CLINICAL STUDY PROTOCOL HGB-205

Eudract No. 2012-000695-42

A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and 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)

Study Sponsor: bluebird bio, Inc.
150 Second Street
Cambridge, MA 02141 USA
Telephone: +1. 339-499-9300
Fax: +1. 339-499-9432

With its EU Legal Representative:
bluebird bio France
155 rue du Faubourg, Saint-Denis
75010 Paris France
(A wholly owned subsidiary of bluebird bio, Inc.)

Responsible Medical Officer: PPD, MD PhD
PPD, Clinical Development
bluebird bio, Inc.
Mobile telephone: PPD
Office telephone(if unavailable by mobile): PPD
E-mail: PPD

Protocol Version: Version 7.0

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[redacted]

SUMMARY OF CHANGES

This protocol (Version 7.0, 19 May 2016) replaces previous Version 6.0, 26 August 2015. The main reason for this amendment was to allow an additional bone marrow harvest to collect bone marrow cells for manufacturing drug product.

Changes were made as follows:

(Note: added text is **bold**; deleted text in ~~strikethrough~~**).**

Section of current document	Rationale	Change
Title page	Change in Sponsor's responsible medical officer	Contact details updated
Section 3.1	Adjusted the acceptable busulfan range based on clinical experience that increased busulfan exposure assists in achieving improved efficacy outcomes	(AUC range ~~800-1100~~ **1000-1300** µM*min for a every-6-hour dosing regimen, or ~~3200-4400~~ **4000-5200** µM*min for a daily dosing regimen)
Sections 3.1, 3.3, 5.2.2, 5.2.3, Table 5-1	Increased the minimum cell number for the dose when bone marrow is used as the cell source, as well as increased the number of bone marrow harvests that may be performed, based on clinical experience suggesting that doses above the current minimum may promote a better clinical response, and on acceptable clinical safety observed during bone marrow harvests in this study to date, for subjects with sickle cell disease (SCD). Additional bone marrow harvests will only be performed at the advisement of the PI and the agreement of the subject, and will not be performed if there is reasonable concern that an additional harvest may jeopardize the safety or well-being of the subject.	For cells procured via apheresis, the minimum dose to be administered is 3.0×10^6 CD34+ cells/kg, and for cells procured by bone marrow harvest, the minimum dose to be administered is ~~1.5~~ **2.0**$\times 10^6$ CD34+ cells/kg. **For subjects with SCD, up to 3 bone marrow harvests may be performed, at the discretion of the Principal Investigator, even if the minimum cell dose is achieved. Additional bone marrow harvests will not be performed if there is reasonable concern that an additional harvest may jeopardize the safety or well-being of the subject. The Principal Investigator should confirm plans for a third bone marrow harvest with the Sponsor prior to scheduling the procedure. Additional bone marrow harvests beyond 3 may only be performed if the requisite minimum CD34+ dose and minimum cell collection for rescue are not attained, or if one or more bone marrow harvests does not result in production of drug product which meets release criteria**
Table 5-1	Clarified the description of the bone marrow cells that are stored for potential rescue, based on the tests (TNC rather than CD34+) performed by the clinical site.	**≥1.0 × 10^8 TNC cells/kg** ~~≥1.5 × 10^6 CD34+ cells/kg~~
Table 6-1 (Schedule of Events) Section 6.3.15, 6.3.16	Provide clarification and details regarding assessments to monitor for clonal dominance, in order to align with recommendations of a Scientific Advisory Board	Table 6-1: Remove ISA at Month 3 and PK/PD at Month 4.5,and add ISA at Month 18 Combined previous sections on ISA analysis of blood and bone marrow into a new Section 6.3.15, and added a new

Section of current document	Rationale	Change
		Section 6.3.16 entitled **"Assessment of Clonal Dominance and/or Suspicion of Leukemia/Lymphoma"**
Sections 2.2.3, 6.2.9 Table 6-1 (SOE)	New tests are being developed to better characterize pharmacodynamics (PD) after treatment of subjects with drug product, and would benefit from additional blood samples for research purposes	Added a new exploratory endpoint **("exploratory methods may be used to evaluate PD endpoints**") Added additional assessment: **A blood sample will be taken for exploratory PD at Months 9, 15, and 21.**
Section 6.2.10	Collection of autopsy material is no longer considered necessary for evaluating this therapy.	Removed request for collection of autopsy material
Section 6.3.17	Safety reporting updated to align with current requirements.	Adverse event definitions updated to align with the CTCAE
Section 7.6	Statistical analyses updated to align with current Statistical Analysis Plan	Safety analysis periods are aligned with those in the SAP

In addition, several minor changes were made to correct typographical errors, and to improve clarity.

CLINICAL STUDY SYNOPSIS

Protocol Title:	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Anemia and 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 Number:	HGB-205
Eudract Number:	2012-000695-42
Objectives:	The primary study objective is to: • Determine the safety, tolerability, and success of engraftment with autologous CD34+ hematopoietic stem cells transduced with LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene and suspended in human serum albumin (HSA) (5% Albunorm™) (i.e., LentiGlobin BB305 Drug Product) after conditioning with Busilvex® (busulfan IV) in subjects with severe sickle cell anemia (sickle cell disease; SCD) or β-thalassemia major (β-Thal$_M$). Secondary objectives are to: • Quantify gene transfer efficiency and expression: o Evaluate expression of $\beta^{A\text{-}T87Q}$-globin chain in whole blood. o Quantify the hematopoietic chimerism resulting from treatment with LentiGlobin BB305 Drug Product (vector copy number [VCN]). • Measure the effects of transplantation with LentiGlobin BB305 Drug Product on the expression of disease-specific biological parameters and clinical events, including the volume of blood transfusions for both severe SCD and β-Thal$_M$ and, for subjects with severe SCD, the number of vaso-occlusive crises (VOCs) and acute chest syndrome (ACS) events in each subject compared with the 2-year pre-treatment period.
Study Design:	This is a Phase 1/2, open label, safety, and efficacy study of the administration of LentiGlobin BB305 Drug Product to subjects with severe SCD, including sickle hemoglobin S (HbS)/β-thalassemia and β-Thal$_M$. This study originally was initiated as Protocol LG001, in which a total of 10 subjects were to be treated with LentiGlobin HPV569 Drug Product. Three subjects with β-Thal$_M$ were treated with LentiGlobin HPV569 Drug Product under Protocol LG001 between September 2006 and November 2011. bluebird bio subsequently developed the new LentiGlobin®BB305 lentiviral vector, which has improved transduction efficiency, and is expected to show improved integrated provirus stability, and safety. In November 2011, the Agence nationale de sécurité du médicament et des produits de santé (ANSM) requested that bluebird bio file a new Clinical Trial Application (CTA) for LentiGlobin BB305 Drug Product; it was understood that a new protocol (eventually designated as HGB-205) would be required that would be the continuation of LG001. Consequently, the remaining 7 subjects will be enrolled and treated with LentiGlobin BB305 Drug Product under the current Protocol HGB-205. Selected subjects must meet the inclusion criteria and have none of the exclusion criteria and have provided informed consent. The study has 4 distinct phases: • Pre-inclusion Screening, Informed Consent, and Eligibility-determining Phase (Day -97 to Day -61) • CD34+ Cell Collection, Genetic Modification, and Release of Modified Cells (Day -60 to Day -11) • Conditioning and Washout Phase (Day -10 through Day 0) and Stem Cell Infusion on Day 1 • Maintenance Phase (Day 1 through Month 24) Prior to cell harvest, subjects with β-Thal$_M$ may undergo a period of hypertransfusion to maintain a pre-transfusion hemoglobin (Hb) of ≥11 g/dL. For subjects with severe SCD, exchange transfusions will be performed before stem cell harvest and

	conditioning in order to reach a sickle hemoglobin HbS proportion lower than 30% and bring the Hb level to ~9-10 g/dL. Subjects with β-Thal$_M$ will undergo hematopoietic stem cell (HSC) procurement by bone marrow harvest or apheresis after mobilization with filgrastim, a granulocyte-colony stimulating factor (G-CSF), alone or in combination with plerixafor (Mozobil®), as decided by the clinical transplant team. Up to 2 cycles of mobilization with up to 3 apheresis procedures per cycle will be permitted to collect enough peripheral blood mononucleated cells (PBMCs) for this treatment. If sufficient cells are not obtained via apheresis, a bone marrow harvest may be performed. Stem cell mobilization by cytokines is contraindicated in SCD, as it could induce a sickle cell crisis; thus, subjects with severe SCD will undergo bone marrow harvest (see Section 5.2.2). A portion of cells will then be cryopreserved for rescue therapy, and another portion will undergo a cell separation process to isolate CD34+ cells to be used in the transduction with the LentiGlobin BB305 lentiviral vector. Of the transduced cells (LentiGlobin BB305 Drug Substance), a portion will undergo release testing and the remainder will be cryopreserved. The LentiGlobin BB305 Drug Substance is frozen (and stored) in cryopreservative solution in the vapor phase of liquid nitrogen until the thawing date, when the cells are washed and suspended in HSA in the final immediate container to produce the LentiGlobin BB305 Drug Product for administration to subjects. Myeloablation of the subject will be initiated after LentiGlobin BB305 Drug Substance release testing is completed, and the LentiGlobin BB305 Drug Substance is dispositioned for clinical use. After LentiGlobin BB305 Drug Substance is dispositioned for clinical use, subjects will be hospitalized and undergo conditioning with busulfan IV to induce myeloablation, a known prerequisite for engraftment. For conditioning, busulfan will be administered at a dose of 3.2 mg/kg/day for 4 consecutive days via intravenous (IV) infusion. (For subjects weighing <35 kg, the busulfan dose regimen should be adjusted to be given every 6 hours.) The dose and schedule of busulfan IV will be monitored daily during the time period of busulfan IV administration, and may be adjusted based upon busulfan plasma levels in order to maintain appropriate levels for myeloablation (AUC range 1000-1300 μM*min for an every-6-hour dosing regimen, or 4000-5200 μM*min for a daily dosing regimen, targetted as the average exposure over the duration of dosing period). After completion of the 4-day course of busulfan IV, monitoring of busulfan levels will continue daily thereafter until no busulfan is detected. The clinical site can then proceed with the Day 0 evaluations. Day 0 is defined as the time prior to the cell infusion; Day 1 begins with the initiation of the LentiGlobin BB305 Drug Product infusion. On Day 1, after thawing, washing, counting, and assessing viability via trypan blue staining, the LentiGlobin BB305 Drug Product will be administered via IV infusion according to applicable standard operating procedures (SOPs) at the clinical site, with vital signs being monitored concurrently. For autologous cells procured via apheresis, the minimum dose to be administered is 3.0×10^6 CD34+ cells/kg, and for autologous cells procured by bone marrow harvest, the minimum dose to be administered is 2.0×10^6 CD34+ cells/kg. Subjects will be followed daily in the transplant unit for AEs, and laboratory parameters will be followed to monitor bone marrow engraftment. The subject will be discharged from the transplant unit once 1) engraftment occurs (as defined as an absolute neutrophil count [ANC] $\geq 0.5 \times 10^9$/L for 3 consecutive days); 2) and the subject is considered medically stable. If platelet count recovery lags behind ANC recovery, the subject may be discharged with regular complete blood count (CBC) monitoring and platelet transfusions, as determined by the treating physicians on a case-by-case basis. Management of post-transplant transfusions for subjects with SCD will follow the institutional standard of care at the clinical site to achieve total Hb and

	HbS proportions appropriate for each individual subject's clinical status. To achieve this, the transfusion program may be progressively reduced as the contribution of the hemoglobin containing β^{A-T87Q} rises. After discharge, subjects will be followed monthly, at a minimum, for 6 months and thereafter every 3 months for the remainder of the 24 months post-transplant. Evaluations will include routine and special biological testing at regular intervals, collection of AEs and concomitant medications, and evaluation of disease specific biological and clinical parameters. Subjects will then be enrolled in a long-term follow-up protocol with annual evaluations for an additional 13 years post-transplant.
Number of Subjects Planned:	The remaining 7 subjects with severe SCD or β-Thal$_M$ will be enrolled.
Study Population:	The population to be enrolled in this study is those subjects with severe, life-threatening SCD or transfusion-dependent β-Thal$_M$ who are eligible for an allogeneic hematopoietic stem cell transplant (HSCT) but do not have a suitable, willing, 10/10 matched human leukocyte antigen (HLA)-identical sibling donor. Refer to Section 1 for conditions regarding enrollment.
Inclusion Criteria:	All subjects must: 1. Be between 5 and 35 years of age, inclusive. • Adult subjects (between 18 and 35 years of age, inclusive, at the time of consent) must be able to provide written consent. • For pediatric subjects (between 5 and 17 years of age, inclusive, at the time of consent), a competent parent or legal guardian must be able to provide written informed consent. When possible, involvement of the child >7 years of age in the decision is highly recommended, and written assent will be obtained and should be clearly documented. • Subjects aged 5 to 14 years require the approval from the Comité de Surveillance prior to enrollment. 2. Have severe SCD or transfusion-dependent β-Thal$_M$, regardless of the genotype (e.g., β^0, β^+, β^E/β^0, β^S/β^S, β^S/β^0, β^S/β^+), with the diagnosis confirmed by Hb studies. Subjects with transfusion-dependent β-Thal$_M$ must be stable and maintained on an appropriate iron chelation regimen. Transfusion dependence is defined as requiring at least 100 mL/kg/year of packed red blood cells (pRBCs). *(Refer to Section 1 for conditions regarding enrollment based on age and diagnosis.)* 3. Be eligible for allogeneic HSCT based on institutional medical guidelines, but without a matched related donor. 4. Be willing and able, in the Investigator's opinion, to comply with the study procedures outlined in the study protocol. If a pediatric subject, the subject's parent/legal guardian also must be willing and able to comply with the study procedures outlined in the study protocol. 5. Have been treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records, including transfusion history. *Subjects with severe SCD also must:* 6. Have failed to achieve adequate clinical benefit following hydroxyurea treatment with sufficient dosage, for at least 4 months unless this treatment was not indicated or not well tolerated. 7. Have 1 or more of the following poor prognostic risk factors: • Recurrent VOC (at least 2 episodes in the preceding year or in the year prior to start of a regular transfusion program). • Presence of any significant cerebral abnormality on magnetic resonance imaging (MRI) (such as stenosis or occlusions). • Stroke without any severe cognitive disability. • Osteonecrosis of 2 or more joints. • Anti-erythrocyte alloimmunization (>2 antibodies).

	• Presence of sickle cell cardiomyopathy documented by Doppler echocardiography. • Acute chest syndrome (at least 2 episodes) defined by an acute event with pneumonia-like symptoms (e.g., cough, fever [>38.5°C], acute dyspnea, expectoration, chest pain, findings upon lung auscultation, tachypnea, or wheezing) and the presence of a new pulmonary infiltrate. Subjects with a chronic oxygen saturation <90% (excluding periods of SCD crisis) or carbon monoxide diffusing capacity (DLco) less than 60% in the absence of an infection should not be included in the study. 8. Subjects with severe SCD and cerebral vasculopathy (defined by overt stroke; abnormal transcranial Doppler [> 170 cm/sec]; or occlusion or stenosis in the polygone of Willis; or presence of Moyamoya disease) may be enrolled only with approval by the Comité de Surveillance after review of safety and efficacy data from ≥2 SCD subjects without cerebral vasculopathy treated with LentiGlobin BB305 Drug Product.
Exclusion Criteria:	All subjects must not meet any of the following: 1. Availability of a willing 10 /10 matched HLA-identical sibling hematopoietic cell donor, unless recommendation for enrollment is provided by the Comité de Surveillance following a review of the case. 2. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV-1 and HIV-2), human T-lymphotrophic virus-1 or 2 (HTLV-1, HTLV-2), vesicular stomatitis virus G (VSV -G) antibody. 3. Clinically significant, active bacterial, viral, fungal, or parasitic infection. 4. Contraindication to anesthesia for bone marrow harvesting. 5. Any prior or current malignancy, myeloproliferative or immunodeficiency disorder. 6. A white blood cell (WBC) count $<3\times10^9$/L and/or platelet count $<120\times10^9$/L. 7. Receipt of an allogeneic transplant. 8. Receipt of erythropoietin within 3 months before HSCT harvest. 9. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to breast, colorectal, ovarian, prostate, and pancreatic cancers). 10. Diagnosis of significant psychiatric disorder of the subject that could seriously impede the ability to participate in the study. 11. Active relapsing malaria. 12. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile subjects. Females of child-bearing potential must agree to use a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection throughout the 27-month study period. 13. History of major organ damage including: 14. Liver disease, with transaminase levels >3× upper limit of normal. **15.** This observation will not be exclusionary if a liver biopsy shows no evidence of extensive bridging fibrosis, cirrhosis, or acute hepatitis. 16. Histopathological evidence of extensive bridging fibrosis, cirrhosis, or acute hepatitis on liver biopsy. 17. Heart disease, with a left ventricular ejection fraction <25%. 18. Kidney disease with a calculated creatinine clearance <30% normal value. 19. Severe iron overload, which in the opinion of the physician is grounds for exclusion. 20. A cardiac T2* <10 ms by magnetic resonance imaging (MRI). 21. Evidence of clinically significant pulmonary hypertension requiring medical intervention. 22. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician.

	23. Participation in another clinical study with an investigational drug within 30 days of screening. 24. Subjects who have the desire to become a parent within the 27-month study period. 25. Prior receipt of gene therapy. 26. An assessment by the Investigator that the subject or parents of the subject will not comply with the study procedures outlined in the study protocol. 27. Hydroxyurea therapy within 3 months before hematopoietic stem cell collection.
Duration of Subject Participation:	Subjects will participate in this study for a total of approximately 27 months, consisting of an up to 97-day pre-transplant period (consisting of a screening period followed by autologous cell harvest, followed by a waiting period during which the harvested cells are transduced and undergo release testing, followed by 4 days of treatment with busulfan IV, and a single infusion of LentiGlobin BB305 Drug Product) and a 24-month post-transplant evaluation period. Following completion of this study, all subjects will be asked to provide consent to participate in a follow-up study for another 13 years, which will focus on long-term safety, with an emphasis on integration site analysis, and long-term efficacy.
Duration of Study:	The total duration of the study is expected to be approximately 4 years (up to 2 years of recruitment and 2 years of follow-up). Long-term follow-up will be performed under a separate protocol for an additional 13 years (up to a total of 15 years post-transplant).
Concomitant Medications/Therapies	Subjects will be permitted to take their usual medications during the course of the study, with the exception of erythropoietin, which is excluded and should be discontinued 3 months prior to treatment. Hemoglobinopathy treatments will continue unchanged, with deferoxamine or another suitable chelator administered for iron overload, and transfusions given according to the hemoglobin and platelet counts. Iron chelation therapy may be suspended following dosing with LentiGlobin BB305 Drug Product until engraftment, at the discretion of the Principal Investigator. Blood products will be filtered and irradiated, as needed. Management of post-transplant transfusions for subjects with SCD will follow the institutional standard of care at the clinical site to achieve total Hb and HbS proportions appropriate for each individual subject's clinical status. To achieve this, the transfusion program may be progressively reduced as the contribution of the hemoglobin containing β^{A-T87Q} rises.
Test Product, Dose and Mode of Administration:	LentiGlobin BB305 Drug Product (autologous CD34+ hematopoietic stem cells transduced with LentiGlobin BB305 lentiviral vector encoding the human β^{A-T87Q}-globin gene and suspended in HSA (5% Albunorm™) in the final immediate container for the intended medical use). All subjects are to receive LentiGlobin BB305 Drug Product on Day 1 via IV infusion according to applicable SOPs, with vital signs being monitored concurrently. For cells procured via apheresis, the minimum dose to be administered is 3.0×10^6 CD34+ cells/kg, and for cells procured by bone marrow harvest, the minimum dose to be administered is 2.0×10^6 CD34+ cells/kg.
Reference Therapy, Dose and Mode of Administration:	Not applicable.

