CLINICAL STUDY PROTOCOL HGB-204

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

Study Sponsor: bluebird bio, Inc.
150 Second Street
Cambridge, MA 02141 USA
Telephone: (001)-339-499-9300
Fax: (001)-339-499-9432

Responsible Medical Officer: PPD, MD
PPD of Hematology
Clinical Development
bluebird bio, Inc.
Mobile telephone: PPD
E-mail: PPD

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SUMMARY OF CHANGES

This protocol (Version 5.0, 29 June 2015) replaces previous (Version 4.0, 06 February 2015). The main reason for this amendment is to collect CCI data from subjects in this study, the addition of further hemoglobin monitoring in between visits, and to update clonal dominance language as advised by a recent Advisory Board convened with experts on this topic.

Changes were made as follows (**new text in bold**):

Section of current document	Rationale	Change
Synopsis, Section 2.2.1, Schedules of Events (SOE), Section 6.2.20	CCI	Add new CCI CCI
Section 3.5.2, Section 6.2.21	Sections updated based on recommendations from a recent Advisory Board assessment of how ISA data should be interpreted with respect to the likelihood of clonal dominance. This Advisory Board was convened by bluebird bio specifically for this purpose and consisted of a number of international experts in the gene therapy field.	Section 3.4.3, Enrollment suspension criteria: Removed the criterion of > 10% clonal contribution and concurrent presence of leukogenesis, and footnoted the criterion of detection of malignancy due to vector-mediated insertional oncogenesis to give more detail. Section 6.2.20, Assessment and Work-up for Clonal Dominance and/or Suspicion of Leukemia/Lymphoma: Replaced previous section on this (previously located in Section 3.4.4) to give more details on how clonal dominance will be determined and investigated.
Section 5.10.5, Appendix 10.4	Consistency of outcomes may be improved by the following similar iron reduction guidelines across institutions	Iron reduction guidelines added
Section 5.10.6	Consistency of outcomes may be improved by the following similar post-transplant transfusion guidelines across institutions	Transfusion guidelines added
Section 6.1	Increased the frequency of hemoglobin (Hb) analyses to provide more information on kinetics of Hb production	Additional CBC assessments added to Table 6-3 such that Hb can be calculated approximately every 4 weeks through the first year and every 8 weeks through the second year post-drug product infusion, resulted in reformatting of this table.
Section 6.1	Aligned with recommendations of recent Advisory board, who advised that integration site analysis (ISA) at	Removed 3M ISA analysis from Table 6-3

Section of current document	**Rationale**	**Change**
	3M provides only data on short-term progenitors, and has no value for predicting integration patterns in long-term progenitors	
Section 6.2.7	Provided more guidance on medical history to be collected, especially on past transfusion parameters	The medical history is to include all prior and current medical history, **including age of first transfusion, age of starting regular transfusions (i.e., age at which transfusion needs stabilized to between once every 4 to 8 weeks), age of starting iron chelation treatment, and spleen size (cm below coastal margin), and additional transfusion details at enrollment.**
Section 4.5, Section 6.2.22.1	Clarified AEs follow-up requirements during study and after discontinuation	Section 4.5: Clarified follow-up rules for subjects who withdraw, either before or after drug product treatment Section 6.2.21.1: Removed rules for follow-up of AEs after discontinuation from study, and cross referenced Section 4.5.
Section 6.2.21	Simplified the presentation of AE information for improved readability	All sections on AEs included as subheadings in a single section 6.2.21
Appendix 10.2, Section 5.2.1	Increased experience with apheresis of subjects with β-thalassemia major has led to more detailed advice on mobilization and apheresis procedures.	Updated with current recommendations for hemoglobin levels during mobilization and apheresis, and updated general recommendations for cell collection by apheresis

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

CLINICAL STUDY PROTOCOL SYNOPSIS

Protocol Title:	A Phase 1/2, Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with β-Thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector (LentiGlobin BB305 Drug Product)
Protocol Number:	HGB-204
Objectives:	• Evaluate the safety of treatment with LentiGlobin BB305 Drug Product in subjects with β-thalassemia major • Evaluate the efficacy of treatment with LentiGlobin BB305 Drug Product in subjects with β-thalassemia major
Study Design:	This is a non-randomized, open label, multi-site, single dose, Phase 1/2 study in up to 18 subjects (including at least 3 adolescents ≥ 12 < 18 years of age) with β-thalassemia major who receive at least 100 mL/kg/year of packed red blood cells (pRBCs) or ≥8 transfusions of pRBCs per year in each of the 2 years preceding enrollment. The study will evaluate the safety and efficacy of autologous hematopoietic stem cell (HSC) transplantation (HSCT) using LentiGlobin BB305 Drug Product (autologous CD34+ HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene and resuspended in cryopreservative solution in the final immediate container for the intended medical use). Initially, treatment will be staggered. The second subject will begin myeloablative conditioning only after the first subject 1) engrafts (defined as an absolute neutrophil count [ANC] $\geq 0.5 \times 10^9$/L for 3 consecutive days); and 2) has no LentiGlobin BB305 Drug Product treatment-related serious adverse event (SAE) unexpected to occur with autologous HSCT. After Subject 2 meets these same criteria, parallel drug product treatment can occur with additional subjects. The study has 4 distinct stages, as follows. **Stage 1:**Screening to determine eligibility **Stage 2:** Autologous CD34+ cell collection, LentiGlobin BB305 Drug Product manufacture and disposition Within the period of 30 days prior to HSC collection, the subject's transfusion regimen may be adjusted to maintain a minimum of 10 g/dL of hemoglobin (Hb) in order to suppress dyserythropoiesis, which can impede the isolation of CD34+ cells enriched for undifferentiated HSC. Each subject will undergo HSC mobilization with filgrastim and plerixafor. Peripheral blood mononuclear cells (PBMCs) will be collected by apheresis using standard methods. A total of 2 mobilization cycles may be performed if needed. Each mobilization cycle may include up to 5 apheresis procedure days, but only 2 consecutive apheresis procedure products may be sent for transduction from any mobilization cycle. The other apheresis procedure products should be used for rescue cells, if they meet minimum requirements as a rescue product. If 2 mobilization cycles are needed to collect sufficient HSCs to meet the requirement of a total dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg, then 2 transductions will be performed: one on the cells collected during Mobilization Cycle 1, and one on the cells collected during Mobilization Cycle 2. Each transduced product from each Mobilization Cycles 1 and 2 is an independent drug product. Thus, subjects who require 2 mobilization cycles to produce the minimum cell dose will be treated with 2 drug products in total. Mobilization cycles must be separated by at least 2 weeks. A bone marrow harvest is also allowed, but only to procure cells for rescue. The harvested cells to be used for transduction will be selected for the CD34+ marker to enrich for HSCs, transduced with LentiGlobin BB305 Lentiviral Vector, and stored under the vapor phase of liquid nitrogen while testing is ongoing.

	Stage 3: Myeloablative conditioning and infusion of LentiGlobin BB305 Drug Product After the transduced cells are dispositioned for clinical use, the subject will undergo myeloablative conditioning with busulfan. Busulfan will be administered intravenously (IV) at a starting dose of 3.2 mg/kg/day or 0.8 mg/kg every 6 hours for 4 consecutive days. The dose of busulfan will be adjusted based upon first dose busulfan pharmacokinetics in order to maintain appropriate levels for myeloablation (area under the curve [AUC] goal of 1000 [range 900 to 1200] µM*min for an every 6 hours [q6h] dosing regimen, or 4000 [range 3600 to 5000] µM*min for a once daily [qd] dosing regimen). Clinical sites that use a test dose of busulfan several days before beginning myeloablation to pre-determine busulfan dose may also do so in this protocol. After completion of the 4-day course of busulfan, there must be a minimum of 72 hours of busulfan washout before drug product infusion. On Day 0, after thawing, the LentiGlobin BB305 Drug Product(s) will be administered via IV infusion at a dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg. Subjects who undergo 2 mobilizations (and subsequent transduction of those cells) to achieve a total dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg will have 2 LentiGlobin BB305 Drug Products, which should be administered in sequence, with the second administered immediately after the first. **Stage 4:** Follow-up, through engraftment and 24 months after drug product infusion Subjects will be followed daily in the transplant unit for adverse events (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 (defined as an ANC $\geq 0.5 \times 10^9$/L for 3 consecutive days); and 2) the subject is considered medically stable. After discharge, subjects will be followed in this protocol for a minimum of 24 months after LentiGlobin BB305 Drug Product infusion.
Data Monitoring Committee:	A Data Monitoring Committee (DMC) comprised of members with appropriate scientific and medical expertise to monitor the study will be convened before the study is opened. The DMC will be charged with review of all unexpected (i.e., unexpected in nature and / or in severity) LentiGlobin BB305 Drug Product treatment-related adverse events, following notification by the Sponsor. The DMC may recommend that the Sponsor stop the study at any time due to concerns for the safety of the subjects.
Number of Subjects Planned:	Up to 18 subjects, including at least 3 adolescents (≥ 12 < 18 years of age) will be treated with drug product. Replacement subjects may be added if subjects withdraw prior to conditioning.
Inclusion Criteria:	1. Subjects between 12 and 35 years of age, inclusive, at the time of consent or assent (as applicable), and able to provide written consent (adults, or legal guardians, as applicable) or assent (adolescents). 2. Diagnosis of β-thalassemia major and a history of at least 100 mL/kg/year of pRBCs or ≥8 transfusions of pRBCs per year for the prior 2 years. 3. Documented baseline, or pretransfusion, Hb level ≤7 g/dL. 4. Clinically stable, have a Karnofsky performance status of ≥ 60, and eligible to undergo HSCT. 5. Treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records, including transfusion history.
Exclusion Criteria:	1. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV-1 or HIV-2), hepatitis B virus (HBV), or hepatitis C virus (HCV). (Note that subjects

