the primary and key secondary efficacy endpoints can be found in Table 4.

Table 4. Estimands for Primary and Key Secondary Efficacy Endpoints

	Primary Efficacy Estimand	Key Secondary Efficacy Estimand	Key Secondary Efficacy Estimand
Treatment	bb1111	bb1111	bb1111
Population	Group C subjects in TPVOE (Group C evaluable subjects in TPVOE- for interim analysis)	Group C subjects in TPVOE (Group C evaluable subjects in TPVOE- for interim analysis)	Group C subjects in TP (Group C evaluable subjects in TP- for interim analysis)
Endpoint (variable)	Complete resolution of adjudicated VOEs between 6 months and 18 months after drug product infusion (i.e., VOE-CR)	Complete resolution of adjudicated severe VOEs between 6 months and 18 months after drug product infusion (i.e., sVOE-CR)	Globin Response, as defined in Section 1.2.6.2
Intercurrent event (ICE)	Discontinues from the study before reaching the 18th month follow-up post-drug product infusion will be considered as a non-responder.	Discontinues from the study before reaching the 18th month follow-up post-drug product infusion will be considered as a non-responder.	Discontinues from the study before achieving Globin Response will be considered as a non-responder. pRBC transfusion is considered an intercurrent event. Starting ≥ 60 days after last pRBC transfusion, a subject should meet Globin Response for a continuous period of at least 6 months after drug product infusion.
Population-level Summary	Proportion of subjects achieving VOE-CR among the subjects who are in the analysis population	Proportion of subjects achieving sVOE-CR among the subjects who are in the analysis population	Proportion of subjects achieving Globin Response among subjects who are in the analysis population

4.4.1. Analysis of Primary Efficacy Endpoint

Analysis for Group C

The primary efficacy endpoint, VOE-CR, is defined as the complete resolution of adjudicated VOE between 6 months and 18 months after drug product infusion. The analysis population can be found in Table 4.

Responders of VOE-CR are subjects who do not have any adjudicated VOEs between 6 months and 18 months (i.e., 183 to 548 days) after drug product infusion.

Non-Responders of VOE-CR are subjects who meet any of the following criteria:

- Has ≥1 adjudicated VOE between 6 months and 18 months after drug product infusion. If a subject has an adjudicated VOE that occurs before 183 days but ends after 183 days after drug product infusion, the subject is considered as a non-responder.
- Discontinues from the study on or before 18 months follow-up post-drug product infusion.

The percentage of subjects who achieve VOE-CR will be presented with a 2-sided exact 95% CI using Clopper-Pearson method for the binomial distribution. The success criterion will be at least 21 out of 35 subjects achieving VOE-CR (i.e., rate of VOE-CR ≥60%), giving a lower bound of the 95% CI greater than 40%, if exactly 35 TPVOE subjects in Group C. If there are fewer than 35 subjects in the analysis population, with the same assumptions such as the null hypothesis as 40% and alternative hypothesis as 80%, the power and success criterion can be found in Appendix 6.3, Table 13.

Supplementary Analyses for Group C

The following Supplementary analyses, but not limited to, will be conducted:

Analysis Population	Supplementary Analysis
TPVOE [1]	The primary analysis will be repeated using protocol VOE instead of adjudicated VOE.
TPVOE [1]	The primary analysis will be repeated using investigator VOE instead of adjudicated VOE.

Analysis Population	Supplementary Analysis
TPVOE [1]	Non-Responders of VOE-CR are subjects who meet any of the following criteria: • Has ≥1 adjudicated VOE between 6 months and 18 months after drug product infusion. If a subject has an adjudicated VOE that occurs before 183 days but ends after 183 days after drug product infusion, the subject is considered as a non-responder. • Has any death related to VOE (adjudicated by independent committee) or drug product/study procedure (assessed by investigator) on or before 18 months post-drug product infusion. Subjects in the TPVOE who don't meet any of the non-responder criteria above will be excluded from the analysis if they discontinued from the study on or before reaching 18 months post-drug product infusion. If there is no death or discontinuation on or before 18 months post-drug product infusion, the results will be the same as for the primary analysis of VOE-CR, so this supplementary analysis will not be performed in this case.

[1] If an interim analysis is performed, subjects should also have or would have at least 18 months of follow up post-drug product infusion.

Analysis for Groups A and B

The number and percentage of subjects achieving VOE-CR will be reported by group for Groups A and B subjects in TPVOE at final analysis, or in TPVOE who have or would have 18 months follow-up at interim analysis. No 95% CI will be presented given the relatively small sample size.

4.4.2. Analysis of Key Secondary Efficacy Endpoints

4.4.2.1. Adjudicated sVOE-CR

Analysis for Group C

One of the key secondary efficacy endpoints, sVOE-CR, is defined as the complete resolution of adjudicated sVOE between 6 months and 18 months after drug product infusion. The analysis population can be found in Table 4.

Responders of sVOE-CR are subjects who do not have any adjudicated sVOEs between 6 months and 18 months (i.e., 183 to 548 days) after drug product infusion.

Non-Responders of sVOE-CR are subjects who meet any of the following criteria:

- Has ≥1 adjudicated sVOE between 6 months and 18 months after drug product infusion. If a subject has an adjudicated sVOE that occurs before 183 days but ends after 183 days after drug product infusion, the subject is considered as a non-responder.
- Discontinues from the study on or before 18 months follow-up post-drug product infusion

The percentage of subjects who achieve sVOE-CR will be presented with a 2-sided exact 95% CI using Clopper-Pearson method for the binomial distribution. The success criterion will be at least 24 out of 35 subjects achieving sVOE-CR (i.e., rate of sVOE-CR ≥69%), giving a lower bound of the 95% CI greater than 50%, if exactly 35 TPVOE subjects in Group C. If there are fewer than 35 subjects in the analysis population, with the same assumptions such as the null hypothesis as 50% and alternative hypothesis as 85%, the power and success criterion can be found in Appendix 6.3, Table 13.

Supplementary Analyses for Group C

The following Supplementary analyses, but not limited to, will be conducted:

Analysis Population	Supplementary Analysis
TPVOE [1]	The analysis will be repeated using protocol sVOE instead of adjudicated sVOE.
TPVOE [1]	Non-Responders of sVOE-CR are subjects who meet any of the following criteria: • Has ≥1 adjudicated sVOE between 6 months and 18 months after drug product infusion. If a subject has an adjudicated sVOE that occurs before 183 days but ends after 183 days after drug product infusion, the subject is considered as a non-responder. • Has any death related to VOE (adjudicated by independent committee) or drug product/study procedure (assessed by investigator) on or before 18 months post-drug product infusion. Subjects in the TPVOE who don't meet any of the non-responder criteria above will be excluded from the analysis if they discontinued from the study on or before reaching 18 months post-drug product infusion. If there is no death or discontinuation on or before 18 months post-drug product infusion, the results will be the same as for the primary analysis of sVOE-CR, so this supplementary analysis will not be performed in this case.

[1] If an interim analysis is performed, subjects should also have or would have at least 18 months of follow up post-drug product infusion.

Analysis for Groups A and B

The number and percentage of subjects achieving sVOE-CR will be reported by group for Groups A and B subjects in TPVOE at final analysis, or in TPVOE who have or would have 18 months follow-up at interim analysis. No 95% CI will be presented given the relatively small sample size.

4.4.2.2. Globin Response

Analysis for Group C

The key secondary efficacy endpoint, Globin Response, defined as meeting the following criteria for a continuous period of at least 6 months after drug product infusion (starting ≥ 60 days after last pRBC transfusion):

a. Weighted average HbA^{T87Q} percentage of non-transfused total Hb ≥30%

   AND

b. Weighted average non-transfused total Hb increase of ≥3 g/dL compared to baseline total Hb, OR weighted average non-transfused total Hb ≥10 g/dL

The detailed definition of Globin Response can be found in Section 1.2.6.2. The analysis population can be found in Table 4.

The weighted average HbA^{T87Q} percentage of non-transfused total Hb is defined as

$$[(t_1 - t_0) \times \frac{(p_0 + p_1)}{2} + (t_2 - t_1) \times \frac{(p_1 + p_2)}{2} + \cdots + (t_k - t_{k-1}) \times \frac{(p_{k-1} + p_k)}{2}] / (t_k - t_0)$$

Where

- t_0 represents the time of the first HbA^{T87Q} assessment post-drug product infusion and there is no pRBC transfusion in the previous 60 days, and $t_1, t_2, \ldots, t_k$ represent continuous time points for HbA^{T87Q} assessments after t_0 and no pRBC transfusion occurs between these timepoints.
- $p_0, p_1, p_2, \ldots, p_k$ represent the HbA^{T87Q} percentage to non-transfused total Hb at each of these time points
- $t_k - t_0$ must be at least 6 months, which is 183 days (with exception below).

This calculation is invariant to the metric used for the time points, e.g., calendar dates or days from drug product infusion, since the consecutive differences in times would always be measured in number of days.