Criteria for Evaluation:	
Safety	• Success and kinetics of HSC engraftment. • Incidence of transplant-related mortality through 100 days post-treatment. • Overall survival. • Detection of vector-derived RCL in any subject. • Characterization of events of insertional mutagenesis leading to clonal dominance or leukemia. • Monitoring of laboratory parameters and frequency and severity of clinical AEs, as assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03.
Efficacy	The effects on the expression of disease-specific biological parameters and clinical events, as measured by: *For all subjects:* • Red blood cell (RBC) transfusion requirements (measured in milliliters [mL] per kilogram [kg]) per month and per year post-transplant. • Number of total in-patient hospitalization days (post-transplant discharge) at 6, 12, and 24 months. *For severe SCD subjects only:* • Number of VOC or acute chest syndrome events at 6, 12, and 24 months. • Evaluation of changes in the nature or frequency of the subject-specific main inclusion criteria.
Pharmacokinetics	Not applicable.
Pharmacodynamics	To quantify both gene transfer efficiency and expression by measuring the following: • Therapeutic globin expression, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin in whole blood, as well as the amount of $\beta^{A\text{-}T87Q}$-globin as a fraction of all β-chains in whole blood. • Average VCN in cell populations from peripheral blood and bone marrow containing the integrated LentiGlobin BB305 lentiviral vector.
Statistical Methods:	All subjects receiving any part of at least 1 injection of the conditioning agent busulfan IV prior to infusion with LentiGlobin BB305 Drug Product will be evaluated for safety. The safety analyses will include evaluation of the incidence of treatment-emergent AEs by preferred term and body system, coded using the Medical Dictionary for Regulatory Activities (MedDRA). Laboratory measures of routine hematology and chemistry parameters will be compared with their corresponding normal ranges, and the incidence of abnormally high and of abnormally low laboratory values will be calculated for each relevant protocol-specified laboratory test. Efficacy outcome measures will be evaluated using descriptive statistics. For categorical variables, summary tabulations of the number and percentage within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the mean, median, standard deviation, minimum and maximum values will be presented. Two-sided 90% confidence intervals will be calculated as appropriate. For clinical events and transfusion requirements, each subject will serve as their own control in that 2 years of pre-transplant hospitalization and biological data will be compared with post-transplant values. No imputation will be performed for missing data elements. For disease-specific biological parameters and clinical events, including transfusion requirements and number of total hospitalization days at 6, 12, and 24 months, baseline will be defined as the average of these parameters over the 2 years prior to study entry. For other change from baseline analyses, baseline will be defined as the value closest to but prior to transplant.

LIST OF ABBREVIATIONS

Abbreviation	Definition
β-HCG	β-Human chorionic gonadotropin
β-$Thal_M$	β-Thalassemia Major, Cooley's anemia
Ab	Antibody
ACTH	Adrenocorticotropic hormone
AE	Adverse event
Afssaps	Agence française de sécurité sanitaire des produits de santé
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
ANSM	Agence nationale de sécurité du médicament et des produits de santé
AST	Aspartate aminotransferase
AUC	Area under the curve
BFU-E	Burst forming units-erythroid
bp	Base pair
BUN	Blood urea nitrogen
Ca	Calcium
CBC	Complete blood count
cHS4	Chromatin insulator DNase I hypersensitive site 4 of the chicken β-globin locus
Cl	Chloride
CMV	Cytomegalovirus
CPP	Comité de protection des personnes
CRE	Conditioning-related event
CRF	Case report form
CTA	Clinical Trial Application

Abbreviation	Definition
DLco	Carbon monoxide diffusing capacity
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
G-CSF	Granulocyte-colony stimulating factor
GGT	Gamma-glutamyl transferase
GMP	Good Manufacturing Practices
GVHD	Graft-versus-host disease
Hb	Hemoglobin
HbA	"Adult" hemoglobin $\alpha_2\beta^A{}_2$
HBcAb	Hepatitis B core antibody
HbS	Sickle hemoglobin S
HBsAb	Hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HCO_3	Bicarbonate
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
HSA	Human serum albumin

Abbreviation	Definition
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
HSV	Herpes simples virus
HTLV-1	Human T-lymphotrophic virus-1
HTLV-2	Human T-lymphotrophic virus-2
ICH	International Conference on Harmonisation
IgG	Immunoglobulin G
IMP	Investigational medicinal products
ITT	Intent-to-treat
IV	Intravenous
K	Potassium
kg	Kilogram
LAM-PCR	Linear amplification-mediated polymerase chain reaction
LCR	Locus control region of the β-globin gene locus
LDH	Lactic dehydrogenase
LH	Luteinizing hormone
LTR	Long terminal repeat
LVEF	Left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
MOI	Multiplicity of infection
MPBSC	Mobilized peripheral blood stem cells
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
Na	Sodium

Abbreviation	Definition
NCI CTCAE	National Cancer Institute Common Terminology Criteria For Adverse Events
NK	Natural killer
PBL	Peripheral blood leukocyte
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PNH	Paroxysmal nocturnal hemoglobinuria
PT	Prothrombin time
PTT	Partial thromboplastin time
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell
RCL	Replication-competent lentivirus
RNA	Ribonucleic acid
RPR	Rapid plasma reagin
RTCGD	Retrovirus Tagged Cancer Gene Database
RT-PCR	Reverse transcriptase-polymerase chain reaction
SAE	Serious adverse event
SC	Subcutaneously
SCD	Sickle cell disease
SCGM	Stem Cell Growth Media
SCID-X1	X-linked severe combined immunodeficiency
SIN	Self-inactivating
SOP	Standard operating procedure
T3	3,5,3′-triiodothyronine
T4	Thyroxine
TNC	Total Nucleated Cells

Abbreviation	Definition
TP	Transplant population
TSH	Thyroid-stimulating hormone
ULN	Upper limit of normal
US	United States
VCN	Vector copy number
VOC	Vaso-occlusive crises
VSV-G	Vesicular stomatitis virus G
VZV	Varicella zoster virus
WBC	White blood cell
X-CGD	X-linked chronic granulomatous disease

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1 INTRODUCTION

This study originally was initiated as Protocol LG001. Enrollment began after appropriate approvals were obtained from the Agence nationale de sécurité du médicament et des produits de santé (ANSM) (formerly the Agence française de sécurité sanitaire des produits de santé [Afssaps]) and the Comité de protection des personnes (CPP) in 2005.

Per Protocol LG001, a total of 10 subjects were to be treated. After treatment of the first 3 subjects, the subsequent 7 subjects may have been enrolled and treated only after 1) the initial 3 subjects treated completed 2 months of post-treatment follow-up; and 2) a review of safety and efficacy data from these 3 subjects was performed by the Comité de Surveillance. Enrollment of the remaining 7 subjects was to commence if 1 of the initial 3 subjects treated showed signs of efficacy (at least 5% expression levels of the therapeutic transgene in circulating peripheral burst forming units-erythroid [BFU-E] and/or reticulocytes), as determined by the Comité de Surveillance.

The initial 3 subjects, all with β-thalassemia major (β-Thal$_M$), were treated with drug product under Protocol LG001 between September 2006 and November 2011; the third subject treated (Subject PPD) completed 24 months of follow-up in January 2014. Consequently, the Comité de Surveillance convened on 19 March 2012; based on the limited data from the 3 subjects treated, the Comité de Surveillance recommended a more conservative approach to enrolling the remaining 7 subjects. The following conditions were instituted:

- Subjects with severe sickle-cell anemia (SCD) are to be enrolled, as initially planned, in addition to subjects with β-Thal$_M$.
- The next 2 subjects enrolled in the study, either with severe SCD or β-Thal$_M$, must be aged ≥15 years.
- After a subject with β-Thal$_M$ is treated, the next subject with β-Thal$_M$ can be treated only after neutrophil engraftment (absolute neutrophil count [ANC] ≥0.5×10^9/L for 3 consecutive days) is achieved in the previous subject.
- If no serious, unexpected, LentiGlobin®BB305 Drug Product-related adverse events occur in the first subject enrolled with severe SCD through 3 months post-transplant and the subject has engrafted and been safely discharged from hospital, the second subject may be treated. Once these criteria have been met for the second subject also, additional subjects with severe SCD may be enrolled at any time thereafter.
    - Subjects with SCD and cerebral vasculopathy may be enrolled only with approval by the Comité de Surveillance after review of safety and efficacy data from ≥2 SCD subjects without cerebral vasculopathy treated with LentiGlobin BB305 Drug Product in this study.
- A pediatric subject with severe SCD who does not have a suitable, willing, 10/10 matched, human leukocyte antigen (HLA)-identical sibling donor may be enrolled at any time, after review of the case and approval by the Comité de Surveillance.

Originally, under Protocol LG001, the LentiGlobin HPV569 lentiviral vector was utilized. Subsequently, bluebird bio improved the LentiGlobin lentiviral vector transduction efficiency; the modified vector, known as LentiGlobin®BB305, to expected to show improved integrated provirus stability, and safety (NC-11-001-R; NC-11-004-R). In November 2011, ANSM requested that bluebird bio file a new Clinical Trial Application (CTA) for LentiGlobin BB305 Drug Product; it was understood that a new protocol would be required. It was agreed to with ANSM that the new protocol would be the continuation of LG001. Consequently, bluebird bio generated the current protocol, Protocol HGB-205, based on the former Protocol LG001. The 3 subjects treated in LG001 were enrolled in the long-term follow-up Protocol, LTF-303. The remaining planned 7 subjects will be enrolled and treated with LentiGlobin BB305 Drug Product under Protocol HGB-205.

The objectives and design of Protocols HGB-205 and LG001 are the same; administrative and other changes made from Protocol LG001 to Protocol HGB-205 are summarized in a separate document.

1.1 The β-hemoglobinopathies as Disease Targets for Gene Therapy

The β-hemoglobinopathies are the most prevalent genetic diseases of humans worldwide and are caused by mutations in the β-globin gene (Ferrone, et al., 2000; Nagel et al., 2000; Olivieri, 1999). While these diseases are rare in the EU, they represent a significant health care burden given the intensive management needed to care for affected patients. The 2 principal subject groups are those with SCD and β-Thal$_M$ (Ferrone et al., 2000). β-Thal$_M$, often referred to as Cooley's anemia, is caused by mutations in both alleles of the β-globin genes that result in a failure to produce functional β-globin, resulting in life-threatening anemia with transfusion-dependency and, as a consequence, a long-term risk of lethal iron overload (Olivieri, 1999). Genetically, these patients are either true homozygotes or double heterozygotes for 2 different mutations, 1 in each gene. The most severe form occurs when no or little expression of β-globin is present.

SCD is caused by a point mutation within the coding region of the β-globin genes that results in the production of an abnormal globin chain (β^S-globin) (Ferrone, et al., 2000; Nagel et al., 2000). The mutated β^S-globin causes hemoglobin (Hb) to polymerize abnormally within the red blood cells (RBCs) of patients, resulting in anemia and major tissue damage (Nagel et al., 2000; Platt et al., 1994; Prasad et al., 2003). Approximately 20% of SCD patients have a very severe form of the disease (Ferrone, et al., 2000; Nagel et al., 2000; Olivieri, 1999; Platt et al., 1994). Overall, the severe forms of SCD and β-Thal$_M$ remain devastating diseases with an unmet medical need. Indeed, the life expectancy of patients with β-Thal$_M$ and the severe forms of SCD is severely shortened, despite currently available treatments (Olivieri, 1999; Platt et al., 1994).

Allogeneic hematopoietic stem cell transplant (HSCT) can cure patients with SCD or β-Thal$_M$, and specific inclusion and exclusion criteria to maximize the success rate and minimize the risk of the procedure are now well established (Platt et al., 1994; Lucarelli et al., 1993; Locatelli et al., 2003; Vermylen et al., 2003). However, only approximately 25% of these patients have a suitable, sibling, HLA-matched donor, and allogeneic HSCT is associated with 5-20% mortality, depending upon the risk category, in large part due to complications from graft versus host disease (GVHD) (Platt et al., 1994; Lucarelli et al., 1993; Locatelli et al., 2003; Vermylen et al., 2003).

The use of intra-familial, partially-matched donors or matched, unrelated donors presents a severe safety risk to patients, and is infrequently employed for SCD or β-Thal$_M$. The population to be enrolled in this study are thus, those patients with severe, life-threatening SCD or β-Thal$_M$, who would be eligible for an allogeneic HSCT, but do not have a HLA-matched sibling donor.

A key element of the rationale for the proposed study is based on the fact that extensive preclinical studies have demonstrated that transfer of the human $\beta^{A\text{-}T87Q}$-globin gene under the control of the β-globin promoter and enhancer elements of the β-globin locus control region (LCR) into hematopoietic stem cells (HSCs) by means of lentiviral vectors reliably results in long-term correction of SCD and β-Thal in relevant mouse models (Pawliuk et al., 2001; Imren et al., 2002; Oh et al., 2004). Similarly, effective ex vivo globin gene transfer into human CD34+ HSCs was observed (Imren et al., 2004). The $\beta^{A\text{-}T87Q}$-globin gene encodes a β-globin variant that has been shown to exhibit strong anti-sickling properties while otherwise maintaining the same functionality as wild-type β-globin. In addition, the in vivo protein level expressed by this variant can be quantified by high performance liquid chromatography (HPLC) and distinguished from that of wild-type β-globin expressed from the endogenous genes or derived from transfused red blood cells (RBCs) (Pawliuk et al., 2001). This property is important for demonstrating a biological effect in subjects with β-Thal$_M$ by detection of engraftment with cells expressing the transferred gene, since subjects may be transfused with normal hemoglobin $\alpha_2\beta^A{}_2$ (HbA)-containing blood during the study.

Treatment of β-hemoglobinopathies with gene therapy is also expected to avoid the major risk of GVHD associated with allogeneic HSCT, since the CD34+ cells are autologous. In addition, in order to reduce complications of immunosuppression associated with the conditioning regimen necessary for allogeneic HSCT, the proposed protocol is designed to use the single agent intravenous (IV) busulfan for myeloablative bone marrow conditioning, and does not rely on immunosuppressive agents such as cyclophosphamide or fludarabine that are required for allogeneic transplant.

Data from nonclinical studies and from clinical studies in subjects with SCD or β-Thal indicate that partial chimerism with the transduced HSCs (animal studies) or allogeneic HSCs (human studies) results in quasi-complete peripheral reconstitution with corrected RBCs, because of the extended life-span of the corrected cells (Lucarelli et al., 1993; Vermylen, 2003; Pawliuk et al., 2001; Imren et al., 2004; Oh et al., 2004; Imren et al., 2002). This is an important observation which underscores the feasibility of the proposed clinical study. Indeed, bone marrow reconstitution with as low as 11% of HSCs containing the therapeutic gene achieves functional and therapeutically beneficial hematopoiesis (Lucarelli et al., 2001; Walters et al., 2001; Alfred & Vora, 2011).

1.2 LentiGlobin BB305

1.2.1 LentiGlobin BB305 Lentiviral Vector

Based on the pioneering work of PPD, MD, PhD, to develop therapeutic applications of gene therapy for β-globin disorders, and in order to provide an effective and safer alternative to allogeneic HSCT, bluebird bio is developing LentiGlobin BB305 Drug Product

(autologous CD34+ hematopoietic stem cells transduced with LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene) for the treatment of $\beta\text{-Thal}_M$ and SCD.

LentiGlobin BB305 lentiviral vector is a replication defective, self-inactivating (SIN), third-generation human immunodeficiency virus-type 1 (HIV-1) based lentiviral vector pseudotyped with the vesicular stomatitis virus-glycoprotein (VSV-G) envelope protein, carrying the human β-globin gene with a single modification at codon 87 [β^{A87} Thr:Gln ($\beta^{A\text{-}T87Q}$)]. The therapeutic $\beta^{A\text{-}T87Q}$ globin gene is under the transcriptional control of the erythroid-specific human β-globin promoter and erythroid-specific enhancer elements (DNase I hypersensitive sites HS2, HS3, and HS4) of the β-globin LCR.

Protocol LG001 utilized an initial lentiviral vector, LentiGlobin HPV569. bluebird bio subsequently developed the new LentiGlobin BB305 vector, which has improved transduction efficiency, and is expected to show improved integrated provirus stability, and safety.(NC-11-001-R; NC-11-004-R) Schematics of LentiGlobin HPV569 lentiviral vector used in Protocol LG001, the improved LentiGlobin BB305 lentiviral vector, and LentiGlobin HPV524 lentiviral vector, the vector from which LentiGlobin BB305 was derived, are presented in Figure 1-1.

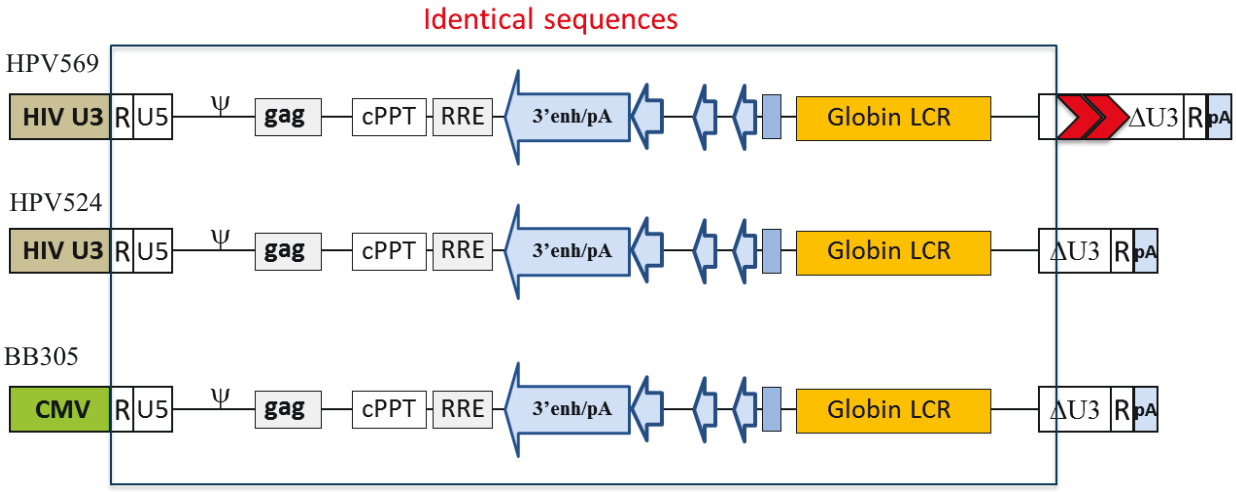


Figure 1-1 Changes Made between Lentiviral Vector LentiGlobin HPV569 and LentiGlobin BB305 Lentiviral Vector

A majority of the integrated provirus sequence of LentiGlobin BB305 lentiviral vector is identical to that of LentiGlobin HPV569 lentiviral vector. With respect to HPV569, the BB305 provirus differs only in that the 2 copies of 250-bp core cHS4 insulator embedded in the SIN U3 of HPV569 have been deleted. The sequences of the $\beta^{A\text{-}T87Q}$-globin gene expression cassette and the vector backbone, which contains the cPPT, RRE, and SIN U3 region, are identical. Of equal importance, the internal globin promoter and LCR, which together drive expression exclusively in the erythroid lineage (Cavazzana-Calvo et al., 2010) remain unchanged. The 5′ human immunodeficiency virus (HIV) U3 promoter/enhancer has been replaced with a cytomegalovirus (CMV) promoter/enhancer. However, the U3 long terminal repeat (LTR) sequences are not packaged as part of the viral ribonucleic acid (RNA) and are not present in the integrated provirus. Also, since CMV is a constitutive promoter, vector production is no longer HIV Tat-dependent,

and a 4 plasmid transfection process (rather than 5, as for LentiGlobin HPV569 lentiviral vector) is used to produce LentiGlobin BB305 lentiviral vector.