	who are positive for anti-HBV antibody [to either core or envelope proteins] or for anti-HCV antibody are eligible as long as they have a negative HBV or HCV viral load by quantitative polymerase chain reaction [qPCR]). Where clinically and/or regionally indicated, one or more of the following tests may be performed, in which case positive results would exclude the subject from participating: human T-lymphotrophic virus-1 (HTLV-1) or HTLV-2, syphilis (RPR), toxoplasmosis, Trypanosoma cruzi, or West Nile Virus. 2. Active bacterial, viral, fungal, or parasitic infection. 3. A white blood cell (WBC) count $< 3 \times 10^9$/L, and / or platelet count $< 100 \times 10^9$/L if not due to hypersplenism. 4. Uncorrected bleeding disorder. 5. Any prior or current malignancy or myeloproliferative or immunodeficiency disorder 6. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome and familial adenomatous polyposis). 7. Prior HSCT. 8. Advanced liver disease, defined as: a. Baseline alanine transaminase or direct bilirubin value $>3 \times$ the upper limit of normal (ULN), or b. Liver biopsy demonstrating cirrhosis, any evidence of bridging fibrosis, or active hepatitis. 9. Baseline estimated glomerular filtration rate (eGFR) < 70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation) for ≥ 18 years of age, and Bedside Schwartz equation calculator for < 18 years of age. (see http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm 10. Uncontrolled seizure disorder. 11. Diffusion capacity of carbon monoxide (DLco) $< 50\%$ of predicted (corrected for hemoglobin and/or alveolar volume, as clinically indicated). 12. A cardiac T2* < 10 ms by magnetic resonance imaging (MRI). 13. Any other evidence of severe iron overload that, in the investigator's opinion, warrants exclusion. 14. Clinically significant pulmonary hypertension, as defined by the requirement for ongoing pharmacologic treatment or the consistent or intermittent use of supplemental home oxygen. 15. Participation in another clinical study with an investigational drug within 30 days of Screening. 16. Failure to obtain appropriate informed consent / assent. 17. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician or investigator 18. Contraindications to the conditioning regimen. 19. Prior receipt of gene therapy. 20. Diagnosis of significant psychiatric disorder of the subject that could seriously impede the ability to participate in the study. 21. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile subjects. Females of child-bearing potential are required to use effective contraception from Screening through at least 6 months after drug product infusion. Male subjects are required to use effective contraception

	(including condoms) from Screening through at least 6 months after drug product infusion. 22. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
Duration of Subject Participation:	Time between Screening and drug product infusion will be variable, and is estimated to be between 2 to 3 months; thereafter subject is planned to remain on study for approximately 24 months after drug product infusion. Eligible subjects will then be enrolled in a long-term follow-up study for another 13 years, for a total of approximately 15 years.
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 resuspended in cryopreservative solution in the final immediate container for the intended medical use). All subjects are to receive LentiGlobin BB305 Drug Product(s) on Day 0 via IV infusion at a total dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg.
Safety endpoints:	Safety will be evaluated by the following: • Success and kinetics of HSC engraftment • Incidence of transplant-related mortality through 100 days post-transplant • 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
Efficacy endpoints:	**Primary Efficacy Endpoint:** • The sustained production of ≥ 2.0 g/dL of HbA containing β^{A-T87Q}-globin for the 6 months between Month 18 and Month 24 post-transplant CCI • CCI • CCI • CCI
Pharmacodynamic endpoints:	Transgene marking and expression will be determined by: • Therapeutic globin expression, as measured by assessing the ratio of β^{A-T87Q}-globin to α-globin and β^{A-T87Q}-globin to all β-like-globin-chains in whole blood • Average VCN in cell populations from peripheral blood and, if collected, bone marrow containing the integrated LentiGlobin BB305 provirus

Statistical Methods:	The primary populations for analysis of both efficacy and safety will consist of those subjects who initiate any study procedures (intent-to-treat; ITT), beginning with mobilization, and those who undergo LentiGlobin BB305 Drug Product treatment (transplant population; TP), should this be a smaller number of subjects. Subjects in the ITT or TP population will be considered treatment failures in the primary endpoint analysis, should they have less than 6 months follow-up after drug product infusion. To evaluate the efficacy of LentiGlobin BB305 Drug Product 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 after drug product infusion. Tabulations will be produced for appropriate demographic, baseline, efficacy, and safety parameters. Descriptive summary statistics will be presented. 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. By-subject listings of data for all completed and discontinued subjects will be provided. For change from baseline analyses, baseline will be defined as the value closest to, but prior to drug product infusion. 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. AEs will be tabulated separately for those that occur 1) after signing the informed consent and prior to conditioning; 2) from the start of conditioning until Day 0 (immediately before the start of LentiGlobin BB305 Drug Product infusion); 3) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through 42 days post-infusion; 4) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through 12 months post-infusion (≥Grade 2 AEs); and 5) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through the entire 24-month study period (SAEs and LentiGlobin BB305 Drug Product-related AEs). Safety parameters will be summarized for each 3-month period, and survival status, AE, SAEs, laboratory abnormalities (values outside of normal ranges) and insertional oncogenesis (insertional mutagenesis resulting in oncogenesis) events will be tabulated. For clinical events and transfusion requirements, each subject will serve as their own control in that 2 years of pre-drug product infusion data will be compared with post-infusion values.

LIST OF ABBREVIATIONS

Abbreviation	Definition
Ab	Antibody
AE	Adverse event
ANC	Absolute neutrophil count
bp	Base pair
CBC	Complete blood count
cHS4	Chicken hypersensitivity site 4
CMV	Cytomegalovirus
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
CCI	
DMC	Data Monitoring Committee
CCI	
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EC	Ethics Committee
ECG	Electrocardiogram
FDA	Food and Drug Administration
CCI	
FSH	Follicle stimulating hormone
CCI	
GCP	Good Clinical Practice
GVHD	Graft-versus-host-disease
Hb	Hemoglobin
HbA	Hemoglobin A
HBcAb	Hepatitis B core antibody
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HLA	Human leukocyte antigen
HPLC	High-pressure liquid chromatography
CCI	
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
HSV	Herpes simplex virus
HTLV-1	Human T-lymphotropic virus type 1
ICH	International Conference on Harmonization
IgA	Immunoglobulin A
IgG	Immunglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board

Abbreviation	Definition
ISA	Integration site analysis
ITT	Intent-to-treat
IV	Intravenous
LCR	Locus control region
LH	Luteinizing hormone
CCI	
LTR	Long terminal repeat
LVEF	Left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NCI	National Cancer Institute
NK	Natural killer
PBL	Peripheral blood leukocyte
PBMC	Peripheral blood mononuclear cell
PBSC	Peripheral blood stem cells
PCR	Polymerase chain reaction
pRBC	Packed red blood cells
qPCR	Qualitative polymerase chain reaction
RBC	Red blood cell
RCL	Replication competent lentivirus
RNA	Ribonucleic acid
RPR	Rapid plasma reagin
RT	Reverse transcriptase
CCI	
SAE	Serious adverse event
SCID-X1	X-linked severe combined immune deficiency
SIN	Self-inactivating
SOP	Standard Operating Procedure
SQUID	Superconducting Quantum Interference Device
T3	3,5,3'-triiodothyronine
T4	Thyroxine
CCI	
TP	Transplant population
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
US	United States
CCI	
VCN	Vector copy number
VSV-G	Vesicular stomatitis virus glycoprotein G
VZV	Varicella zoster virus
WBC	White blood cell

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1 INTRODUCTION

β-Thalassemia is a rare hereditary blood disorder found most commonly in persons of Mediterranean, Middle Eastern, Indian, and South Asian descent (Colah et al., 2010). Due to the rarity of this disease in North America, published research on the prevalence of β-thalassemia in the United States (US) is limited. In California, it is estimated that 1.8 in 100,000 births are affected by β-thalassemia (Michlitsch et al., 2009), based on the mandatory screening for hemoglobinopathies in this state. Adjusting from this state-wide rate and assuming the incidence of milder forms, it is estimated that the prevalence of β-thalassemia in the US population is ~5500 patients. However, this is likely an over-estimate as it assumes a normal life expectancy, which is not the case for the majority of patients (Delea et al., 2007), and immigration from Asia to California has likely led to a local increase in the state. The Thalassemia Clinical Research Network of the National Heart, Lung, and Blood Institute developed a registry and surveyed non-Network sites resulting in an estimate of ~1000 patients in the US and Canada with β-thalassemia. In brief, it appears that the estimated prevalence of β-thalassemia in the US is approximately 1,000 patients.

β-Thalassemia results from a biochemical imbalance in the monomeric globin chains of the hemoglobin tetramer. Normally existing at an approximate 1:1 ratio, causative mutations impair the production of β-globin and lead to a relative excess of α-globin. Unable to form hemoglobin (Hb) tetramers on their own, the excess α-globin chains become insoluble and form a precipitate that damages the developing red blood cell (RBC). The clinical implications of the α-globin/β-globin imbalance are 2-fold: 1) patients lack sufficient RBCs and Hb to effectively transport oxygen throughout the body; and 2) RBCs suffer an elevated rate of hemolysis that leads to morbidity through chronic vasculature damage.

The clinical course of β-thalassemia correlates with the degree of globin chain imbalance and is most severe in patients with β-thalassemia major. These patients require >100 mL/kg/year of packed RBCs (pRBC) or ≥8 transfusions per year, and produce little to no β-globin. In terms of genotype, most patients with the β^0/β^0 genotype and many with the β^E/β^0 genotype suffer from β-thalassemia major. β-Thalassemia major is diagnosed in infancy, and affected patients have profound hypochromic, microcytic anemia, producing as little as 1 to 7 g/dL of total Hb (Rachmilewitz and Giardina, 2011; Olivieri, 1999). In many developing countries where necessary chronic blood transfusions are not readily available, children with β-thalassemia major have a poor prognosis and experience growth retardation, hepatosplenomegaly, and skeletal deformities resulting from extra-medullary hematopoiesis. Ultimately, most die in childhood (Adams, III and Coleman, 1990).

Where adequate treatment is available, patients with β-thalassemia major receive chronic blood hypertransfusion regimens aimed at maintaining steady-state Hb levels of about 9-10 g/dL. This regimen consists of infusions with 3 units of pRBCs every 3 to 5 weeks, and is highly effective at preventing the hallmark symptoms of childhood β-thalassemia major. However, chronic transfusions introduce a large amount of elemental iron into the body (Cao et al., 1996), and over time lead to mortality through iron overload-associated heart and liver toxicity (Vichinsky et al., 2005; Cao et al., 1996). In order to prevent iron overload associated risks, patients must adhere to iron chelation regimens administered orally (Hoffbrand et al., 2003; Cappellini et al., 2006) or subcutaneously (Olivieri and Brittenham, 1997). However, compliance remains a key

challenge, and even with current therapies, overall survival until the age of 30 is only 55% (Modell et al., 2000; Delea et al., 2007).

