STATISTICAL ANALYSIS PLAN

A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with β-thalassemia Major by Transplantation of Autologous $CD34^+$ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-globin Vector (LentiGlobin BB305 Drug Product)

Protocol HGB-204

Protocol Number:	HGB-204
Protocol Version and Date:	Version 5.0, 29 June 2015
Name of Test Drug:	LentiGlobin BB305
Phase:	Phase 1/2
Methodology:	Open-label, Safety, and Efficacy
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Analysis Plan Date:	23 August 2017
Analysis Plan Version:	Version 2.0

bluebirdbio

APPROVAL OF STATISTICAL ANALYSIS PLAN
SIGNATURE PAGE

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
AE	Adverse event
ANC	Absolute neutrophil count
ATC	Anatomic therapeutic class
AUC	Area under the curve
CBC	Complete blood count
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CRF	Case report form
CS	Clinically significant
CSR	Clinical study report
CCI	[redacted]
DMC	Data Monitoring Committee
DP	Drug product
EC	Ethics Committee
ECG	Electrocardiogram
eGFR	Estimated glomerular filtration rate
CCI	[redacted]
CCI	[redacted]
G-CSF	Granulocyte-colony stimulating factor
GRR	Global reference range
GVHD	Graft-versus-host disease
Hb	Hemoglobin
HbA	Hemoglobin A
CCI	[redacted]
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
ICF	Informed consent form
ICH	International Conference on Harmonisation
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
IS	Insertion site
ISA	Integration Site Analysis
ITS	Interrupted Time Series
ITT	Intent-to-treat
IV	Intravenous
CCI	[redacted]
LVEF	Left ventricular ejection fraction

Abbreviation	Definition
CCI	[redacted]
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
NCI CTCAE	National Cancer Institute Common Terminology Criteria For Adverse Events
NCS	Not clinically significant
NE	Neutrophil Engraftment
PBL	Peripheral blood leukocyte
CCI	[redacted]
pRBC	Packed RBCs
CCI	[redacted]
RBC	Red blood cell
RCL	Replication competent lentivirus
Rel Day	Relative study day
CC	[redacted]
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SE	Successful engraftment
SI	International system of units
SOC	System organ class
SQUID	Superconducting quantum interference device
TDT	Transfusion dependent β-thalassemia
CCI	[redacted]
TP	Transplant population
TSH	Thyroid stimulating hormone
US	United States
CC	[redacted]
VCN	Vector copy number
WBC	White blood cell
WHO	World Health Organization

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-204, A Phase 1/2, Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with β-Thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral βA-T87Q-Globin Vector (LentiGlobin BB305 Drug Product). It's based on protocol version 5.0 dated 29 June 2015.

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 trial.

1.1.2. Study Objectives

The study objectives are to:

1. Evaluate the safety of treatment with LentiGlobin BB305 Drug Product in subjects with β-thalassemia major (also referred to as transfusion dependent thalassemia, TDT; this document will use this abbreviation throughout).
2. Evaluate the efficacy of treatment with LentiGlobin BB305 Drug Product in subjects with TDT.

1.2. Study Design

1.2.1. Synopsis of Study Design

This is a single-arm, multi-site, single dose, Phase 1/2 study in up to 18 subjects (including at least 3 adolescents between 12 and 17 years of age, inclusive) with TDT who receive at least 100 mL/kg/year of pRBCs or ≥8 transfusions of pRBCs per year in each of the 2 years preceding enrollment, as defined by a subject or designee signing an Informed Consent Form (ICF) to participate.

The study will evaluate the safety and efficacy of autologous hematopoietic stem cell transplant (HSCT) using LentiGlobin BB305 Drug Product (DP) (autologous CD34+ cell-enriched population that contains cells transduced with lentiviral vector encoding human $\beta^{A\text{-}T87Q}$-globin and resuspended in cryopreservative solution in the final immediate container for the intended medical use).

The study has 4 distinct stages, as follows:

Stage 1: Screening to determine eligibility.

Stage 2: Autologous $CD34^{+}$ cell collection, LentiGlobin BB305 DP manufacture and disposition.

Stage 3: Myeloablative conditioning and infusion of LentiGlobin BB305 DP.

Stage 4: Follow-up, through engraftment and 24 months after DP infusion.

1.2.2. Randomization Methodology

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

1.2.3. Stopping Rules and Unblinding

Unblinding is not applicable to this open-label study.

1.2.4. Enrollment Suspension Criteria:

Enrollment in this study may be stopped at any time for safety reasons. It will be the responsibility of the Data Monitoring Committee (DMC) to determine if there is reasonable cause for suspending enrollment. The DMC, comprised of members with appropriate scientific and medical expertise to monitor the study, will be convened before the study is opened. The Sponsor will inform the regulatory authorities and the investigators; each site's Institutional Review Board (IRB) / Ethics Committee (EC) and other appropriate institutional regulatory bodies will be promptly notified if a decision to suspend enrollment is made. In the event enrollment is suspended, no new mobilization/conditioning or DP infusion of subjects will be initiated, but subjects who have already been infused with LentiGlobin BB305 DP will continue in the study. If mobilization has been initiated, cell collection will be completed at the investigator's discretion. Likewise, if the study is halted while a subject is undergoing conditioning, conditioning will be completed at the investigator's discretion, and every effort will be taken to restart the study prior to the scheduled infusion. However, a subject may be infused with rescue cells following conditioning if the study cannot be restarted in time.

Enrollment and treatment with DP will be temporarily suspended for any of the following reasons pending review and recommendations from the DMC and appropriate communication with the relevant regulatory agency(ies).

Death, until the cause of the death is determined.

Detection of leukemia/lymphoma due to vector-mediated insertional oncogenesis.

Detection of vector-derived RCL in any subject.

Failure to achieve reconstitution with transduced cells in 1 subject, requiring use of backup cells.

Determination of unexpected, clinically significant, or unacceptable risk to subjects (e.g., development of study treatment-related grade 3 or 4 toxicities in at least 3 subjects).

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. Efficacy, Pharmacodynamics, and Safety Parameters

The endpoints stated in the protocol are included, but additional endpoints intended to support regulatory submission and to allow consistent evaluation across multiple studies using LentiGlobin BB305 DP to treat TDT have been added.

1.2.6.1. Efficacy Parameters

The efficacy endpoints include effects on the expression of disease-specific biological parameters and clinical events, as follows:

1.2.6.2. The primary efficacy endpoint from the protocol is:

The sustained production of ≥2.0 g/dL of hemoglobin A (HbA) containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}) for the 6 months between Month 18 and Month 24 post Drug Product (DP) infusion

Subjects who discontinue with less than the minimum required follow-up to evaluate sustained production of HbA^{T87Q} between Month 18 and Month 24 will be considered failures.

1.2.6.3. The primary efficacy endpoint for regulatory submission is:

The proportion of subjects who meet the definition of "transfusion independence" (TI), defined as a weighted average hemoglobin (Hb) ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after DP infusion. See Section 4.4.2 for details on characterization of TI.

A point estimate of at least 70% of non-β^0/β^0 subjects demonstrating TI is considered to be clinically meaningful, and hence will be used as the success criterion for regulatory submission.

1.2.6.4. Secondary efficacy endpoints

Characterization of TI

Duration of TI.

Time from DP infusion to last RBC transfusion prior to becoming TI.

Time from DP infusion to becoming TI.