The decision to delete the insulator sequences was driven by data generated from 1 subject (Subject PPD) treated under Protocol LG001, which indicated that 1 of the copies of the core insulator in the U3 region was frequently deleted. As described in Cavazzana-Calvo, et al. (Cavazzana-Calvo et al., 2010), the lentiviral vector used to transduce CD34+ cells for Subject PPD (LentiGlobin HPV569 lentiviral vector) was a SIN vector, with 2 copies of the 250-base pair (bp) core of the cHS4 chromatin insulator inserted in the U3 LTR region. Tests conducted on this subject's transduced cells demonstrated that the integrated vector was intact, except for the presence of only 1 of the 2 250-bp cHS4 insulator cores at each end. Southern blot analysis of transduced cells indicated that up to one-third of integrated vector copies contained a deletion of 1 of the cHS4 cores within the 3′ U3 LTR, and two-thirds of integrated vector copies contained a deletion of 1 of the cHS4 cores within the 5′ U3 LTR. Data from a more recent publication (Uchida, et al, 2011) demonstrated that inclusion of HS4 elements lowers the viral titers, reduces efficiency of transduction, and produces minimal effect on transgene expression among human hematopoietic cell in vitro and in vivo.

In addition, polymerase chain reaction (PCR) was performed across the insulator regions to determine whether in transduced mice, as in the treated subject, recombination of the insulator sequences had occurred leading to loss of 1 of the copies. Ronen, et al, demonstrated that in all mice in their experiment, a single copy of the insulator was the major form detected at both the 5′ and 3′ LTRs, and in 3 mice, the double copy form was barely detectable. Thus, the insulator was mostly lost from the vector in mice as well (Ronen, et al, 2011).

A recent publication reported that the flanking copy of the insulator does not have the proposed insulation effect and suggested that the cHS4 insulators have negligible barrier function in hematopoietic cell progeny transduced with lentiviral vectors (Uchida, et al, 2011). CCI [redacted]

Further, the overexpressed HMGA2 messenger ribonucleic acid (mRNA) transcripts from Subject PPD 's cells were sequenced (Cavazzana-Calvo et al., 2010). The HMGA2 mRNA was truncated by alternative splicing of the third intron of HMGA2, with a cryptic 3' splice signal (GTAT(C)6AG) located within the cHS4 insulator core and cleavage/polyadenylation within the adjacent region of the 5′ LTR. CCI [redacted]

CCI [redacted]

1.2.1.1 Rationale for the Globin Gene Used

The β-globin gene used in this study is the wild-type human β-globin gene with 1 single modification at amino-acid position 87 [β^{A87} Thr:Gln ($\beta^{A\text{-}T87Q}$)]. This change encodes 1 amino-acid residue from the normal human δ- and γ-globin genes within a stretch of amino acids highly conserved in both human β^{A}-, δ- and γ- globin chains. This ensures the lack of untoward immunogenicity, which is less likely in the presence of bone marrow chimerism with transduced HSCs, since expression of foreign protein in HSCs generally results in a state of long-term tolerance for the expressed protein, whereas this would not be the case if the foreign protein was expressed in peripheral tissues (Andersson et al., 2003; Down and White-Scharf, 2003). Furthermore, expressing the wild-type human β-globin protein in β-Thal would raise similar questions, since these subjects were born without expression of normal β-globin (Olivieri, 1999). As corroborating evidence, bluebird bio has shown that mice transplanted after busulfan conditioning without induced immunosuppression with a lentiviral vector expressing either human β^{A}- or $\beta^{A\text{-}T87Q}$-globin do express these human proteins long-term without detectable evidence of rejection (Pawliuk et al., 2001; Imren et al., 2002).

This gene has several advantages over the normal β-globin gene for the gene therapy of both SCD and $\beta\text{-Thal}_{M}$:

- $\beta^{A\text{-}T87Q}$-globin has been shown in vitro and in vivo to have anti-sickling properties similar to those of the human γ-globin chain and, thus, should be more active in preventing sickling in SCD subjects than the wild-type β-globin gene, which does not actively inhibit sickling (Pawliuk et al., 2001).
- Both $\beta^{A\text{-}T87Q}$-globin and Hb containing this chain can be distinguished from wild-type human β-globin and HbA by HPLC (Pawliuk et al., 2001; Imren et al., 2002). This is especially important in this study, as the ability to accurately detect the engraftment of transduced cells will not be impaired in subjects who require transfusion with normal HbA-containing blood post-transplant.

1.2.1.2 Prevention of Vector-related Toxicity

Lentiviral vectors are retroviruses which integrate into the chromosomal deoxyribonucleic acid (DNA) of genetically modified cells (transduced cells). The risk of this type of vector is insertional mutagenesis that can activate or inactivate a gene in the chromosomal DNA of hematopoietic stem cells or cells derived from these cells. The risk of mutagenesis for this study is limited to the hematopoietic cell compartment, since the lentiviral vector is designed not to mobilize after integration into the chromosomal DNA of HSCs. The activation of an oncogene by the integrated provirus can lead to myelodysplasia and leukemogenesis of the hematopoietic system, which are long-term risks of this study.

Clinical trials for severe combined immunodeficiency due to adenosine deaminase deficiency (Aiuti, et al., 2009) or IL2RG-deficiency (X-linked severe combined immunodeficiency [SCID-X1]) (Hacein-Bey-Abina, et al., 2010; Gaspar, et al., 2004) have shown that gene therapy with retrovirally transduced HSC results in long-term clinical efficacy. However, gene modification by γ-retroviral vectors has resulted in insertional mutagenesis in the clinical trials

for SCID-X1 (Hacein-Bey-Abina, et al., 2008; Howe, et al., 2008) and chronic granulomatous disease (Stein, et al., 2010). Consequent to insertional mutagenesis, 5 of 20 subjects with SCID-X1 treated with re-injection of HSCs genetically modified ex vivo with a γ-retroviral vector derived from murine Moloney leukemia virus have developed an abnormal proliferation of T lymphocytes associated with the development of T-cell leukemia. One of these 5 subjects succumbed to leukemia; the remaining subjects have been treated, are in remission, and maintain transgene correction of their immunodeficient state. In the gene therapy study to treat X-linked chronic granulomatous disease (X-CGD), two young adults with X-CGD were treated with autologous HSC transduced with g-retroviral vector expressing the $gp91^{phox}$ gene. Insertional activation of *MDS1–EVI1,* the (*PRDM16*) and SET-binding protein 1 (*SETBP1*) triggered a threefold to fivefold increase in the number of gene-transduced cells in the peripheral blood of both subjects, leading to oligoclonal hematopoiesis, monosomy 7 and the development of a myelodysplastic syndrome.(Stein et al., 2010) It also has been reported that 2 children with Wiskott-Aldrich Syndrome have developed leukemia after transplantation of HSCs genetically-modified with a γ-retroviral vector (Boztug et al., 2010).

The different nature of the LentiGlobin BB305 lentiviral vector should minimize but not completely eliminate the risk of oncogenesis due to insertional mutagenesis. Unlike the γ-retroviral vectors that led to leukemia, the LentiGlobin BB305 lentiviral vector is considered to be safer since it carries a SIN LTR and is a lentiviral vector (which integrates less frequently near transcription start sites of genes compared with γ-retroviruses). Thus, SIN lentiviral vectors, such as LentiGlobin BB305 lentiviral vector, may represent a substantial improvement in terms of safety profile while offering a more robust transduction and better transcriptional control of transgene expression in HSC (Montini, et al., 2006). Indeed, unlike the γ-retroviral vectors that generated leukemias in the X-SCID and CGD trial, the LentiGlobin BB305 lentiviral vector expression is restricted specifically in the erythroid lineage, and is therefore less likely to generate oncogenesis in more sensitive early hematopoietic progenitors. In addition, SIN lentiviral vectors provide significant safety improvements over γ-retroviral vectors used in earlier studies (Rivière et al., 2012). SIN lentiviral vectors lack the strong enhancer/promoter LTR sequences of gamma-retroviral vectors, and unlike gamma-retroviral vectors they do not preferentially integrate near gene promoter regions. Therefore, lentiviral vectors are less likely to transactivate oncogenes, and have demonstrated a significantly curtailed probability of oncogenic transformation in vitro and in vivo.

1.2.2 Nonclinical Data

Nonclinical studies have been performed to support the use of LentiGlobin BB305 lentiviral vector in clinical studies; refer to the Investigator's Brochure for summaries of study results.

The transduction efficiency of late and early clonogenic progenitors derived from transduced SCD (β^S/β^{+Thal}) CD34+ cells is higher with LentiGlobin lentiviral vectors BB305 and HPV524 than with HPV569 (NC-11-004-R). There was a trend of increased proportion of genetically modified progenitors with LentiGlobin lentiviral vectors BB305 and HPV524 in comparison with HPV569, but this difference was not statistically significant. At a multiplicity of infection (MOI) of 25, the total amount of therapeutic globin in erythroid colonies was significantly higher with LentiGlobin

lentiviral vectors BB305 or HPV524 than with HPV569. The frequency of late (CFC) and early (LTC-IC) progenitors was similar with the LentiGlobin lentiviral vectors and the control (Mock) transduction. Overall, LentiGlobin lentiviral vector BB305 demonstrated improved transduction efficiency, as measured by VCN and globin expression in transduced cells.

The oncogenic potential of lentiviral constructs were analyzed in 2 independent in vitro immortalization assays (Medizinische Hochschule Hannover, Technical Report NC-12-016-R). Compared to the positive control vectors RSF91.GFPgPRE and RRL.PPT.SF.eGFP.pre*, which have known in vivo and in vitro oncogenic potential, the test vectors (LentiGlobin lentiviral vectors HPV569, HPV524, and BB305) showed a strongly reduced risk of in vitro immortalization of murine HSCs and, hence were considered to be significantly less genotoxic. Furthermore, there was no significant cytotoxicity associated with high titer virus transduction with the test vectors.

Overall, results of completed nonclinical efficacy and safety studies clearly demonstrate the comparable safety of LentiGlobin lentiviral vectors BB305 and HPV569 as well as the improved transduction efficiency of LentiGlobin BB305 lentiviral vector compared to LentiGlobin HPV569 lentiviral vector. CCI [redacted] and the comparable safety profile with LentiGlobin HPV569 lentiviral vector used in clinical study LG001 provides support for the clinical use of LentiGlobin BB305 lentiviral vector to transduce autologous CD34+ cells.

1.2.3 Clinical Data

At the time this protocol was initiated there were no clinical data with LentiGlobin BB305 Drug Product. However, 3 subjects had been treated using LentiGlobin HPV569 Drug Product under Protocol LG001: Subject PPD on PPD at a dose of 0.93×10^6 cells/kg; Subject PPD on PPD at a dose of 4.92×10^6 cells/kg; and Subject PPD on PPD at a dose of 4.3×10^6 cells/kg. All 3 treated subjects completed Study LG001 (i.e., completed the 2-year Safety Follow-up Visit; Clinical Study Report LG001,v1, 27 October 2014), and consented to enroll in the longterm follow-up Study LTF-303 (no further treatment with drug product, an additional 13 years of follow-up only, for a total of 15 years follow-up after drug product infusion).

Clinical benefit of treatment with LentiGlobin HPV569 Drug Product was obtained in 1 of the 3 treated subjects. Subject PPD sustained clinical benefit (as evidenced by transfusion-independence) that has been sustained through at least 7 years post-transplant (from receiving LentiGlobin HPV569 Drug Product on PPD through PPD). Transduced cells successfully engrafted in Subject PPD but this subject did not achieve sustained clinical benefit and remains transfusion-dependent at least 2 years post-transplant. Transduced cells did not successfully engraft in Subject PPD; this subject received back-up stem cells for rescue, and remained transfusion dependent at 7 years post-transplant (Cavazzana-Calvo et al., 2010 and 2014; refer to the Investigator's Brochure for more details).

Treatment with LentiGlobin HPV569 Drug Product was well tolerated, with no significant, long-term sequelae of treatment observed. In particular, no subject has developed HIV positivity,

vector-derived RCL, leukemia, or lymphoma. In 1 subject (Subject PPD), partial clonal dominance was observed with a myeloid-based repopulating cell bearing an integrated vector in the third intron of the HMGA2 gene. The presence of the HMGA2 clone has not affected homeostasis of RBCs or nucleated cells in peripheral blood or bone marrow (Cavazzana-Calvo et al., 2010; Cavazzana et al., 2014; refer to the Investigator's Brochure for more details).

1.2.3.1 Vector Integration within the HMGA2 Gene

As discussed in Section 1.2.3, in 1 subject treated with LentiGlobin HPV569 Drug Product (Subject PPD), partial clonal dominance (at less than 3% level of all circulating nucleated cells) of a common myeloid progenitor following the integration of the vector in the third intron of the HMGA2 gene was observed (Cavazzana-Calvo et al., 2010). Refer to the Investigator's Brochure for more details regarding this event in Subject PPD .

Overexpression of a truncated HMGA2 mRNA has been reported in several benign cell proliferations (e.g., lipomas). The findings in Subject PPD bear certain similarities with paroxysmal nocturnal hemoglobinuria (PNH), a clonal yet most often benign hematopoietic expansion. PNH is often associated with multilineage overexpression of a truncated HMGA2 mRNA together with an inactivated PIGA gene. Several subjects with PNH and with complete or extensive HMGA2 clonal dominance have been observed for up to 18 years without evidence of leukemic progression (Murakami et al., 2012), although broad transgenic forced expression of HMGA2 can result in myelodysplasia (Ikeda et al., 2011). This contrasts with overexpression of the full-length HMGA2 mRNA found in several malignancies which have been attributed to the loss of let-7 microRNAs, which control the degradation of multiple oncogenic mRNAs that include MYC and RAS (Viswanathan et al., 2009). Interestingly, several retroviral and lentiviral human gene therapy studies have now reported vector integration within or near HMGA2 without, as of yet, the emergence of any malignancy, including studies in SCID-X1 (Wang et al., 2010), adrenoleukodystrophy (PPD and PPD , personal communication), and Wiskott Aldrich syndrome (Boztug et al., 2010), and MGMT-based selection in glioblastomas (Adair et al., 2011).

1.3 Rationale for Subject Population

Subjects with either β-Thal$_M$ or severe SCD between the ages of 5 and 35 years will be included in this study. The first 3 subjects enrolled under Protocol LG001 were required to be at least 15 years of age and sexually mature at the time of study entry to allow for the option of sperm /testicular tissue or oocyte banking. The rationale for the subject population was determined as follows:

- β-Thal$_M$ and severe SCD have similar therapeutic requirements. In particular, both require blood transfusions over the long-term. Subjects with either disease suffer from the same complications induced by multiple transfusions (e.g., iron overload). Accordingly, there is a convergence of therapeutic goals between the 2 types of transfused subjects, who could benefit from the same therapeutic gene autotransplantation protocol and conditioning, evaluation of therapeutic efficiency (e.g., hematopoietic chimerism, amount of therapeutic Hb, and ultimately reduction or interruption of blood transfusions), and safety assessments.

- In the absence of chronic transfusions, transplantation is indicated for SCD when severe vaso-occlusive crises (VOCs) require frequent hospitalizations, upon repetitive acute chest syndromes, and other complications, as listed in the inclusion criteria (see Section 4.2).
- Based on the efficacy and safety profile of LentiGlobin HPV569 Drug Product seen in the first 3 subjects treated under Protocol LG001, inclusion of pediatric subjects in this study may be enrolled, with conditions, as described in Section 1. The minimum age (5 years) allows for a 2-year observation period pre-transplant and initiation of transplantation procedures. Inclusion of subjects up to 35 years old was set to limit the impact of chronic disease complications on transplant outcomes.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

The primary study objective is to:

- Determine the safety, tolerability, and success of engraftment with autologous CD34+ hematopoietic stem cells transduced with LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene and suspended in human serum albumin (HSA) (Albunorm™ 5%) (i.e., LentiGlobin BB305 Drug Product) after conditioning with busulfan IV in subjects with severe SCD or $\beta\text{-Thal}_M$.

Secondary objectives are to:

- Quantify gene transfer efficiency and expression:
  - Evaluate expression of $\beta^{A\text{-}T87Q}$-globin chain in whole blood.
  - Quantify the hematopoietic chimerism resulting from treatment with LentiGlobin BB305 Drug Product (VCN).
- Measure the effects of transplantation with LentiGlobin BB305 Drug Product on the expression of disease-specific biological parameters and clinical events, including the volume of blood transfusions for both severe SCD and $\beta\text{-Thal}_M$ and, for subjects with severe SCD, the number of VOC and acute chest syndrome (ACS) events in each subject compared with the 2-year pre-treatment period.

2.2 Study Endpoints

2.2.1 Safety Endpoints

Safety endpoints are:

- Success and kinetics of HSC engraftment.
- Incidence of transplant-related mortality through 100 days post-treatment.
- Overall survival.
- Detection of vector-derived RCL in any subject.

- Characterization of events of insertional mutagenesis leading to clonal dominance or leukemia.
- Monitoring of laboratory parameters and frequency and severity of clinical adverse events (AEs), as assessed by the United States (US) National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03.

2.2.2 Efficacy Endpoints

The effects on the expression of disease-specific biological parameters and clinical events will be measured, as follows:

For all subjects:

- RBC transfusion requirements (measured in milliliters [mL] per kilogram [kg]) per month and per year post-transplant.
- Number of total in-patient hospitalization days (post-transplant discharge) at 6, 12, and 24 months.

For severe SCD subjects only:

- Number of VOC or acute chest syndrome events at 6, 12, and 24 months.
- Evaluation of changes in the nature or frequency of the subject-specific main inclusion criteria.