The only cure for β-thalassemia major is allogeneic hematopoietic stem cell transplant (HSCT). For pediatric subjects with available human leukocyte antigen (HLA)-identical sibling donors (< 25% of cases), outcomes from this potentially curative procedure are promising. A recent large-scale allogeneic HCT study in 179 pediatric subjects (Sabloff et al., 2011) reported overall and disease-free survival rates of 91% and 88%, respectively. Approximately 9.5% of subjects suffered graft failure, and 38% and 13% experienced acute or chronic graft-versus-host-disease (GVHD), respectively. Unfortunately, the experience with allogeneic HSCT in adult subjects is considerably worse, with a 27% transplant-related mortality and only a 67% rate of thalassemia-free survival (Gaziev et al., 2005). In another study in 68 adults with thalassemia receiving HSCT using a matched, unrelated donor, overall rates of survival, disease-free survival, rejection, and transplant-related mortality were 79%, 66%, 14%, and 21%, respectively (Lucarelli et al., 2012). Therefore, in adults, HSCT is not a commonly prescribed approach to treatment and these subjects have a limited life-expectancy.

Based on the pioneering work of Philippe Leboulch, MD, PhD to develop therapeutic applications of gene therapy for β-globin disorders (Cavazzana-Calvo et al., 2010), 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 for the treatment of β-thalassemia major.

The LentiGlobin BB305 lentiviral vector is a replication defective, self-inactivating (SIN), human immunodeficiency virus type 1 (HIV-1) based lentiviral vector encoding a single codon variant of the β-globin gene, $\beta^{A\text{-}T87Q}$, which conserves the protein's function while allowing for quantification relative to other globin species. Expression of the $\beta^{A\text{-}T87Q}$ gene is driven by the erythroid lineage specific globin locus control region (LCR) to correct the β-globin/α-globin imbalance in erythrocytes.

Gene therapy with LentiGlobin BB305 Drug Product involves the isolation of autologous mobilized hematopoietic CD34+ cells followed by transduction ex-vivo to introduce the $\beta^{A\text{-}T87Q}$-globin therapeutic gene. After undergoing myeloablation, subjects receive LentiGlobin BB305 Drug Product via intravenous (IV) infusion, leading to peripheral reconstitution with corrected erythrocytes. As LentiGlobin BB305 Drug Product consists of genetically-modified autologous cells, the risks of GVHD and graft rejection are eliminated.

A previous LentiGlobin lentiviral vector (LentiGlobin HPV569 lentiviral vector) was utilized in a clinical study, LG001, in which 3 subjects with β-thalassemia major were treated (Cavazzana-Calvo et al., 2010). bluebird bio has designed LentiGlobin BB305 lentiviral vector to improve the transduction efficiency, integrated provirus stability, and safety of this earlier LentiGlobin lentiviral vector (NC-11-001-R; NC-11-004-R). (See Section 3.2.1 for details.)

Nonclinical studies have been performed to support the use of LentiGlobin BB305 lentiviral vector in clinical studies. Results of such studies showed transduction efficiency of CD34+ cells to be higher with LentiGlobin BB305 lentiviral vector than with HPV569. The improved transduction efficiency resulted in a greater amount of therapeutic globin produced in erythroid colonies with LentiGlobin BB305 lentiviral vector than with HPV569, while the level of expression per copy remained unchanged (NC-11-001-R; NC-11-004-R).

The potential for insertional oncogenesis (defined throughout this document as insertional mutagenesis resulting in oncogenesis) with lentiviral constructs were analyzed in 2 independent in vitro immortalization assays (NC-12-016-R). Compared to the positive control vectors, which have known potential for insertional oncogenesis, LentiGlobin BB305 lentiviral vector demonstrated a significantly reduced risk of in vitro immortalization of murine hematopoietic stem cells (HSCs). Furthermore, there was no significant cytotoxicity associated with transduction with the test vector.

No clinical data with LentiGlobin BB305 Drug Product were available at the time this protocol was first approved. However, treatment with autologous CD34+ HSCs transduced with LentiGlobin HPV569 lentiviral vector had been investigated in 3 subjects with β-thalassemia major, all of whom required significant transfusion support prior to treatment (Cavazzana-Calvo et al., 2010). The 3 subjects were treated between September 2006 and November 2011 in Study LG001, a Phase 1/2 study in France.

Among the 3 subjects treated, LentiGlobin HPV569 Drug Product has been well tolerated, with no significant, long-term sequelae of treatment observed. In particular, no subject has developed HIV positivity, vector-derived replication competent lentivirus (RCL), leukemia, or lymphoma. In one subject (Subject PPD) treated with LentiGlobin HPV569 Drug Product, 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 RBC or nucleated cell homeostasis in peripheral blood or bone marrow, and the levels of this clone have gradually decreased over time. Clinical benefit has been demonstrated in this same subject (Subject PPD), as evidenced by transfusion-independence and stable Hb levels by 1 year post-transplant that have remained stable for >4 years thereafter despite regular phlebotomies. (See the Investigator's Brochure for additional details regarding these 3 subjects.)

On the basis of these encouraging results, this clinical study will investigate LentiGlobin BB305 Drug Product for the treatment of β-thalassemia major.

1.1 Potential Risks

1.1.1. Oncogenesis by Insertional Mutagenesis

Lentiviral vectors are retroviruses, which integrate into the chromosome of target cells upon transduction. A potential risk of this type of vector is insertional mutagenesis leading to oncogenesis. 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 (i.e., it is self-inactivating [SIN]). Gene transfer with γ-retroviral vectors has resulted in oncogenesis in the clinical trials for X-linked severe combined immune deficiency (SCID-X1) (Hacein-Bey-Abina et al., 2008; Howe et al., 2008), chronic granulomatous disease (Stein et al., 2010), and Wiskott-Aldrich Syndrome (Boztug et al., 2010). Five of 20 subjects in the SCID-X1 trial developed acute lymphocytic leukemia; one of the 5 subjects succumbed to leukemia, while the remaining 4 were successfully treated. In the Wiskott-Aldrich study, 4 subjects developed leukemia (Paruzynski A, 2012).

The different nature of the LentiGlobin BB305 lentiviral vector should minimize but not completely eliminate the risk of oncogenesis by insertional mutagenesis. Unlike the γ-retroviral vectors that led to leukemia, SIN lentiviral vectors, such as LentiGlobin BB305 Lentiviral Vector, may represent a substantial improvement in terms of safety (Montini et al., 2006;

Riviere et al., 2012). SIN lentiviral vectors lack the strong enhancer/promoter long terminal repeat (LTR) sequences of γ-retroviral vectors, and, unlike γ -retroviral vectors, 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 (Biffi et al., 2011). To date, it is estimated that more than 50 subjects have been treated in gene therapy studies involving lentiviral vectors and HSCs, with no published cases of therapy-related leukemia or lymphoma in any patient (Scaramuzza et al., 2012; Montini E et al., 2012; Cavazzana-Calvo et al., 2010; Cartier et al., 2012)(data on file).

Supporting evidence for the safety of LentiGlobin BB305 Drug Product comes from 3 patients treated in Study LG001; see Section 1 and the Investigator's Brochure for details.

1.1.2. Vector Integration within the HMGA2 Gene

Integration site (IS) analysis in nucleated blood cells for Subject PPD in Study LG001 revealed that 1 dominant myeloid cell clone contained an integrated vector in the third intron of the HMGA2 gene. Vector integration resulted in transcriptional activation of this gene, producing a truncated messenger ribonucleic acid (mRNA) with loss of the sites for binding and degradation by let7 family microRNAs in the fourth and fifth exons by virtue of a cryptic splice acceptor in the 5′ LTR of the vector. The HMGA2 mRNA and protein are expressed in erythroblasts, but not in neutrophils or monocytes. Through last follow-up, the clone has remained under homeostatic control, with a peak at ≤ ~4% and gradually decreasing levels to approximately 1.5% of total nucleated blood cells at 5 years post-transplant. The clone is not present in lymphocytes, suggesting cell expansion may be restricted to the myeloid cell lineage. Despite declining levels of this clone, the amount of $\beta^{A\text{-}T87Q}$-globin produced in Subject PPD has remained stable, indicating that the observed therapeutic benefit is not dependent on this specific clonal expansion.

The insertion of retroviral vectors in the HMGA2 gene has been observed in other gene therapy studies, with no published cases of therapy-related leukemia or lymphoma in any patient (Scaramuzza et al., 2012; Montini E et al., 2012; Cavazzana-Calvo et al., 2010; Cartier et al., 2012). Truncation of HMGA2 mRNA has also been observed in benign tumors, such as lipomas, and has been observed for many years in 2 patients with paroxysmal nocturnal hemoglobinuria without malignant transformation (Murakami et al., 2012; Inoue et al., 2006). The truncated HMGA2 mRNA expression may provide an advantage with regard to proliferation, but may not be oncogenic, per se.

1.1.3. Use of a Lentiviral Vector Derived from HIV-1

Because the LentiGlobin BB305 lentiviral vector was derived from HIV-1, a potential risk is mobilization or recombination with wild-type HIV-1. This risk is considered to be very low for several reasons: the lentiviral vector is replication incompetent, the probability of producing a recombinant virus from a multi-plasmid transfection is very low, the transduction is ex-vivo so there is no opportunity for the vector to be exposed to HIV, and a highly sensitive, validated assay is used prior to transduction to detect RCL. To date, none of the 3 subjects treated with LentiGlobin HPV569 Drug Product in Study LG001 have had RCL detected.

Subjects treated with LentiGlobin BB305 Drug Product will be monitored from Baseline for 24 months under the current protocol, and then 13 years under the separate, longterm follow-up

protocol to ensure that mobilization of the lentiviral vector has not occurred by testing for RCL. If a vector-derived RCL is detected in any subject, enrollment in the study will be suspended until a full investigation by the Data Monitoring Committee (DMC) and consultation with the appropriate regulatory authorities.

1.1.4. Other Risks

The risks of mobilization with filgrastim and plerixafor and of conditioning with Busulfex® (busulfan) are described in the product package inserts.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

The study objectives are to:

- evaluate the safety of treatment with LentiGlobin BB305 Drug Product in subjects with β-thalassemia major, and
- evaluate the efficacy of treatment with LentiGlobin BB305 Drug Product in subjects with β-thalassemia major.

2.2 Study Endpoints

2.2.1 Efficacy Endpoints

The primary efficacy endpoint is:

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

CCI

- CCI
- CCI
- CCI

2.2.2 Pharmacodynamic Endpoints

Transgene marking and expression will be determined by:

- therapeutic globin expression, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin and $\beta^{A\text{-}T87Q}$-globin to all β-like-globin-chains in whole blood, and
- average vector copy number (VCN) in cell populations from peripheral blood and, if collected, bone marrow containing the integrated LentiGlobin BB305 provirus.