After drug product infusion, HbA^{T87Q} will be assessed at Month 1, 2, 3, 4.5, 6, and then every 3 months through Month 24. In the situation where there are three consecutive scheduled HbA^{T87Q} assessments in a 6-month period of time (i.e., every 3-month) and scheduled Hb^{AT87Q} assessments are within visit window but are less than 6 months apart, then the ≥6 months requirement will not be needed, however, the requirement of starting ≥60 days after the last pRBC transfusion still needs to be met. Scheduled visits for assessments are defined in Appendix 6.2, Table 12. The weighted average may be considered as an average AUC calculation for HbA^{T87Q} percentage. Note that the weighted non-transfused total Hb can be calculated in a similar way.

A TP subject will be considered as a non-responder if the subject does not achieve Globin Response before discontinuing from the study.

The percentage of subjects who achieve Globin Response will be presented with a 2-sided exact 95% CI using the Clopper-Pearson method for the binomial distribution. The success criterion at

the final analysis will be at least 24 out of 41 subjects achieving Globin Response (i.e., rate of Globin Response ≥59%), giving a lower bound of the 95% CI greater than 40%, if exactly 41 TP subjects in Group C. If there are fewer than 41 subjects in the analysis population, with the same assumptions such as the null hypothesis as 40% and alternative hypothesis as 70%, the power and success criterion can be found in Appendix 6.3, Table 13.

Analysis for Groups A and B

The number and percentage of subjects reaching Globin Response will be reported by group for Groups A and B subjects in TP at final analysis.

No 95% CI will be presented given the relatively small sample size.

4.4.3. Analysis of Other Efficacy Endpoints

4.4.3.1. Characterization of Globin Response (only for Group C subjects who have achieved Globin Response)

- **Proportion of subjects who meet the definition of Globin Response at Month 24**
  - The numerator is the number of subjects who continuously meet Globin Response criteria from the first date of Globin Response initiating (t_0) to Month 24 visit of HPLC assessment
  - The denominator is the number of subjects who have achieved Globin Response.

If after achieving Globin Response, a subject discontinues from the study before having the Month 24 visit of HPLC assessment, then the subject will also be considered as a non-responder.

The percentage of subjects who maintain Globin Response at Month 24 will be presented with a 2-sided 95% CI using Clopper-Pearson method.

Supplementary Analyses

The following Supplementary analyses will be conducted:

Analysis Population	Supplementary Analysis
TP	The primary analysis will be repeated, but after achieving Globin Response, subjects with death or discontinuation unrelated to the drug product will be considered as not evaluable. If after achieving Globin Response but before having the Month 24 visit of HPLC assessment, there is death or discontinuation related to the drug product, then the subject will be considered as a non-responder.

- **Duration of Globin Response**

Duration of Globin Response is from the first date of Globin Response initiating (t_0) to the date of last HPLC assessment such that the weighted average of HbA^{T87Q} percentage of non-transfused total Hb, non-transfused total Hb, and non-transfused total Hb increase from the baseline total Hb (if baseline total Hb is available) continuously meet Globin Response criteria

during this time period. Duration of Globin Response (in months) and these weighted average values during Globin Response will be summarized.

4.4.3.2. Hematologic Endpoints

All hematologic endpoints indicated below will be based on subjects in the TP and summary tables will be presented by group.

- **Weighted average of the following at Month 6, 12, 18 and 24**:
  - non-transfused total Hb
  - HbS percentage of non-transfused total Hb
  - HbS percentage of non-transfused total Hb ≤ 70%, ≤ 60%, ≤ 50%
  - HbA^{T87Q} percentage of non-transfused total Hb
  - non-HbS percentage of non-transfused total Hb

Descriptive summary statistics or frequency distribution of the weighted average of the endpoints above at Month 6, 12, 18 and 24 will be provided. Data starting ≥60 days after the last pRBC transfusion will be utilized. The weighted average will be based on the assessments obtained up to an approximately 6-month period and can be calculated in a similar way as the weighted total Hb in the Globin Response in Section 4.4.2. For example, for the weighted average at Month 6, all assessments post-drug product infusion up to Month 6 Visit will be used; for the weighted average at Month 12, all assessments obtained between Month 6 Visit and Month 12 Visit will be utilized for the calculation.

- **Assessment of the following over time:**
  - non-transfused total Hb
  - HbS percentage of non-transfused total Hb
  - HbA^{T87Q} percentage of non-transfused total Hb
  - non-HbS percentage of non-transfused total Hb

Descriptive summary statistics will be provided for the endpoints above over time per SOE. If there are multiple assessments within the same SOE time window, the average values will be presented in the tables. Change from baseline in non-transfused total Hb will also be presented, where baseline total Hb (as defined in Section 1.2.6.2) will serve as the baseline for non-transfused total Hb.

- **Hemolysis markers, markers of iron stores, and markers for stress erythropoiesis**

Descriptive summary statistics will be provided for these biomarkers over time and change from baseline will also be presented. Baseline is defined the first assessment on or after Informed Consent but before initiation of stem cell collection.

- **Change from baseline in annualized frequency and volume of pRBC transfusions between 6 months and 24 months after drug product infusion**

Absolute and percentage change from baseline in annualized frequency and volume (in mL/kg) of pRBC transfusions between 6 months (i.e., 183 days) to 24 months post-drug product infusion will be presented for Group C subjects in summary table. Baseline is defined as annualized frequency and volume of pRBC transfusions in the 24 months prior to Informed Consent and it is

only collected for Group C subjects. The final analysis will be based on Group C subjects in the TP. The summary statistics may be repeated for TPVOE subjects in Group C.

At the time of analysis, the day of the last available follow-up to the Month 24 visit (inclusive) will be used. For example, if a subject has only 700 days of follow-up post-drug product infusion, the annualized frequency or volume of pRBC will be adjusted by 700 days. Using volume as an example, it will be defined as:

$$\frac{\text{Total volume (mL/kg) received during 183 to 700 days post drug product infusion}}{(700 - 183 + 1)/365.25}$$

For volume of pRBC (in mL/kg) calculation, the weight (in kg) at or closest (in absolute value) to the date of the transfusion will be used. For example, if there are 2 weight assessments, one is 45 kg and 30 days before the transfusion, the other is 43 kg and 5 days after the transfusion, and no other weight assessments that are closer to the transfusion have been recorded, then 43 kg will be used for the calculation. The imputation rules for partial information of pRBC transfusion can be found in Appendix 6.1, Table 11. The weight (in kg) at screening will be used for the calculation in annualized pRBC volume (in mL/kg) at baseline.

Change from baseline in annualized volume of pRBC transfusion will be presented in the following categories:

- Increased
- No change
- >0 and <50% reduction
- ≥50% reduction
- ≥60% reduction
- ≥75% reduction
- ≥90% reduction
- 100% reduction

The pRBC transfusions in Groups A and B subjects during the study will be presented in the listing without change from baseline calculation.

4.4.3.3. Clinical and Disease Evaluation Endpoints

The following endpoint will be only for subjects who have at least 4 protocol VOEs in the 24 months prior to Informed Consent (TPVOE).

- **Change in the annualized number of VOEs in the 24 months post-drug product infusion compared to the 24 months prior to Informed Consent**

The formula for annualized number of VOE on or post-drug product infusion is:

$$\frac{\text{Number of VOEs started on or post drug product infusion}}{(\text{Number of days post drug product infusion})/365.25}$$

If a VOE starts before drug product infusion but ends after drug product infusion, this event will not be included in annualized number of VOE post-drug product infusion calculation.

The number of adjudicated VOEs for each subject will be based on the number of unique VOE ID. The number of protocol or investigator VOEs for each subject will be based on the number of unique CRF page numbers.

Descriptive summary statistics of annualized number of VOEs at baseline, in 24 months post-drug product infusion, absolute and percentage change in the annualized number of VOEs compared to the 24 months prior to Informed Consent will be provided. Change from baseline in annualized number of VOE will be also presented in the following categories:

- Increased
- No change
- >0 and <25% reduction
- ≥25% and <50% reduction
- ≥50% reduction
- ≥60% reduction
- ≥75% reduction
- ≥90% reduction
- 100% reduction

Summary statistics for annualized number and change from baseline for VOEs between 6 months and 18 months, and between 6 months and 24 months after drug product infusion may also be presented.

The following endpoints will be only for Group C subjects who have at least 4 protocol VOEs in the 24 months prior to Informed Consent (TPVOE).

- **sVOE-CR24**

The number and percentage of subjects with sVOE-CR24 will be presented for Group C subjects with a 2-sided 95% CI using the Clopper-Pearson method.

Responders of sVOE-CR24 are subjects who do not have any adjudicated sVOE in 6 to 24 months post-drug product infusion (i.e., 183 days post-drug product infusion to 731 days post-drug product infusion or the Month 24 visit, whichever is earlier).

Non-Responders of sVOE-CR24 are subjects who meet either of the following situations:

- Have ≥1 adjudicated sVOE in 6 to 24 months post-drug product infusion. If a subject has an adjudicated sVOE that occurs before 183 days post-drug product infusion but ends after 183 days post-drug product infusion, the subject is considered as a non-responder.
- Discontinued before completing the study.

- **VOE-CR24**

VOE-CR24 will be derived in a similar way as sVOE-CR24. The number and percentage of subjects with VOE-CR24 will be presented for Group C subjects with a 2-sided 95% CI using the Clopper-Pearson method.

- **sVOE-75**

The number and percentage of subjects with sVOE-75 will be presented for Group C subjects with a 2-sided 95% CI using the Clopper-Pearson method.