2.2.3 Pharmacodynamic Endpoints

Gene transfer efficiency and expression will be quantified by measurement of the following:

- Therapeutic globin expression, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin in whole blood, as well as the amount of $\beta^{A\text{-}T87Q}$-globin as a fraction of all β-chains in whole blood.
- Average VCN in cell populations from peripheral blood and bone marrow containing the integrated LentiGlobin BB305 lentiviral vector.

Additionally, exploratory methods may be used to evaluate PD endpoints.

3 INVESTIGATIONAL PLAN

3.1 Overall Design and Plan of the Study

This is a Phase 1 / 2, open label, safety, and efficacy study of the administration of LentiGlobin BB305 Drug Product to subjects with severe SCD, including sickle hemoglobin S (HbS)/β-thalassemia and β-$Thal_M$.

This study originally was initiated as Protocol LG001, in which a total of 10 subjects were to be treated with LentiGlobin HPV569 Drug Product. Three subjects with β-$Thal_M$ were were treated with LentiGlobin HPV569 Drug Product under Protocol LG001 between September 2006 and November 2011. bluebird bio subsequently developed the new LentiGlobin BB305 lentiviral vector which has improved LentiGlobin transduction efficiency, and is expected to show

improved integrated provirus stability, and safety. In November 2011, ANSM requested that bluebird bio file a new CTA for LentiGlobin BB305 Drug Product; it was understood that a new protocol would be required that would be the continuation of LG001. Consequently, the remaining 7 subjects will be enrolled and treated with LentiGlobin BB305 Drug Product under the current Protocol HGB-205.

Selected subjects must meet the inclusion criteria, have none of the exclusion criteria, and have provided informed consent.

The study has 4 distinct phases:

- Pre-inclusion Screening, Informed Consent, and Eligibility-determining Phase (Day -97 to Day -61)
- CD34+ Cell Collection, Genetic Modification, and Release of Modified Cells (Day -60 to Day -11)
- Conditioning and Washout Phase (Day -10 through Day 0) and Stem Cell Infusion on Day 1
- Maintenance Phase (Day 1 through Month 24)

Prior to cell harvest, subjects with β-Thal$_M$ may undergo a period of hypertransfusion to maintain a pre-transfusion Hb of ≥ 11 g/dL. For subjects with severe SCD, exchange transfusions will be performed before conditioning in order to reach a sickle hemoglobin HbS proportion lower than 30% and bring the Hb level to ~9-10 g/dL.

Since stem cell mobilization by cytokines is contraindicated in SCD, as it could induce a sickle cell crisis, subjects with severe SCD will undergo bone marrow harvest (see Section 5.2.2). Subjects with β-Thal$_M$ will undergo HSC procurement by apheresis after mobilization with either filgrastim, a G-CSF, alone or in combination with plerixafor (Mozobil®), as decided by the clinical transplant team. Up to 2 cycles of mobilization (with up to 3 apheresis procedures per cycle) will be permitted to collect enough peripheral blood mononucleated cells (PBMCs) for this treatment. If apheresis is not successful, a bone marrow harvest may be performed. A portion of cells will then be cryopreserved for rescue therapy, and another portion will undergo a cell separation process to isolate CD34+ cells to be used in the transduction with the LentiGlobin BB305 lentiviral vector. Of the transduced cells (LentiGlobin BB305 Drug Substance), a portion will undergo release testing and the remainder will be cryopreserved. The cells will be stored in cryopreservative solution in the vapor phase of liquid nitrogen until the thawing date, when the cells are washed and suspended in HSA in the final immediate container to produce LentiGlobin BB305 Drug Product for administration to subjects.

After LentiGlobin BB305 Drug Substance is dispositioned for clinical use, subjects will be hospitalized and undergo conditioning with busulfan IV to induce myeloablation, a known prerequisite for engraftment.

For conditioning, busulfan will be administered at a dose of 3.2 mg/kg/day for 4 consecutive days via IV infusion on Days -10, -9, -8, and -7 pre-transplant. (For subjects weighing <35 kg, the busulfan dose regimen should be adjusted to be given every 6 hours.) The dose and schedule of busulfan IV administration will be monitored daily during the time period of administration, and

may be adjusted based upon busulfan plasma levels in order to maintain appropriate levels for myeloablation (AUC range 1000 - 1300 μM*min for an every-6-hour dosing regimen, or 4000 - 5200 μM*min for a daily dosing regimen, targeted as the average exposure over the duration of dosing period). After completion of conditioning, monitoring of busulfan levels will continue daily thereafter until no busulfan is detected. The clinical site can then proceed with the Day 0 evaluations.

Day 1 is the date of LentiGlobin BB305 Drug Product infusion. LentiGlobin BB305 Drug Product will not be infused into subjects until all release testing is completed, and the LentiGlobin BB305 Drug Substance is dispositioned for clinical use. All subjects are to receive LentiGlobin BB305 Drug Product on Day 1 via IV infusion according to applicable standard operating procedures (SOPs) at the clinical site, with vital signs being monitored concurrently. For cells procured via apheresis, the minimum dose to be administered is 3.0×10^6 CD34+ cells/kg, and for cells procured by bone marrow harvest, the minimum dose to be administered is 2.0×10^6 CD34+ cells/kg.

Subjects will be followed daily in the transplant unit for AEs, and laboratory parameters will be followed to monitor bone marrow engraftment. The subject may be discharged from the transplant unit once 1) engraftment occurs (as defined as an ANC $\geq 0.5 \times 10^9$/L for 3 consecutive days); and 2) and the subject is considered medically stable. If recovery of platelet counts lags behind ANC recovery, the subject may be discharged, with regular complete blood count (CBC) monitoring and platelet transfusions, as decided by the treating physicians on a case-by-case basis. Management of post-transplant transfusions for subjects with SCD will follow the institutional standard of care at the clinical site to achieve total Hb and HbS proportions appropriate for each individual subject's clinical status. To achieve this, the transfusion program will be progressively reduced as the contribution of the hemoglobin containing β^{A-T87Q} rises. After discharge, subjects will be followed monthly, at a minimum, for 6 months and thereafter every 3 months for the remainder of the 24 months post-transplant.

Evaluations will include routine and special biological testing at regular intervals, collection of AEs and concomitant medications, and evaluation of disease specific biological and clinical parameters. After completion of the Month 24 visit, if appropriate consent is obtained, subjects will be followed for an additional 13 years under a separate follow-up protocol. The long-term follow-up study will focus on long-term safety, with an emphasis on integration site analysis, and long-term efficacy.

This study will end when the last subject completes the Month 24 visit or discontinues from the study.

3.2 Justification of the Study Design

Because of the invasive nature of the bone marrow harvest and the potential toxicity of busulfan, no treatment control group is included in this Phase 1/2 open label study. In addition, since 2 years of retrospective hematological data will be collected for each subject in the study, each subject may serve to provide reference data for evaluation of transfusion requirements, number of VOC, and the evaluation of changes in the nature or frequency of the subject-specific main inclusion criterion.

3.3 Rationale for the Dose Selected

The LentiGlobin BB305 Drug Product doses are as follows:

Origin	Minimum dose of CD34+ cells
Mobilized cells:	3.0×10^6 CD34+ cells/kg
Bone marrow harvest:	2.0×10^6 CD34+ cells/kg

The number of cells will be quantified at the end of the transduction procedure, immediately before cryopreservation. Together with results from completed LentiGlobin BB305 Drug Substance release testing, the number of cells and viability will be used to determine if it is appropriate to proceed with myeloablation, or whether further cell harvesting is required. The cell dose actually administered will be determined based on cell number in the LentiGlobin BB305 Drug Product as per release criteria.

The minimum dose of CD34+ cells to be administered is based on accepted safe practice to achieve rapid and robust hematopoietic reconstitution with long-term engraftment after autologous transplantation. Although the theoretical objective is to provide as many CD34+ cells as possible with doses up to 15×10^6 cells/kg (Jillella & Ustun, 2004), this is rarely achievable with mobilized peripheral blood stem cells (MPBSC) and impossible to obtain with bone marrow collection from a single harvest due to inherent limitations in cell collection techniques. In addition, hyper-erythroid marrow seen in the β-hemoglobinopathies potentially reduces the yield of CD34+ cells further.

There is a consensus in the field that the minimum CD34+ dose of MPBSC associated with favorable engraftment kinetics is $\geq2.5\times10^6$ cells/kg (Jillella & Ustun, 2004). While lower cell counts result in engraftment, there are delays in neutrophil and platelet recovery relative to higher doses. Therefore, the minimum dose of MPBSC selected for the study is 3.0×10^6 CD34+ cells/kg. For subjects with SCD, bone marrow harvest remains the preferred modality due to the risk posed by cytokine injection and other mobilizing agents in these subjects (Fitzhugh et al., 2009). With bone marrow, the yield of CD34+ cells is typically lower than with MPBSC (Larghero et al., 2008). This is why the minimum CD34+ dose of bone marrow origin to be administered in this protocol is set at 2.0×10^6 cells/kg. However, every effort will be made to obtain as many CD34+ cells as possible for each subject following a period of hypertransfusion, and all collected CD34+

cells will be submitted to transduction with LentiGlobin BB305 lentiviral vector. For subjects with SCD, up to 3 bone marrow harvests may be performed, at the discretion of the Principal Investigator, even if the minimum cell dose is achieved. Additional bone marrow harvests will not be performed if there is reasonable concern that an additional harvest may jeopardize the safety or well-being of the subject. The Principal Investigator should confirm plans for a third bone marrow harvest with the Sponsor prior to scheduling the procedure. Additional bone marrow harvests beyond 3 may only be performed if the requisite minimum CD34+ dose and minimum cell collection for rescue are not attained, or if one or more bone marrow harvests does not result in production of drug product which meets release criteria.

If LentiGlobin BB305 Drug Substance fails to meet release criteria, then mobilization and apheresis or bone marrow harvest, as applicable, and additional transduction, may be repeated at the discretion of the Principal Investigator with approval of the Sponsor (Medical Monitor; see also Section 5 for further details of HSC collection).

3.4 Treatment Discontinuation and Enrollment Suspension Criteria

3.4.1 Stopping Rules for Individual Subjects for Busulfan IV Conditioning Regimen

- Any Grade 4 clinical or laboratory toxicity (with the exception of the intended hematologic consequences [i.e., pancytopenia] of myeloablation), as determined using the US NCI CTCAE, version 4.03, considered by the Investigator to be at least possibly related to busulfan IV treatment.
- Withdrawal of consent.
- Any medical condition which, in the opinion of the investigators, would put the subject at risk for continuing treatment with busulfan.

If such an event puts the subject at risk with continued busulfan treatment, the Medical Monitor should be contacted immediately. In situations in which busulfan conditioning has not been completed per protocol, LentiGlobin BB305 Drug Product should not be given, and it is likely that rescue therapy with back up cells will be required.

3.4.2 Stopping Rules for LentiGlobin BB305 Drug Product

3.4.2.1 Individual Subject Stopping Rules

- Withdrawal of consent prior to transplantation of LentiGlobin BB305 Drug Product.
- Failure of LentiGlobin BB305 Drug Substance to be dispositioned for clinical use. The Investigator, in consultation with the subject and approval from the Sponsor, may decide to proceed with an additional HSC harvest.
- Any medical condition that, in the opinion of the Investigators, would put the subject at risk for transplantation or active participation in follow-up.

3.4.2.2 Study Stopping Rules

- Death: If there is any death during the study, further enrollment and treatment with study drug product will be temporarily suspended to evaluate whether the cause of death is related to

LentiGlobin BB305 Drug Product. This evaluation will be made by the Comité de Surveillance (see Section 8.2). If it is not related to LentiGlobin BB305 Drug Product, the study may continue. If related to LentiGlobin BB305 Drug Product, the study will be stopped.

- Detection of leukemia/lymphoma due to vector-mediated oncogenesis by insertional mutagenesis. If there is any such event, enrollment will be temporarily suspended and the event assessed by the Comité de Surveillance. After evaluation, the Comité de Surveillance will make a recommendation regarding whether enrollment may recommence.
- Detection of confirmed vector-derived RCL in any subject.
- Failure to achieve reconstitution with transduced cells in 2 subjects, requiring use of backup cells. If there are such events, enrollment will be temporarily suspended and the events assessed by the Comité de Surveillance. After evaluation, the Comité de Surveillance will make a recommendation regarding whether enrollment may recommence.
- Determination of unexpected, clinically significant, or unacceptable risk to subjects (e.g., development of LentiGlobin BB305 Drug Product-related Grade 3 or 4 toxicities in at least 3 subjects). In such cases, enrollment will be temporarily suspended and the case(s) evaluated. After evaluation of the case(s), the Investigators and Sponsor, in consultation with the Comité de Surveillance, will determine whether enrollment may continue.

4 STUDY POPULATION

This study will be performed in subjects selected from a population with at least 2 years of follow-up at a specialized center. Selected subjects must meet the inclusion and exclusion criteria and have given their informed consent. Subjects who are not surgically sterile or females who are not postmenopausal must use reliable methods of birth control for the duration of the study. If the number of eligible subjects is greater than the number of subjects to be enrolled, lots will be drawn to determine entry into the study.

Refer to Section 1 for conditions regarding enrollment.

4.1 Number of Subjects

A total of 10 subjects with β-Thal$_M$ or severe SCD were planned to be treated in Study LG001. Three subjects were treated under Study LG001; the remaining 7 subjects will be enrolled and treated under the current protocol, Study HGB-205 (see Section 1).

4.2 Subject Inclusion Criteria

1. Be between 5 and 35 years of age, inclusive.
    - Adult subjects (between 18 and 35 years of age, inclusive, at the time of consent) must be able to provide written consent.
    - For pediatric subjects (between 5 and 17 years of age, inclusive, at the time of consent), a competent parent or legal guardian must be able to provide written informed consent. When possible, involvement of the child >7 years of age in the decision is highly recommended, and written assent will be obtained and should be clearly documented.

- Subjects aged 5 to 14 years require the approval from the Comité de Surveillance prior to enrollment.

2. Have severe SCD or transfusion-dependent β-Thal$_M$, regardless of the genotype (e.g., β^0, β^+, β^E/β^0, β^S/β^S, β^S/β^0, β^S/β^+), with the diagnosis confirmed by hemoglobin studies. Subjects with transfusion-dependent β-Thal$_M$ must be stable and maintained on an appropriate iron chelation regimen. Transfusion dependence is defined as requiring at least 100 mL/kg/year of packed RBCs.

(Refer to Section 1 for conditions regarding enrollment based on age and diagnosis.)

3. Be eligible for allogeneic HSCT based on institutional medical guidelines, but without a matched related donor.
4. Be willing and able, in the Investigator's opinion, to comply with the study procedures outlined in the study protocol. If a pediatric subject, the subject's parent/legal guardian also must be willing and able to comply with the study procedures outlined in the study protocol.
5. Have been treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records, including transfusion history.

Subjects with severe SCD also must:

6. Have failed to achieve adequate clinical benefit following hydroxyurea treatment with sufficient dosage, for at least 4 months, unless this treatment was not indicated or not well tolerated.
7. Have 1 or more of the following poor prognostic risk factors:
    - Recurrent VOC (at least 2 episodes in the preceding year or in the year prior to start of a regular transfusion program).
    - Presence of any significant cerebral abnormality on magnetic resonance imaging (MRI) (such as stenosis or occlusions).
    - Stroke without any severe cognitive disability.
    - Osteonecrosis of 2 or more joints.
    - Anti-erythrocyte alloimmunization (>2 antibodies).
    - Presence of sickle cell cardiomyopathy documented by Doppler echocardiography.
    - Acute chest syndrome (at least 2 episodes) defined by an acute event with pneumonia-like symptoms (e.g., cough, fever [>38.5°C], acute dyspnea, expectoration, chest pain, findings upon lung auscultation, tachypnea, or wheezing) and the presence of a new pulmonary infiltrate. Subjects with a chronic oxygen saturation <90% (excluding periods of SCD crisis) or carbon monoxide diffusing capacity (DLco) less than 60% in the absence of an infection should not be included in the study.

8. Subjects with severe SCD and cerebral vasculopathy (defined by overt stroke; or abnormal transcranial Doppler [> 170 cm/sec]; or occlusion or stenosis in the polygone of Willis; or the presence of Moyamoya disease) may be enrolled only with approval by the Comité de Surveillance after review of safety and efficacy data from ≥2 SCD subjects without cerebral vasculopathy treated with LentiGlobin BB305 Drug Product.

4.3 Subject Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for inclusion in this study.

1. Availability of a willing, 10 /10-matched HLA-identical sibling hematopoietic cell donor, unless recommendation for enrollment is provided by the Comité de Surveillance following a review of the case.
2. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV1 or HIV-2), human T-lymphotrophic virus-1 or -2 (HTLV-1 or HTLV-2), VSV-G antibody.
3. Clinically significant, active bacterial, viral, fungal, or parasitic infection.
4. Contraindication to anesthesia for bone marrow harvesting.
5. Any prior or current malignancy, myeloproliferative or immunodeficiency disorder.
6. A white blood cell (WBC) count $<3\times10^9$/L and/or platelet count $<120\times10^9$/L.
7. Receipt of an allogeneic transplant.
8. Receipt of erythropoietin within 3 months before HSCT harvest.
9. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to breast, colorectal, ovarian, prostate, and pancreatic cancers).
10. Diagnosis of significant psychiatric disorder of the subject that could seriously impede the ability to participate in the study.
11. Active relapsing malaria.
12. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile subjects. Females of child-bearing potential must agree to use a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection throughout the 27-month study period.
13. History of major organ damage including:
    - Liver disease, with transaminase levels >3× upper limit of normal.
        - This observation will not be exclusionary if a liver biopsy shows no evidence of extensive bridging fibrosis, cirrhosis, or acute hepatitis.
    - Histopathological evidence of extensive bridging fibrosis, cirrhosis, or acute hepatitis on liver biopsy.
    - Heart disease, with a left ventricular ejection fraction <25%.
    - Kidney disease with a calculated creatinine clearance <30% normal value.

- Severe iron overload, which in the opinion of the physician is grounds for exclusion.
- A cardiac T2* <10 ms by MRI.
- Evidence of clinically significant pulmonary hypertension requiring medical intervention.

14. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician.
15. Participation in another clinical study with an investigational drug within 30 days of screening.
16. Subjects who have the desire to become a parent within the 27-month study period.
17. Prior receipt of gene therapy.
18. An assessment by the Investigator that the subject or parents of the subject will not comply with the study procedures outlined in the study protocol.
19. Hydroxyurea therapy within 3 months before hematopoietic stem cell collection.

4.4 Subject Identification and Registration

Prior to the Screening Phase, the Principal Investigator will identify candidates potentially meeting the study eligibility criteria, based on review of medical records and clinical test findings performed routinely as standard of care for the treatment of the subject. The competent custodial parent(s) of subjects who are less than 18 years old and are determined by the Investigator to be potentially eligible will be informed of the potential to participate in the study.

If the subject / subject's competent custodial parent is willing to have the subject potentially participate in the study, then the consent process will commence. Written informed consent and, if applicable, assent must be obtained before the conduct of any screening tests not performed routinely in the treatment of the subject.