2.2.3 Safety Endpoints

Safety will be evaluated by the following:

- success and kinetics of HSC engraftment,

- incidence of transplant-related mortality through 100 days post-transplant,
- overall survival,
- detection of vector-derived RCL in any subject,
- characterization of events of insertional mutagenesis leading to clonal dominance or leukemia, and
- monitoring of laboratory parameters and frequency and severity of clinical adverse events (AEs).

3 INVESTIGATIONAL PLAN

3.1 Overall Design and Plan of the Study

This is a non-randomized, open label, multi-site, single dose, Phase 1/2 study in up to 18 subjects (including at least 3 adolescents ≥ 12 < 18 years of age) with β-thalassemia major who receive at least 100 mL/kg/year of packed red blood cells (pRBCs) or ≥8 transfusions of pRBCs per year in each of the 2 years preceding enrollment. The study will evaluate the safety and efficacy of autologous hematopoietic stem cell (HSC) transplantation (HSCT) using LentiGlobin BB305 Drug Product (autologous CD34+ HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human β^{A-T87Q}-globin gene and resuspended in cryopreservative solution in the final immediate container for the intended medical use).

Initially, treatment will be staggered. The second subject will begin myeloablative conditioning only after the first subject 1) engrafts (defined as an absolute neutrophil count [ANC] ≥ 0.5×10^9/L for 3 consecutive days); and 2) has no LentiGlobin BB305 Drug Product treatment-related serious adverse event (SAE) unexpected to occur with autologous HSCT. After Subject 2 meets these same criteria, parallel drug product treatment can occur with additional subjects.

The study has 4 distinct stages, as follows.

Stage 1: Screening to determine eligibility

Stage 2: Autologous CD34+ cell collection, LentiGlobin BB305 Drug Product manufacture and disposition

Within the period of 30 days prior to HSC collection, the subject's transfusion regimen may be adjusted to maintain a minimum of 10 g/dL of hemoglobin (Hb) in order to suppress dyserythropoiesis, which can impede the isolation of CD34+ cells enriched for undifferentiated HSC.

Each subject will undergo HSC mobilization with filgrastim and plerixafor. Peripheral blood mononuclear cells (PBMCs) will be collected by apheresis using standard methods. A total of 2 mobilization cycles may be performed if needed. Each mobilization cycle may include up to 5 apheresis procedure days, but only 2 consecutive apheresis procedure products may be sent for transduction from any mobilization cycle. The other apheresis procedure products should be used for rescue cells, if they meet minimum requirements as a rescue product. If 2 mobilization cycles are needed to collect sufficient HSCs to meet the requirement of a total dose of ≥3.0×10^6 CD34+ cells/kg, then 2 transductions will be performed: one on the cells collected during Mobilization Cycle 1 and one on the cells collected during Mobilization Cycle 2. Each transduced product from each Mobilization Cycles 1 and 2 is an independent drug product.

Thus, subjects who require 2 mobilization cycles to produce the minimum cell dose will be treated with 2 drug products in total. Mobilization cycles must be separated by at least 2 weeks. A bone marrow harvest is also allowed, but only to procure cells for rescue.

The harvested cells to be used for transduction will be selected for the CD34+ marker to enrich for HSCs, transduced with LentiGlobin BB305 Lentiviral Vector, and stored under the vapor phase of liquid nitrogen while testing is ongoing.

Stage 3: Myeloablative conditioning and infusion of LentiGlobin BB305 Drug Product

After the transduced cells are dispositioned for clinical use, the subject will undergo myeloablative conditioning with busulfan. Busulfan will be administered intravenously (IV) at a starting dose of 3.2 mg/kg/day or 0.8 mg/kg every 6 hours for 4 consecutive days. The dose of busulfan will be adjusted based upon first dose busulfan pharmacokinetics in order to maintain appropriate levels for myeloablation (area under the curve [AUC] goal of 1000 [range 900 to 1200] µM*min for an every 6 hours [q6h] dosing regimen, or 4000 [range 3600 to 5000] µM*min for a once daily [qd] dosing regimen). Clinical sites that use a test dose of busulfan several days before beginning myeloablation to pre-determine busulfan dose may also do so in this protocol. After completion of the 4-day course of busulfan, there must be a minimum of 72 hours of busulfan washout before drug product infusion.

On Day 0, after thawing, the LentiGlobin BB305 Drug Product(s) will be administered via IV infusion at a dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg. Subjects who undergo 2 mobilizations (and subsequent transduction of those cells) to achieve a total dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg will have 2 LentiGlobin BB305 Drug Products, which should be administered in sequence, with the second administered immediately after the first.

Stage 4: Follow-up, through engraftment and 24 months after drug product infusion

Subjects will be followed daily in the transplant unit for adverse events (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 (defined as an ANC $\geq 0.5 \times 10^9$/L for 3 consecutive days); and

2) the subject is considered medically stable.

After discharge, subjects will be followed in this protocol for a minimum of 24 months after LentiGlobin BB305 Drug Product infusion.

3.1.1 Data Monitoring Committee

A DMC comprised of members with appropriate scientific and medical expertise to monitor the study will be convened before the study is opened. A charter describing the composition and conduct of the DMC will be issued by the Sponsor and agreed to by all DMC members prior to the DMC's initial meeting.

A DMC chairperson will be appointed who will be responsible for the overall operation of the DMC. Minutes from each meeting will be recorded and archived.

The DMC will meet by teleconference at regular intervals, approximately once every 3 months, or more or less frequently if needed, and depending on speed of subject enrollment and amount of new data generated. The DMC will be charged with review of all unexpected LentiGlobin Drug Product treatment-related SAEs following notification by the Sponsor.

It will be the responsibility of the Sponsor or Sponsor's designee to promptly contact the chairperson of the DMC about all unexpected AEs (i.e., unexpected in nature and / or in severity).

The DMC will have the right to recommend halting the study at any time due to concerns for the safety of the subjects. (Refer to Section 3.5.2 for the enrollment suspension criteria).

3.2 Rationale for the Study

Allogeneic HSCT is associated with a high rate of transplant-related mortality, particularly in adults, where it is not considered a standard of care for β-thalassemia major. In order to provide a potentially curative treatment to adults with β-thalassemia major, bluebird bio is developing LentiGlobin BB305 Drug Product (autologous CD34+ HSCs transduced with LentiGlobin BB305 lentiviral vector encoding the human $\beta^{A\text{-}T87Q}$-globin gene) for the treatment of β-thalassemia major.

Nonclinical study findings have shown that the therapeutic $\beta^{A\text{-}T87Q}$-globin gene is under the transcriptional control of the erythroid-specific human β-globin promoter and β-globin Locus Control Region elements (DNase I hypersensitive sites HS2, HS3, and HS4) (Pawliuk et al., 2001; Oh et al., 2004; Imren et al., 2002). Similarly effective ex vivo globin gene transfer into human CD34+ HSCs was observed (Imren et al., 2002). The $\beta^{A\text{-}T87Q}$-globin protein level expressed 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 RBCs (Pawliuk et al., 2001). This property is important for demonstrating a biological effect in subjects with β-thalassemia major 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.

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.

Treatment of β-hemoglobinopathies with gene therapy is also expected to eliminate the major risk of GVHD associated with allogeneic HSCT, since the CD34+ cells transduced with the lentiviral vector are autologous. In addition, in order to avoid complications of extensive immunosuppression associated with the conditioning regimen necessary for allogeneic HSCT, the proposed protocol is designed to use the single agent 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 hemoglobinopathies, including β-thalassemia, 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 their extended life-span (Vermylen, 2003; Pawliuk et al., 2001; Oh et al., 2004; Lucarelli et al., 1993; 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 is likely to result in clinical benefit to the patients (Walters et al., 2001; Lucarelli et al., 2001).

There was no clinical data with LentiGlobin BB305 Drug Product when this study initiated. However, as stated previously, 3 subjects were treated using LentiGlobin HPV569 Drug Product under Protocol LG001. All 3 subjects treated were adults with β-thalassemia major who

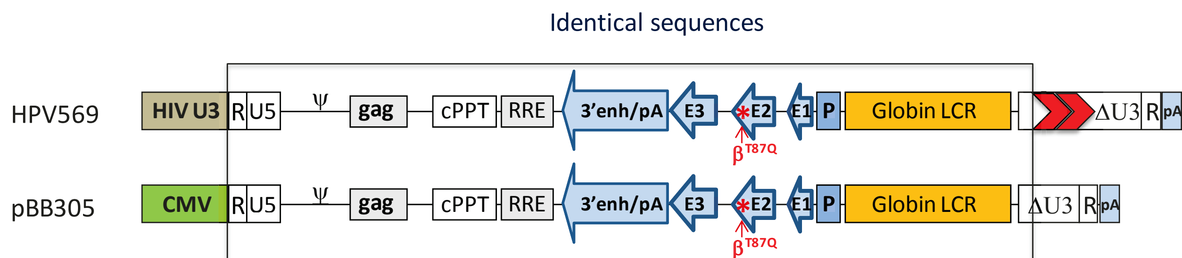
required significant transfusions (≥100 mL of pRBCs/kg/year). Treatment with autologous CD34+ HSCs transduced with LentiGlobin HPV569 lentiviral vector (LentiGlobin HPV569 Drug Product) has been well tolerated, with no significant, long-term sequelae of treatment seen. In particular, no subject has developed HIV positivity, vector-derived RCL, leukemia, or lymphoma. In 1 subject (Subject PPD) partial clonal dominance of a myeloid-biased repopulating cell was observed 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. Clinical benefit has been demonstrated in 1 of 3 subjects treated, as evidenced by transfusion independence and stable Hb levels observed 1 year post-transplantation that have remained stable for >4 years thereafter despite regular phlebotomies (Cavazzana-Calvo et al., 2010). Refer to the Investigator's Brochure for more information regarding these subjects.

3.2.1 History of LentiGlobin Lentiviral Vectors HPV569 and BB305

LentiGlobin BB305 lentiviral vector is a replication defective, SIN, third-generation 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 β-globin Locus Control Region elements (DNase I hypersensitive sites HS2, HS3, and HS4).