Responders of sVOE-75 are subjects who have ≥75% reduction from baseline in annualized number of adjudicated sVOE in up to 24 months post-drug product infusion.

Non-Responders of sVOE-75 are subjects who meet either of the following situations:

  - Have < 75% reduction from baseline in annualized number of adjudicated sVOE in up to 24 months post-drug product infusion
  - Discontinued before completing the study

- **Change in the annualized number of severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent**

The annualized number of severe VOEs will be calculated in the same way as specified in annualized number of VOEs. For Group C subjects in the analysis population, a 1-sided 97.5% CI for the percentage change in the annualized number of sVOEs in the 24 months post-drug product infusion compared to the 24 months prior to Informed Consent will be provided. The detailed method for the 1-sided of 97.5% CI can be found in Appendix 6.6.

4.4.3.4. SCD Burden and Chronic Complications Assessments

These SCD burden and chronic complications assessments will be presented in table summaries by group for all subjects in TP. All data will be presented in listings.

The absolute change at each visit from baseline will be summarized by group.

- **Change from baseline in renal function as measured by eGFR**

  eGFR will be calculated by CKD-EPI formula for subjects ≥18 years old and Schwartz formula for subjects <18 years old. The formulas can be found in Appendix 6.5.

- **Change from baseline in cardiac-pulmonary function via TRJV, PFTs and LVEF**
  - TRJV over time for each subject will be classified into 2 categories: < 2.5 m/s versus ≥ 2.5 m/s. A shift table will be presented to show the change from baseline in TRJV classification. The change from baseline values in TRJV will be presented in a listing.
  - Pulmonary Function Tests (PFTs) over time of each subject will be classified into one of the five categories indicated in Table 5. A shift table will be presented to show the change from baseline in PFTs classification.

Table 5. Classification of Pulmonary Function

Category of Pulmonary Function	% of Predicted value					
	FEV1	FVC	TLC[1]	RV	DLco	FEV1/ FVC
Normal	≥ 80%	≥ 80%	≥ 80%	≥ 80%	≥ 80%	≥70%
Obstructive	<80%	<80%	≥ 80%	≥ 80%	≥ 80%	<70%
Restrictive: meet any situation below						

Category of Pulmonary Function	% of Predicted value					
	FEV1	FVC	TLC[1]	RV	DLco	FEV1/ FVC
situation 1	<80%	<80%	<80%		<80%	≥70%
situation 2	≥ 80%	≥ 80%	<80%	<80%	≥ 80%	≥70%
situation 3			<80%		<80%	
Mixed obstructive and restrictive			<80%	<80%	≥ 80%	<70%
Isolated low DLco	≥ 80%	≥ 80%	≥ 80%	≥ 80%	<80%	≥70%

DLco = carbon monoxide diffusing capacity; FEV1 = forced expiratory volume in 1 second; FVC = forced vital capacity; RV = respiratory volume; TLC = total lung capacity.
[1] % predicted FEV1, FVC, RV and DLco are directly collected in the CRF. Predicted values for TLC are derived using the formulas below, and height (meter) is subject's height at the time of the assessment or at the closest time to the assessment.
Male (age 4 – 17 years): 10.0**(-2.0018 +2.5698*log10(HEIGHT*100))/1000.
Male (age ≥18 years): 0.066*(HEIGHT*100)-5.79
Female (age 4 – 17 years): 10.0**(-2.033 +2.5755*log10(HEIGHT*100))/1000
Female (age ≥18 years): 0.0799*(HEIGHT*100)-7.08

- LVEF over time and change from baseline will be presented in summary table and listing.

- **Change from baseline in meters walked during 6-minute walk test (6MWT)**

Descriptive statistics will be provided for the meters walked during 6MWT at baseline and visits post-drug product infusion. The absolute change from baseline will also be presented.

4.4.3.5. Hospitalizations and Quality of Life

The final analysis will be based on subjects in the TP. If an interim analysis is performed for the hospitalization endpoint before the final analysis, the analysis will be based on the evaluable subjects defined in Section 3.12; the quality of life endpoints will be presented based on all data available as of the data cut-off.

- **Change from baseline in annualized hospital admissions and days**

The number of VOE-related hospital admissions and in-patient VOE-related hospitalization days from post-drug product infusion discharge through last follow-up will be annualized and compared to the corresponding annualized values during the 2 years prior to Informed Consent. All hospitalizations for 3 groups will also be included data listings. The imputation rules for partial date of admission and discharge can be found in Appendix 6.1, Table 11.

- **Change from baseline in patient-reported quality of life, as measured by PROMIS**

PROMIS-57 is used for subjects ≥18 years old and has 7 domains + 1 pain intensity question. PROMIS-49 (PROMIS Pediatric Profile 49 or PROMIS Parent Proxy Profile 49) is used for subjects <18 years old (from Group C only) and has 6 domains + 1 pain intensity question. Each domain has 8 questions. Standardized score ("T score") will be derived per the raw values from the answers to the questions and corresponding T score reference table.

The descriptive statistics for PROMIS-57 or PROMIS-49 T scores in each domain and pain intensity over time will be presented separately. Baseline is defined the first assessment on or after Informed Consent but before initiation of stem cell collection. Change from baseline will be based on T scores in each domain and raw value in pain intensity question. The number and percentage of subjects with change from baseline of T scores ≥5 or ≤-5 (Terwee et al. 2021), depending on the direction of each domain, will be summarized over time. Likewise, the number and percentage of subjects with change from baseline ≤-2 will be presented for raw values in pain intensity questions. If different version of PROMIS is used at baseline and post-drug product infusion, change from baseline in some domains will not be calculated if it is considered as incomparable per PROMIS user manual. For example, if the baseline is based on the 8 questions of "Satisfaction with Participation in Social Roles 8a", and the post-drug product infusion is based on the 8 questions of "Ability to participate in social roles and activities 8a", then no change from baseline will be calculated although both domains are for social roles.

The results from each individual question and T scores from each domain will be also presented in the listing.

4.4.4. Analysis of Exploratory Efficacy Endpoints

All exploratory endpoints indicated below will be based on subjects in the TP and summary tables will be presented by group.

- **Change from baseline in cerebral vasculature and prior brain parenchymal injury evaluation at Month 12 and Month 24**

Cerebral MRA/MRI data at Baseline, Month 12, and Month 24 will be presented in a shift table using the following categories: Normal, Abnormal- not clinically significant (NCS), and Abnormal- clinically significant (CS).

TCD (cm/s) data at Baseline, Month 12, and Month 24 may be presented in a listing or a shift table using the following categories: <170, ≥170 and <200, and ≥200.

- **Change from baseline in bone mineral density (BMD) using DXA at Month 24**

Descriptive statistics for BMD Z scores (g/cm^2) and the corresponding change from baseline will be presented by visit for different locations (such as lumbar spine (L1-L4), right and left proximal femur, whole body less head, and other) for subjects in Group C.

Overall interpretation of BMD at Month 24 will be presented using the following categories: No significant change from baseline, Significant change from Baseline, and Clinically significant Findings.

- **Change from baseline in brain natriuretic peptide (BNP)**

Descriptive statistics will be provided for BNP observed values and change from baseline. Listing will be provided for BNP assessments over time.

- **Change from baseline in patient-reported outcome (PRO) measures**

Descriptive statistics will be provided for the following PRO measures for subjects in Group C. If data are spare, only listings will be provided.

  - Overall health, as measured by EuroQol-5D (EQ-5D-3L or EQ-5D-Y)

Descriptive statistics for each parameter over time will be presented for EQ-5D-3L and EQ-5D-Y, separately. EQ-5D index score will be derived only for EQ-5D-3L. Change from baseline will be summarized over time for Health State Today and EQ-5D index.

- Work productivity, as measured by the Work productivity and Activity Impairment Questionnaire-General Health (WPAI-GH or Caregiver WPAI-GH)

  Descriptive statistics and change from baseline over time will be provided for each parameter.

- Cognitive function, as measured by PROMIS Short Form 6a (PROMIS-6a)

  Descriptive statistics for T scores and change from baseline will be presented over time. The number and percentage of subjects with change from baseline of T scores >= 5 (Terwee et al. 2021) will be summarized for each timepoint.

- **Evaluation of chronic pain using AAPT**

Chronic pain will be assessed per the SOE and includes evaluation of the AAPT Diagnostic Criteria for Pain Associated with SCD. Descriptive statistics will be provided. If data are sparse, only listings will be presented.

- **Change from baseline in pain medication use**

Pain medication is defined as opioids if WHO Drug Anatomical Therapeutic Chemical (ATC) level 3 equals to "Opioid". Number and percentage of subjects who have opioids medication use at baseline and post- drug product infusion by time interval (Day 1 to Day 30, Day 31 to Day 60, Day 61 to Day 90, >3-6 months, >6-12 months, >12-18 month and >18 months post-drug product infusion) will be provided for subjects in Group C. Baseline is defined as within 30 days prior to Informed Consent. Opioid medications will be further categorized as non-oral opioids and oral opioids. All opioid pain medications will be presented in listings.