Once consent has been obtained, the subject will be registered and assigned a unique subject number. Once a subject number has been assigned, it cannot be reused, and the number stays with the subject even if the subject is subsequently determined to be ineligible for the study.

The Investigator will further evaluate the subject for study eligibility to ensure all entrance criteria are satisfied.

Additional details regarding the identification and registration of subjects, including details regarding the informed consent process, will be provided by the Sponsor in the Study Operations Manual.

4.5 Subject Withdrawal from the Study

Subjects have the right to withdraw from the study at any time for any reason. Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible.

After giving informed consent subjects may withdraw or be withdrawn from study related procedures and treatments (e.g., bone marrow harvest, busulfan IV conditioning) under the following conditions:

- NCI CTCAE Grade 4 adverse event or laboratory toxicity, which is thought to be at least possibly related to any procedure or treatment (with the exception of the intended hematologic consequences of myeloablation).
- Failure of transduced cells to be dispositioned for clinical use.
- Withdrawal of consent.
- Any medical condition which, in the opinion of the investigators, would put the subject at risk for continuing treatment or follow-up studies.

Once subjects have been treated with LentiGlobin BB305 Drug Product, they will continue follow-up for safety assessments for the duration of the protocol except where:

- Informed consent is withdrawn, and the subject refuses further follow-up.
- The subject is unable to comply with protocol-defined visits.
- The subject has undetectable VCN (< 0.0003 copies per cell) in peripheral blood cells for 2 consecutive measurements at least 1 month apart and at least 12 months after drug product infusion.

Although subjects have the right to withdraw from the study at any time, withdrawal after the start of conditioning (Day -10 to -2) and before administration of LentiGlobin BB305 Drug Product by infusion will be strongly discouraged, as this would be considered deleterious to the subject. In such cases, the subject's stored HSCs (rather than transduced cells) will be infused.

Seven subjects are expected to receive treatment under HGB-205. Subjects withdrawn from the study prior to treatment with LentiGlobin BB305 Drug Product will be replaced. Subjects who receive LentiGlobin BB305 Drug Product but are subsequently withdrawn will not be replaced. Where subjects have received all or part of the conditioning regimen with busulfan IV, LentiGlobin BB305 Drug Product, or rescue therapy with cryopreserved autologous cells, attempts will be made to continue safety follow-up procedures for the duration of the study.

The subject's reason for and date of withdrawal from the study is to be recorded in the case report form (CRF).

5 STUDY TREATMENTS

5.1 Description of LentiGlobin BB305 Lentiviral Vector and LentiGlobin BB305 Drug Product

LentiGlobin BB305 Lentiviral Vector: LentiGlobin BB305 lentiviral vector used for transduction of autologous CD34+ hematopoietic stem cells, is a replication defective, SIN, third generation HIV-1 based lentiviral vector pseudotyped with the VSV-G envelope protein, carrying the human β-globin gene with a single modification at codon 87 [β^{A87} Thr:Gln ($\beta^{A\text{-}T87Q}$)].

LentiGlobin BB305 Drug Product: LentiGlobin BB305 Drug Product is defined as autologous CD34+ hematopoietic stem cells transduced with the LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene and suspended in HSA (5% Albunorm™) in the final immediate container for the intended medical use.

5.2 Summary of Treatments to be Performed or Administered

The apheresis for β-Thal$_M$ subjects, harvesting of bone marrow and separation of autologous CD34+ hematopoietic stem cells for subjects with β-Thal$_M$ or severe SCD, the transduction of CD34+ cells with LentiGlobin BB305 lentiviral vector, the conditioning regimen with busulfan IV, and infusion of LentiGlobin BB305 Drug Product or cryopreserved rescue cells are to be performed/administered only to subjects who have provided informed consent.

All cell manipulation procedures will be performed according to the transduction facility's SOPs.

Table 5-1 outlines the source of the subject cells, usage, and the minimum dose of LentiGlobin BB305 Drug Product or rescue for β-Thal$_M$ and SCD. Additional details are provided in the following subsections.

Table 5-1 Cell Source, Usage, and Minimum Dose of LentiGlobin BB305 Drug Product or Rescue for β-Thal$_M$ and SCD

Indication	Source[1]	Usage	Minimum Dose
β-Thal$_M$	Apheresis	Drug Product	≥3.0 × 10^6 CD34+ cells/kg
	Apheresis	Rescue	≥1.0 × 10^8 TNC/kg
	Bone marrow	Drug Product	≥2.0 × 10^6 CD34+ cells/kg
	Bone marrow	Rescue	≥1.0 × 10^8 TNC/kg
SCD[2]	Bone marrow	Drug Product	≥2.0 × 10^6 CD34+ cells/kg
	Bone marrow	Rescue	≥1.0 × 10^8 TNC/kg

Abbrev.: TNC, total nucleated cells

1 Cells may be procured by more than one mobilization cycle or bone marrow harvest.

2 For subjects with SCD, cells are procured via bone marrow harvest. Subjects may undergo up to 3 bone marrow harvests which result in DP manufacture that meets specification, at the discretion of the Principal Investigator. More than 3 bone marrow harvests may only be attempted if minimum dose of drug product and rescue are not met, or if one or more harvests fails to pass release criteria for reasons other than cell dose.

5.2.1 Mobilization and Apheresis Procedure (for β-Thal$_M$ Subjects)

5.2.1.1 Mobilization

Mobilization and apheresis (see Section 5.2.1.2) are to be performed for subjects with β-Thal$_M$ only. Up to 2 cycles of mobilization (with up to 3 apheresis procedures per cycle) will be permitted if needed to collect sufficient peripheral blood mononucleated cells (PBMCs) for treatment. Mobilization is not recommended in subjects with severe SCD, as it could induce a sickle cell crisis.

After evaluation by a physician from the apheresis facility, subjects will be treated with either filgrastim (Neupogen®) alone or in combination with plerixafor (Mozobil®), at the discretion of

the transplant team. Filgrastim will be administered at a dose of 5 µg/kg/dose administered every 12 to 24 hours subcutaneously (SC) for 5-6 days. Plerixafor may be administered after the subject has received filgrastim for 4 days. Plerixafor is to be administered via SC injection; the recommended dose is 0.24 mg/kg.

CBCs will be performed each day of filgrastim for monitoring leukocytosis and the dosage will be adapted if WBC is $>70\times10^9$/L. Equally, a measurement of CD34+ cells in peripheral blood will be performed the morning(s) before the apheresis.

Refer to the prescribing information for product details regarding filgrastim and plerixafor, including adverse events associated with each agent.

5.2.1.2 Apheresis Procedure

Apheresis is to be performed for subjects with β-Thal$_M$ only.

PBMCs will be collected per SOP at the clinical site. The goal is to collect a total of at least 10×10^6 CD34+ cells/kg per subject for transduction from all HSC harvest procedures. Up to 3 apheresis procedures per mobilization cycle may be performed using standard methods to collect cells. If the number of cells from each apheresis is inadequate, then the cells will be processed and cryopreserved. If sufficient cells are not procured following 3 apheresis collections, the Principal Investigator can either proceed with bone marrow harvest or, another round of mobilization and apheresis, after an interval of >2 weeks . The PBMCs collected will be transported to the transduction facility.

Back-up cells for use in case of engraftment failure may be obtained by either apheresis or bone marrow harvest. Back-up cells will be stored per SOP at the clinical site.

Potential toxicities of filgrastim, plerixafor, and apheresis will be discussed with subjects before signing the Informed Consent for the study.

5.2.2 Bone Marrow Harvest Procedure (All SCD Subjects and Some β-Thal$_M$ Subjects)

Once a subject has signed the informed consent, met entry criteria, and undergone screening procedures, they will undergo a bone marrow harvest under general anesthesia. Subjects with SCD will receive an exchange transfusion within 24 hours prior to the harvest in order to lower the HbS content below 30% and to bring the Hb level to ~9-10 g/dL.

Bone marrow harvest will be performed according to SOP at the clinical site.

The maximum amount of bone marrow aspirated is recommended not to exceed 20 mL/kg and the total amount of nucleated cells harvested should be $>3\times10^8$ cells/kg. The subject may undergo a second or third harvest procedure, each spaced from each other by at least 1 month, to collect additional cells if the minimum requirements for cell dose or the minimum required for back-up are not met, or if drug product does not meet release criteria, or at the discretion of the principal investigator. The Principal Investigator should confirm plans for a third bone marrow harvest with the Sponsor prior to scheduling the procedure. Additional bone marrow harvests will not be performed if there is reasonable concern that an additional harvest may jeopardize the safety or well-being of the subject. In the case where the second or third harvest is required only for the

collection of back-up cells, any additional $CD34^+$ cells collected over the minimum required for back-up may be used for additional drug substance production.

5.2.3 Drug Product Manufacturing Process

All cell manipulation procedures will be performed according to SOP at the clinical site.

An unmanipulated (untransduced) amount of procured cells containing a minimum of 1.0×10^8 TNC/kg will be cryopreserved and given as rescue therapy in the event that medullary aplasia persists after infusion of transduced, manipulated cells. Bone marrow stem cells may be collected, cryopreserved, and given as rescue therapy if an insufficient number of PBMCs are collected via apheresis for rescue or if apheresis is contraindicated.

Prestimulation

The procured cells will first be subjected to a platelet wash (or Ficoll gradient in the case of bone marrow harvest), followed by CD34+ selection using the Miltenyi CliniMACS® system. The CD34+ stem cells will be incubated in Stem Cell Growth Media (SCGM), with human stem cell factor, Fms-related tyrosine kinase 3 ligand, thrombopoietin, and interleukin-3 cytokines for 24-48 hours in cell culture bags.

Transduction

The CD34+ cells will be incubated in a new cell culture bag and with the same additives (cytokines) as used for the pre-stimulation in the presence of protamine sulfate and LentiGlobin BB305 lentiviral vector. A minimum of 3×10^6 $CD34^+$ cells/kg will be transduced with the LentiGlobin BB305 lentiviral vector according to the transduction facility's SOPs. Following transduction, a portion of cells and supernatant will be removed for release testing, and the remainder cryopreserved in a volume of 20-50 mL. LentiGlobin BB305 Drug Substance for an individual subject must be dispositioned for clinical use prior to the subject undergoing myeloablative conditioning with busulfan IV.

Dose of CD34+ Cells per Subject

For cells procured via apheresis, the minimum dose to be administered is 3.0×10^6 CD34+ cells/kg, and for cells procured by bone marrow harvest, the minimum dose to be administered is 2.0×10^6 CD34+ cells/kg.

The dose of autologous CD34+ cells to be administered IV to the subject (after the transduction procedure and release testing) is based on accepted safe practice of rapid and robust hematopoietic reconstitution with long-term engraftment after allogeneic and autologous transplantation with bone marrow CD34+ cells.

5.2.4 Conditioning

Busulfan IV is a bifunctional alkylating agent with a chemical name of 1,4-butanediol, dimethanesulfonate. Busulfan IV is intended for IV administration. It is supplied as a clear, colorless, sterile, solution in 10 mL single use ampoules. Busulfan IV is intended for dilution with 0.9% Sodium Chloride Injection, EP or 5% Dextrose Injection, EP prior to IV infusion (see prescribing information).

Conditioning will only begin after LentiGlobin BB305 Drug Substance has completed release testing; the Quality Person at the manufacturing site and bluebird bio are responsible for the final clinical disposition of the LentiGlobin BB305 Drug Substance. The Quality Person at the manufacturing site is responsible for the disposition for clinical use of Drug Product. Prior to transplantation with LentiGlobin BB305 Drug Product, busulfan will be administered at a dose of 3.2 mg/kg/day for 4 consecutive days via IV infusion. (For subjects weighing <35 kg, the busulfan dose regimen should be adjusted to be given every 6 hours.) The dose and schedule of busulfan IV administration will be monitored daily during the period of administration, and may be adjusted based upon busulfan plasma levels in order to maintain appropriate levels for myeloablation. After completion of the 4-day conditioning regimen, monitoring of busulfan levels will continue daily thereafter until no busulfan is detected.

Dose preparation procedures should be performed under aseptic techniques in a vertical laminar flow safety hood while wearing gloves and protective clothing per SOP at the clinical site. Please see the prescribing information for detailed instructions regarding busulfan dose preparation.

5.2.4.1 Rationale for the Use of Busulfan IV for Conditioning

Busulfan IV will be used as a single conditioning agent. Busulfan IV offers the advantage of being almost purely myeloablative as it induces very little immunosuppression (Lucarelli et al., 1993). Regimens using busulfan IV have resulted in short times to neutrophil and platelet recovery (15 days), few treatment-related complications and stable donor engraftment (Lucarelli et al., 1993). Busulfan IV is typically used in conjunction with cyclophosphamide, etoposide, or fludarabine for conditioning prior to allogeneic stem cell transplant (Lucarelli et al., 1993). Because this protocol involves autologous stem cells, immunosuppression is dispensable and busulfan IV is used as a single agent. Human HSCs from normal individuals do not have a spontaneous selective advantage in vivo over HSCs from β-Thal$_M$ or SCD subjects; therefore it is essential to maximize the myeloablative regimen in gene therapy protocols for these diseases. This protocol therefore uses the myeloablative dose of busulfan IV, 12.8 mg/kg, as defined in the Manufacturer's prescribing information.

Refer to the prescribing information for product details regarding busulfan IV, including associated adverse events.

5.2.5 Infusion Procedures

LentiGlobin BB305 Drug Product is to be given up to 7 days after completion of the busulfan conditioning regimen .

Prior to administration, the transduced autologous CD34+ cells are thawed, washed, resuspended in 5% HSA, counted, and tested for viability. LentiGlobin BB305 Drug Product is then ready for administration to the subjects.

LentiGlobin BB305 Drug Product will be administered on Day 1 via IV infusion according to SOP at the clinical site, with vital signs being monitored concurrently. (The LentiGlobin BB305 Drug Product dose is specified in Section 5.2.3.)

5.3 Storage and Stability

5.3.1 LentiGlobin BB305 Lentiviral Vector

LentiGlobin BB305 lentiviral vector is not administered to subjects; it is used to transduce the subject's CD34+ cells.

The clinical-grade LentiGlobin BB305 lentiviral vector is to be shipped and stored at ≤-65°C.

The LentiGlobin BB305 lentiviral vector is to be thawed per SOP at the transduction facility.

Each LentiGlobin BB305 lentiviral vector final product container is labeled with a product label. Final product labels are made within the production facility at SAFC according to SOP. All labels are checked for accuracy and released by SAFC quality assurance unit.

LentiGlobin BB305 lentiviral vector final product containers are controlled at SAFC according to SOP. Upon completion of production, labeled final product containers of LentiGlobin BB305 lentiviral vector are placed in an appropriately labeled freezer equipped with a remote temperature alarm. Long-term storage is at a qualified packaging and distribution facility, where the vector is shipped and stored under Good Manufacturing Practices (GMP).

5.3.2 LentiGlobin BB305 Drug Product

The subject's CD34+ hematopoietic stem cells transduced with LentiGlobin BB305 lentiviral vector are frozen and stored in cryopreservative solution in the vapor phase of liquid nitrogen until the thawing date.

The LentiGlobin BB305 Drug Product is prepared when the subject's transduced cells are thawed, washed, and suspended in 5% HSA in the final immediate container for the intended medical use.

All procedures involving LentiGlobin BB305 Drug Product must be performed using aseptic techniques by trained personnel according to SOP at the clinical site.

5.4 Method of Assigning Subjects to Treatment

All subjects entered will be assigned to the single treatment group in this open-label study.

5.5 Blinding, Packaging, and Labeling

5.5.1 Blinding and Breaking the Blind

This is an unblinded, open-label study.

5.5.2 LentiGlobin BB305 Lentiviral Vector Packaging, Labeling, Shipment, and Export-Import

At the written request of bluebird bio, the GMP storage facility will package and ship LentiGlobin BB305 lentiviral vector according to SOP. The LentiGlobin BB305 lentiviral vector will be shipped in compliance with applicable regulatory requirements. All export-import laws and regulations will be followed, and a specialized export-import service for biological material will be utilized following predetermined SOP.

Each shipment will include a packing list specifying the number of units contained in the shipment and storage conditions. The receiving Responsible Pharmacist at the clinical site will document

receipt of number of containers and condition of shipment and return a copy as per directions on the form. Traceability (shipment, receipt, storage, usage, and disposal) of the LentiGlobin BB305 lentiviral vector will be controlled by SOP at the clinical site.

5.5.3 LentiGlobin BB305 Drug Product Packaging and Labeling

LentiGlobin BB305 Drug Product consists of autologous CD34+ hematopoietic stem cells transduced with the LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene and suspended in HSA (5% Albunorm™) in the final immediate container for the intended medical use (infusion bag). LentiGlobin BB305 Drug Product will be labeled in accordance with SOP at the clinical site.

5.6 Duration of Subject Participation

Each subject will remain on study for approximately 27 months from time of consent and then will be asked to consent for a follow-up study for another 13 years as recommended by regulatory agencies.

Subjects who enroll in the study but discontinue prior to myeloablation should be followed for at least 1 month after completion of harvesting, or until resolution of any study procedure-related adverse events, whichever is later. In the rare case a subject undergoes myeloablation but does not undergo LentiGlobin BB305 Drug Product infusion, follow up should continue on study for at least 3 months, or until resolution of any study-procedure related adverse events, whichever is later.

Subjects meeting the vector copy number (VCN) discontinuation criterion (see Section 4.5) will be discontinued from the study without further follow-up. The VCN discontinuation criterion is met when VCN is undetectable (< 0.0003 copies per cell) in peripheral blood cells for 2 consecutive measurements at least 1 month apart and at least 12 months after drug product infusion.

5.7 Assessment of Treatment and Study Compliance

Treatment compliance will not be an issue in this study because eligible subjects will be treated as in-patients and thus will be monitored by hospital personnel.

Subject compliance with the subsequent post-transplant study visits will also be assessed through Month 24.

5.8 Product Accountability

5.8.1 LentiGlobin BB305 Lentiviral Vector

Detailed records will be maintained to allow for accurate accountability of LentiGlobin BB305 lentiviral vector. bluebird bio will provide direction to the manufacturing site on how unused material is to be handled.

5.8.2 LentiGlobin BB305 Drug Product

LentiGlobin BB305 Drug Product accountability and traceability is ultimately the responsibility of the Investigator. However, this responsibility may be delegated to a suitably qualified

investigator who has had appropriate study-specific training and that has been appropriately listed on the Delegation of Responsibility Log for this task.

Detailed records will be maintained to allow for accurate accountability of the LentiGlobin BB305 Drug Product.

These records will include details of storage and use of the LentiGlobin BB305 Drug Product. Transfer of LentiGlobin BB305 Drug Product from the transduction facility through administration to subjects will be recorded.

The Investigator will ensure that the LentiGlobin BB305 Drug Product is used only in accordance with this protocol. Drug accountability records indicating the LentiGlobin BB305 Drug Product's inventory at the clinical site, use by each subject, and disposal will be maintained by the clinical site. These records will adequately document that the subjects were provided the dose as specified in the protocol and should reconcile all LentiGlobin BB305 Drug Product received. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and subject numbers. The Sponsor or its designee will review LentiGlobin BB305 Drug Product accountability at the clinical site on an ongoing basis during monitoring visits.