Weighted average Hb during period of TI.

Proportion of subjects who meet the definition of TI at Month 18 and Month 24 visits.

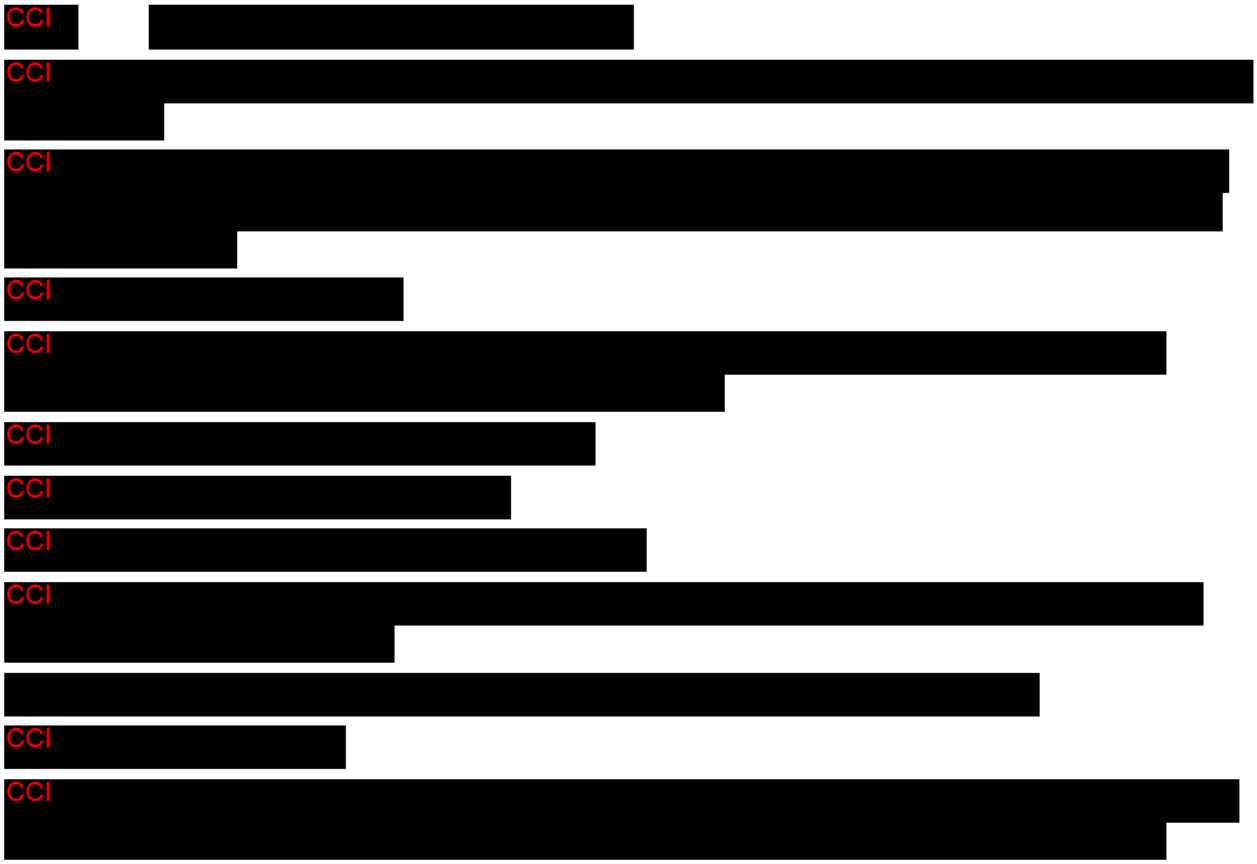
Characterization of transfusion reduction (TR)

The proportion of subjects with a reduction of: <50, ≥50%, ≥60%, ≥75%, ≥90% or ≥100% in average annual pRBC transfusion volume from 6 months (183 days post DP infusion) through Month 24 Visit compared to the average annual pRBC transfusion volume requirement during the 2 years prior to enrollment.

Frequency of pRBC transfusions from 6 months (183 days post DP infusion) through Study Visits at Months 18, and 24 (normalized to a 12-month period) compared to the annual frequency of transfusions during the 2 years prior to enrollment.

Average annual pRBC transfusion volume (mL/kg/year) from 6 months (183 days post infusion) through Study Visits at Months 18, and 24, including change and percent change from baseline.

Weighted average nadir Hb from 6 months (183 days post DP infusion) through Study Visits at Months 18, and 24 compared to weighted average nadir Hb during the 2 years prior to enrollment.



1.2.8. Pharmacodynamic Parameters

The pharmacodynamic endpoints of gene transfer efficiency and expression will be quantified by measurement of the following:

Therapeutic globin expression, as measured by HbA^{T87Q} in peripheral blood and the ratio of α-globin and to all β-like-globin.

Vector copy number (VCN) in cell populations from peripheral blood and, if collected, bone marrow.

Correlations between HbA^{T87Q} expression at Month 6 and HbA^{T87Q} expression at Months 12, and 24.

Correlations between HbA^{T87Q} expression and Peripheral Blood VCN.

Relationship between measures of myeloablation and pharmacokinetic and pharmacodynamic parameters as well as further exploration of associations between gene transfer and expression parameters will be explained in a separate pharmacodynamic SAP.

1.2.9. Safety Parameters

The safety endpoints are:

Success and kinetics of HSC engraftment.

Incidence of transplant-related mortality through 100 and 365 days post DP infusion.

Overall survival.

Detection of vector-derived Replication Competent Lentivirus (RCL) in any subject.

Integration Site Analysis (ISA) to determine the presence of clonal dominance.

Changes in laboratory parameters and frequency and severity of clinical AEs.

2. SUBJECT POPULATION

2.1. Population Definitions

The following subject populations will be evaluated and used for presentation and analysis of the data:

Intent-to-Treat (ITT) Population: All subjects who initiate any study procedures, beginning with mobilization by G-CSF with or without plerixafor.

Transplant Population (TP): All subjects in the ITT population who undergo LentiGlobin BB305 DP infusion.

Successful Engraftment Population (SEP): All subjects who have successful neutrophil engraftment (NE) after LentiGlobin BB305 DP infusion.

The ITT population is the primary population for the analysis of safety parameters. The TP is the primary population for the analysis of efficacy and pharmacodynamic parameters. The TP is also the primary population for transplant-related safety parameters (e.g., success and kinetics of engraftment and incidence of transplant-related mortality through 100 days and 365 days post DP infusion) in the event that TP and ITT Population are not identical. The SEP will be used to provide supportive evidence for subjects who successfully engraft, defined as 3 consecutive absolute neutrophil count [ANC] laboratory values $\geq 0.5 \times 10^9$/L obtained on different days. If the populations are the same, separate analyses will not be performed.

2.2. Protocol Deviations

All protocol deviations will be presented in a data listing; major deviations will be indicated.

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

Up to 18 subjects, including at least 3 adolescents (between 12 and 17 years of age, inclusive), will be treated with the drug product. Replacement subjects may be added if subjects withdraw/ are withdrawn prior to conditioning.

Sample size was not determined by formal statistical methods, but is sufficient to demonstrate a robust effect on the binary response endpoint, where a responder is defined as a subject with production of ≥2.0 g/dL of HbA^{T87Q} for the time period between the Month 18 visit and Month 24 visit post DP infusion. If 70% of all 18 treated subjects met this criterion, a 1-sided lower 95% confidence bound on the rate of response will be 50%.