Protocol LG001 utilized LentiGlobin HPV569 Lentiviral Vector. The vector used in the current study, LentiGlobin BB305 Lentiviral Vector, is expected to demonstrate improved transduction efficiency as well as improved integrated provirus stability and safety (NC-11-001-R; NC-11-004-R). Schematics of LentiGlobin HPV569 lentiviral vector used in Protocol LG001 and the improved LentiGlobin BB305 lentiviral vector are presented in Figure 3-1.

Figure 3-1 Illustration of LentiGlobin HPV569 and BB305 Lentiviral Vectors



Key: The box denotes the boundaries of identical sequences for the 2 lentiviral vectors. The red angle brackets in the HPV569 plasmid denote the position of the 2 cHS4 core insulators (250bp) sequences. HIV U3, unique 3' eukaryotic Tat-dependent promoter from HIV-1; CMV, cytomegalovirus eukaryotic constitutive promoter; R, repeat; U5, unique 5´; Ψ, packaging signal; gag, HIV-1 partial gag sequence; cPPT, central polypurine tract; RRE, rev response element; 3´ enh/pA, 3´ enhancer/poly-adenylation signal from β-globin gene; E, exon; P, β-globin promoter; Globin LCR, human globin locus control regions; ΔU3, promoter/enhancer-deleted unique 3´; pA, synthetic polyadenylation signal. The position of T87A variation is indicated as red asterisk. Note that the HIV U3 and CMV promoters are a part of the transfer vector plasmids that drive expression of the packaged transcript in the packaging cell line and are not a part of the provirus that integrates into the recipient genome.

Source: Data on file (HPV569 and BB305 Vector Sequence Diagram vf 20121202 and Legend and accompanying text_569_305 dated 02 December 2012).

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-base pair (bp) core chicken hypersensitivity site

4 (cHS4) insulators 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 central polypurine tract, rev response element, 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′ HIV U3 promoter/enhancer has been replaced with a cytomegalovirus (CMV) promoter. However, the 5′ U3 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-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). Due to the inherent instability of 2 copies of core insulator, bluebird bio decided to delete insulators completely to create LentiGlobin BB305 Lentiviral Vector, as shown in Figure 3-1.

Further, the overexpressed HMGA2 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. This leads to an RNA with increased stability due to deletion of the let7 microRNA binding sites. Therefore, in order to improve safety of the vector, the cHS4 containing the cryptic splice site was deleted to greatly reduce or eliminate the splicing in the event of HMGA2 integration of the vector.

3.2.2 Rationale for the β-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 gene has an advantage over the normal β-globin gene for the gene therapy of β-thalassemia in that 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 a clinical study setting, 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.

Immunogenicity with $\beta^{A\text{-}T87Q}$ is not anticipated. β-globin proteins are notoriously poorly immunogenic, as evidenced by the fact that patients with β^0/β^0 thalassemia do not develop anti-hemoglobin antibodies despite receiving multiple transfusions (Olivieri, 1999). In addition, efforts as published by Reichlin to identify HbA antibody in multiply transfused patients with sickle cell disease yielded only negative results (Reichlin, 1975).

3.3 Rationale for the Study Design

Because of 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 as his/her own control for evaluation of transfusion requirements and the evaluation of changes in the nature or frequency of the subject-specific main inclusion criterion.

3.4 Rationale for the Dose Selected

Cellular doses of $\geq 0.75 \times 10^6$ CD34+ cells/kg are reported to provide acceptable engraftment, although the rate and response of engraftment are related to the dose (Perez-Simon et al., 1998). For apheresis-procured cells, the dose of $\geq 3 \times 10^6$ cells/kg was chosen because this number of cells is often obtainable with one mobilization cycle and two collection procedures (which are typically well tolerated by subjects) and are anticipated to provide the desired engraftment kinetics. For bone marrow harvest procured cells, the dose of $\geq 1.5 \times 10^6$ cells/kg was chosen based on consideration of the lower yield expected from this collection method and the risk associated with the collection procedures. In particular, the risks associated with a bone marrow harvest are significantly greater than the risks associated with a peripheral mobilization and apheresis, primarily due to the inherent toxicities of general anesthesia. The potential risk associated with a cell dose between 1.5 and 3.0×10^6 CD34+ cells/kg, as compared to a cell dose $\geq 3.0 \times 10^6$ CD34+ cells/kg, is a delay in engraftment which is likely to be less than 2 days (Miyamoto et al., 2004). Given these data, and the fact that rescue cells are non-transduced and therefore should have the best engraftment characteristics, Table 3-1 outlines the source of subject cells, usage, and minimal dose to be received.

Table 3-1 Dose of LentiGlobin BB305 Drug Product or Rescue for β-Thalassemia Major

Usage	Dose
Drug Product	$\geq 3.0 \times 10^6$ $CD34^+$ cells/kg[a]
Rescue Cells (obtained by apheresis)	$\geq 2.0 \times 10^6$ $CD34^+$ cells/kg
Rescue Cells (obtained by bone marrow harvest)	$\geq 1.5 \times 10^6$ CD34+ cells/kg or $>1.0 \times 10^8$ TNC/kg

TNC, total nucleated cells
[a] If 2 transductions are performed, the total dose of the 2 drug products together must meet this criterion.

If LentiGlobin BB305 Drug Product fails to be dispositioned for clinical use, collection does not yield adequate cells for drug product manufacture, or manufacturing does not yield sufficient cells to meet the minimum dose criteria, then mobilization, apheresis, and additional transduction may be repeated.

Myeloablation should not begin on a subject until it is confirmed that Drug Product for that subject has been dispositioned for clinical use and rescue cells are available.

3.5 Treatment Discontinuation and Enrollment Suspension Criteria

See Section 4.5 for “subject withdrawal from the study”.

3.5.1 Stopping Rules for Busulfan

Once myeloablation with busulfan has begun, there are no stopping rules for busulfan. In the anticipated very rare event of consent withdrawal during conditioning or the development of a new medical condition that, in the investigator’s opinion, puts the subject at risk with continued busulfan treatment, the Medical Monitor should be contacted immediately. In such 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 will be required.

3.5.2 Enrollment Suspension Criteria

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

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

- Death, until the cause of the death is determined*

- Detection of leukemia/lymphoma due to vector-mediated insertional oncogenesis**
- Detection of vector-derived RCL in any subject
- Failure to achieve reconstitution with transduced cells in 1 subject, requiring use of backup cells
- Determination of unexpected, clinically significant, or unacceptable risk to subjects (e.g., development of study treatment-related Grade 3 or 4 toxicities in at least 3 subjects)

*Any death of a subject with β-thalassemia major on Studies HGB-204 or HGB-205 after receiving LentiGlobin BB305 Drug Product will result in a hold of further enrollment and treatment with drug product until an investigation into the cause of death is performed. If it is determined that the death was not related to the drug product, then enrollment/treatment with drug product may restart. If the relationship between the death and the drug product is not clear, or it appears that the death may be related to the study drug, enrollment will be held until the DMC assessment and recommendations as described above. In cases in which the cause of death is still under investigation, subjects that have already received conditioning may receive either back-up cells or BB305 Drug Product on a case-by-case basis in consultation with the DMC.

**If a subject is diagnosed with leukemia or lymphoma after receiving LentiGlobin BB305 in any clinical study, enrollment will be held until determination is made as to whether the malignancy was related to a vector-mediated insertion. Once this assessment occurs, if the malignancy is not related to a vector-mediated insertion, enrollment may resume. If the relationship between the malignancy and an insertion is not clear or it appears they may be related, enrollment will be held until the DMC assessment and recommendations as described above.

4 STUDY POPULATION

4.1 Number of Subjects

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

4.2 Inclusion Criteria

Subjects must meet all of the following criteria to be considered eligible for enrollment in the study.

1. Subjects between 12 and 35 years of age, inclusive, at the time of consent or assent (as applicable), and able to provide written consent (adults, or legal guardians, as applicable) or assent (adolescents).
2. Diagnosis of β-thalassemia major and a history of at least 100 mL/kg/year of pRBCs or ≥8 transfusions of pRBCs per year for the prior 2 years.
3. Documented baseline, or pretransfusion, hemoglobin level of ≤ 7 g/dL.
4. Clinically stable, have a Karnofsky performance status of ≥ 60, and eligible to undergo HSCT.
5. Treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records, including transfusion history.

4.3 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study.

1. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV-1 or HIV-2), hepatitis B virus (HBV), or hepatitis C virus (HCV). (Note that subjects who are positive for anti-HBV antibody [to either core or envelope proteins] or for anti-HCV antibody are eligible as long as they have a negative HBV or HCV viral load by quantitative polymerase chain reaction [qPCR]. Where clinically and/or regionally indicated, one or more of the following tests may be performed, in which case positive results would exclude the subject from participating: human T-lymphotrophic virus-1 (HTLV-1) or HTLV-2, syphilis (RPR), toxoplasmosis, Trypanosoma cruzi, or West Nile Virus).
2. Active bacterial, viral, fungal, or parasitic infection.
3. A WBC count $<3 \times 10^9$/L, and / or platelet count $<100 \times 10^9$/L not related to hypersplenism.
4. Uncorrected bleeding disorder.
5. Any prior or current malignancy or myeloproliferative or immunodeficiency disorder.
6. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome and familial adenomatous polyposis).
7. Prior HSCT.
8. Advanced liver disease, defined as:

   a. Baseline alanine transaminase or direct bilirubin value > 3 × the upper limit of normal (ULN), or

   b. Liver biopsy demonstrating cirrhosis, any evidence of bridging fibrosis, or active hepatitis.
9. Baseline estimated glomerular filtration rate (eGFR) < 70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation for ≥ 18 years of age, and Bedside Schwartz equation calculator < 18 years of age (see http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm).
10. Uncontrolled seizure disorder.
11. Diffusion capacity of carbon monoxide (DLco) < 50% of predicted (corrected for hemoglobin and/or alveolar volume, as clinically indicated).
12. A cardiac T2* < 10 ms by magnetic resonance imaging (MRI)
13. Any other evidence of severe iron overload that, in the investigator's opinion, warrants exclusion.
14. Clinically significant pulmonary hypertension, as defined by the requirement for ongoing pharmacologic treatment or the consistent or intermittent use of supplemental home oxygen.
15. Participation in another clinical study with an investigational drug within 30 days of Screening.
16. Failure to obtain appropriate informed consent.
17. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician or investigator.