All material containing LentiGlobin BB305 Drug Product will be treated and disposed of as hazardous waste in accordance with governing regulations. In the event that drug product cannot be administered due to triggering of stopping rules or other reasons, drug product will be kept cryopreserved in the vapor phase of liquid nitrogen until further written instruction by the Sponsor. The sponsor will instruct the site staff to destroy the drug product via their institutional procedures before the end of the study.

5.9 Prior and Concomitant Treatment

All concomitant treatments, including transfusions or phlebotomies, will be recorded in the CRF for any subject who was deemed eligible for bone marrow harvest or mobilization.

Hemoglobinopathy treatments given prior to study start, with the exception of erythropoietin, will continue as needed after treatment with LentiGlobin BB305 Drug Product. These include exchange transfusions to prevent VOC in SCD subjects, transfusions of blood or platelets given according to Hb and platelet counts, and deferoxamine administered for iron overload. Blood products for transfusion will have been irradiated, if required.

Erythropoietin is excluded and should be discontinued 3 months prior to treatment, as it may prevent engraftment of the transduced cells.

Other concomitant therapies during conditioning may include hyperdiuresis, prophylactic or therapeutic administration of ondansetron or alizapride for nausea and vomiting, clonazepam for prevention of seizures, and ursodeoxycholic acid to prevent liver dysfunction (see Section 5.9.1).

5.9.1 Concomitant Treatments during Conditioning

Permitted concomitant treatments during conditioning may be given at the Investigator's discretion, as follows:

- Hyperdiuresis, beginning 12 hours before initiating conditioning and continuing through 24 hours thereafter.
- Prevention or treatment of nausea and vomiting:
  - Prophylactic administration of ondansetron.
  - Alizapride for the treatment of nausea and vomiting.
- Clonazepam for the prevention of seizures.
- Liver complications will be prevented with ursodeoxycholic acid.
- Prior to cell harvest, subjects with β-Thal$_M$ may undergo a period of hypertransfusion to maintain a Hb of ≥11 g/dL.
- For subjects with severe SCD, exchange transfusions will be performed before conditioning in order to reach an HbS proportion lower than 30% and bring the Hb level to ~9-10 g/dL.
- Hemoglobinopathy treatments will continue unchanged, with deferoxamine or another suitable chelator administered for iron overload, and transfusions given according to the Hb and platelet counts. Blood products will be filtered and irradiated, if required.
- Defibrotide for prophylaxis and treatment of veno-occlusive disease is to be administered at the Investigator's discretion.
- Subjects will be isolated in single rooms and appropriate precautions will be taken if the polynuclear leukocyte count is $<0.5\times10^9$/L. Broad-spectrum antibiotic treatment will be administered according to the usual procedures for febrile neutropenia.

6 STUDY ASSESSMENTS

6.1 Schedule of Events

Table 6-1 provides the schedule of events to be conducted during the study. Detailed descriptions of the efficacy, pharmacodynamics, and safety procedures to be conducted during this study are provided in the following sections. Additional details, including administrative information, regarding the efficacy, pharmacodynamic, and safety procedures, will be provided by the Sponsor in the Study Operations Manual.

Study treatments and evaluations can be considered as 4 distinct phases:

- Pre-inclusion Screening, Informed Consent, and Eligibility-determining Phase (Day -97 to Day -61)
- CD34+ Cell Collection, Genetic Modification, and Release of Modified Cells (Day -60 to Day -11)
- Conditioning and Washout Phase (Day -10 through Day 0) and Stem Cell Infusion on Day 1
- Maintenance Phase (Day 1 through Month 24)

The study treatments are described in detail in Section 5.2.

Note: Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or as deemed necessary by the Investigator. Evaluations and procedures identified in the Schedule of Events may be performed at unscheduled visits, as clinically indicated, at the Investigator's discretion in consultation with the sponsor.

Table 6-1 Schedule of Events

	Treatment Phase: Transduction, Conditioning, Treatment, and Engraftment											Follow-Up									
	Inclusion / Screening	Mobilization	CD34+ Harvest	Transduction, and Cryopreservation	Release Testing and Pre-Conditioning[1]		Conditioning / Busulfan Monitoring	Post-infusion Monitoring of Busulfan Levels	CD34+ Cell Infusion			M 2	M 3	M 4.5	M 6	M 9	M 12	M 15	M 18	M 21	M 24
	The actual time from Screening to CD34+ cell infusion on Day 0 may be >75 days due to the duration of Release Testing; however, the sequence and duration of other activities are to be maintained.																				
Parameter	D -97 to -61[2]	D -60 to -55	D -54, -53, -52[3]	D -51 to -48	D -47 to -11		D -10 to -7	D -6 to -1	D0 / D1 to 14	D 15	D 30	D 60	D 90	D 135	D 180	D 270	D 360	D 450	D 540	D 630	D 720
Visit window (weeks)	-	-	-	-	-		-		-	-	*±1*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*+8*
Pre-inclusion Screening, Informed Consent, and Eligibility-determining Procedures																					
Informed consent	+					*The subject will be admitted for conditioning only after a Certificate COA for study treatment is issued.*															
Physical examination[4]	+	+[5]	+[6]				+[7]	+[7]	+[7,8]												
Vital signs	+	+[9]	+[10]		+		+[11]	+[11]	+[11]												
Demographics and medical history	+																				
Confirmation of eligibility	+	+	+				+[12]														
Adverse events (from consent through end of study)	+	+	+	+	+		+	+	+												
Concomitant medications	+	+	+	+	+		+	+	+												
Hematology[13]	+	+[14]	+[15]		+		+[11,13]	+[11,13]	+[11,13]												
Peripheral blood CD34^{+} count		+	+[16]																		
Clinical chemistry[18]	+						+[11]	+[11]	+[11]												
Urinalysis	+																				
Serology panel[19]	+	+																			
Microbiological testing									+[20]												
Serum pregnancy test[21]	+						+[22]														
Sperm / testicular tissue or oocyte banking[23]	+																				
Chest X-ray	+																				

	Inclusion / Screening	Treatment Phase: Transduction, Conditioning, Treatment, and Engraftment										Follow-Up									
		Mobilization	CD34+ Harvest	Transduction, and Cryopreservation	Release Testing and Pre-Conditioning[1]		Conditioning / Busulfan Monitoring	Post-infusion Monitoring of Busulfan Levels	CD34+ Cell Infusion												
	The actual time from Screening to CD34+ cell infusion on Day 0 may be >75 days due to the duration of Release Testing; however, the sequence and duration of other activities are to be maintained.											M 2	M 3	M 4.5	M 6	M 9	M 12	M 15	M 18	M 21	M 24
Parameter	D -97 to -61[2]	D -60 to -55	D -54, -53, -52[3]	D -51 to -48	D -47 to -11		D -10 to -7	D -6 to -1	D0 / D1 to 14	D 15	D 30	D 60	D 90	D 135	D 180	D 270	D 360	D 450	D 540	D 630	D 720
Visit window (weeks)	-	-	-	-	-		-		-	-	*±1*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*+8*
Pre-inclusion Screening, Informed Consent, and Eligibility-determining Procedures (continued)																					
Pulmonary DLco	+					*The subject will be admitted for conditioning only after a Certificate COA for study treatment is issued.*															
Doppler Echocardiography	+																				
ECG	+																				
Cardiac MRI	+																				
Liver MRI (Fe assessment)	+																				
Liver biopsy, if needed[24]	+																				
Blood typing; HLA typing[25]	+																				
Hormonal testing[26]	+																				
Immunologic testing[27]	+																				
Urine creatinine and Cr / Cl (calculated)	+																				
Blood for: • VCN by qPCRin cell populations	+																				
• Globin chains by HPLC	+																				
• RCL test	+																				
• Exploratory biomarkers/Samples for storage[28]	+																				
• SCD tests, if applicable	(+)																				

Footnotes for Pre-inclusion Screening, Informed Consent, and Eligibility-determining Procedures appear on the following page.

[redacted]

Footnotes for Pre-inclusion Screening, Informed Consent, and Eligibility-determining Procedures

Key: D=day, D0= Day of infusion ***PRIOR*** to infusion; D1=Day of Infusion from Time of Start of Infusion and beyond;
M=month, AE=adverse events, PBMC= peripheral blood mononuclear cells (leukocytes

1 If the transduced cells fail to be dispositioned for clinical use, then mobilization and apheresis may be repeated at the Investigator's discretion after approval by the Sponsor (Medical Monitor). In such cases, all study procedures will be repeated, starting with Day -60. Additionally, windows may be extended if there are delays in drug product dispositioning.

2 Evaluations other than those listed (eg, dental imaging, sinus imaging) may be performed during the period between Screening and the start of mobilization, as deemed necessary by the Investigator.

3 A third day of CD34+ harvest will only be done if needed.

4 Physical examination will include measurement of weight at all visits and height and performance status at Screening only.

5 A physical examination should be performed within 5 days prior to or on the first day of every mobilization cycle.

6 A physical examination should be performed on each apheresis day, prior to the apheresis procedure and again after the completion of each apheresis procedure. If the subject undergoes a bone marrow harvest, a physical examination should be performed on the day of the harvest but prior to the harvest and then again prior to discharge.

7 A physical examination should be performed at least twice a week until discharge.

8 A physical examination is to be performed and weight documented just prior to transplant on Day 0.

9 Vital signs should be measured on the first day of every mobilization cycle.

10 Vital signs should be measured on each apheresis day, prior to the apheresis procedure then again after the apheresis procedure is completed. If the subject undergoes a bone marrow harvest, vital signs should be performed on the day of the bone marrow harvest prior to the harvest and then again prior to discharge.

11 Clinical chemistry to be assessed as per standard of care during hospitalization for conditioning, engraftment, and follow-up. For adult subjects, this is usually daily; for pediatric subjects, this can be every other day due to blood volume concerns. Additional assessments should be obtained as clinically indicated (e.g. AE evaluation).

12 Subject will be admitted to transplant unit. All testing for D-10 is to be performed in the time between admission and the subject receiving the first dose of busulfan.

13 Hematology will include a CBC, platelets, erythroblasts, and reticulocyte at all visits; prothrombin time (PT) / partial thromboplastin time (PTT) at screening, Day-10 and study termination; iron, ferritin, transferrin, iron saturation, serum transferrin receptor at screening, Day 90, 180, 360 and study termination; plasma busulfan monitoring post-infusion through Day 0; erythropoietin at Screening, then every 6 months post- infusion. Hematology will be assessed as per standard of care during the hospitalization for conditioning, engraftment, and follow up. Additional assessments should be obtained as clinically indicated (e.g. AE evaluation).

14 A CBC should be performed on every day of each mobilization cycle.

15 A CBC should be performed on every day an apheresis procedure is performed.

16 Peripheral blood $CD34^+$ count should be performed prior to the anticipated Apheresis Procedure Day 1.

18 Chemistry will include sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3), blood urea nitrogen (BUN), creatinine, calcium, phosphate, albumin, total protein, uric acid, lactic dehydrogenase (LDH), liver enzymes (gamma-glutamyl transferase [GGT], alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin), glucose at every visit.

19 The serology panel will be performed using standard methods and will assay for HIV-1 and HIV-2; hepatitis A antibody (Ab); hepatitis B core antibody (HBcAb); hepatitis B surface antibody (HBsAb); hepatitis B surface antigen (HBsAg); hepatitis C virus (HCV) Ab; HTLV-1 and HTLV-2; CMV immunoglobulin G (IgG) Ab; CMV; Epstein-Barr virus (EBV) Ab; Herpes simplex virus (HSV) type I/II Ab; toxoplasmosis IgG Ab; varicella zoster virus (VZV) IgG Ab; syphilis and VSV-G Ab. In certain circumstances, additional testing may be required depending on the subject's history and/or the characteristics of the subject's cells (e.g., malaria, toxoplasma, Trypanosoma cruzi).

20 Microbiological testing will include surveillance blood cultures twice weekly and CMV and aspergillus antigenemia once weekly from Day 1 to ANC $>0.5\times10^9$/L.

21 For female subjects of child-bearing potential only.

22 To be performed for female subjects of child-bearing potential on -Day 10 prior to initiation of busulfan.

23 Sperm or testicular tissue banking for males or oocyte banking for females will be offered; harvesting and long-term storage, when indicated. Cryopreservation will be performed outside of the study, and is done at the discretion of the subject. Procedures related to oocyte removal should be completed by Day -30.

24 A liver biopsy, if needed, should be performed, as clinically indicated, to assess fibrosis and inflammation. Liver biopsy should be performed within 6 months before study entry.

25 Blood typing will include ABO blood typing, Rh, and atypical agglutinins, if potential sibling-match is available. If previous results are available, need not be repeated during Screening.

26 Hormonal testing will include: thyroid stimulating hormone (TSH), 3,5,3'-triiodothyronine (T3), thyroxine (T4), follicle stimulating hormone (FSH), luteinizing hormone (LH), and estrogen or testosterone level. In addition, subject will be screened with AM cortisol levels and adrenocorticotropic hormone (ACTH) for adrenal insufficiency.

27 Immunological testing will include quantification of T cell subsets (CD4, CD8), B cells, and natural killer (NK) cells.

28 Plasma, serum, blood, DNA, and RNA from PBLs are to be stored.

Table 6-1 Schedule of Events (Continued)

	Inclusion / Screening	Treatment Phase: Transduction, Conditioning, Treatment, and Engraftment										Follow-Up									
		Mobilization	CD34+ Harvest	Transduction, and Cryopreservation	Release Testing and Pre-Conditioning[1]		Conditioning / Busulfan Monitoring	Post-infusion Monitoring of Busulfan Levels	CD34+ Cell Infusion			M 2	M 3	M 4.5	M 6	M 9	M 12	M 15	M 18	M 21	M 24
	The actual time from Screening to CD34+ cell infusion on Day 0 may be >75 days due to the duration of Release Testing; however, the sequence and duration of other activities are to be maintained.																				
Parameter	D -97 to -61[2]	D -60 to -55	D -54, -53, -52[3]	D -51 to -48	D -47 to -11		D -10 to -7	D -6 to -1	D0 / D1 to 14	D 15	D 30	D 60	D 90	D 135	D 180	D 270	D 360	D 450	D 540	D 630	D 720
Visit window (weeks)	-	-	-	-	-		-		-	-	*±1*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*+8*
Autologous CD34+ Cell Collection, Modification, and Release, Conditioning, and Transplant Phase Procedures																					
HSC mobilization		+				*The subject will be admitted for conditioning only after a Certificate COA for study treatment is issued.*															
HSC harvest			+																		
CD34+ stem cell transduction & cryopreservation				+																	
Subject admitted to transplant unit							+[12]														
Conditioning agent infusion							+														
Monitor of conditioning agent levels							+	+													
Release testing					+[1]																
Infusion								+[29]													

1 If the transduced cells fail to be dispositioned for clinical use, then mobilization and apheresis may be repeated at the Investigator's discretion. In such cases, all study procedures will be repeated, starting with Day -60.

2 Evaluations other than those listed (eg, dental imaging, sinus imaging) may be performed during the period between Screening and the start of mobilization, as deemed necessary by the Investigator.

3 A third day of CD34+ harvest will only be done if needed.

12 Subject will be admitted to transplant unit. All testing for D-10 is to be performed in the time between admission and the subject receiving the first dose of busulfan.

29 LentiGlobin BB305 Drug Product may be infused if no busulfan has been detected for the prior 2 consecutive days.

[redacted]

Table 6-1 Schedule of Events (Continued)

	Inclusion / Screening	Treatment Phase: Transduction, Conditioning, Treatment, and Engraftment										Follow-Up									
		Mobilization	CD34+ Harvest	Transduction, and Cryopreservation	Release Testing and Pre-Conditioning[1]		Conditioning / Busulfan Monitoring	Post-infusion Monitoring of Busulfan Levels	CD34+ Cell Infusion			M 2	M 3	M 4.5	M 6	M 9	M 12	M 15	M 18	M 21	M 24
	The actual time from Screening to CD34+ cell infusion on Day 0 may be >75 days due to the duration of Release Testing; however, the sequence and duration of other activities are to be maintained.																				
Parameter	D -97 to -61[2]	D -60 to -55	D -54, -53, -52[3]	D -51 to -48	D -47 to -11		D -10 to -7	D -6 to -1	D0 / D1 to 14	D 15	D 30	D 60	D 90	D 135	D 180	D 270	D 360	D 450	D 540	D 630	D 720
Visit window (weeks)	-	-	-	-	-		-		-	-	*±1*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*+8*
Maintenance Phase, Efficacy, and Exploratory Procedures																					
Record transfusions												+	+	+	+	+	+	+	+	+	+
Record hospitalizations												+	+	+	+	+	+	+	+	+	+
Cardiac MRI																					+
Liver MRI (Fe assessment)																					+
Blood for:																					
• VCN by qPCR in cell populations											+	+	+		+	+	+	+	+	+	+
• Necker VCN assay development											+	+	+		+	+	+	+	+	+	+
• Globin chains by HPLC											+	+	+		+	+	+	+	+	+	+
• Exploratory PD																+		+		+	
• Exploratory biomarkers /Samples for storage[28]											+	+	+		+	+	+		+		+
Bone marrow:																					
• VCN by qPCR – $CD34^+$															30	30	30	30	30	30	30
• Globin chains by HPLC																	31				31
• Cellularity																	32				32
SCD tests, if applicable													(+)		(+)		(+)		(+)		(+)

28 Plasma, serum, blood, DNA, and RNA from PBLs will be stored.
30 To be performed if there is any evidence of clonal skewing in PBLs, or at the investigator's discretion.
31 To be performed in bone marrow only if a bone marrow sample is collected.
32 At investigator's discretion

Table 6-1 Schedule of Events (Continued)

	Inclusion / Screening	Treatment Phase: Transduction, Conditioning, Treatment, and Engraftment										Follow-Up									
		Mobilization	CD34+ Harvest	Transduction, and Cryopreservation	Release Testing and Pre-Conditioning[1]		Conditioning / Busulfan Monitoring	Post-infusion Monitoring of Busulfan Levels	CD34+ Cell Infusion												
	The actual time from Screening to CD34+ cell infusion on Day 0 may be >75 days due to the duration of Release Testing; however, the sequence and duration of other activities are to be maintained.											M 2	M 3	M 4.5	M 6	M 9	M 12	M 15	M 18	M 21	M 24
Parameter	D -97 to -61[2]	D -60 to -55	D -54, -53, -52[3]	D -51 to -48	D -47 to -11		D -10 to -7	D -6 to -1	D0 / D1 to 14	D 15	D 30	D 60	D 90	D 135	D 180	D 270	D 360	D 450	D 540	D 630	D 720
Visit window (weeks)	-	-	-	-	-		-		-	-	*±1*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*±2*	*+8*
Maintenance Phase, Safety Procedures																					
Physical examination[4]										+	+	+	+	+	+	+	+	+	+	+	+
Vital signs										+	+	+	+	+	+	+	+	+	+	+	+
Hematology[13]										+	+	+	+	+	+	+	+	+	+	+	+
Clinical chemistry[18]										+											
Hormonal testing[26]																	+				+
Immunology tests													+		+						
ECG																					+
RCL test													+		+		+				+
LAM-PCR & sequencing in whole blood (Integration site analysis)[33]															+		+		+		+
Adverse event assessment[34]										+	+	+	+	+	+	+	+	+	+	+	+
Concomitant medications										+	+	+	+	+	+	+	+	+	+	+	+
End of Study																					+

Table footnotes for Maintenance Phase, Safety Procedures appear on the following page.