Among these 18 subjects who were treated with LentiGlobin BB305 DP, 10 have a non-β^0/β^0 genotype. If 7 (70%) of the non-β^0/β^0 subjects meet the primary endpoint of TI and hence meet the success criterion for regulatory submission, a 1-sided lower 95% confidence bound will be 39%.

3.2. General Methods

All output will be incorporated into Microsoft Word or Excel files, or Adobe Acrobat PDF files, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Summary tabulations will be produced for appropriate demographic, baseline, efficacy, and safety parameters as described in Section 4. For categorical variables, summary tabulations of the number and percentage of subjects within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the number of subjects, mean, standard deviation (SD), median, minimum, and maximum values will be presented. A 1-sided 95% confidence interval will be calculated for the primary endpoint from the protocol and the primary endpoint for regulatory submission using the 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.

This study is primarily descriptive in nature; therefore, there are no formal statistical hypothesis tests planned. Data will be presented by subject and summarized overall within each analysis population.

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 should be rounded to the nearest day (ie, the calculated value at 18 months, 547.88, would be rounded to 548 days).

All data listings that contain an evaluation date will contain a relative study day (Rel Day). Pre- and post DP infusion study days are numbered relative to the day of infusion which is designated as Day 1. Note that day of DP infusion was designated as Day 0 in the Protocol and Schedule of Events; this is changed to Day 1 for SAP analyses to be consistent with CDISC guidelines and to allow for cross study comparisons.

3.3. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software Version 9.3 or higher, unless otherwise noted. Medical history and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 19.0 or higher). Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary (March 2016 or later).

3.4. Baseline Definitions

Two years of retrospective pre-enrollment data will be collected for each subject in the study, so that each subject may serve as his/her own control for the parameters of pRBC transfusion requirements (mL/kg/year, number of pRBC transfusions/year). Baseline will be defined as the average of the transfusion requirements over the 2 years prior to study enrollment (date of informed consent).

For other efficacy parameters as well as for pharmacodynamic parameters and CCI baseline will be defined as the most recent measurement prior to conditioning. The conditioning start date will be defined as the first date of busulfan administration.

For safety including key laboratory (hematology and chemistry) parameters, the most recent value prior to mobilization will be used as the baseline assessment. The mobilization start date will be defined as the first date of G-CSF administration.

3.5. Methods of Pooling Data

For purposes of the summary tabulations, subject data will be pooled across all study sites.

3.6. Adjustments for Covariates

No formal statistical analyses that adjust for possible covariate effects are planned.

3.7. Multiple Comparisons/Multiplicity

Multiplicity is not of concern for this study with a descriptive interpretation.

3.8. Subpopulations

Additional selected tables, including demographics and baseline characteristics, and primary efficacy and safety data, will also be summarized by genotype category (β^0/β^0 versus non-β^0/β^0 genotype). CCI [redacted] Drug product and Peripheral blood VCN and HbA^{T87Q} expression over time as well as selected safety tables, such as incidence of adverse events (AE), treatment-emergent adverse events (TEAE), and AE maximum severity, will be summarized by age group (<18 versus ≥18), gender, and race in addition to genotype category. Disposition status will also be summarized by investigational site, with investigational site defined as the site where the LentiGlobin BB305 DP infusion was performed. Additionally, Mobilization/Apheresis details will be summarized by splenectomy status at baseline.

3.9. Withdrawals, Dropouts, Loss to Follow-up

Subjects withdrawn from the study prior to conditioning (myeloablation) will be replaced. Subjects who begin conditioning but are subsequently withdrawn will not be replaced.

3.10. Missing Data

3.10.1. Transfusion Information

If a subject is missing a pRBC volume (mL) when it is known a transfusion took place, but the number of pRBC units is reported, then the standard volume per unit for that transfusion provided on the CRF will be substituted and normalized for subject weight in kg. If neither number of units nor volume is reported, then the imputed volume, for transfusions rendered before study drug infusion, will be the mean volume that the patient has received in the two years prior to study enrollment. If the unknown blood volume was transfused after study drug infusion, then the imputed volume will be the mean volume that the patient received between study drug infusion and most recent study visit; if no other transfusions have been given during this time frame, then the pre-study enrollment mean volume will be used. If all volumes are reported in units and there is no standard volume per unit provided, then 300 mL/unit will be imputed.

After DP infusion, subjects who do not have a documented Hb value for any period greater than 6 months will be analyzed as follows for TI: if the values before and after the gap are both ≥9 g/dL and there are no transfusions during the gap, they are evaluable for TI during this period. If either value is <9 g/dL, this period cannot be counted towards the definition of TI.

Subjects must have a minimum of 12 months' pre-enrollment transfusion data available to be included in the analysis of reduction of transfusion requirements.

3.10.2. Partial Dates

When tabulating AE, partial onset/start dates will be handled as follows. 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 DP infusion and the event stop date is equal to or after the date of DP 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 DP infusion. 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 DP infusion and the event stop date is equal to or after the date of DP infusion. In this case, the event onset will be coded to the day of DP infusion to conservatively report the event as treatment-emergent. A missing onset date will be coded as the day of DP infusion. For AE end dates, an event missing the day of the month will be set to the last day of the month, and an end date missing both day and month will be set to missing.

Partial dates for diagnosis of β-Thalassemia Major or TDT will be handled as follows: if the day of the month is missing, the onset day will be set to the first day of the month. If the onset day and month are both missing, the day and month will be assumed to be January 1. If imputation of partial date results in a date of diagnosis less than the date of birth, then the date of birth is used as the date of diagnosis; age at diagnosis will be zero for these subjects.

3.11. Visit Windows

It is expected that all visits should occur according to the protocol schedule. All data will be tabulated per the evaluation visit as recorded on the CRF even if the assessment is outside of the visit window. If the evaluation visit is missing in the database but there is data from an unscheduled or additional visit that is inside a pre-specified midpoint window, the data from the unscheduled or additional visit will be used in data summaries. For subjects with multiple evaluations within the midpoint window, the evaluation closest to the target visit date will be used.

By visit summary tables and figures of VCN and hemoglobin fractions will use the following midpoint windows based on scheduled visits:

Table 1: Hemoglobin Fractions and Vector Copy Number: Midpoint Windows for by Visit Summaries

>D1 - D45 M1	D46 - D75 M2	D76 - D135 M3	D136 - D225 M6	D226 - D315 M9	D316 - D405 M12	D406 - D495 M15	D496 - D585 M18	D586 - D675 M21	D676 - D750 M24

Windows for time course distributions of AEs, clinical laboratory, and concomitant medications summaries are based on the following time periods relative to DP infusion:

Date of informed consent until date of initiation of mobilization (ICF to <M) *

Date of initiation of mobilization until date of initiation of conditioning (M to <C)

Date of initiation of conditioning until the date of NE (C to <NE)

Date of NE through Month 24 visit (NE to M24)

Day 1 (date of LentiGlobin BB305 DP infusion) through Month 24 visit (D1 to M24)

Date of informed consent through Month 24 visit (ICF to M24) *

* These periods are excluded from laboratory assessments.

The end of Study HGB-204 will be defined as the last visit for the last subject treated with LentiGlobin BB305.

In data listings, the day relative to LentiGlobin BB305 DP infusion will be presented.