18. Contraindications to the conditioning regimen.
19. Prior receipt of gene therapy.
20. Diagnosis of significant psychiatric disorder of the subject that could seriously impede the ability to participate in the study.
21. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile subjects. Females of child-bearing potential are required to use effective contraception from Screening through at least 6 months after drug product infusion. Male subjects are required to use effective contraception (including condoms) from Screening through at least 6 months after drug product infusion.
22. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.

4.4 Subject Identification and Registration

Prior to the Screening Phase, the 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. Subjects who are determined by the investigator to be potentially eligible will be informed of the potential to participate in the study.

If the subject is willing to participate in the study, then the site will begin the consent process as per institutional practices, according to Good Clinical Practice (GCP). Written informed consent must be obtained before the conduct of any Screening tests not performed routinely in the treatment of the subject.

Upon signing the informed consent, the potential 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; a new subject number will be assigned if the subject re-enrolls into the trial.

4.5 Subject Withdrawal from the Study

Subjects have the right to withdraw from the study at any time for any reason. After giving informed consent, subjects may withdraw or be withdrawn from study-related procedures and treatments (e.g., apheresis, busulfan conditioning) under the following conditions:

- withdrawal of consent,
- the subject is unable to comply with protocol-defined visits or other requirements of the protocol,
- any medical condition which, in the opinion of the investigators, would put the subject at risk for continuing treatment or follow-up studies, or
- adequate cells are not collected during harvests, or failure of transduced cells to be dispositioned for clinical use.

In addition, after subjects have been treated with LentiGlobin BB305 Drug Product, they may be withdrawn from the study under the following additional condition:

- the subject has undetectable VCN (< 0.0003 copies per diploid genome) 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 and before administration of LentiGlobin BB305 Drug Product by infusion will be strongly discouraged, because this would be considered deleterious to the subject. In such cases, the subject's stored unmanipulated HSCs (rather than transduced cells) will be infused.

For subjects who withdraw consent or assent (if relevant), no further data will be collected on the subject.

For subjects who withdraw for reasons other than withdrawal of consent , any AEs open at the time of discontinuation should be followed-up for 30 days, or until resolution or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es), whichever is later. Additionally for these subjects:

- If withdrawal is before drug product infusion, subjects should remain on study for at least 30 days after any invasive study procedure (e.g., mobilization, liver biopsy) before withdrawal. In the rare case a subject undergoes myeloablation and receives back up cells instead of LentiGlobin BB305 Drug Product, subject should remain on the study for at least 3 months post myeloablation.
- If withdrawal is after drug product infusion, subjects will be asked to complete the same assessments as specified in the Schedule of Events (SOE) for Month 24 (Early Termination Visit assessments) and will be asked to enroll in the longterm follow-up study LTF-303.

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

5 STUDY TREATMENTS

5.1 Description of LentiGlobin BB305

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 resuspended in cryopreservative solution [redacted] in the final immediate container for the intended medical use.

5.2 Summary of Treatments to be Performed or Administered

After confirmation of eligibility, HSCs must be collected from the subject for 2 purposes – transduction for LentiGlobin BB305 Drug Product and for rescue cells (to be used if the subject fails to engraft with transduced cells). Table 3-1 outlines the source of the subject cells, usage, and the dose of LentiGlobin BB305 Drug Product or rescue.

Additional details are provided in the following subsections.

5.2.1 Mobilization and Apheresis Procedure

Within the period of 30 days prior to and during the period of mobilization and apheresis, the subject's transfusion regimen may be adjusted to maintain a minimum of 10 g/dL of Hb in order to suppress dyserythropoiesis, which can impede the isolation of CD34+ cells enriched for undifferentiated HSC.

A total of 2 mobilization cycles may be performed if needed. Each mobilization cycle may include up to 5 apheresis procedure days, but only 2 consecutive apheresis procedure products may be sent for transduction for each drug product. Apheresis procedure products are also to be used for rescue cells. Mobilization cycles must be separated by at least 2 weeks. A bone marrow harvest is also allowed, but only to procure cells for rescue. If 2 mobilization cycles are needed to collect enough HSCs to meet the requirement of a total dose of $\geq 3.0 \times 10^6$ $CD34^+$ cells/kg, then 2 transductions will be performed, 1 on the cells collected during Mobilization Cycle 1 and another on the cells collected during Mobilization Cycle 2. Each transduced product from each Mobilization Cycles 1 and 2 is an independent drug product. Thus, subjects who require 2 mobilization cycles to produce the minimum cell dose will be treated with 2 drug products in total.

Potential toxicities of filgrastim, plerixafor, and apheresis are included in the Informed Consent form.

5.2.1.1 Mobilization

Recent published data (Yannaki et al., 2013) has indicated that the combination of plerixafor and filgrastim may be the most appropriate mobilization strategy for subjects with β-thalassemia. The mobilization approach below follows this recent data. Modifications to this approach are allowed only after consultation with the medical monitor.

Table 5-1 Mobilization for subjects who have NOT undergone splenectomy

Mobilization day	Filgrastim dose[a]	Plerixafor dose (evening)	Complete Blood Count (CBC)[c]	Peripheral $CD34^+$ count	Apheresis
1	10 ug/kg				
2	10 ug/kg		X		
3	10 ug/kg		X		
4	10 ug/kg	0.24 mg/kg	X		
5	10 ug/kg	0.24 mg/kg	X	X[d]	X
6 (if needed)	10 ug/kg	[b]	X	X	X
7 (if needed)	[b]	[b]	X	X	X
8 (if needed)	[b]	[b]	X	X	X
9 (if needed)			X	X	X

[a] Dosage of filgrastim should be decreased if WBC > 100×10^9/L prior to initiation of apheresis
[b] Mobilization regimen after 2 days of apheresis should be discussed with the Medical Monitor.

[c] Recommended but can be deferred in case of difficulty in obtaining CBC (e.g., weekend) at the discretion of the site PI.
[d] $CD34^+$ count is done prior to apheresis.

Table 5-2 Mobilization for subjects who have undergone a splenectomy

Mobilization day	Filgrastim dose[a]	Plerixafor dose (evening)	Complete Blood Count (CBC)[c]	Peripheral CD34+ count	Apheresis
1	5 ug/kg				
2	5 ug/kg		X		
3	5 ug/kg		X		
4	5 ug/kg	0.24 mg/kg	X		
5	5 ug/kg	0.24 mg/kg	X	X[d]	X
6 (if needed)	5 ug/kg	[b]	X	X	X
7 (if needed)	[b]	[b]	X	X	X
8 (if needed)	[b]	[b]	X	X	X
9 (if needed)			X	X	X

[a] Dosage of filgrastim should be decreased if WBC > 100×10^9/L prior to initiation of apheresis
[b] Mobilization regimen after 2 days of apheresis should be discussed with the Medical Monitor.
[c] Recommended but can be deferred in case of difficulty in obtaining CBC (e.g., weekend) at the discretion of the site PI.
[d] $CD34^+$ count is done prior to apheresis.

After completion of Mobilization Cycle 1, the clinical site will be informed by the sponsor as to whether the total dose of drug product had been attained. This notification should come within approximately 7 days of the completion of Mobilization Cycle 1. Only if additional HSCs are needed either for drug product manufacture, or for rescue cells (i.e., if < 2.0×10^6 $CD34^+$ cells/kg have been collected and stored at the site for rescue cells), subject should begin Mobilization Cycle 2 no sooner than 2 weeks after the completion of Mobilization Cycle 1. Mobilization Cycle 2 should follow the same procedures as Mobilization Cycle 1, although the dose of plerixafor and filgrastim and the apheresis schedule may be modified based on the subject's experience from Mobilization Cycle 1.

5.2.1.2 Apheresis

As described above, typically a subject's first apheresis will occur on Mobilization Day 5. However, after discussion with the Medical Monitor, it may occur on Mobilization Day 4 or 6 if the subject has a particularly robust or sluggish response to mobilization, respectively. On each day of apheresis, the subject should have a physical examination and vital signs performed prior to beginning apheresis and again after completion of apheresis. Apheresis will be performed per SOP at the clinical site. Recommendations for apheresis are also provided in Section 10.2.

The following examples are given as possible scenarios to show how cells for both drug product manufacture and rescue could be obtained from Mobilization Cycle 1. There should be close consultation between the Medical Monitor and the investigator during apheresis.

Apheresis product from Mobilization Cycle 1: recommended evaluation and management:

- If the Apheresis Procedure Day 1 collection is $\geq 10\times10^6$ $CD34^+$ cells/kg, this collection should be sent to the transduction facility (as per the Study Operations Manual) no later than the next business day. The subject should return for as many additional apheresis procedures required (up to 5 total collections may be performed as part of any 1

mobilization cycle) to collect $CD34^+$ cells for rescue, which should be stored indefinitely at the clinical site or per local procedures.

- If the Apheresis Procedure Day 1 collection is between 1×10^6 and 10×10^6 $CD34^+$ cells/kg, this collection should be held at an overnight, controlled storage facility at the clinical site (should be shipped immediately to the transduction facility if storage is not possible), and the subject should return for Apheresis Procedure Day 2. If Day 1 collection was stored overnight, the collection from Apheresis Procedure Day 1 and Apheresis Procedure Day 2 should be sent together to the transduction facility immediately following the completion of the Apheresis Day 2 procedure (as per the Study Operations Manual). The subject should then return for as many additional apheresis procedures required (up to 5 total collections may be performed as part of any one mobilization cycle) to collect $CD34^+$ cells for rescue, which should be stored indefinitely at the clinical site or per local procedures.
- If the Apheresis Procedure Day 1 collection is $< 1\times10^6$ $CD34^+$ cells/kg, this collection should be stored towards collecting sufficient rescue cells and the subject should return for Apheresis Procedure Day 2.
  - o If the Apheresis Procedure Day 2 collection is $\geq 2 \times 10^6$ $CD34^+$ cells/kg, consult with Medical Monitor to determine how to proceed. In this scenario, it is possible that the subject will return for Apheresis Procedure Day 3, in which case the collections from Apheresis Procedure Days 2 and 3 would be sent together to the transduction facility.
  - o If the Apheresis Procedure Day 2 collection is $< 2 \times 10^6$ $CD34^+$ cells/kg, then it should be stored towards collecting sufficient rescue cells, to be stored indefinitely at the clinical site or per local procedures. Plans for an additional mobilization cycle should be discussed with the Medical Monitor.