[redacted]

Table Footnotes, Maintenance Phase, Safety Procedures

Key: D=day, M=month, AE=adverse events, PBLs= peripheral blood leukocytes.

Note: Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or as deemed necessary by the Investigator. Evaluations and procedures identified in the Schedule of Events may be performed at unscheduled visits, as clinically indicated, at the Investigator's discretion in consultation with the sponsor.

4 Physical examination will include measurement of weight at all visits.

13 Hematology will include a CBC, platelets and reticulocyte at all visits; PT / PTT at screening, Day-10 and study termination; iron, ferritin, transferrin, iron saturation, serum transferrin receptor at Screening, Day 90, 180, 360 and study termination; erythropoietin at Screening, then every 6 months post- infusion. Hematology will be assessed as per standard of care during the hospitalization for conditioning, engraftment, and follow up. Additional assessments should be obtained as clinically indicated (e.g. AE evaluation).

18 Chemistry will include Na, K, Cl, HCO_3, BUN, creatinine, calcium, phosphate, albumin, total protein, uric acid, LDH, liver enzymes (GGT, ALP, ALT, AST, and total bilirubin), glucose at every visit.

26 Hormonal testing will be include: TSH, T3, T4, FSH, LH, and estrogen or testosterone level. In addition, subject will be screened with AM cortisol levels and ACTH for adrenal insufficiency.

33 Proviral integration site analysis will be performed in whole blood and, if evidence of clonal skewing is observed, in bone marrow by LAM-PCR and sequencing to monitor for the development of clonal dominance.

34 AEs are to be monitored and collected as follows: all ≥Grade 1 AEs through 30 days after LentiGlobin BB305 Drug Product infusion; ≥Grade 2 AEs and SAEs through 12 months after LentiGlobin BB305 Drug Product infusion; and LentiGlobin BB305 Drug Product-related AEs through 24 months after LentiGlobin BB305 Drug Product infusion. For subjects who discontinue from the study for reasons other than withdrawal of consent before the performance of any invasive study procedure (eg, liver biopsy, mobilization), follow-up for AEs is required for 30 days post-discontinuation.

6.2 Efficacy and Pharmacodynamic Measures

6.2.1 Transfusions

Interval transfusions required (mL packed RBC/kg) are to be documented as per the Schedule of Events.

6.2.2 In-patient Hospitalizations

All in-patient hospitalizations occurring after post-transplant discharge from the clinical site are to be documented. In-patient hospitalization is defined as treatment by a physician in a hospital for at least 24 hours.

6.2.3 Hemoglobinopathy Markers in Blood

Blood samples are to be collected for globin and hemoglobin analyses per the Schedule of Events and may include the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin in whole blood, as well as the amount of $\beta^{A\text{-}T87Q}$-globin as a fraction of all β-chains in whole blood.

6.2.4 Hemoglobinopathy Markers in Bone Marrow

If bone marrow samples are collected for other purposes, such samples also are to be used for determination of $\beta^{A\text{-}T87Q}$-globin by HPLC, as indicated in the schedule of events.

6.2.5 Vector Copy Number in Blood

Blood samples will be collected for VCN determination in cell populations from peripheral blood, using quantitative polymerase chain reaction (qPCR) according to the schedule of events; collection details are included in the Study Operations Manual. Lineage fractionation will be performed for VCN analyses on specific lineages, as needed.

Blood will also be collected for VCN assay development at Necker laboratories.

6.2.6 Vector Copy Number in Bone Marrow

If bone marrow samples are collected for other purposes, such samples also are to be used for determination of VCN in bone marrow by qPCR, as indicated in the Schedule of Events.

6.2.7 Sickle Cell Disease-specific Laboratory Tests

Blood samples are to be collected from subjects with sickle cell disease (SCD) only, per the Schedule of Events for SCD-specific pharmacodynamic laboratory tests, as follows:

- RBC quality and function (e.g., 2,3 diphosphoglycerate levels, RBC density distribution curve, phosphatidyl serine levels, and sickling)
- Hemolysis markers (e.g., reticulocyte count, indirect bilirubin, haptoglobin level, cell-free Hb level, and lactate dehydrogenase)
- Inflammation, as measured by CRP

All these SCD-specific tests are to be performed by a central laboratory. Blood sample collection details are included in the Laboratory Manual.

6.2.8 Exploratory bone marrow aspirate

At the investigator's discretion, a bone marrow aspirate and biopsy can be performed at the 12 Month and 24 Month Visits. CCI [redacted]

6.2.9 Exploratory PD

Blood samples will be taken at 9, 15, and 21 Month Visits. Blood sample collection details are included in the Laboratory Manual. These samples will be shipped to the Sponsor and used to evaluate PD after drug product infusion using exploratory techniques (CCI [redacted]).

6.2.10 Exploratory biomarkers/ Samples for Storage

Optional blood samples will be collected per the Schedule of Events for future research. CCI [redacted] Such samples may be stored until the samples are exhausted or till the repository is discontinued. The Sponsor will be the custodian of the samples in the repository and any unused samples will be destroyed at the Sponsor's discretion. Leftover samples from protocol procedures (e.g., drug product manufacture, blood draw for integration site analysis) may also be stored (optional) for potential future analyses as described above.

Note that samples collected as part of the manufacture of the drug product may be used to study the manufacturing process. In particular these samples may be used to understand how the process may be improved or made more robust. These possible studies are not optional, and may be performed at the Sponsor's discretion. Other uses of these samples (for non-manufacturing improvement research) is optional and discussed in the above paragraph.

Collection and storage of the samples described above will be subject to discretionary approval from the research center's IEC and the subject's specific written consent. Samples will be labeled with a unique identification number that includes no subject identifying information.

6.3 Safety Measures

6.3.1 Demographics and Medical History

Subject demographic data such as gender, age, race, and ethnicity, will be obtained during Screening. A complete medical history also will be obtained during Screening. The medical history is to include all prior and current medical history.

6.3.2 Clinical/Physical Examination

A complete physical examination (including general appearance; head, eyes, ears, nose, and throat; cardiovascular; dermatologic; abdominal; genitourinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological) is to be conducted as per the Schedule of Events.

Subject's weight (in kilograms) and performance status are to be measured at every physical examination; subject's height is to be included only at Screening Visit.

The weight measurement on Day 0 prior to infusion will be used in the calculation of the subject's dose.

6.3.3 Vital Signs

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

Vital signs will be measured and recorded as per the Schedule of Events.

6.3.4 Electrocardiogram

A 12-lead electrocardiogram (ECG) will be obtained as per the Schedule of Events.

6.3.5 Imaging Studies

A chest x-ray will be obtained as per the Schedule of Events.

Cardiac Doppler echocardiography, including the assessment of left ventricular ejection fraction (LVEF), and liver and cardiac MRI will be performed as per the Schedule of Events.

6.3.6 Blood Typing

Blood samples will be collected for ABO, Rh, and HLA blood typing and determination of atypical agglutinins, for subjects with a potential HLA-matched sibling donor, as per the Schedule of Events.

6.3.7 Serology and Microbiological Testing

Screening serology will be evaluated using standard methods as per the Schedule of Events. The serology panel includes HIV-1 and HIV-2; hepatitis A Ab; HBcAb; HBsAb; HBsAg; HCV Ab; HTLV-1, HTLV-2, CMV IgG Ab; CMV; EBV Ab; HSV type I/II Ab; toxoplasmosis IgG Ab; VZV IgG Ab; syphilis; and VSV-G Ab. In certain circumstances, additional testing may be required depending on the subject's history and/or the characteristics of the subject's cells (e.g., malaria, toxoplasma, Trypanosoma cruzi).

After drug product infusion, microbiological testing is to be performed twice weekly and CMV and *Aspergillus* antigenemia testing is to be performed once weekly until the subject's ANC is $>0.5\times10^9$/L.

6.3.8 Hormone Testing

Hormonal testing, including measurement of TSH, T3, T4, FSH, LH, and estrogen or testosterone, as applicable, is to be performed as per the Schedule of Events. In addition, subjects will be screened with AM cortisol levels and ACTH for adrenal insufficiency.

6.3.9 Immunological Testing

Immunological testing, including T cell subsets (CD4, CD8), B cells, and NK cells, will be performed as per the Schedule of Events.

6.3.10 Sperm / Testicular Tissue or Oocyte Banking

Sperm or testicular tissue banking for males or oocyte aspiration following ovarian stimulation and cryopreservation for females will be done at the discretion of the subject. Procedures related to oocyte removal should be complete by Day –30. All cryopreserved gamete samples are for use

only by the subject from whom they were obtained and will be destroyed upon the death of the subject, unless requested otherwise by the individual subject or subject's parent or legal guardian.

Ovarian tissue graft (or even graft of entire ovary) may not be offered as standard care. However, it may be offered in the context of biomedical research. It may be discussed with the subject and/or their guardian(s) in this context, separately from clinical study HGB-205.

6.3.11 Liver Biopsy and Imaging

MRI of the liver is to be performed for assessment of fibrosis, inflammation, and iron content, and a liver biopsy also is to be performed, if clinically indicated, as per the Schedule of Events.

6.3.12 Pregnancy Testing

For female subjects of child-bearing potential only, a serum pregnancy test (β-human chorionic gonadotropin [β-HCG]) will be obtained as per the Schedule of Events.

6.3.13 Clinical Safety Laboratory Tests

Clinical laboratory tests, including hematology, coagulation studies, clinical chemistries, and urinalysis, will be performed as specified below, and in the Schedule of Events. Blood volumes to be collected from children with low body weight may be adjusted lower as necessary.

Clinical laboratory tests are to be performed and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner).

6.3.13.1 Hematology and Clinical Chemistry

Blood samples for all hematology parameters, coagulation studies, and clinical chemistries, are to be collected as per the Schedule of Events.

The following clinical laboratory parameters are to be determined:

Hematology	**Iron Studies**
CBC with differential	Iron
Platelet count	Ferritin
Reticulocyte count	Serum transferrin receptor
Erythroblast count	Transferrin
	Iron saturation
Coagulation studies	**Erythropoietin**
PT/PTT	
Serum Chemistry and Liver Function	
Na	BUN
K	Creatinine
Cl	Glucose
HCO_3	Ca
Albumin	Uric acid
Total protein	Phosphate
LDH	Bilirubin (total)
ALT	ALP
AST	GGT

Additional clinical laboratory tests may be performed at the Investigator's discretion.

6.3.13.2 Urinalysis

A urinalysis will be performed as per the Schedule of Events. In addition, creatinine and creatinine clearance (calculated) will be measured from the urine sample obtained as per the Schedule of Events.

6.3.14 Testing for Replication Competent Lentivirus (RCL)

Blood samples for RCL testing will be collected, as specified in the Schedule of Events. Blood samples for RCL testing will be collected and tested using a RCL screening assay. If the RCL screening assay is positive, a test to assess the presence of RCL in PBLs will be performed. This latter test relies upon the culture of the subject's viable PBLs with a permissive cell line, and allows the amplification and detection of RCL in vitro. Pre-transplant RCL samples will be collected, but will only be tested if post-transplant samples are positive

The occurrence of RCL positivity would lead to suspension of the inclusion of any new subjects in the study. Further, RCL positivity will be considered an SAE and will be handled and reported as described in Section 6.3.17.3.

6.3.15 Proviral Integration Site Analysis in Blood and Bone Marrow by LAM-PCR

Blood or bone marrow samples for proviral integration site analysis by LAM-PCR and sequencing are to be collected according to the Schedule of Events; collection details are included in the Central Laboratory Manual that is appended to the Study Operations Manual.

An integration site analysis (ISA) may not be performed if VCN is less than 0.01.

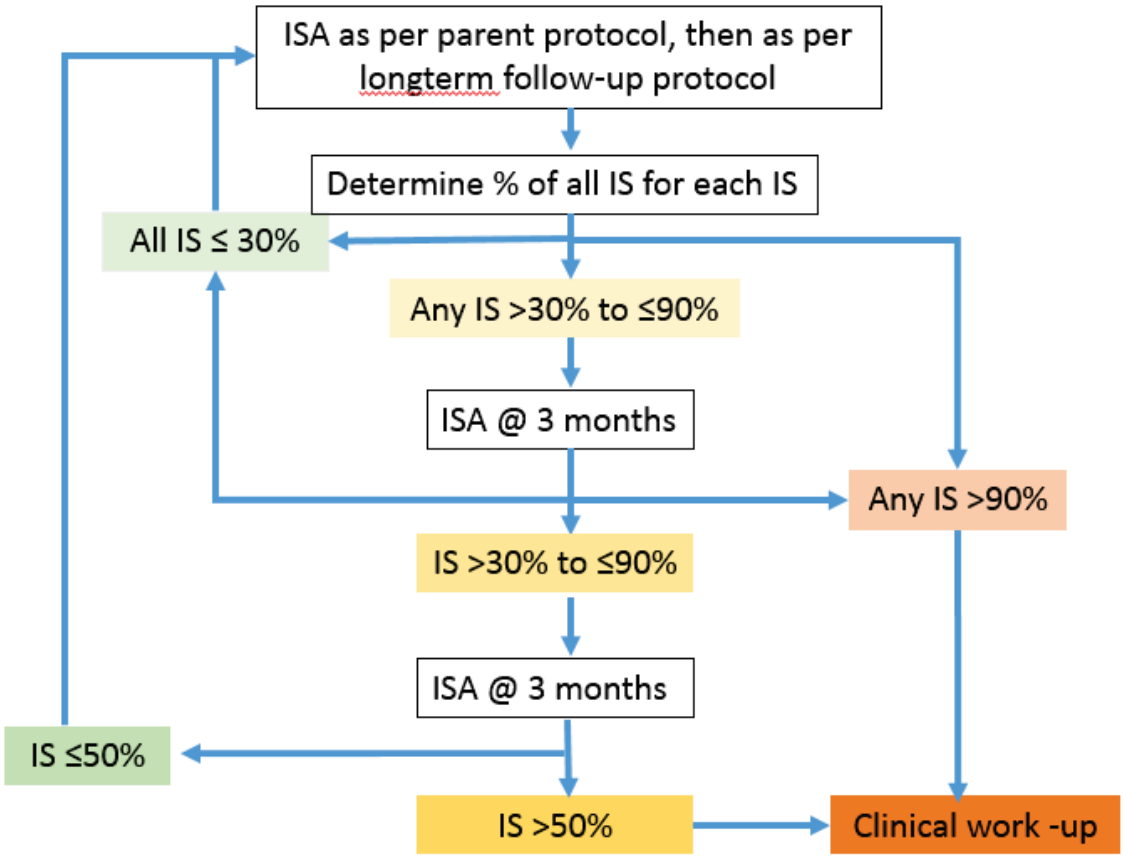
6.3.16 Assessment of Clonal Dominance and/or Suspicion of Leukemia/Lymphoma

6.3.16.1 Assessment of Clonal Dominance

The frequency of the ISA should be increased for subjects with VCN of ≥0.3 if the ISA demonstrates that a mappable insertion site (IS) contributes >30% to the total number of retrieved IS as follows (see also schematic in Figure 6-1), or at any time at the discretion of the investigator and sponsor:

- If ISA detects an IS contributing >30% to the total IS, ISA should be repeated twice, each test approximately 3 months apart.
    - If result is ≤30% clonal contribution at either repeat ISA, monitoring of the subject returns to the protocol-defined schedule.
    - If result is >30% and ≤90% clonal contribution at the first repeat, and ≤50% clonal contribution at the second repeat, monitoring of the subject also returns to the protocol-defined schedule.
    - If result is >30% and ≤90% clonal contribution at the first repeat, and >50% clonal contribution at the second repeat, clonal dominance criteria are met and clinical work-up for malignancy should be initiated.
- If ISA result is >90% clonal contribution at any time, clonal dominance criteria are met and clinical work-up for malignancy should be initiated.

Figure 6-1 Schematic for Assessment of Clonal Dominance



6.3.16.2 Other Criteria that can Trigger Clinical Work-up for Malignancy

- Any clinical suspicion of malignancy including leukemia or lymphoma
- Unexplained WBC count > 30,000 (cells/µL) on two consecutive measurements
- After achievement of a WBC count within the normal range post-drug product infusion and engraftment of gene-modified cells, the development of a WBC < 1000 (cells/µL) on two consecutive measurements

6.3.16.3 Clinical Work-up for Malignancy

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

- Physical exam
- Complete blood count (CBC) with differential and lymphocyte subsets
- Studies to rule out infectious cause
- Studies to rule out autoimmune disease
- Imaging studies, as appropriate
- Bone marrow analysis

If clinical results indicate a diagnosis of a malignancy or myelodysplasia, enrollment into this study will be suspended, and further analyses will be determined by the Sponsor, in consultation with the DMC. It should be noted it may not be possible to distinguish the source of malignancy, e.g. arising from transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional mutagenesis, and all efforts should be made to confirm the source of malignancy before determining to halt or alternatively to resume the study.

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

6.3.17 Adverse Events

Monitoring of AEs will be conducted throughout the study. AEs, as defined in Section 6.3.17.1, will be monitored and recorded in the CRFs from the time informed consent is signed through the following timepoints for any subject deemed eligible to start mobilization or bone marrow harvest:

- ≥Grade 1 AEs: through 30 days after LentiGlobin BB305 Drug Product infusion.
- ≥Grade 2 AEs and SAEs: through 12 months after LentiGlobin BB305 Drug Product infusion.
- LentiGlobin BB305 Drug Product-related AEs: through 24 months after LentiGlobin BB305 Drug Product infusion.

All AEs should be monitored until they are resolved or are determined to be due a stable or chronic condition.

For subjects who discontinue from the study for reasons other than withdrawal of consent before the performance of any invasive study procedure (eg, liver biopsy, mobilization), monitoring of AEs is required through 30 days post-discontinuation.

6.3.17.1 General Definitions

Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in subjects, whether or not considered drug related. An AE may include a change in physical signs, symptoms, and/or clinically significant laboratory change occurring in any phase of a clinical study. This definition includes intercurrent illnesses or injuries, and exacerbation of pre-existing conditions. A preexisting condition is a clinical condition (including a condition being treated) that is diagnosed before the subject signs the informed consent form and is documented as part of the subject's medical history.

For the purposes of this study, engraftment failure is defined as an AE and is to be reported as such.

Unexpected Adverse Events

An AE is considered unexpected with LentiGlobin BB305 Drug Product if it is not consistent in nature or severity with the LentiGlobin reference safety information which is contained in the current Investigator's Brochure provided to the Investigator by the Sponsor.