- **Exploratory assays to assess change from baseline in sickle cell characteristics and bone marrow pathophysiology**

The exploratory assays, such as single cell Western Blot for β-globins (determining $\%\beta^{A\text{-}T87Q}$+ RBCs), single colony assays (determining %LVV+ colonies), single cell LVV (determining PB %LVV+ Cells), or ex-Vivo Sickling (determining AUC of sickled RBCs) may be presented, and by-subject results over time may be presented. Depending on results, these results may also be included in correlation analyses. Kinetics including by-subject spaghetti plots may be presented as pharmacodynamics, whereas proportion of subjects with these parameters may be summarized as efficacy.

Bone marrow morphology read locally by investigator will be presented in a by-subject listing. Bone marrow smear morphology analyzed centrally will be summarized by alpha-globin genotype and for Group C subjects in TP, for each time period (<Day 1, ≥Day 1). The parameters include the number of subjects with dyserythropoiesis noted, abnormal erythroid precursors (%), the number of subjects with dysgranulopoiesis noted, abnormal myeloid precursors (%), the number of subjects with dysmegakaryopoiesis noted, abnormal megakaryocyte precursors (%), erythroid (%) and category (<30%, ≥30%), Myeloid:Erythroid ratio (%) and category (≤1.1:1, >1.1:1). Details will be provided in listings.

4.4.5. Analysis of Correlation

The following exploratory correlation analyses, but may not be limited to, will be conducted:

- VOE-CR (Yes or No) (in TPVOE for Group C and Overall) versus:
  - Globin Response (Yes or No)
  - VOE-CR24 (Yes or No)
  - sVOE-CR24 (Yes or No)
  - non-transfused HbA^{T87Q} (% of non-transfused total Hb) at Month 6
  - DP VCN (c/dg)
  - DP %LVV+ cells
  - Total CD34+ cells (10^6/kg) infused
  - PB VCN (c/dg) at Month 6
  - PB %LVV+ cells at Month 6

- sVOE-CR (Yes or No) (in TPVOE for Group C and Overall) versus:
  - Globin Response (Yes or No)
  - sVOE-CR24 (Yes or No)
  - non-transfused HbA^{T87Q} (% of non-transfused total Hb) at Month 6
  - DP VCN (c/dg)
  - DP %LVV+ cells
  - Total CD34+ cells (10^6/kg) infused
  - PB VCN (c/dg) at Month 6
  - PB %LVV+ cells at Month 6

- Globin response (Yes or No) (in TP for Group C and Overall) versus:
  - VOE-CR24 (Yes or No)
  - sVOE-CR24 (Yes or No)
  - non-transfused HbA^{T87Q} (% of non-transfused total Hb) at Month 6
  - DP VCN (c/dg)
  - DP %LVV+ cells
  - Total CD34+ cells (10^6/kg) infused
  - PB VCN (c/dg) at Month 6
  - PB %LVV+ cells at Month 6

- HbA^{T87Q} (% of non-transfused total Hb in TP for Group C and Overall) at Month 6 versus:
  - Change (%) from baseline in annualized number of adjudicated sVOEs
  - Change (%) from baseline in annualized number of adjudicated VOEs

- DP VCN (c/dg)
- DP %LVV+ cells
- Total CD34+ cells (10^6/kg) infused
- PB VCN (c/dg) at Month 6
- PB %LVV+ Cells at Month 6
- HbS (% of non-transfused total Hb) at Month 6

Scatter plots for DP VCN (c/dg) and DP %LVV+ Cells versus HbA^{T87Q} (% of non-transfused total Hb) at Month 6 for TP Group C and Overall will be provided to explore whether there is a positive correlation trend between these drug product characteristics and HbA^{T87Q} (%) at Month 6. For this analysis, data may be transformed as appropriate.

- Non-HbS (% of non-transfused total Hb in TP for Group C and Overall) at Month 6 versus:
  - Change (%) from baseline in annualized number of adjudicated sVOEs
  - Change (%) from baseline in annualized number of adjudicated VOEs

- HbA^{T87Q} (% of non-transfused total Hb in TP for Group C and Overall) at Month 6 versus:
  - Change from baseline in total bilirubin at Month 6
  - Change from baseline in indirect bilirubin at Month 6
  - Change from baseline in lactate dehydrogenase at Month 6
  - Change from baseline in reticulocytes at Month 6

- Non-HbS (% of non-transfused total Hb in TP for Group C and Overall) at Month 6 versus
  - Change from baseline in total bilirubin at Month 6
  - Change from baseline in indirect bilirubin at Month 6
  - Change from baseline in lactate dehydrogenase at Month 6
  - Change from baseline in reticulocytes at Month 6

For each correlation analysis, all specified subjects will be included in the analysis, providing they have non-missing data in both endpoints for the correlation. For correlation between 2 binary outcomes, between 2 continuous outcomes, and between 1 continuous and 1 binary outcome, the Kappa score, Pearson correlation coefficient, and Mann-Whitney test, respectively, will be performed.

4.5. Pharmacodynamic Analysis and Correlation Analysis

Analyses will be conducted using the TP and SEP (if different than TP), or subgroups of TP as applicable, and will include summary tables with descriptive statistics and figures (all values versus time on x-axis), and displaying the items below:

- PB VCN over time will be summarized per time point, and by-subject spaghetti plots presented. Bone marrow and lineage cells VCN will be included in a listing only, if available.
- By-subject over time summaries and spaghetti plots for Group C will also be presented for exploratory analyses:
  - %LVV+ colonies over time
  - % $\beta^{A\text{-}T87Q}$+ RBCs over time
- The following exploratory correlation analyses plots, but may not be limited to, will be conducted in TP:
  - Weighted average DP %LVV+ Cells vs weighted average DP VCN (TP overall)
  - PB VCN at Month 6 vs weighted average DP VCN (TP Group C)
- Hemoglobin fractions (including HbA^{T87Q}, HbA, HbA_2, HbS and HbF, as relevant, calculated using ratio data from HPLC and total Hb) are summarized by time point. Additionally, each fraction over time will be presented in a by-subject spaghetti plots separately for all subjects in TP. The ratios and total Hb used to derive the fractions will also be included in a by-subject listing.
  - HbA^{T87Q} = $\beta^{A\text{-}T87Q}$-Globin to All β-Like-Globin-Chains/100×total Hb
  - HbA = β^{A}-Globin to All β-Like-Globin-Chains/100×total Hb
  - HbA_2 = δ-Globin to All β-Like-Globin-Chains/100×total Hb
  - HbS = β^{S}-Globin to All β-Like-Globin-Chains/100×total Hb
  - HbF = (γG-Globin to All β-Like-Globin-Chains + γA-Globin to All β-Like-Globin-Chains)/100×total Hb
  - Ratio of α-globin to total of all β-like globins if data available

4.6. Safety Analysis

The safety analyses identified below will be performed on subjects in all 3 Groups in the ITT population or TP, as appropriate. Supportive figures will be provided as needed.

Because the safety profile will be assessed for several different time intervals (see Section 3.11) there may be subjects in the ITT population who are not eligible for analyses during some of these intervals. For example, if a subject has conditioning-regimen related events and does not receive drug product, that subject would not be considered in the analyses of safety data post-drug product infusion (i.e., Day 1 through Month 24 Visit).

4.6.1. Adverse Events

AE collection windows for Groups A and B are different than those for Group C, and the details can be found in Section 1.2.1. All AEs will be coded via MedDRA coding system (version 23.0 or higher) and displayed in tables and listings using SOC and PT. The safety analyses will include evaluation of all AEs and of treatment-emergent AEs. The terminology "treatment-emergent" is reserved for events that occur during or after drug product infusion.

Summaries of AEs related to drug product will be based on the investigator's assessment; an assessment of 'Possibly Related', 'Related' or missing (if AE occurred after drug product

infusion) will be considered related to drug product. AEs will be summarized by the time intervals mentioned in Section 3.11. For treatment-emergent AEs, only the following time intervals will be presented: "D1 to <NE" which is from drug product infusion to the latest date prior to initiation of neutrophil engraftment, "NE to M24", and "D1 to M24". The number of subjects "at risk" in each time interval will also be provided in summary tables. Subjects who are lost to follow-up, withdrew from the study, or died prior to the beginning of the time interval will be excluded from the number "at risk" in the time interval.

Summary information (the number and percentage of subjects by SOC and PT, separated by group and by time interval) will be tabulated for:

- All AEs
- Treatment-emergent AEs
- Serious AEs (SAEs)
- Treatment-emergent SAEs
- Grade 3 or higher AEs
- Grade 3 or higher treatment-emergent AEs
- Treatment-emergent AEs related to drug product
- Treatment-emergent SAEs related to drug product
- AEs attributed to SCD
- AEs attributed to SCD post Month 6
- SAEs attributed to SCD
- SAEs attributed to SCD post Month 6
- AEs attributed to cell procurement (bone marrow harvest [1] and mobilization/apheresis [1])
- Grade 3 or higher AEs onset within 7 days post plerixafor
- AEs attributed to conditioning [1]
- AEs attributed to study procedure [1]
- AEs from Informed Consent to Month 24 visit by selected subgroups (as specified in Section 3.8 as appropriate), to be presented in two time intervals: "ICF to M24" and "D1 to M24".
- AEs by maximum severity (overall and by selected subgroups)
- Events of interest (search criteria in Appendix 6.7)

[1]. *Based on attribution on CRF for events as reported by investigators*

A summary for all AEs will also be tabulated by PT, separated by group and time interval. The AEs will be presented according to descending frequency PT in overall group.