Apheresis product from Mobilization Cycle 2: recommended evaluation and management:

- If Mobilization Cycle 2 is for the procurement of rescue cells only, the subject should undergo as many apheresis procedures (up to 5) needed to collect the minimum dose of rescue cells required, which should be stored indefinitely at the clinical site or per local procedures.
- If Mobilization Cycle 2 is for the procurement of additional HSCs for drug product, the management of the collection from each Apheresis Procedure day will be discussed with the Medical Monitor on a case-by-case basis prior to Apheresis Procedure Day 1, as it is dependent upon how many cells were collected and transduced from Mobilization Cycle 1.

5.2.2 Bone Marrow Harvest Procedure

If sufficient cells for rescue are not procured after 2 Mobilization Cycles, the investigator can proceed with a bone marrow harvest. Bone marrow harvest will be performed according to standard operating procedures at the clinical site.

5.2.3 Conditioning

5.2.3.1 General considerations

Myeloablative conditioning of the subject will be performed using busulfan.

Conditioning will only begin after the following criteria are met:

1. All LentiGlobin BB305 Drug Product to be used for a particular subject has been release tested, dispositioned for clinical use, and is stored at the clinical site;
2. The subject has undergone an interim medical history, AE and concomitant medications assessments, physical examination, vital signs, and laboratory tests as per the SOE and continues to meet the eligibility criteria based on these results.
3. Iron chelation therapy will be stopped 7 days prior to start of the conditioning.

Busulfan will be administered at a starting dose of 3.2 mg/kg/day for 4 consecutive days via IV infusion on Days -7 through -4. The preferred schedule is busulfan via a central venous line (CVL) as a 3-hour infusion once daily for 4 consecutive days for a total of 4 doses. Divided dosing is permitted. In this case, busulfan should be administered at 0.8 mg/kg as a 2-hour infusion every 6 hours for 4 consecutive days for a total of 16 doses. Please note that the timing of busulfan pharmacokinetics (PK) sample collection is based on whether a 2-hour or 3-hour infusion is used.

Clinical sites that use a test dose of busulfan several days before myeloablation to pre-determine the busulfan dose may also do so in this protocol.

Anti-seizure prophylaxis should begin at least 12 hours before initiating busulfan, and should continue for at least 24 hours after completion of the four days of busulfan administration. All drugs other than phenytoin are allowed for anti-seizure prophylaxis. Additional supportive medications are permitted as per site standard practices.

5.2.3.2 Pharmacokinetics for Busulfan Dose Adjustments

Target first dose PK should be performed in all subjects regardless of busulfan dosing schedule. Daily dosing should target an AUC of 4000 (range 3600 to 5000) μM*min. Every 6 hour dosing should target an AUC of 1000 (range 900 to 1200) μM*min.

Recommendations for Q24 hr dosing: Samples for busulfan PK analysis should be collected at the end of the first 3 hour infusion, 195 minutes after **start** of infusion, and 4, 5, 6, and 8 hours after the start of the first infusion. Samples should not be drawn from the lumen used to infuse busulfan.

Recommendations for Q6hr dosing: Samples for busulfan PK analysis should be collected at the end of the first 2 hour infusion, 135 minutes after start of infusion, 150 minutes after start of infusion, and 3, 4, 5, and 6 hours after the start of the first infusion. Samples should not be drawn from the lumen used to infuse busulfan.

5.2.4 Infusion Procedures, Dose, and Administration

LentiGlobin BB305 Drug Product is to be given after a minimum of 72 hours after completion of the busulfan conditioning regimen in order to achieve complete washout of busulfan.

Pre-hydration and pre-medication of the subject are not required but sites should follow local procedures for HSC infusion.

Prior to administration, the drug product(s) is (are) thawed at 37°C and the infusion of the drug product should be completed immediately after thawing and within a maximum of 4 hours of its thawing. All procedures involving LentiGlobin BB305 Drug Product must be performed using aseptic techniques by trained personnel.

LentiGlobin BB305 Drug Product will be administered on Day 0 via IV infusion at the clinical site. The dose to be administered is $\geq 3.0 \times 10^6$ CD34+ cells/kg. Subjects who undergo 2 mobilization cycles (and subsequent transduction of those cells) to achieve a total dose of $\geq 3.0 \times 10^6$ CD34+ cells/kg will have 2 LentiGlobin BB305 Drug Products, which should be administered in sequence, with the second administered immediately after the first.

Vitals signs are to be monitored concurrently during LentiGlobin BB305 Drug Product infusion no less frequently than at the start, once during, and upon completion of the infusion. Infusion reactions, including anaphylaxis, will be managed according to the medical judgment of the physician overseeing the infusion.

5.3 Transduction Process

All cell manipulation procedures will be performed in transduction facilities in accordance with Current Good Manufacturing Processes following process-specific procedures.

5.4 Storage and Stability of LentiGlobin BB305 Drug Product

The subject's CD34+ HSCs transduced with LentiGlobin BB305 lentiviral vector are frozen and stored in cryopreservative solution [redacted] in the vapor phase of liquid nitrogen at the transduction facility until release testing and dispositioned for clinical use.

The LentiGlobin BB305 Drug Product is thawed at 37°C at the clinical site, and infused within 4 hours thereafter.

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

Refer to the Study Operations Manual (SOM) for details regarding traceability.

5.5 Product Accountability

LentiGlobin BB305 Drug Product accountability and traceability is ultimately the responsibility of the investigator and Sponsor. However, this responsibility may be delegated to a suitably qualified investigator listed on Food and Drug Administration (FDA) Form 1572 who has had appropriate study-specific training and whose name 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 as per applicable sponsor and clinical site procedures.

These records will include details of storage of the LentiGlobin BB305 Drug Product, transfer of LentiGlobin BB305 Drug Product from the transduction facility, administration to subjects, and disposal of remaining materials.

All material containing LentiGlobin BB305 Drug Product will be treated and disposed of as hazardous waste in accordance with governing regulations and clinical site procedures.

5.6 Method of Assigning Subjects to Treatment

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

5.7 Blinding, Packaging, and Labeling

5.7.1 Blinding and Breaking the Blind

This is an unblinded, open-label study.

5.7.2 Packaging and Labeling

LentiGlobin BB305 Drug Product consists of autologous $CD34^+$ HSCs transduced ex vivo with the LentiGlobin BB305 lentiviral vector and resuspended in cryopreservative solution in the final infusion bag.

LentiGlobin BB305 Drug Product will be labeled by the transduction facility according to Good Manufacturing Practices.

5.8 Duration of Subject Participation

Time between Screening and HSC-infusion will be variable, and is estimated to be between 2 to 3 months; thereafter subject is planned to remain on study for approximately 24 months after HSC infusion. Eligible subjects will then be enrolled in a long-term follow-up study for another 13 years, for a total of approximately 15 years.

5.9 Assessment of Treatment Compliance

Eligible subjects will receive LentiGlobin BB305 Drug Product by IV administration on a single day as in-patients and thus will be monitored by hospital personnel.

5.10 Prior and Concomitant Medications

5.10.1 Prior Medications

All medications taken within 30 days prior to the Screening Visit are to be recorded in the Prior and Concomitant Medication CRF.

5.10.2 Concomitant Medications and Therapies: General

All concomitant treatments, including transfusions, will be recorded in the Prior and Concomitant Medication CRF.

Iron chelation therapy should be discontinued at least 7 days prior to conditioning. Iron chelation therapy may be restarted at the discretion of the treating physicians no sooner than 30 days after bone marrow engraftment (defined as an ANC $\geq 0.5 \times 10^9$/L for 3 consecutive days).

5.10.3 Concomitant Treatments: During Conditioning

Permitted concomitant treatments during may include:

- Hyperdiuresis, beginning 12 hours before initiating conditioning and continuing through 24 hours thereafter
- Prevention or treatment of nausea and vomiting
    - o Prophylactic administration of ondansetron

  - Metoclopramide for the treatment of nausea and vomiting

- Seizure prophylaxis. All drugs other than phenytoin are allowed for anti-seizure prophylaxis during conditioning.
- Ursodeoxycholic acid for prevention of hepatic veno-occlusive disease/ hepatic sinusoidal obstruction syndrome.
- Transfusions should be given according to Hb and platelet counts. All blood products will be filtered and irradiated.
- Subjects will be isolated in single rooms and appropriate precautions will be taken if the polynuclear leukocyte count is $<0.5 \times 10^9$/L. Broad-spectrum antibiotic treatment will be administered according to the usual procedures for febrile neutropenia.

5.10.4 Concomitant Medications: Infection Surveillance and Prophylaxis

The LentiGlobin BB305 Drug Product consists of CD34+ cells and is functionally T cell-depleted. Thus, opportunistic infection surveillance and prophylaxis is recommended. Below is a suggested approach to infection surveillance and prophylaxis. Alternatives that follow institutional practices may be employed.

- In subjects with past exposure to HBV, regular monitoring of HBV DNA by PCR for up to 3 months after drug product infusion, with pre-emptive lavimudine advised for any increase in HBV DNA titer (HBV reactivation).
- Herpes simplex virus (HSV) prophylaxis: Acyclovir prophylaxis is recommended for at least 3 months after drug product infusion for patients who are sero-positive for HSV or VZV.
- Pneumocystis pneumonia prophylaxis: Trimethoprim-sulphamethoxazole or an equivalent drug is recommended for at least 3 months after drug product infusion.
- Fungal prophylaxis: Anti-fungal prophylaxis is recommended with agents such as Fluconazole for at least 3 months after drug product infusion.
- Cytomegalovirus (CMV) surveillance: CMV should be tested using the PCR based methods or CMV antigenemia for at least 3 months after drug product infusion. Antiviral therapy for CMV reactivation should be commenced pre-emptively if CMV testing reveals a high or rising viral load. If CMV reactivation occurs at or before engraftment, foscarnet may be considered to prevent marrow suppression.
- Adenovirus surveillance: Testing for adenovirus infection is recommended in the event of symptoms suspicious for infection such as diarrhea, hepatic dysfunction, respiratory symptoms, etc. If diagnosed with an active systemic infection, therapy should be instituted with cidofovir or other active agents.
- Epstein-Barr virus (EBV) surveillance: EBV monitoring by PCR is recommended for at least 3 months after drug product infusion. In the event of persistent EBV viremia or signs/symptoms consistent with EBV-related post-transplant lymphoproliferative disorder (adenopathy, fever, etc.) therapy such as Rituximab is recommended.
- Intravenous Immune Globulin (IgG) may be administered for 3 months to maintain IgG levels >500 per institutional guidelines.

- Follow institutional post-transplantation guidelines for re-immunization of subjects.