Conditioning-related Events

Busulfan IV is a cytotoxic drug that causes profound myelosuppression. Accordingly, subjects will experience intended hematologic events (eg, neutropenia, thrombocytopenia, anemia) and non-hematologic events (eg, mucositis [stomatitis], nausea, vomiting, alopecia, pyrexia) as a result of receiving busulfan IV. For the purposes of this protocol, these events, which are familiar to transplant physicians and are described in the busulfan prescribing information, are considered conditioning-related events (CREs).

The intended profound myelosuppression (manifested by neutropenia, thrombocytopenia, and/or anemia) and events that frequently occur after the initiation of busulfan IV conditioning and are considered to be the direct consequence of busulfan conditioning are to be reported as AEs, and also indicated as a CRE on the appropriate CRF.

If there is any doubt whether the information constitutes an AE, the information is treated as an AE for the purposes of this protocol.

All AEs will be monitored until resolution or, if the AE is determined to be chronic, a cause is identified. The time of the onset, intensity, relationship to Lenti-Globin BB305 Drug Product will also be recorded. If an AE remains unresolved at the conclusion of the study, a clinical assessment will be made by the Investigator and medical monitor as to whether continued follow-up of the AE is warranted.

The **relationship** of the study treatment to an AE will be determined by the Investigator based on the following definitions:

- Not Related: Exposure to the investigational product did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the investigational product (LentiGlobin BB305 Drug Product).
- Unlikely Related: The study treatment and the AE were not closely related in time, and/or the AE could be explained more consistently by causes other than exposure to the investigational product (LentiGlobin BB305 Drug Product).
- Possibly Related: The study product administration and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the investigational product (LentiGlobin BB305 Drug Product).
- Related: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study product than by other causes, or the investigational product (LentiGlobin BB305 Drug Product) was the most likely cause of the AE.

For the purposes of expedited safety reporting, events assessed as 'Not Related' and 'Unlikely Related' will be considered as Not drug related; and events assessed as 'Possibly Related' and 'Related' will be considered as Drug related.

The **severity** of AEs will be assessed according to NCI CTCAE, version 4.03. The following definitions should be used for toxicities that are not defined in NCI CTCAE, version 4.03:

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

The diagnosis rather than the signs/symptoms should be reported as the adverse event, when possible. If no diagnosis is available, report only the signs and symptoms that met AE criteria as individual AE terms.

6.3.17.2 Serious Adverse Events

An SAE is defined as any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires hospitalization or prolongation of existing in-patient hospitalization,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect,
- is an important medical event that may jeopardize the subject and may require medical or surgical intervention to prevent an outcome listed above.
    - An important medical event is an event that may not result in death, be life threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs.
    - For the purposes of this study, the confirmed presence of RCL in any subject's sample or the occurrence of leukemia/lymphoma related to vector-mediated oncogenesis by insertional mutagenesis would constitute an SAE
    - For the purposes of this protocol, Grade 3 and Grade 4 lab values (per CTCAE criteria) that are related to myeloablative conditioning (i.e. busulfan) will not be reported as an SAE unless they meet the requirement of being immediately life threatening.

6.3.17.3 Procedures for SAE Reporting

All SAEs must be reported to the Sponsor's agent (see below) within 24 hours of the Investigator's knowledge of the event.

Contact Information
CTI Safety
SAE Hotline: +33 (0) 800.90.17.58
Efax (France): +33 (1) 77.72.38.70
E-mail: CTISafety@ctifacts.com

A written report of any SAE will be submitted as per the instructions in the Study Operations Manual on the appropriate SAE form. The initial SAE report should contain the clinical site and subject numbers, date of birth, date of event, SAE diagnosis (or list of symptoms if diagnosis not yet available), brief description of event, and Investigator's assessment of relationship to LentiGlobin BB305 Drug Product. Supporting source documents should also be provided (hospital notes, discharge summaries, laboratory and procedure results, identified by the subject's initials and study number and with the subject's name and any other identification details removed). The Investigator or designee will then make an accurate and adequate report to the Sponsor's agent, and to the CPP, on any serious and unexpected AE that may reasonably be regarded as caused by or associated with LentiGlobin BB305 Drug Product and which was not previously anticipated (in nature, severity, or degree of incidence) in the written information or Investigator's Brochure provided to the Investigator by the Sponsor. In addition, all SAEs that occur at a particular clinical site regardless of whether they are deemed related or unexpected must also be reported to the presiding CPP. Any related and unexpected SAE that occurs when busulfan IV is administered must also be reported by the Investigator to the manufacturer of busulfan IV.

Copies of each report will be kept in the Investigator's study file and adequate documentation will be provided to the Sponsor, including documentation that the CPP has been notified of such SAEs.

Reports of all serious and unexpected AEs associated with the use of LentiGlobin BB305 Drug Product must be submitted to the study sites, ANSM, and the CPP within **15 calendar days** after their disclosure. The Sponsor and the Sponsor's agent will be responsible for this. However, fatal or life-threatening AEs associated with the use of the study drug must be reported by the Sponsor to the study sites, ANSM, and CPP within **7 calendar days** of disclosure.

Additional information regarding the subject's subsequent course must be submitted to the Sponsor's agent as per the instructions in the Study Operations Manual until the event has been resolved or until an acceptable medical endpoint has been reached.

6.3.18 Pregnancy

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which requires reporting as an SAE). However, all pregnancies occurring during this study (in subjects or female partners of subjects) are to be reported in the same time frame as SAEs. Any reported pregnancy occurring after initiation of dosing with busulfan IV must be immediately submitted (within 24 hours) on the specified Pregnancy Form to the Sponsor's agent as per the instructions in the Study Operations Manual. The course of all

pregnancies, including perinatal and neonatal outcome, regardless of whether the subject has discontinued participation in the study, will be followed until resolution, including follow-up of the health status of the newborn to 6 weeks of age.

All subjects, male and female, will be urged to use effective contraception during the entire study. Busulfan has been shown in animal studies to be teratogenic. The effects of administration of LentiGlobin BB305 Drug Product on the pregnant female or the developing fetus are unknown.

6.3.19 Unscheduled Visits

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

6.3.20 Long-Term Follow-Up Protocol

All subjects will be followed for 24 months post-transplant under this protocol. Then, if appropriate consent is obtained, subjects will be followed for an additional 13 years under a separate long-term follow-up protocol (LTF-303). This follow-up includes recording of SAEs, and archiving of peripheral blood leukocyte cell samples for RCL and clonality testing.

6.4 Appropriateness of Measurements

The safety measures used in this study are standard for the industry. RCL testing of the LentiGlobin BB305 Drug Product will be performed in parallel with administration to the subject and in the subjects' blood samples following treatment; this level of monitoring is specifically required for gene therapy protocols in order to detect potential recombination events that may result in RCLs.

The special laboratory tests, including measurements for the proportion of a given cell population containing the LentiGlobin BB305 lentiviral vector provirus and expression level of the $\beta^{A\text{-}T87Q}$-globin gene are sensitive, quantitative measures of the correction of the genetic defects that cause the clinical syndromes associated with SCD and β-Thal$_M$. The measurement of these special laboratory parameters, particularly the measurement of the percentage of Hb containing $\beta^{A\text{-}T87Q}$-globin, should correlate directly with the clinical endpoints of transfusion requirement and number of hospitalizations. Since transfusion and hospitalization data will be collected for each subject for 2 years prior to study entry, each subject will serve as their own control for these measures post-transplant.

7 STATISTICAL PROCEDURES

7.1 Sample Size Estimation

The sample size for this study was not determined by formal statistical methods, but was based on extent and availability of data.

7.2 Populations for Analysis

The primary population for analysis of both efficacy and safety will consist of those subjects who initiate any study procedures, beginning with mobilization (subjects with β-Thal$_M$) or anesthesia

for bone marrow harvest (subjects with SCD); this population will be denoted as the intent-to-treat (ITT) population. All subjects must have at least 2 years of follow-up at a specialized center, in order to be used as their own control for key measurements of clinical events and transfusion requirements. Additionally, analysis will be performed on those who undergo the gene therapy transplantation; should this be a smaller number of subjects, this population will be denoted as the transplant population (TP). It is anticipated that the ITT and TP groups will be identical.

Since it is of interest to evaluate the efficacy of the gene therapy in subjects who are successfully transplanted and are followed for sufficient time to evaluate clinical benefit, an evaluable population will be defined as those subjects who successfully engraft and have sufficient study visit compliance to acquire primary efficacy data through 24 months post-treatment. Subjects in this population must be compliant with the visit window for the Month 24 evaluations.

7.3 Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. Subjects in the ITT or TP analysis groups will be considered treatment failures in the primary analysis of stabilization, if they have less than 24 months post-transplant follow-up.

7.4 Statistical Methods

7.4.1 General Methods

Tabulations will be produced for appropriate demographic, baseline, efficacy, and safety parameters. For categorical variables, summary tabulations of the number and percentage within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the mean, median, standard deviation, minimum and maximum values will be presented. Two-sided 90% confidence intervals will be calculated as appropriate.

Descriptive summary statistics as well as 2-sided, 90% confidence intervals will be presented on selected parameters, as described in the sections below. By-subject listings of data for all completed and discontinued subjects will be provided.

For disease-specific biological parameters and clinical events, including RBC transfusion requirements and number of total hospitalization days at 6, 12, and 24 months, baseline will be defined as the average of these parameters over the 2 years prior to study entry. For other change from baseline analyses, baseline will be defined as the value closest to, but prior to transplant. Longitudinal data (collected serially over time on study and follow-up) will be presented by appropriate time intervals, such as monthly, quarterly and so forth, depending on the nature of the data.

7.4.2 Disposition of Subjects

A tabulation of the disposition of subjects will be presented, including the number enrolled, the number with any post-transplant data available for analysis, and the extent of data available. Tables and listings will be provided for subjects in each analysis data set, including the distribution of subjects according to the β-hemoglobinopathy type. Subject data will also be displayed by site. The number of subjects completing the study through 2 years post-transplant and reasons for study

discontinuation will be reported. Deviations from protocol treatment and assessment specifications will be tabulated and listed.

7.4.3 Demographic and Baseline Characteristics

The following demographic and baseline characteristic factors will be summarized: age (current and age at diagnosis), country of origin, race and ethnicity, time from diagnosis of β-hemoglobinopathy type to confirmation for inclusion in the study, the presence of any significant co-morbid conditions, and the time from diagnosis of β-hemoglobinopathy type to treatment.

7.5 Efficacy Analysis

The following parameters will be evaluated using descriptive statistics.

For clinical events and RBC transfusion requirements, each subject will serve as their own control, as 2 years of pre-transplant hospitalization and biological data will be compared with post-transplant values.

7.5.1 For All Subjects

- Therapeutic globin expression, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin as well as the amount of $\beta^{A\text{-}T87Q}$-globin as a fraction of all β-chains in whole blood.
- Average VCN in cell populations from peripheral blood and bone marrow containing the integrated LentiGlobin BB305 lentiviral vector.
- RBC transfusion requirements (mL) at 6, 12, and 24 month periods.
- Number of in-patient hospitalization days (post-transplant discharge) at 6, 12, and 24 months.

7.5.2 For SCD Subjects

- The number of VOC or acute chest syndrome events at 6, 12, and 24 months.
- Evaluation of changes in the nature or frequency of the subject-specific main inclusion criterion.

7.6 Safety Analysis

All eligible subjects enrolled in this study will be evaluated for safety. The safety analyses will include evaluation of the incidence of AEs by preferred term and body system coded using the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be summarized for those events that occur 1) after signing the informed consent and prior to mobilization; 2) start of mobilization through start of conditioning; 3) from the start of conditioning until through 43 days after drug product infusion; and 4) from the start of LentiGlobin BB305 Drug Product infusion on Day 1 through Day +365 (≥Grade 2 AEs); and 5) from the start of LentiGlobin BB305 Drug Product infusion on Day 1 through the Month 24 Visit (SAEs and LentiGlobin BB305 Drug Product-related AEs). Laboratory measures will be compared with their corresponding normal ranges and the incidence of abnormal laboratory values will be calculated for each relevant protocol-specified laboratory test. Replication-competent lentivirus testing will be performed and any positive results will be confirmed. Banked leukocytes will be assayed for insertional mutagenesis by LAM-PCR and sequencing in the event that a malignancy is observed.

7.7 Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original statistical analysis plan will be provided in the final clinical study report.

8 ADMINISTRATIVE AND REGULATORY REQUIREMENTS

8.1 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki.

The selected CPP will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the subjects. The study will only be conducted at clinical sites where CPP approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the subjects (including diary cards), safety updates, annual progress reports, and any revisions to these documents will be provided to the CPP by the Principal Investigator or designee.

During the study, the Principal Investigator or designee is responsible for: 1) notifying the CPP in writing of any serious or other significant AEs, 2) informing the CPP of the study progress periodically as required by the CPP, and 3) obtaining periodic (at least annual) CPP re-approval. The CPP must also be notified of study completion within 30 days of the final visit of the last subject and should be provided with a summary of the results of the study by the Principal Investigator or designee.

bluebird bio must have on file, prior to study initiation, documentation of a recommendation of approval of the study and a letter from ANSM and the CPP granting approval of the study.

8.2 Comité de Surveillance

This is a scientific and medical committee that will be composed of at least 4 voting members, including a Chairman and at least 3 other independent reviewers who are experts in β-Thal$_M$ and/or SCD, or gene therapy. These individuals will have no involvement with the planning or operational aspects of the clinical study. They may not have a financial equity interest in bluebird bio. Non-voting participants may include the Principal Investigator, the Study Director, certain Co-investigators, including the study Biostatistician, and Sponsor representatives, including the bluebird bio Chief Medical Officer and Chief Scientific Officer. Non-voting members are not entitled to participate in "closed" sessions of the CdS. A charter has been developed under which the Comité de Surveillance will conduct itself.

This Committee met after the 3 subjects treated under Protocol LG001 completed at least 2 months post-transplant to review all safety, laboratory and efficacy data, as described in Section 3.1., and after 2 subjects enrolled under Protocol HGB-205 had completed at least 3 months post-transplant. Treatment of additional subjects may commence upon approval from the Comité de Surveillance. The Committee will also meet on a regular basis during the study to review the progress of subjects

on the study, as well as all SAEs, safety laboratory values, and special hematology testing, in addition to making recommendations on study conduct.

8.3 Subject Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from either the subject or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

8.4 Subject Confidentiality

In order to maintain subject privacy, all CRFs, LentiGlobin BB305 lentiviral vector and LentiGlobin BB305 Drug Product accountability records, study reports, and communications accessible to the sponsor will identify the subject by initials and their assigned subject number. The Principal Investigator/Coordinator and associated investigators will grant monitor(s) and auditor(s) from bluebird bio or its designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

8.5 Protocol Compliance

The Principal Investigator /Coordinator and associated investigators will conduct the study in compliance with the protocol provided by bluebird bio, and given approval/favorable opinion by the CPP and the appropriate regulatory authority(ies). Modifications to the protocol should not be made without agreement of both the Principal Investigator and bluebird bio. Changes to the protocol will require written CPP and approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The CPP may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the CPP. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the Principal Investigator will contact the bluebird bio responsible medical officer, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the CRF and source documentation.

8.6 Direct Access to Source Data

Monitoring and auditing procedures developed by bluebird bio will be followed, in order to comply with GCP guidelines.

The study will be monitored by bluebird bio or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the CRFs for completeness and clarity, cross-checking with source documents, and clarification of administrative matters. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, e-mail, telephone, and fax).

Regulatory authorities, the CPP, and/or bluebird bio's clinical quality assurance group may request access to all source documents, CRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Principal Investigator and Coordinator and associated investigators, who must provide support at all times for these activities.

8.7 Case Report Form Completion

bluebird bio will provide the clinical sites with a CRF for each subject.

CRFs will be completed for each study subject. It is the Principal Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

The Principal Investigator or designated representative, should complete the CRF pages in a timely manner. Post screening, CRFs should be completed as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Principal Investigator must sign and date the Investigator's Statement at the end of the CRF to endorse the recorded data.

8.8 Record Retention

The Principal Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval, or 2 years after formal discontinuation of the clinical development of the investigational product, or according to applicable regulatory requirement(s). If the Principal Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. bluebird bio must be notified in writing if a custodial change occurs.

bluebird bio has full rights over any invention, discovery, or innovation, patentable or not, that may occur when performing the study, unless otherwise agreed to in writing.

8.9 Liability and Insurance

bluebird bio has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

8.10 Publication of Study Findings and Use of Information

All information regarding LentiGlobin BB305 lentiviral vector, or LentiGlobin BB305 Drug Product supplied by bluebird bio to the Principal Investigator is privileged and confidential information. The Principal Investigator agrees to use this information to accomplish the study and

will not use it for other purposes without consent from bluebird bio. It is understood that there is an obligation to provide bluebird bio with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of LentiGlobin BB305 Drug Product and may be disclosed to regulatory authority(ies), other investigators, corporate partners, or consultants as required, as authorized by bluebird bio.

8.10.1 Publications

The first authors of any published paper(s) will be individuals who have directly participated in the establishment of the study protocol, its conduct, or data analysis. Associated investigators will be mentioned as participants in the study group. PPD and PPD will be the "senior authors", either first and last, or co-first or co-last. PPD and PPD will decide jointly on the composition and order of the co-authors.

8.10.2 Presentations

It is anticipated that data from this study will be presented at scientific meetings. Any materials (e.g., slides, abstracts, scripts of oral presentations) are to be provided to bluebird bio for review and approval at least 15 days prior to presentation / submission for presentation. bluebird bio will make every effort to provide any revisions in advance of the scheduled presentation date.

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Protocol Title:	A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β-Hemoglobinopathies (Sickle Cell Disease and 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 Number:	HGB-205 Version 7.0 (19 May 2016)

INVESTIGATOR STATEMENT

I have read, understood, and agree to abide by all the conditions and instructions contained in this protocol.

I understand that all documentation provided to me by bluebird bio or its designated representative(s) concerning this study that has not been published previously will be kept in the strictest confidence. This documentation includes the study protocol, investigator brochure, case report forms, and other scientific data.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any subjects, or any persons used as controls, that the Drug Product is being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent, as per local regulations and under Good Clinical Practice (GCP), are met.

I agree to report to the Sponsor adverse events that occur in the course of the investigation(s) in accordance with this protocol and as required by local regulations and under GCP.

I have read and understand the information in the investigator's brochure, including the potential risks and side effects of the Drug Product.

I agree to maintain adequate and accurate records and to make those records available for inspection in accordance with local regulations and under GCP.

I will ensure that an ethics committee that complies with all local regulations and GCP requirements will be responsible for the initial and continuing review and approval of the clinical investigation.

I also agree to promptly report to the ethics committee all changes in the research activity and all unanticipated problems involving risks to human subjects or others.

I agree that this study will not commence without the prior approval of the appropriate national health authorities together with a properly constituted ethics committee. I agree that no changes will be made to the study protocol without the prior written approval of bluebird bio and the aforementioned regulatory bodies, as applicable in the relevant laws and regulations.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

Investigator Name	Investigator Signature	Date

Investigational site or name of institution and location (printed)