Listings will also be provided for all AEs, deaths, SAEs, AEs related to drug product, AEs leading to discontinuation, AEs attributed to study procedure, AEs attributed to BMH, AEs attributed to mobilization/apheresis, AE attributed to conditioning, and events of interest.

Events related to malignancy

The number and percentage of subjects with insertional oncogenesis (e.g., myelodysplasia, leukemia, lymphoma), in which LVV-insertion is demonstrated as likely to have contributed to the root cause of the malignancy will be summarized over time. Details will be presented in a by-subject listing. Malignancies will be reviewed via the bluebird bio safety governance process to determine the root cause and if any event meets the insertional oncogenesis endpoint.

Acute and/or chronic GVHD assessed by investigator

Number and percentage of subjects with acute and/or chronic GVHD assessed by investigator will be summarized. Details will be presented in a by-subject listing.

4.6.2. Engraftment

All analyses in this section will be based on the TP unless otherwise specified.

Neutrophil engraftment (NE)

- Incidence of successful NE: defined as having 3 consecutive ANC values that are $\geq 0.5\times10^9$/L obtained on different days after the initial post-transplant nadir by Day 43, without receiving back-up cells at any time during the neutropenic phase.
- Time to NE: defined as relative days from drug product infusion date to the first day of the 3 consecutive ANC laboratory assessments that meet NE criteria. It will only be calculated among subjects who had successful NE.

The proportion of subjects who achieved NE will be provided. The Rel Day of NE will be descriptively summarized. A scatter plot with regression line will be provided for Time to NE (in Days) against total cell dose of CD34+cells to explore their correlation.

Platelet engraftment (PE)

- Incidence of successful PE: defined as having 3 consecutive platelet values that are $\geq50\times10^9$/L obtained on different days after the initial post- transplant nadir, without receiving any platelet transfusions for 7 days immediately preceding and during the evaluation period
- Time to PE: defined as relative days from drug product infusion date to the first day of the 3 consecutive platelet assessments that meet platelet engraftment criteria. It will only be calculated among subjects who had successful PE.

The proportion of subjects who achieved PE will be provided. The Rel Day of PE will be descriptively summarized. A scatter plot with regression line will be provided for Time to PE (in Days) against total cell dose of CD34+cells to explore their correlation.

Supplementary Analyses for Time to PE

The following Supplementary analyses will be conducted:

Analysis Population	Supplementary Analysis
TP or TPVOE	For subjects who took eltrombopag before achieving PE, the Time to PE will be extended to one day after the end day of eltrombopag administration.

4.6.3. Laboratory Data

Clinical laboratory values will be expressed using the International System of Units (SI), except g/dL and 10^9/L will be used for hemoglobin and nucleated erythrocytes, respectively. Values will be summarized for each clinical laboratory parameter, including hematology, clinical chemistries, and iron studies, as specified below, and in the schedule of events.

The laboratories' own reference ranges are used as the default. In the absence of these ranges, refer to the Global Reference Range (GRR) Sources identified in Table 6. Age- and sex-specific ranges will be used to flag out of range values and to indicate CTCAE (version 4.03) grade, where applicable.

The versions of the GRRs being utilized for this study are footnoted below. If revised GRRs are published during the study, the version will not automatically update.

Table 6. Global Reference Range Sources

Source	Version	Purpose
Kratz A, et al. "Laboratory Reference Values". N Engl J Med. 2004;351: 1548-63.	N Engl J Med. 2004;351: 1548-63	Adult Ranges
Mayo Clinic	Updated regularly online[1]	Children Ranges
Brown A. NHS Blood Sciences Department of Immunology and Immunogenetics. "Immunology Age Related Reference Ranges". QPR No. IMMQ0066.	Version No. 1.4	Immunology Age-Related Reference Ranges
Shearer W, et al. "Lymphocyte subsets in health children from birth through 18 years of age: The Pediatric AIDS Clinical Trials Group P1009 study." J Allergy Clin Immunol. 2003; 112: 973-80.	J Allergy Clin Immunol. 2003; 112: 973-80.	Lymphocyte Subsets in Pediatrics ages 0 to 18

[1] https://www.mayocliniclabs.com/test-info/pediatric/refvalues/index.html

Descriptive statistics on the laboratory parameters listed in Table 7 below, but may not be limited to, will be presented by visit, and where applicable, change from baseline will also be provided.

Table 7. List of Laboratory Parameters

Hematology	**Iron Contents**
• Complete blood count (CBC) with differential • Haptoglobin • Nucleated erythrocytes • Platelet count • Reticulocyte count • Methemoglobin • Red cell distribution width (RDW)	• Iron saturation (transferrin saturation) • Serum ferritin • Serum transferrin receptor • Serum transferrin • Liver iron content • Cardiac iron content (if assessed at baseline)
Serum Chemistry and Liver Function	
• Albumin • Bicarbonate • Blood urea nitrogen • Calcium • Chloride • Creatinine • C-reactive protein (CRP) • eGFR • Erythropoietin • Glucose • Magnesium	• ALP (Alkaline phosphatase) • ALT (Alanine aminotransferase) • AST (Aspartate aminotransferase) • GGT (Gamma-glutamyl transferase) • LDH (Lactate dehydrogenase) • Total, direct and indirect bilirubin • Phosphate • Potassium • Sodium • Total protein • Uric acid
Immunology	**Hormonal**
• CD3 • CD4 • CD8 • CD19 • CD16/CD56	• Estradiol (females only) • Total testosterone (males only) • TSH, Free T3, Free T4 • Cortisol • ACTH • FSH • LH
	Coagulation
	• Prothrombin time (PT) • Activated partial thromboplastin time (aPTT) • D-Dimer

Urinalysis including protein, microalbumin, glucose, ketones, pH, specific gravity, and occult blood will be listed. Additional clinical laboratory tests may be performed at the Investigator's discretion and will be listed.

Shift tables from baseline which indicate abnormally high or abnormally low laboratory parameter value grades based on CTCAE criteria will be performed using the highest or lowest abnormal value in the following time period intervals: "MB to < C", "C to < NE", "NE to M12", ">M12 to M24", "D1 to < NE", and "D1 to M24" (see definitions of the time period intervals in Section 3.11).

The parameters included in the CTCAE shift tables and the direction for CTCAE can be found in Table 8.

Table 8. Selected Laboratory Tests – Direction (Increase or Decrease) for CTCAE

Category	Laboratory test	Direction for CTCAE
Hematology	Absolute neutrophils count	Decrease
	Hemoglobin	Both
	Leukocytes	Decrease
	Lymphocyte	Both
	Platelets	Decrease
Chemistry	Albumin	Decrease
	ALP	Increase
	ALT	Increase
	AST	Increase
	Calcium	Both
	Creatinine (serum creatinine)	Increase
	eGFR	Decrease
	GGT	Increase
	Glucose	Both
	Magnesium	Both
	Phosphate	Decrease
	Potassium	Both
	Sodium	Both
	Total Bilirubin	Increase
Coagulation	Activated thromboplastin time	Increase

Hematology and chemistry parameters will be also assessed for potentially clinically significant (PCS) abnormal criteria. Lab results that meet the PCS criteria will be listed and summarized based on lab time periods indicated in Section 3.11. The PCS thresholds used are listed in Table 9.

Summary of ≥Grade 3 cytopenia (i.e., decreased platelets, neutrophils or hemoglobin) will be presented for the TP subjects at risks on or after Day 60, and on or after Day 100.

Laboratory values for selected hematology and chemistry parameters may be presented graphically as appropriate. If data allow, boxplots may be provided as well.

All laboratory data will be provided in data listings. A subset listing will be presented for all subjects with any laboratory values ≥Grade 3 based on CTCAE version 4.03 criteria.

Table 9. Potential Clinically Significant Abnormal Criteria for Hematology and Chemistry Parameters

Category	Test Name	Potentially CS – Low if observed value is:	Potentially CS – High if observed value is:
Hematology			
	ANC	$<1.5 \times 10^9$/L	$>13.5 \times 10^9$/L
	Basophils		$>1.6 \times 10^9$/L
	Eosinophils		$>1.6 \times 10^9$/L
	Erythrocytes	$\leq 3.5 \times 10^{12}$/L	$\geq 6.4 \times 10^{12}$/L
	Hemoglobin	< 8 g/dL	>16 g/dL
	Leukocytes	$<3.0 \times 10^9$/L	$\geq 16 \times 10^9$/L
	Lymphocytes	$<0.8 \times 10^9$/L	$>12 \times 10^9$/L
	Monocytes		$>2.5 \times 10^9$/L
	Platelets	$\leq 75 \times 10^9$/L	$\geq 700 \times 10^9$/L
Chemistry			
Liver	ALT		≥3 ×ULN
	AST		≥3 ×ULN
	ALP		≥3 ×ULN
	Total bilirubin		≥34.2 umol/L
	Direct bilirubin		≥7.0 umol/L
Renal	Creatinine (serum creatinine)		≥176.8 umol/L
	Urea Nitrogen		≥10.7 mmol/L
Electrolytes	Sodium	≤126 mmol/L	≥156 mmol/L
	Potassium	≤3 mmol/L	≥6 mmol/L
	Chloride	≤90 mmol/L	≥118 mmol/L
Other	Glucose	≤2.22 mmol/L	≥9.71 mmol/L

4.6.4. Vital Signs, Physical Examination and Performance Status

Vital signs include systolic/diastolic blood pressure, heart rate, respiration rate, and temperature, and will be performed in accordance with institutional standards and as per the SOE.