3.12. Interim Analyses

No formal interim analysis is planned for this study; however, multiple data extracts throughout the length of the study are planned for publication purposes.

3.13. Final Analyses

A final analysis will be performed per protocol when all subjects treated have been followed for 24 months or discontinued from the study.

4. STUDY ANALYSES

4.1. Subject Disposition

A tabulation of the disposition of subjects will be presented, overall and by investigational site, including the number of subjects who initiate mobilization, the number of subjects infused with LentiGlobin BB305 DP, the number of subjects who achieve successful NE, the number of subjects completing 2 years of study post DP infusion, and the number of subjects who discontinued from the study with reasons for discontinuation. Tables and listings will be provided for subjects in each analysis data set, including the distribution of subjects according to the β-thalassemia genotype.

4.2. Demographic and Baseline Characteristics

The following demographic and baseline characteristic factors will be summarized: age (age at diagnosis, at first transfusion, at starting regular transfusions, CCI [redacted] age at informed consent or assent, and age at DP infusion [<12, ≥12-<18, and ≥18 years]), specific thalassemia-related mutations (including mutations on both *HBB* alleles using both HGVS terminology as well as β+/β0 terminology, grouped by genotype and including any other known thalassemia-related non-*HBB* genetic results), genotype category (β^0/β^0 and non-β^0/β^0) mutation, country of birth, race, ethnicity, gender, and presence of spleen (yes/no).

In addition, baseline data from the 2-year retrospective collection (RBC transfusion requirements) will be summarized. The mean total volume and number of pRBCs transfused in the 2 years prior to the date of consent will be summarized in mL/kg/year.

Other data to be summarized from the 2-year retrospective data collection include the following where available:

Hb transfusion trigger (i.e., the Hb concentration that the investigator designated as the value that would "trigger" a pRBC transfusion during the 2 years prior to study enrollment).

Weighted average nadir Hb during the 2 years prior to study enrollment.

CCI [redacted]

Additional screening results to be summarized will include the following:

Echocardiogram status (normal, abnormal (not clinically significant [NCS]), abnormal (clinically significant [CS]), not done).

Left ventricular ejection fraction (LVEF) %.

CCI [redacted]

CCI [redacted]

Estimated glomerular filtration rate (eGFR).

Subjects who opted in for fertility preservation (Y/N/unknown).

Summary tabulations will be produced for the ITT population, TP, and SEP, if they differ. Demographic and baseline data for each subject will be provided in data listings.

4.3. Mobilization, Transplant and Conditioning Details

Information to be tabulated for mobilization cycles includes:

Number of mobilization cycles / subject (1 or 2).

Average granulocyte-colony stimulating factor (G-CSF; µg/kg) and plerixafor (mg/kg) used per subject per day using the weight at screening.

Number of apheresis procedures performed per mobilization cycle.

Number of subjects requiring bone marrow harvest.

Number of nucleated cells collected (cells × 10^8).

Total blood volume processed during apheresis (mL) (for subjects with more than one mobilization cycle, data will be averaged across cycles first).

Number of CD34+ cells sent for transduction (cells × 10^6/kg).

Number of CD34+ cells sent for rescue (cells × 10^6/kg).

This information will be reported by splenectomy status as well as overall across subjects in TP.

Dosing details to be summarized include the following:

Time in months from informed consent to DP infusion.

Duration of hospitalization (from initiation of conditioning to post DP infusion discharge).

Number of DP lots infused (1 or 2 depending on the number of mobilization cycles).

Total number of infused CD34+ (cells × 10^6/kg).

VCN of DP (average per subject if more than one drug product lot, and average per lot).

Time to NE (defined as the day on which the first of 3 consecutive ANC laboratory values obtained on different days was ≥0.5x10^9/L after a post DP infusion value <0.5 × 10^9/L).

For NE, if ANCs are not collected on a day but the white blood cell (WBC) count is less than 0.75 × 10^9 cells/L, the ANC is considered to be <0.5 × 10^9/L for the purposes of calculating time to neutrophil recovery.

Time to platelet engraftment (defined as the first of 3 consecutive unsupported platelet counts of ≥20 × 10^9/L obtained on different days while no platelet transfusions were administered for 7 days immediately preceding and during the evaluation period); to be summarized as a continuous measure as well as categorized into ≤30 days, >30 to ≤60 days, >60 days to ≤90 days, and >90 days.

Incidence of successful NE (achieving NE by Day 43).

Incidence of successful platelet engraftment (achieving platelet engraftment at any time during the study).

The use of medications for myeloablative conditioning (busulfan as well as any prophylactic and empiric anti-convulsive, antifungal, and antibiotic treatments, and other supportive care usage for the preparative regimen) will be included in a data listing. For busulfan, the total dose in mg, , the average daily dose (mg/kg/day), the individual and daily estimated average busulfan area

under the curve (AUC) (µM*min) will be included in a data listing; the average daily dose (mg/kg/day) and estimated average busulfan AUC will additionally be summarized. The daily estimated average AUC is defined as the average AUC including both observed and derived AUC, where derived AUC is calculated as the observed AUC per busulfan dose (if AUC is collected on more than 1 day, AUC per busulfan dose will be calculated for each day and then averaged) multiplied by observed busulfan dose when AUC is not collected. The closest weight prior to conditioning will be used for analysis.

Time to NE and platelet engraftment will be plotted against total DP dose and estimated busulfan AUC using a scatter plot.

Transfusions of any blood products (platelets, pRBCs) will be reported in a listing. The volume of each type of blood product transfusion (in mL/kg) will also be reported. If the amount of transfusion is reported in 'units,' volume will be calculated as indicated in Section 3.10.1.

4.4. Efficacy Evaluation

Statistical methods will be primarily descriptive in nature; a lower 1-sided 95% CI will be calculated for the primary efficacy endpoint from the protocol and the primary efficacy endpoint for regulatory submission. All efficacy information will be presented in data listings.

All efficacy analyses will be performed by genotype (β^0/β^0, non-β^0/β^0, and overall).

The TP will be used for primary conclusions of LentiGlobin BB305 DP infusion efficacy, with supportive analyses performed on the SEP if it differs from the TP.

4.4.1. Primary Efficacy Endpoint from the Protocol

The primary efficacy endpoint from the Protocol is the sustained production of ≥2.0 g/dL of HbA^{T87Q} for the period between Month 18 and Month 24 post DP infusion.

The number and percentage of subjects that meet the criterion of ≥2.0 g/dL of HbA^{T87Q} will be summarized at Months 18 and 24, along with the number of subjects with sustained production throughout the period between the Month 18 and Month 24 visits (i.e., at Months 18, 21, and 24). The Clopper-Pearson exact method will be used to calculate the lower 1-sided 95% CI for the proportion of subjects meeting this criterion. The denominator at each time point will be the number of subjects with available data at that time point or discontinued prior. Subjects who discontinue with less than the minimum required follow-up to evaluate sustained production of HbA^{T87Q} between Month 18 and Month 24 will be considered failures for calculation of the primary endpoint.

4.4.2. Primary Efficacy Endpoint for Regulatory Submission

The primary efficacy endpoint for regulatory submission is TI, defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after DP infusion, where:

Calculation of time period of TI will start when subjects achieve an Hb ≥9 g/dL with no transfusions in the preceding 60 days.

To meet the initial TI criteria, the weighted Hb must be ≥9 g/dL at the end of the 12-month period.