A summary table of the number and percentage of subjects with PCS abnormal criteria for vital signs and physical examination parameters at any point during the study will be presented for

pre- and post-infusion time periods. The criteria for determining PCS abnormal criteria values in vital signs can be found in Table 10.

Table 10. Potential Clinically Significant Abnormal Criteria for Vital Signs and Physical Examinations

Variable Name	PCS – Low if:			PCS – High if:		
	Observed Value is:	AND	Decrease from Baseline is:	Observed Value is:	AND	Increase from Baseline is:
SBP	<90 mmHg		≥20 mmHg	>160 mmHg		≥20 mmHg
DBP	<50 mmHg		≥10 mmHg	>95 mmHg		≥10 mmHg
Heart Rate	<50 bpm		≥15 bpm	>120 bpm		≥15 bpm
Weight	≥ 10% decrease from baseline			≥ 10% increase from baseline		
Height				≥ 2 inches increase from baseline		

DBP = diastolic blood pressure; SBP = systolic blood pressure

Performance status, Karnofsky score, or Lansky score will be assessed at multiple time points prior to drug product infusion and at all scheduled follow-up visits. A listing will be provided. Tanner staging will only be presented in data listings.

4.6.5. Transplant-Related Mortality

Transplant-related mortality will be determined by the Investigator and summarized for the following intervals: from the Informed Consent through 100 days post-drug product infusion, and from the Informed Consent through 365 days post-drug product infusion.

4.6.6. Overall Survival

Overall Survival (OS) is defined as time from date of drug product infusion (Day 1) to date of death. Overall survival will be censored at the date of last follow-up if subject is alive. If there is any death due to any reason occurring during the study, OS rate at 12 months and 24 months by Kaplan-Meier method will be reported.

4.6.7. Persistent Oligoclonality and Integration Site Analysis (ISA)

Persistent oligoclonality is defined as RelFreq of ≥ 10% for the same IS at two consecutive timepoints or RelFreq ≥ 5% for the same two or more IS at two consecutive timepoints.

Persistent oligoclonality at any time is defined as a subject meeting the criteria of persistent oligoclonality at any time during the study, including cases when the criteria are no longer met in later follow-up assessments.

Current persistent oligoclonality is defined as a subject meeting the criteria of persistent oligoclonality at the last two assessments as of the data cut-off. It is a subset of persistent oligoclonality at any time.

Current oligoclonality is defined as RelFreq ≥ 10% for at least 1 IS or RelFreq ≥ 5% for two or more IS at the last assessment as of the data cut-off.

ISA results from bone marrow may also be considered in the above algorithm. If an IS is measured from multiple blood or tissue samples (i.e., concurrent peripheral blood and bone marrow samples), the higher IS RelFreq will be used. Samples taken more than 30 days apart

will be considered distinct consecutive timepoints. Additionally, IS-VCN results collected from IS-specific qPCR per historical protocols will not be considered in the above algorithm.

The number and percentage of subjects meeting persistent oligoclonality at any time, current persistent oligoclonality, and current oligoclonality will be summarized.

IS RelFreq overtime will be plotted for subjects with IS meeting oligoclonality (persistent oligoclonality at any time, current persistent oligoclonality and/or current oligoclonality will be indicated).

Top 10 RelFreq results for each subject overtime will be presented in listings.

In addition, the total number of unique mappable IS at each assessment, as well as the highest RelFreq and highest total number of unique mappable IS within subjects across all assessment, will be summarized. Additional analysis may be performed as appropriate.

4.6.8. Replication Competent Lentivirus (RCL)

Blood will be tested for RCL at Month 3, 6, 12, and 24 Visits. If RCL screening tests are negative up to and including the Month 12 Visit, RCL screening will cease, and the Month 24 co-culture RCL sample only will be collected and archived. Results will be listed as detected, detected but not quantified, and not detected, and by co-culture assays (if applicable) detected or not detected, for each visit.

4.6.9. Dose-Limiting Toxicities (DLT) for Plerixafor for Subjects in Group B2

Three subjects in Group B2 including the two subjects re-assigned to Group C (PPI PPI and PPI) were required for evaluation of DLT at each dose of plerixafor. A listing for DLT for Plerixafor occurred in this study will be provided.

4.6.10. Chromosomal Abnormality or Genetic Mutation

Genetic testing by FISH (Fluorescence in situ hybridization), conventional cytogenetics (karyotyping) and NGS (next generation sequencing) will be summarized and presented in listings. The number of subjects with at least one abnormality will be summarized by test type (FISH, conventional cytogenetics, NGS, and all tests), sample type (blood, bone marrow, and both) and by time period (<Day 1, ≥Day 1 and overall). Listings will be presented separately for each type of genetic test. They will include date sample collected, sample type (bone marrow or blood), local or central laboratory assessment, and details about abnormalities.

5. CHANGES TO PLANNED ANALYSES

Any changes to the analyses from procedures outlined in this SAP will be summarized in the associated interim or final CSR. Decisions to deviate from planned analyses will be documented at the time they are made.

5.1. Changes from Analyses Specified in Study Protocol

Changes from Protocol (Version 13.0) to SAP (Version 3.0) are listed below:

Protocol Section	Section Title	Text in Protocol	Change from Protocol in SAP and Rationale
			No change

5.2. Changes from Previous SAP (Version 2.0)

Section Number	Section	Changes
4.4.1	Analysis of Primary Efficacy Endpoint	Clarified that only the supplementary analyses for Group C subjects based on the protocol VOEs and the investigator VOEs in TPVOE will be performed for the final CSR. Removed the supplementary analysis in the ITT population with ≥4 protocol VOEs in the 24 months prior to Informed Consent. The analysis was performed for the interim CSR, in case the agency wanted the analysis based on the ITT population. The result was however not of the agency's interest. Moreover, this analysis was heavily weighted by subjects who discontinued prior to receiving the benefit of treatment.
4.4.2.1	Adjudicated sVOE-CR	Clarified that only the supplementary analysis for Group C subjects based on the protocol sVOEs in TPVOE will be performed for the final CSR. Removed the supplementary analysis in the ITT population with ≥4 protocol VOEs in the 24 months prior to Informed Consent. See the change above made in Section 4.4.1 for the rationale.
4.4.2.2	Globin Response	Removed the imputation rules of missing HPLC assessments due to COVID-19 pandemic. The imputation rules were never implemented, because of no such cases in data by the end of the pandemic.

Section Number	Section	Changes
		Removed the supplementary analysis in the ITT population with ≥4 protocol VOEs in the 24 months prior to Informed Consent. See the change above made in Section 4.4.1 for the rationale.
4.4.3.1	Characterization of Globin Response	Added a supplementary analysis. In the definition, added "After achieving Globin Response, subjects with death or discontinuation unrelated to the drug product will be considered as not evaluable." This is to exclude the subject who discontinued due to sudden death which was unrelated to the drug product, according to communication with the agency.
4.4.3.2	Hematologic Endpoints	Removed "starting from Month 1 post-drug product infusion".
4.4.4	Analysis of Exploratory Efficacy Endpoints	Added the analyses of bone marrow smear.
4.4.5	Analysis of Correlation	Added the correlation between HbAT87Q vs. HbS (% of non-transfused total Hb) at Month 6
4.5	Pharmacodynamic Analysis and Correlation Analysis	Deleted the spaghetti plots of PB %LVV+ and AUC of sickled RBCs, and the correlation analyses related to the two parameters.
4.6.1	Adverse Events	Added AE/SAE attributed to SCD post Month 6
4.6.2	Engraftment	Added a supplementary analysis for time to PE. This is to extend the time to PE for subjects who took eltrombopag before achieving PE to align with the label.
4.6.3	Laboratory Data	Added hematology RDW test. Added the analysis of cytopenia.
4.6.7	Persistent Oligoclonality and Integration Site Analysis (ISA)	Updated the algorithm used for ISA to align with Protocol Version 13.0.
6.1	Imputation Rules for Missing Data	Updated the imputation rule for AE end date by replacing data cut-off date with last follow-up date. Updated the imputation rule for missing adjudicated VOE end date.
6.2	Analysis Visit Windows	Added analysis visit window for RDW.
6.7	Search Strategy for Events of Interest	Added thromboembolic events

6. APPENDIX

6.1. Imputation Rules for Missing Data

Table 11. Imputation Rules for Missing Data

Situations	Imputation rules
AE	
Start/onset date is incomplete	– If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as study drug treatment (i.e., drug product infusion). In this case, in order to conservatively report the event as treatment-emergent, the onset date will be assumed to be the date of study drug treatment, except in cases where this will lead to a start date being after stop date. In these situations, the original rule will be applied. – If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study drug treatment. In this case, the event onset will be coded to the day of study drug treatment to conservatively report the event as treatment-emergent, except in cases where this would lead to a start date being after stop date. In these situations, the original rule will apply. – A missing onset date will be coded as the day of study drug treatment, except when this would lead to a start date being after the stop date. In these situations, the start date will be set to the first day of the month of the AE end date.
End date is incomplete	– If the day is missing, it will be set to the last day of the month or the last follow-up date, whichever occurs first. – If both the day and the month are missing, it will be set to December 31 or the last follow-up date, whichever occurs first. – If the end date is completely missing, it will be set to the last follow-up date
Prior/Concomitant and Non-drug treatment start and end date to support analyses of (s)VOE, VOC/ACS, (s)VOE start and end date	– If the imputed start date is after the stop date, then the imputed start date will be one day prior to the stop date. – If the imputed end date is after the end of study date or DCO (data cut-off date), then the imputed date will be set as set to DCO or end of study date, whichever occurs earlier.