To remain in the TI state beyond the 12-month period, the treated subject needs to maintain a weighted Hb of ≥9 g/dL from that point forward, without receiving a pRBC transfusion.

The weighted average Hb for determining TI will be defined as follows. Let t_0, t_1, t_2,, … represent the consecutive time points for assessment of Hb, where t_0 denotes the time when Hb is first ≥9 g/dL with no transfusions in the preceding 60 days, and where the t_i are continuing as long as no transfusions are given. Further, let h_0, h_1, h_2, … represent the Hb level at each of these time points. Then the weighted average Hb is defined as:

$$[(t_1-t_0)x((h_0+h_1)/2)+ (t_2-t_1)x((h_1+h_2)/2)+\ldots+ (t_k-t_{k-1})x((h_{k-1}+h_k)/2)]/(t_k-t_0)$$

where t_k represents the time point such that (t_k-t_0) represents at least 12 consecutive months. This calculation is invariant to the metric used for the time points, eg, calendar dates or days from DP infusion, since the consecutive differences in times would always be measured as a number of days. Note that the weighted average may be considered as an average AUC calculation for Hb. To determine if a subject remains TI beyond 12 months, the calculation of weighted average Hb will always start at t_0. If a subject loses TI status, defined as starting transfusion again or weighted Hb falls below 9 g/dL, a new t_0 will be identified to determine future TI status. The calculation of the duration of TI will begin with t_0.

The primary efficacy endpoint of TI will be analyzed as a point-estimate of the proportion of subjects achieving TI, with a lower 1-sided 95% CI calculated using the Clopper-Pearson exact method. Subjects who discontinue after LentiGlobin BB305 DP infusion will be considered as failures unless TI was reached prior to discontinuation.

As a sensitivity analysis, transfusions of pRBCs for any single acute events (e.g., surgery, trauma, parvovirus infection, or sepsis) will not be counted towards the definition of TI. This analysis will not be performed if no such transfusions exist.

A second sensitivity analysis to evaluate the effect of early discontinuation and transfusion post-TI will also be performed. In this analysis, subjects who achieved TI and then discontinued early or received any pRBC transfusions will be considered failures.

For the calculation of the weighted Hb when an allowed transfusion has occurred, the Hb that triggered the transfusion would be carried forward for 60 days and Hb values during those 60 days would be imputed by the carried-forward value. Post 60 days, the actual Hb drawn would again be used in the calculation of TI.

4.4.3. Secondary Efficacy Endpoints

The following secondary endpoints will be descriptively analyzed.

Characterization of subjects achieving TI:

Duration of TI.

Time from DP infusion until last pRBC transfusion prior to becoming TI.

Time from DP infusion to becoming TI.

Weighted average Hb during TI.

Proportion of subjects who meet the definition of TI at Month 18 and Month 24 Visits.

These will be displayed in summary tables using descriptive statistics and plotted using horizontal bar graphs.

The proportion of subjects meeting the definition of TI will be tabulated with a Clopper-Pearson exact lower 95% CI.

Characterization by TR:

The proportion of subjects with a reduction of: <50%, ≥50%, ≥60%, ≥75%, ≥90% or ≥100% in average annual pRBC transfusion volume from 6 months (183 days post DP infusion) through Month 24 Visit compared to the average annual pRBC transfusion requirement during the 2 years prior to enrollment.

Number of pRBC transfusions from 6 months (183 days post DP infusion) through Study Visits at Months 18, and 24 (normalized to a 12-month period) compared to the annual frequency of transfusions during the 2 years prior to enrollment.

Volume of pRBC transfusions (mL/kg/year) from 6 months (183 days post DP infusion) through Study Visits at Months 18, and 24 (normalized to a 12-month period) compared to the annual frequency of transfusions during the 2 years prior to enrollment.

The weight at or closest to the date of transfusion will be used in calculations of pRBC transfusion requirements. The annualized change per subject from 6 months post DP infusion to Month 18 and Month 24 visits will be summarized in a table and reported in a listing.

The weighted average nadir Hb during 24 months prior to enrollment compared to weighted average nadir Hb from 6 months (183 days post DP infusion) through Month 18 and 24 Visits, where nadir is defined as the most recent Hb level on or within 3 days prior to each pRBC transfusion. If there is a period of more than 60 days without a pRBC transfusion, all Hb records between Day 61 and day of last visit or next transfusion (inclusive) will also be considered as nadir.

The annualized change in weighted nadir Hb per subject from 6 months post DP infusion to the Month 18 and Month 24 Visits will be summarized in a table.

The weighted average of the nadir Hb concentrations during the 2 years prior to enrollment will be compared to weighted average of the nadir Hb concentrations from 12 months post-DP infusion through the Month 24 Visit.

The measurements per year will be standardized at the reporting intervals to account for variability in the number of days observed relative to Day 183. For example, if a subject has 18 months of observation with Month 18 Visit on Day 548, the standardized value of pRBC volume at 12 months will be defined as *(total volume/548)**365.25. In addition to the standardized values, the actual number of transfusions and total volume pRBC at the time points above will be reported.

pRBC transfusion requirement over time will also be analyzed by interrupted time series (ITS, Kontopantelis et al., 2015) for β^0/β^0 subjects. The monthly pRBC transfusion volume during each of the 48 months (24 months prior to DP infusion and 24 months post DP infusion) will be calculated for each subject first. The mean pRBC transfusion volume over all β^0/β^0 subjects will then be calculated for each of these 48 months. Due to the expected high volume of transfusion

around DP infusion, the month immediately prior to and after the DP infusion will be excluded from this analysis. The basic ITS model will be applied, i.e., time will be the only covariate. This is equivalent to a linear regression model with a shift in intercept and slope at time 0 (DP infusion). A summary table will be provided for the estimated parameters. The fitted model will also be plotted along with the calculated monthly transfusions.

CCI [redacted]

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4.5. Pharmacokinetic and Pharmacodynamic Evaluations

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

VCN of drug product (average by subject and average by lot), VCN of cell populations from peripheral blood and, if collected, bone marrow will be tabulated. Figures will be presented only for VCN in peripheral blood versus time.

The ratio of α-globin to all β-like-globin-chains.

Hemoglobin fractions over time (including HbA^{T87Q}, HbA, HbA_2, HbE, and HbF, as relevant, calculated using ratio data from HPLC and total Hb), by subject (g/dL) and overall summaries by time point (will include by subject figures with all fractions for a given subject in one plot). The ratios and total Hb used to derived the fractions will also be included in a data listing.

HbA^{T87Q} = $\beta^{A\text{-}T87Q}$-globin to all β-like-globin *total Hb

HbA = β^{A}-globin to all β-like-globin *total Hb

HbA_2 = δ-globin to all β-like-globin *total Hb

HbE = β^{E} to all β-like-globin *total Hb

HbF = (γG-globin to all β-like-globin + γA-globin to all β-like-globin) *total Hb

For the Hb fraction analysis, the globin sample (within the midpoint 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 sample will be merged with the closest Hb result with no transfusion in between. 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. A midpoint window will be applied for by visit summary tables and figures (Table 1). If there are multiple fractions for a subject within a given midpoint window, an average of the fractions and Hb used to derive the factions will be calculated and used in summary tables and figures.

Relationships between measures of myeloablation and pharmacokinetic and pharmacodynamic parameters as well as further exploration of associations between gene transfer and expression parameters will be explained in a separate pharmacodynamic SAP.

4.6. Safety Analyses

The safety of treatment will be summarized through the longitudinal evaluation of AEs and laboratory assessments. Analyses will be performed in the TP on rates of failure to engraft, and adverse effects of the transplant procedure or preparation for the procedure.

Since the safety profile will be assessed for several different time intervals relative to mobilization, conditioning, and post DP infusion, there may be subjects in the ITT population who are not candidates 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 DP infusion. Safety parameters will be summarized over time.

4.6.1. Adverse Events

All AEs will be coded using the MedDRA coding system and displayed in tables and data listings.

Incidence of AEs will be summarized by preferred term and body system coded using MedDRA. Some AEs reported to the Investigations SOC will be recoded to their synonyms under Blood and Lymphatic System Disorders. A listing of these recoded PTs will be provided in the CSR. The relationship of AEs to LentiGlobin BB305 DP or Study Procedure will be based on the investigator's assessment. For the assessment of relationship to LentiGlobin BB305 DP, a classification of 'Possibly Related' or 'Related' will be classified as related to DP. In the event relationship to LentiGlobin BB305 DP is missing, this will also be classified as related to DP.

Incidence of AEs will be summarized for these periods as defined in Section 3.11: ICF to <M, M to <C, C to <NE, NE to M24, D1 to M24, and ICF to M24.

For the above periods, the appropriate denominators for rates of events would consist of the number of subjects "at risk" in each interval; the number at risk is defined as subjects who enter the period.

The terminology "treatment-emergent" would be reserved for events that occur during or after the DP infusion (i.e., D1 to M24 period). For treatment-emergent AEs only the following periods will be assessed: "D1 to <NE," "NE to M24," and "D1 to M24." For AEs in which there is ambiguity as to whether it was treatment-emergent, it will be conservatively classified as treatment-emergent. Summaries will be provided for the following by period.

Incidence of all AEs

Incidence of all serious AEs (SAEs)

Incidence of Grade 3 or higher AEs

Incidence of all study drug product related AEs*

Incidence of all study drug product related SAEs*

Incidence of all AEs by System Organ Class (SOC) frequency

Incidence of all AEs by Preferred Term (PT) frequency

Incidence of all AEs from ICF to M24 by selected subgroups (genotype, gender, age at informed consent, race)

Incidence of all AEs by maximum severity

Incidence of all study drug product related AEs by maximum severity*

AEs attributed to Mobilization/Apheresis**

AEs attributed to Conditioning**

Events of Interest*

* Treatment-emergent events only.
**Based on investigator attribution on CRF for events not related to study drug.

By-subject listing for all AEs occurring on study will be provided, and in addition, by-subject listings will be provided for subject deaths, SAEs, and AEs related to drug product.

AEs designed on the CRF to be attributed to any study procedures will also be summarized.

Events of Interest

The clinical database will be searched for the following treatment-emergent lab results and/or reported AEs as events of interest. The definition for each event of interest will be provided in the CSR. Results will be presented both as a by-subject listing as well as summarized with incidence.

Event of Interest
≥Grade 3 Thrombocytopenia AE / platelet count decreased on/after neutrophil engraftment (from AE and Lab)
Neutrophil engraftment failure
HIV infection
Autoimmune disorders
Lack of efficacy
Malignancies

Engraftment

4.6.2. Laboratory Data

The incidence of ANC and platelet engraftment failure will be calculated and tabulated.
Laboratory Data

Clinical laboratory values will be expressed using the International System of Units (SI), with the exception of Hb summaries and figures which will use g/dL. Values will be summarized for each clinical laboratory parameter, including hematology, coagulation studies, clinical chemistries, CCI and urinalysis, will be performed as specified below, and in the Schedule of Events.

Internationally accepted ranges published by the New England Journal of Medicine and the Mayo Clinic are utilized. For purposes of this plan, these ranges are referred to as Global Reference Ranges (GRRs). Age-specific (age at informed consent/assent as applicable) and gender specific ranges (i.e., adult or pediatric, male or female) will be used to flag out of range values and to categorize into CTCAE (version 4.03) grades where applicable.

The versions of the GRRs being utilized for this study are as footnoted below. If updated GRRs are published during the course of the study, the version used will not be automatically updated.

Source	Version	Purpose
New England Journal of Medicine	2004;351:1548-63.	Adult Ranges
Mayo Clinic	(As provided by bluebird bio on 04-AUG-2014)	Children Ranges

These sources along with any additional sources and/or additional ranges will be maintained in a separate document and provided as an appendix in the CRF.

The following clinical laboratory parameters are to be evaluated:

Hematology

- Complete blood count (CBC) with differential
- Platelet count
- CCI
- CCI

CCI

- CCI
- CCI
- CCI
- CCI
- CCI

Serum Chemistry and Liver Function

- Sodium
- Potassium
- Chloride
- Bicarbonate
- Albumin
- Total protein
- Alanine transaminase
- Aspartate transaminase
- Gamma glutamyl transferase
- Blood urea nitrogen
- Creatinine
- Glucose
- Calcium
- Phosphorus
- Bilirubin (total)
- Alkaline phosphatase
- Lactic dehydrogenase

* Laboratory parameter evaluated for efficacy.

Additional clinical laboratory tests may be performed at the investigator's discretion. Urinalysis was performed as described in the SOE. In addition, creatinine clearance will be derived if data are available.

The eGFR will be determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation) for ≥18 years of age, and Bedside Schwartz equation calculator for <18 years of age as follows:

CKD-EPI

$eGFR = 141 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] _ 1.159 [if black], where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κor 1, and max indicates the maximum of Scr/κ or 1.

Bedside Schwartz

eGFR =0.413 x (height/Scr) where height is expressed in centimeters, where Scr is serum creatinine (mg/dL).

Shift tables which will indicate abnormally high or abnormally low changes in laboratory parameter grade based on CTCAE criteria from baseline will be performed using the most abnormal value in the following periods: M to <C, C to <NE, NE to M24, D1 to M24. The parameters included in the CTCAE shift tables are Hb, ANC, platelets, WBC, serum creatinine, AST, ALT, total bilirubin, AP, calcium, phosphorus, and potassium.

Haematology	Haemoglobin	Both
	White blood cell count (WBC)	Both
	Neutrophils	Decrease
	Platelets	Decrease
Chemistry	Alanine aminotransferase (ALT)	Increase
	Aspartate aminotransferase (AST)	Increase
	Alkaline phosphatase (AP)	Increase
	Calcium	Both
	Creatinine	Increase
	Phosphorus	Decrease
	Potassium	Both
	Total bilirubin (TBL)	Increase

Laboratory values for selected hematology and chemistry parameters will be presented graphically. By visit summary figures will be provided for the following parameters: neutrophils, leukocytes, platelets, Hb, creatinine, total bilirubin, AST, ALT, ALP and LDH. Summary figures will present median (range) for each visit (baseline through M24), with N=value under each visit.

By-patient figures will be provided for Hb (pRBC transfusions noted), and platelets (with platelet transfusions noted) for each visit (baseline through M24).

Figures for CCI [redacted] reticulocytes/erythrocyte (%), and nucleated RBC will be included in efficacy reporting, both as summary figures with either median (range) with N=values, and with all subjects (by genotype), for each visit (baseline through M24).

Immunology parameters will be descriptively summarized by visit by genotype.