Situations	Imputation rules
Start date is missing or partial	– if month is missing, use January – if day is missing, use the first day of the month under consideration – if year is missing, use year of the Informed Consent date – if entire date is missing, use Informed Consent date - 1 (not applicable to VOE or sVOE start date)
End date is missing or partial	– if month is missing, use December – if day is missing, use the last day of the month under consideration – if year or the entire date is missing, set to DCO or end of study date, whichever occurs earlier – for adjudicated VOE, impute the missing entire VOE end date as below: • If adjudicated VOE start date is on or after conditioning start date, then the imputed date will be set to data cut-off date or end of study date, whichever occurs earlier • If adjudicated VOE start date is before conditioning start date, then the imputed date will be one day prior to the conditioning start date
SCD diagnosis date or Medical History diagnosis date	
(1) Only Day is missing	Set to 01 Month Year or DOB (date of birth), which occurred last
(2) Both Day and Month are missing	Set to 01 January Year or DOB, which occurred last
(3) Completely missing	Set to DOB
Missing age (years) at Diagnosis of SCD	– If DOB and SCD diagnosis date are complete, calculate the age = (SCD diagnosis date – DOB)/365.25, and round down to the integer – Otherwise, if at least Month and Year available in DOB and SCD diagnosis date, replacing the missing Day by 01 and calculate the age as the same formula above; if the imputed age is negative, assign it as 0. – If only Year is available in DOB or SCD diagnosis date, the age = Year of SCD diagnosis date – Year of DOB

Situations	Imputation rules
Missing age (years) at starting regular transfusion	– If DOB and starting regular transfusion date are complete, imputed the age = (starting regular transfusion date – DOB)/365.25, and round down to the integer – Otherwise, if at least Month and Year available in DOB and starting regular transfusion date, replacing the missing Day by 01 and calculate the age as the same formula above; if the imputed age is negative, assign it as 0. – If only Year is available in DOB or in starting regular transfusion date, the age = Year of starting regular transfusion date – Year of DOB – If starting regular transfusion date is completely missing, the age is missing
Hospital or ER dates (date admitted, or date discharged)	o If Day is missing: - If the month of discharge is after the month of admission, and day of discharge is missing, then set as 01 for the missing day - If the month of discharge is after the month of admission, and both day of discharge and admission are missing, then set as 01 for the missing day - If the month of discharge is the same the month of admission, then duration of hospital = 1 o If both Month and Day are missing in either date, no imputation for duration of hospital. o At interim analysis, if a subject has not been discharged from the hospital for drug product infusion, the duration of hospital will be the days between the last follow up date and admission date
Transfusion information	o If it is known a transfusion took place with number of pRBC unit but pRBC volume (mL) is missing: the standard volume per unit (300 mL) will be substituted o If it is known a transfusion took place, but neither number of unit nor volume is reported: - For transfusion recorded before drug product infusion: mean volume that the subject received in the 2 years prior to enrollment will be substituted - For transfusion recorded on or after drug product infusion: a) If there are transfusions on or after drug product infusion and before the record with the missing value, the mean volume that the subject received during this time frame will be substituted. b) If no other transfusions have been given during this time frame, then the mean volume from ICF to pre-study drug will be used. c) If all volumes are reported in units and there is no standard volume per unit provided, then 300 mL/unit will be imputed

6.2. Analysis Visit Windows

Table 12. Analysis Visit Windows for Assessments

- **Hematology, Serum Chemistry, Haptoglobin, and Central Laboratory for Globin and VCN**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M1	D30	1	45
	M2	D60	46	75
	M3	D90	76	113
	M4.5	D135	114	158
	M6	D180	159	225
	M9	D270	226	315
	M12	D360	316	405
	M15	D450	406	495
	M18	D540	496	585
	M21	D630	586	675
	M24	D720	676	*

* Last visit day on the study, if it is > 676

- **Chronic Pain Assessment, Performance Status, Tanner Staging (for Subjects <18 years of age), and Central Laboratory for ISA**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M6	D180	1	270
	M12	D360	271	450
	M18	D540	451	630
	M24	D720	631	*

* Last visit day on the study, if it is > 631

- **Cerebral MRA/MRI (and TCD if ≤16 years of age), PFT, 6MWT, Local Laboratory for Hormonal Testing, Urinalysis, Coagulation analysis, Brain Natriuretic Peptide, Erythropoietin, Karyotyping and NGS as applicable**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M12	D360	1	540
	M24	D720	541	*

* Last visit day on the study, if it is > 541

- **Iron Metabolism Studies**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M3	D90	1	180
	M9	D270	181	315
	M12	D360	316	450
	M18	D540	451	630
	M24	D720	631	*

* Last visit day on the study, if it is > 631

- **Immunological Studies**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M3	D90	1	135
	M6	D180	136	225
	M9	D270	226	315
	M12	D360	316	540
	M24	D720	541	*

* Last visit day on the study, if it is > 541

- **Exploratory PD analysis: ex vivo sickling**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M6	D180	1	225
	M9	D270	226	315
	M12	D360	316	405
	M15	D450	406	495
	M18	D540	496	585
	M21	D630	586	675
	M24	D720	676	*

* Last visit day on the study, if it is > 676

- **Other exploratory PD analysis**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
			Start	End
Follow-up Post-drug product Infusion	M3	D90	1	113
	M4.5	D135	114	158
	M6	D180	159	225
	M9	D270	226	315
	M12	D360	316	405
	M15	D450	406	495
	M18	D540	496	585
	M21	D630	586	675
	M24	D720	676	*

- **Hematology: RDW**

Timepoint	Month	Target Day	Analysis Visit Window (Study Day)	
Follow-up Post-drug product Infusion			Start	End
	M24	D720	1	*

* Last visit day on the study, if it is > 1

6.3. Power and Success Criteria for VOE-CR, sVOE-CR and Globin Response

For the primary efficacy endpoint of VOE-CR, the power and success criterion in the table below are calculated based on the null hypothesis as 40% and alternative hypothesis as 80% at 1-sided alpha of 0.025 using Exact Test Per EAST® (Version 6). Likewise, for sVOE-CR, the null hypothesis is assumed to be 50% and alternative hypothesis be 85%. For Globin Response, the calculation is performed under the assumption of null hypothesis as 40% and alternative hypothesis as 70%.

Table 13. Power and Success Criteria for VOE-CR, sVOE-CR and Globin Response

Endpoint	Number of Subjects in the Analysis Population	Power [1]	Success Criteria: Number of Subjects Achieve the Endpoint
VOE-CR	35 (currently planned)	>99%	21
	34	>99%	20
	33	>99%	20
	32	>99%	19
	31	>99%	19
	30	>99%	18
	29	>99%	18
sVOE-CR	35 (currently planned)	>99%	24
	34	>99%	24
	33	>99%	23
	32	98%	23
	31	99%	22
	30	99%	21
	29	98%	21
Globin Response	41 (currently planned)	96%	24
	40	97%	23
	39	95%	23
	38	96%	22
	37	94%	22
	36	95%	21
	35	93%	21

[1] at 1-sided alpha of 0.025, using Exact Test Per EAST® (Version 6).

6.4. Definitions of VOE and sVOE

Table 14. Definitions of VOE and sVOE

	Definitions
Investigator VOE	In the opinion of the investigator, the event meets any of the following VOE categories: o Acute pain with no medically determined cause other than VOE (e.g., VOC) o Acute chest syndrome o Acute hepatic sequestration o Acute splenetic sequestration o Acute priapism These are all VOEs, irrespective of the severity, as reported by the investigator and can include VOEs managed at home.
Protocol VOE	It is an investigator reported VOE and meets at least one criterion below: 1. The VOE category =”Acute pain with no medically determined cause other than VOE (e.g., VOC)” and Yes for all the sub-questions below: - Duration > 2 hours? - Require care at a medical facility? 2. The VOE category =”Acute chest syndrome” and Yes for all sub-questions below: - Pneumonia-like symptoms (e.g., chest pain, fever [>38.5°C], tachypnea, wheezing or cough, or finding upon lung auscultation)? - Presence of a new pulmonary infiltration consistent with ACS? - Requiring oxygen treatment and/or blood transfusion? 3. The VOE category =”Acute hepatic sequestration” and Yes for all sub-questions below: - Sudden enlargement of the liver with pain in the RUQ? - Abnormal results of LFTs not due to biliary disease? - Reduction in Hb concentration by at least 2 g/dL below the baseline value? 4. The VOE category =”Acute splenic sequestration” and Yes for all the sub-questions below: - Sudden enlargement of the spleen? - Reduction in Hb concentration by at least 2 g/dL below the baseline value? 5. The VOE category =”Acute priapism” and Yes for all the sub-questions below:

	Definitions
	- Lasting more than 2 hours? - Require care at a medical facility?
Adjudicated VOE	It is a VOE as determined by adjudication committee after referring to protocol VOE definition and based upon the preponderance of the evidence, clinical knowledge, and expertise.
Protocol sVOE	It is a protocol VOE and any of the following is Yes o Hospitalization ≥ 24 hours? o ER observation ≥ 24 hours? o At least 2 visits to a day unit or ER over 72 hours with both visits requiring IV treatment? o Priapism lasting more than 2 hours requiring medical facility visit?
Adjudicated sVOE	It is a sVOE as determined by adjudication committee after referring to protocol sVOE definition and based upon the preponderance of the evidence, clinical knowledge, and expertise.