All laboratory data will be provided in data listings, with listings including results for immunological testing, serology and hormonal testing. Additionally, change and percentage change from baseline will also be provided in data listings, where baseline is defined as the most recent value prior to mobilization. A subset listing of will be presented for all subjects with any laboratory values ≥Grade 3 based on CTCAE version 4.03 criteria.

4.6.3. Transplant-Related Mortality

Transplant-related mortality will be determined by the investigator and will be provided in a data listing.

4.6.4. Vital Signs, Karnofsky Performance Status, and Physical Examination

Vital signs to be measured include systolic/diastolic blood pressure, pulse, respiration rate, and temperature, and will be performed in accordance with institutional standards, as per the Schedule of Assessments.

Additionally, a summary table of the number and percent of subjects with potentially clinically significant (CS) vital signs parameters at Day 1 of the study will be presented. The following criteria will be used to determine potentially CS values:

	Potentially CS – Low if:			Potentially CS – High if:		
Variable Name	Observed Value is:	AND	Decrease from Baseline is:	Observed Value is:	AND	Increase from Baseline is:
Systolic Blood Pressure	<90 mmHg		≥20 mmHg	>180 mmHg		≥20 mmHg
Diastolic Blood Pressure	<50 mmHg		≥10 mmHg	>105 mmHg		≥10 mmHg
Heart Rate	<50 bpm		≥15 bpm	>120 bpm		≥15 bpm

Abbreviations: CS=clinically significant.

Karnofsky score will be assessed at multiple time points prior to DP infusion, and at all scheduled follow-up visits. Average Karnofsky scores will be summarized by visit for the TP and SEP, along with change scores from baseline.

Vital sign measurements, weight, height, Tanner staging, where relevant, and Karnofsky performance status will be presented for each subject in data listings.

4.6.5. Concomitant Medications and Procedures

Concomitant medications will be coded using the WHO Drug Dictionary and results will be listed with the assigned anatomic therapeutic class (ATC) and preferred term. Medications will be assigned to one or more study periods indicated in Section 4.6.1, with the exception of an additional < ICF period, based on the medication start and stop dates relative to the study periods. The assigned period(s) will be included in the data listing.

Concomitant treatments/procedures (including transfusions) will also be displayed in a listing.
CCI [redacted]

4.6.6. Overall Survival

Overall survival is defined as time from date of DP infusion (Day 1) to date of death. Overall survival will be censored at the date of last visit if subject is alive.

A by-subject listing of time from Day 1 to date of death or censorship will be provided.

4.6.7. Integration Site Analysis

An ISA will be performed on PBLs approximately every 6 months after the infusion of DP, and more frequently if clonal dominance is suspected (see protocol for details).

Clonal dominance is defined as an ISA result >90% of the total IS at any time and a VCN ≥0.3, or an initial ISA result of >30% of the total IS with a VCN ≥0.3 followed by a result >30% and ≤90% at first repeat and a result >50% at second repeat. The number and percentage of subjects who meet the ISA clonal dominance criteria will be summarized over time.

By-subject listings of number of unique mappable integration sites in PBLs at each applicable visit will be provided. Additional analysis may be performed as appropriate.

4.6.8. Replication Competent Lentivirus

Blood will be tested for RCL at Month 3, 6, 12, and 24. Results will be summarized and listed as RCL screen detected, detected but not quantified, and not detected, and by co-culture assay (if applicable) detected or not detected, for each visit.

5. CHANGES TO PLANNED ANALYSES

All changes from procedures outlined in the protocol and procedures outlined in this SAP will be summarized in the study report. Decisions to deviate from planned analyses will be documented at the time they are made.

Changes from protocol to SAP:

Protocol Section	Section Title	Text in Protocol	Change from Protocol in SAP and Rationale
2.2.1	Efficacy Endpoints		The endpoints in the protocol were retained, but additional endpoints were added for consistency of evaluation across multiple studies using LentiGlobin BB305 to treat TDT, and to support marketing authorization. Specifically, the primary efficacy endpoint for regulatory submission, secondary efficacy endpoints, and additional exploratory efficacy endpoints were added.
2.2.1	Efficacy Endpoints	RBC transfusion requirements (mL/kg) per month	Per month analysis is provided in a by-subject graph only and includes all pRBC transfusions
2.2.3	Safety Endpoints	Monitoring of laboratory parameters and frequency and severity of clinical AEs.	"Monitoring of" is changed to "Changes in." Integration site analysis (ISA) is added to clarify that this analysis will be done to allow evaluation of clonal dominance (another endpoint).
7.1	Sample Size Estimation		Using a 1-sided 95% CI to justify sample size and conduct primary efficacy endpoint analysis from protocol

Protocol Section	Section Title	Text in Protocol	Change from Protocol in SAP and Rationale
7.2	Populations for Analysis	The ITT population is the primary population for the analysis of efficacy parameters and safety parameters. .... an evaluable population will be defined as those subjects who 1) receive LentiGlobin BB305 Drug Product; 2) engraft, defined as an ANC ≥0.5 × 109/L for 3 consecutive days; and 3) have sufficient study visit compliance to acquire clinical laboratory and transfusion data for a minimum of 24 months after drug product infusion. Subjects in this population must be compliant with the visit window for the Month 24 evaluation.	The ITT population is the primary population for the analysis of safety parameters. The TP is the primary population for the analysis of efficacy parameters, because this is the population that were treated with the drug product. Evaluable Population (EP) is replaced by Successful Engraftment Population (SEP), as the name better reflects the criteria for inclusion in this population.
7.3	Procedures for Handling Missing, Unused, and Spurious Data	No imputation will be performed for missing data elements.	Details of missing data handling rules are added, e.g., rules needed for analysis of secondary endpoints and partial AE dates.
7.4.1	General Methods	For other change from baseline analyses, baseline will be defined as the value closest to, but prior to transplant.	For CCI CCI [redacted] and efficacy laboratory parameters, baseline will be defined as the most recent measurement prior to conditioning. For safety including other key laboratory (hematology and chemistry) parameters, the most recent value prior to mobilization will be used as the baseline assessment. Changes were made to provide most clinically relevant baseline.
7.4.2	Disposition of Subjects	The number of subjects completing the study through 2 years post-transplant and reasons for study discontinuation will be reported.	Disposition according to study population (number mobilized, number infused, and number with successful NE) was added.