RUQ = right upper quadrant.

6.5. eGFR Formula

eGFR is reported as ml/min/SSA (x); Age is reported as years (x).
For purpose of reporting, 1.73m^2 has been given the unit of SSA, which is short for Standard Surface Area.

- **For subjects ≥ 18 years old, CKD-EPI creatinine equation for eGFR as below**

Conventional: Serum creatinine is reported as mg/dL (x.xx) and the CKD-EPI equation (Levey et al. 2009) is below:

Race	Sex	Serum Creatinine (mg/dL)	Equation for eGFR by CKD-EPI
Black	Female	≤0.70	$166\times(\text{Serum Creatinine}/0.7)^{-0.329}\times(0.993)^{\text{Age}}$
Black	Female	>0.70	$166\times(\text{Serum Creatinine}/0.7)^{-1.209}\times(0.993)^{\text{Age}}$
Black	Male	≤0.90	$163\times(\text{Serum Creatinine}/0.9)^{-0.411}\times(0.993)^{\text{Age}}$
Black	Male	>0.90	$163\times(\text{Serum Creatinine}/0.9)^{-1.209}\times(0.993)^{\text{Age}}$
White or Other	Female	≤0.70	$144\times(\text{Serum Creatinine}/0.7)^{-0.329}\times(0.993)^{\text{Age}}$
White or Other	Female	>0.70	$144\times(\text{Serum Creatinine}/0.7)^{-1.209}\times(0.993)^{\text{Age}}$
White or Other	Male	≤0.90	$141\times(\text{Serum Creatinine}/0.9)^{-0.411}\times(0.993)^{\text{Age}}$
White or Other	Male	>0.90	$141\times(\text{Serum Creatinine}/0.9)^{-1.209}\times(0.993)^{\text{Age}}$

SI: Serum creatinine is reported as umol/L (x.xx), and the CKD-EPI equation is below:

Race	Sex	Serum Creatinine (umol/L)	Equation for eGFR by CKD-EPI
Black	Female	≤61.9	$166\times(\text{Serum Creatinine}\times 0.011312/0.7)^{-0.329}\times(0.993)^{\text{Age}}$
Black	Female	>61.9	$166\times(\text{Serum Creatinine}\times 0.011312/0.7)^{-1.209}\times(0.993)^{\text{Age}}$
Black	Male	≤79.6	$163\times(\text{Serum Creatinine}\times 0.011312/0.9)^{-0.411}\times(0.993)^{\text{Age}}$
Black	Male	>79.6	$163\times(\text{Serum Creatinine}\times 0.011312/0.9)^{-1.209}\times(0.993)^{\text{Age}}$
White or Other	Female	≤61.9	$144\times(\text{Serum Creatinine}\times 0.011312/0.7)^{-0.329}\times(0.993)^{\text{Age}}$
White or Other	Female	>61.9	$144\times(\text{Serum Creatinine}\times 0.011312/0.7)^{-1.209}\times(0.993)^{\text{Age}}$
White or Other	Male	≤79.6	$141\times(\text{Serum Creatinine}\times 0.011312/0.9)^{-0.411}\times(0.993)^{\text{Age}}$
White or Other	Male	>79.6	$141\times(\text{Serum Creatinine}\times 0.011312/0.9)^{-1.209}\times(0.993)^{\text{Age}}$

Note: Conversion factor for serum creatinine umol/L × 0.011312 = mg/dL

- **For subjects < 18 years old, Bedside-Schwartz equation for eGFR (Schwartz et al 2019; Schwartz and Work 2019) as below**

Conventional:

eGFR by Schwartz = 0.413 × (Height/Enzymatic serum creatinine), where:

  - Enzymatic serum creatinine is reported in mg/dL (x.xx)
  - Height is reported in cm (x.x)

SI:

eGFR by Schwartz = 0.413 × (Height/Enzymatic serum creatinine × 0.011312), where:

  - Enzymatic serum creatinine is reported in umol/L (x.xx)
  - Height is reported in cm (x.x)

6.6. Statistical Methods for Confidence Interval of Percentage of Reduction in Annualized sVOE or VOE

This section presents the 1-sided of 97.5% CI for percentage reduction in annualized sVOE in the 24 months after drug product infusion from baseline. The same statistical methods will be used for the corresponding annualized number of VOE analysis.

sVOE is count outcome. Hence, it is reasonable to assume the targeted patient sub-population has an underlying distribution Poisson (λ).

Assuming the sVOE data consist of $(N_{i0}, N_{i1}, T_{i0}, T_{i1}), i = 1, \cdots, k$, where N_{i0} *and* T_{i0} are the number of sVOEs and follow-up time prior to Informed Consent, serving as baseline data, respectively, and N_{i1} *and* T_{i1} are the number of sVOEs and follow-up time after the treatment, respectively. In Study HGB-206, $T_{i0} = 2$ years for every subject.

We assume that given λ_{i0} and λ_{10}, $N_{i0} \sim Poisson(\lambda_{i0}T_{i0})$, and $N_{i1} \sim Poisson(\lambda_{i1}T_{i1})$ are two conditionally independent variables with a common ratio

$$r = \frac{\lambda_{i1}}{\lambda_{i0}}, i = 1, \cdots, k,$$

where r is the parameter of interest. Also note that sVOE reduction can be noted as θ, where $\theta = (1 - r)$.

Under this model, according to (Krishnamoorthy and Thomson 2004),

$$N_{i1}|N_i \sim Binomal\left(N_i, \frac{rT_{i1}}{T_{i0} + rT_{i1}}\right),$$

where $N_i = N_{i0} + N_{i1}$.

The following test statistics can be used to construct a 1-sided confidence interval (CI) for r:

$$T = \sum_{i=1}^{k} N_{i1}.$$

For any given value $r_0 > 0$, one can assume the observed test statistics is t. Thus, the p-value can be calculated as

$$p(r_0) = P(T \leq t | r = r_0).$$

Clearly, $p(r)$ can be calculated easily with Monte Carlos method: we only need to simulate

$$T^* \sim \sum_{i=1}^{k} Binom\left(N_i, \frac{rT_{i1}}{T_{i0} + rT_{i1}}\right)$$

by a large number of times and calculate

$$p(r) = B^{-1} \sum_{b}^{B} I(T_b^* \leq t).$$

In the end, a 1-sided 97.5% confidence interval for r can be constructed as $\{r: p(r) \geq 0.025\}$.

Note that our model has very general assumptions although we assume N_{i0} and N_{i1} follow a conditional Poisson distribution. This assumption is quite general because the baseline incidence rate, λ_{i0}, can vary from patient to patient and does not have any distribution requirement. As a special case, assuming λ_{i0} and λ_{i1} follow a gamma distribution across all patients, then N_{i0} and N_{i1} follow a negative binomial distribution. This means that our model is still valid even if there is a big variation in baseline sVOE.

6.7. Search Strategy for Events of Interest

Event of Interest	Search Strategy
HIV	MedDRA HLT = Acquired immunodeficiency syndromes, Retroviral infections
Autoimmune Disease/ Immunogenicity/ long latency hypersensitivity	MedDRA HLGT = Autoimmune disorders MedDRA HLT = Autoimmunity analyses, Anaemias haemolytic immune MedDRA PT= Acute graft versus host disease, Acute graft versus host disease in intestine, Acute graft versus host disease in liver, Acute graft versus host disease in skin, Acute graft versus host disease oral, Chronic graft versus host disease, Chronic graft versus host disease in eye, Chronic graft versus host disease in intestine, Chronic graft versus host disease in liver, Chronic graft versus host disease in skin, Chronic graft versus host disease oral, Graft versus host disease, Graft versus host disease in eye, Graft versus host disease in gastrointestinal tract, Graft versus host disease in liver, Graft versus host disease in lung, Graft versus host disease in skin, Transfusion associated graft versus host disease
Malignancies	MedDRA SMQ = Malignant tumors, Malignant lymphomas, Myelodysplastic syndrome, Blood premalignant disorders
Infections	MedDRA SOC = Infections and infestations
Thromboembolic Events	MedDRA SMQ = Embolic and Thrombotic Events

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