Protocol Section	Section Title	Text in Protocol	Change from Protocol in SAP and Rationale
7.4.5	Safety Analysis	AEs will be summarized for those events that occur 1) after signing the informed consent and prior to conditioning; 2) from the start of conditioning until Day 0 (immediately before the start of LentiGlobin BB305 Drug Product infusion); 3) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through 42 days post-infusion;	Incidence of AEs will be summarized as follows to align periods analyzed with those of regulatory submission: • after signing the ICF and prior to mobilization; • start of mobilization through start of conditioning (all AEs, AEs related to the mobilization/collection regimen); • from the start of conditioning until date prior to ANC engraftment (all AEs, AEs related to the conditioning regimen, AEs related to drug product); • from date of ANC engraftment through last follow up visit • from the start of LentiGlobin BB305 Drug Product infusion on Day 1 through the Month 24 Visit Added categories for summarizing AEs related to study procedures, mobilization/ apheresis, and conditioning

Changes between SAP versions:

Section Number	Section	Changes from Prior SAP Version
Change in SAP Version 1.0 to Version 2.0		
1.2.5.1	Efficacy Parameters	Adjusted primary efficacy endpoint from protocol to read as per protocol (removed 12 to 18 month endpoint); added conditions for subjects who discontinue early
1.2.5.1	Efficacy Parameters	Added primary efficacy endpoint of TI for regulatory submission; added success criterion for this endpoint
1.2.5.1	Efficacy Parameters	Moved TR, characterization of TI, Hb nadir endpoints from additional exploratory endpoints to secondary endpoints (removed Month 12 from TR and weighted nadir Hb analyses)
1.2.5.1	Efficacy Parameters	Hb nadir analyses will use the weighted average nadir Hb
1.2.5.2 and 4.5	Pharmacodynamic Parameters and Pharmacokinetic and Pharmacodynamic Evaluations	Made reference to separate pharmacodynamic statistical analysis plan
2.1	Population Definitions	Replaced Evaluable Population with Successful Engraftment Population, defined as all subjects who have successful neutrophil engraft after drug product infusion
3.1	Sample Size Justification	Updated to use 1-sided 95% CI
3.2	General Methods	Added "Analyses will be stratified by genotype category β^0/β^0 vs non-β^0/β^0 where appropriate"
3.2	General Methods	Changed CI method from Wilson to exact, using lower 1-sided 95% CI and removed all other CI not used for primary outcomes
3.2	General Methods	Updated definitions of month and years used in calculations from 30.4 and 365 to 30.4375 and 365.25, respectively.
3.2 and 4.6.6	General Methods and Overall Survival	Time to event analyses (Kaplan-Meier) have been removed
3.3	Computing Environment	Updated MedDRA version number from 16.0 to 19.0 or higher
3.8	Subpopulations	Added detail to explicitly state what subgroups are being analyzed and for which parameters

Section Number	Section	Changes from Prior SAP Version
Change in SAP Version 1.0 to Version 2.0		
3.10	Missing Data	Removed sensitivity analyses from this section; was previously titled "Missing Data and Sensitivity Analyses"
3.10.1	Missing Data; Transfusion Information	Removed 21/90 day Hb rule from this section; added 300 mL imputation conditions for missing volume in units (previously in section 4.2)
3.11	Visit Windows	Updated AE evaluation windows to be ICF to <M, M to <C, C to <NE, NE to M24, D1 to M24, and ICF to M24
3.11	Visit Windows	Updated lab shift evaluation windows to be same as updated AE windows excluding ICF to <M and ICF to M24
3.11	Visit Windows	Updated conmed evaluation windows to be same as AE evaluation windows
3.11	Visit Windows	Added midpoint windowing to be used to summarize Hb fractions and VCN
3.12	Interim Analyses	Replaced M18 interim analysis statement with "No formal interim analysis is planned for this study; however, multiple data extracts throughout the length of the study are planned for publication purposes."
3.13	Final Analysis	Added section 3.13 to state a final analysis will occur per protocol when all subjects have been followed up for 24 months or discontinued
4.1	Subject Disposition	Protocol deviations are no longer being tabulated but provided in a listing only
4.2	Demographic and Baseline Characteristics	Removed tabulations of PFT
4.2	Demographic and Baseline Characteristics	Added "andor" between biopsy and MRI and between MRI and SQUID given subjects will often have biopsy and either MRI or SQUID
4.3	Mobilization, Transplant and Conditioning Details	G-CSF and plerixafor will be tabulated as an average used per subject per day using weight at screening
4.3	Mobilization, Transplant and Conditioning Details	Removed time from diagnosis to DP infusion
4.3	Mobilization, Transplant and Conditioning Details	Removed definition of footnote for subjects receiving G-CSF post-infusion

Section Number	Section	Changes from Prior SAP Version
Change in SAP Version 1.0 to Version 2.0		
4.3	Mobilization, Transplant and Conditioning Details	Changed from incidence of failure to engraft to incidence of successful engraftment
4.3	Mobilization, Transplant and Conditioning Details	Added categories of time to Platelet engraftment
4.3	Mobilization, Transplant and Conditioning Details	Added scatter plots of time to NE and Platelet engraftment vs DP and busulfan AUC
4.3	Mobilization, Transplant and Conditioning Details	Moved definition of platelet engraftment from 4.6.1 to here
4.3	Mobilization, Transplant and Conditioning Details	Updated to report busulfan dosing as total dose in mg, average daily dose in mg/kg/day, and the estimated average busulfan AUC using both the derived and observed AUC values
4.4.1	Primary Efficacy Endpoint from the protocol	Updated from Wilson to Clopper-Pearson Exact method; from lower 90% to lower 95%
4.4.2	Primary Efficacy Endpoint for Regulatory Submission	Revised to include pRBC for single acute events to be removed only in a sensitivity analysis. Second sensitivity analysis to evaluate the effect of early discontinuation and transfusion post-TI added.
4.4.2	Primary Efficacy Endpoint for Regulatory Submission	Updated weighted nadir Hb algorithm to use closest Hb on or within 3 days prior to transfusion, and to use all Hb for a gap of 60 days between transfusions
4.4.3	Secondary Efficacy Endpoint	Updated to use the weight at or closest to the pRBC transfusion to calculate volume transfused for all pRBC transfusions (previously used screening weight for 2-year prior pRBC transfusions)
4.4.3	Secondary Efficacy Endpoint	Added ITS analyze method to analyze pRBC transfusion requirements over time for β^0/β^0 subjects
4.4.4.4	CCI [redacted]	CCI [redacted]
4.4.4.5	CCI [redacted]	CCI [redacted]
4.5	Pharmacokinetic and Pharmacodynamic Evaluations	Added (…(average by subject and average by lot), ….)

Section Number	Section	Changes from Prior SAP Version
Change in SAP Version 1.0 to Version 2.0		
4.5	Pharmacokinetic and Pharmacodynamic Evaluations	Added ratio of α-globin to all β-like-globin-chains
4.5	Pharmacokinetic and Pharmacodynamic Evaluations	Add calculations for each Hb fraction
4.5	Pharmacokinetic and Pharmacodynamic Evaluations	Removed all correlation analyses to be describe in PD SAP (removed Month 18 correlations)
4.5	Pharmacokinetic and Pharmacodynamic Evaluations	Updated Hb fraction merging algorithm to use ±7 day window, exclude if transfusions occurred between globin sample and Hb sample, and added detail on if there are multiple observations on same date or within same window
4.6	Safety Analyses	Change from ITT to TP for rate of failure to engraft
4.6.1	Adverse Events	Added incidence of all AE by SOC, incidence of all AE by PT, incidence of all AE by subgroups, incidence of all AE by maximum severity
4.6.2	Laboratory Data	Added change from baseline evaluations will be provided as listings
4.6.2	Laboratory Data	Added that additional lab reference ranges and resources will be documented in an appendix in the CRF
4.6.3	Transplant-Related Mortality	Deleted KM analysis looking at relationship between drug product infusion and mortality

6. REFERENCES

E. Kontopantelis, T. Doran, DA. Springate, I. Buchan and D. Reeves. Regression based quasi-experimental approach when randomisation is not an option: interrupted time series analysis. BMJ 2015;350:h2750

7. CLINICAL STUDY REPORT APPENDICES

The list of statistical tables, figures, and data listings, along with a complete set of reporting shells, will be provided in a separate document.