5.10.5 Concomitant Medications: Iron Reduction guidelines

Chelation should stop 7 days prior to starting busulfan.

Post-transplant chelation guidelines are provided in Appendix 10.4.

5.10.6 Concomitant Medication: Transfusion Guidelines

Recommendations for transfusions are made for 3 separate periods during treatment:

1. Within the period of 30 days prior to and during mobilization and apheresis, the subject's transfusion regimen may be adjusted to maintain a minimum of 10 g/dL of Hb in order to suppress dyserythropoiesis, which can impede the isolation of CD34+ cells enriched for undifferentiated HSC.
2. During conditioning and hospitalization supporting engraftment: follow institutional guidelines.
3. During follow-up: the goal is to maintain Hb ≥ 9 g/dL. Transfusions should be avoided for Hb ≥ 9 g/dL, unless the need is medically justified (e.g. as a pre-requirement for surgery). It is recommended that subjects should receive pRBC transfusions for any Hb < 7.0 g/dL, and for clinically symptomatic anemia, irrespective of Hb level.

6 STUDY ASSESSMENTS

6.1 Schedule of Events

The study has 4 distinct stages, as follows.

- Stage 1: Screening and eligibility assessments
- Stage 2: Autologous CD34+ cell collection and LentiGlobin BB305 Drug Product manufacture and disposition
- Stage 3: Myeloablative conditioning and infusion of LentiGlobin BB305 Drug Product
- Stage 4: Follow-up through Month 24

The SOE to be performed is outlined in Table 6-1 for Stages 1 and 2, Table 6-2 for Stage 3, and Table 6-3 for Stage 4.

Detailed descriptions of the efficacy, pharmacodynamics, and safety procedures to be conducted during this study are provided in the following subsections. Additional details, including administrative information, will be provided in the Study Operations Manual.

Evaluations and procedures identified in the SOE 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: Screening and CD34+ Cell Harvest

Procedure	Screening (Up to approximately 35 days before mobilization)	Mobilization[1]	Days of harvest
Signing of informed consent form (ICF)	X		
Demographics and medical history	X		
Physical examination	X[2]	X[2]	X[2]
Vital signs	X	X[3]	X[3]
Local lab: Blood for clinical laboratory tests	X[4]	X[5]	X[5]
CrCl estimate	X		
Local lab: Urinalysis	X		
Local lab: Blood for serology	X[6]		
Local lab: Blood for immunological testing	X[7]		
Local lab: Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (WOCBP; serum pregnancy test)	X		
Local lab: hormonal testing	X[8]		
Central lab: Blood for RCL, VCN, and globin analysis	X		
Blood for storage: potential biomarker analysis (optional)	X		
Peripheral blood CD34+ count			X[9]
Sperm / testicular tissue or oocyte banking commencement, if requested	X		
Liver biopsy (CCI)	X		
12-lead ECG	X		
Imaging studies: Chest X-ray, cardiac Doppler echocardiology (including LVEF), cardiac MRI, liver MRI/SQUID	X		
	CCI		
Transfusion regimen		X[11]	
Adverse event collection	Continuous from ICF signing		
Prior and concomitant medications	Continuous from ICF signing		

[1] Up to 2 cycles of mobilization are permitted. If needed, a bone marrow harvest may be performed to obtain rescue cells. See also Section 5.2.1.1 for recommendations on dosing for mobilization days.

[2] Includes weight and height for adolescents, and weight for adults (except height also included at Screening only for adults). A physical examination should be performed within 5 days prior to or on the first day of every mobilization cycle, and on every day of apheresis. Additionally, Tanner staging should be performed during puberty, if relevant, for adolescents.

[3] Vital signs should be performed on the first day of every mobilization cycle, and 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.

[4] Includes hematology (including CBC, platelets, CCI), CCI , serum chemistry, liver function tests, prothrombin and partial thromboplastin time

[5] CBC should be performed during mobilization and every day of apheresis.

[6] Including testing for presence of HIV-1, HIV-2, hepatitis B, and hepatitis C. Blood may also be drawn for additional serology testing if subject has risk factors or clinical evidence of infection with other communicable disease agents or disease. Tests should be done according to country-specific and institutional guidelines. See exclusion criteria for details.

[7] T cell subsets (CD4, CD8), B cells (CD19), natural killer cells (CD16 or CD56); immunoglobulins (IgG, IgM, and IgA)

[8] Includes TSH, T3, T4, FSH, LH, estrogen or testosterone level, as applicable. For adolescents, include also growth hormone (IGF-1 and IGF BP-3).

[9] Peripheral blood CD34+ count should be performed prior to apheresis

[10] CCI

[11] To maintain Hb ≥10g/dL prior to mobilization

Table 6-2 Schedule of Events: Conditioning and Drug Product Infusion

	Preconditioning	Conditioning	Infusion	Post-infusion
	Up to 7 days before busulfan	Day -7 to -4 inclusive	Day 0	Day 1 until discharge
Interval medical history	X			
Physical examination	X	X	X[12]	X[13]
Vital Signs	X	X	X[14]	X[15]
Stop iron chelation	X[16]			
Local lab: Blood for clinical laboratory tests	X[17]	X[17]	X[17]	X[13,17]
Local lab: Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (WOCBP; serum pregnancy test)	X			
Re-confirmation of eligibility	X[18]			
Anti-seizure prophylaxis		X[19]		
Busulfan chemotherapy (daily or q6h regimen, for 4 days)		X		
Local laboratory: Blood for busulfan pharmacokinetics[20]	X	X		
Infusion of LentiGlobin BB305 Drug Product			X	
Adverse event collection		Continuous from ICF signing		
Concomitant medication collection		Continuous from ICF signing		

[12] To be performed on Day 0 before drug product administration. For adolescents, include height, and Tanner test to monitor puberty if relevant.
[13] At least twice a week during hospitalization
[14] To be measured on Day 0 no less frequently than at the start, once during, and upon completion of the infusion
[15] To be performed daily until discharge
[16] Iron chelation must be stopped at least 7 days before initiating busulfan
[17] CBC; serum chemistry; liver function tests; and any other tests required by institutional guidelines, including for example testing for CMV, EBV, HSV and VZV IgG. CBC and clinical chemistry should be performed at least every second day until neutrophil engraftment is obtained, and frequency should increase if any abnormality of clinical significance is detected.
[18] Review CBC, serum chemistry, liver function tests, physical examination, and interval medical history; verification that LentiGlobin BB305 Drug Product has been clinically dispositioned, is available on site and that rescue cells are available.
[19] Anti-seizure prophylaxis to start at least 12 hours before initiating busulfan and to continue at least 24 hours after completion of 4 day busulfan course
[20] A test dose of busulfan several days before beginning myeloablation to pre-determine busulfan dose is also permitted.

Table 6-3 Schedule of Events: Follow-up

Procedure	D30 M1 (±7)	D60 M2 (±7)	D90 M3 (±7)	D135 M4.5 (±14)	D180 M6 (±14)	D210 M7 (±14)	D240 M8 (±14)	D270 M9 (±14)	D300 M10 (±14)	D330 M11 (±14)	D360 M12 (±30)	D420 M14 (±30)	D450 M15 (±30)	D480 M16 (±30)	D540 M18 (±30)	D600 M20 (±30)	M21 (±30)	D660 M22 (±30)	D720 M24 (+-30)
	Follow-Up: Day (D), Month (M) (Visit Window, days)																		
Physical examination[21]	X	X	X	X	X			X			X		X		X		X		X
Vital signs	X	X	X	X	X			X			X		X		X		X		X
Local lab: Blood for clinical laboratory tests[22]	X	X	X	X	X			X			X		X		X		X		X
Local lab: Blood for CBC only						X	X		X	X		X		X		X		X	X
Local lab: hormonal testing[23]											X								X
[redacted] CCI			[redacted]								[redacted]								[redacted]
Local lab: Blood for immunology[25]			X		X			X			X								
Central lab: Blood for globin, VCN	X	X	X		X			X			X		X		X		X		X
Central lab: Blood for RCL			X		X						X								X
Central lab: Blood for ISA					X						X				X				X
Blood for storage: potential biomarker analysis (optional)					X						X								X
Bone marrow for globin, VCN[26]											X								(X)
CCI [redacted]											[redacted]								[redacted]
CCI [redacted]											[redacted]								[redacted]
[redacted] CCI											[redacted]								[redacted]
Cardiac MRI & echocardiology											X								X
CCI [redacted]					[redacted]						[redacted]				[redacted]				[redacted]
Record transfusions	Continuous from ICF signing																		
Record hospitalizations	Continuous from ICF signing																		
Adverse event collection	Continuous from ICF signing																		
Concomitant medication (incl. CCI [redacted])	Continuous from ICF signing																		

[21] Include weight and spleen size at every visit, height and performance status every 6 months after drug product infusion. Tanner staging should be performed every 6 months during puberty, if relevant.
[22] Hematology (CBC, platelets, CCI [redacted]); CCI [redacted] (CCI [redacted]), serum chemistry and liver function tests.
[23] Includes TSH, T3, T4, FSH, LH, estrogen or testosterone level, as applicable. For adolescents, include also growth hormone (IGF-1 and IGF BP-3).
[redacted]
[25] T cell subsets [CD4, CD8], B cells (CD19), and natural killer cells (CD16 and/or CD56); immunoglobulins (IgG, IgM, and IgA)
[26] VCN in bone marrow to be performed at any time if there is evidence of clonal skewing in peripheral blood leukocytes (PBLs); at D360 if sufficient sample available when collecting for CCI [redacted] or at the investigator's discretion; globin analysis of erythroid burst forming units may be done at the investigator's discretion
[redacted]
[redacted]

6.2 Assessments

6.2.1 Transfusions

Interval transfusions required (mL/kg of RBC) are to be documented as per the SOE, along with the most recent reticulocyte and Hb values taken prior to the transfusion. Where available, this data should also be collected for the 2 years prior to drug product enrollment (see also Medical History, Section 6.2.7).

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 analyses per the SOE for determination of the following by HPLC:

- Whole blood: ratio of $\beta^{A\text{-}T87Q}/\alpha$-globin, and ratio of $\beta^{A\text{-}T87Q}/$ all β-globin chains.

CCI

CCI

CCI

6.2.5 Testing for Vector Copy Number (VCN), Replication Competent Lentivirus (RCL), and Integration Site Analysis (ISA)

Blood samples will be collected according to the SOE for assessments of the following:

- Vector copy number (VCN)
- Replication competent lentivirus (RCL)
- Integration site analysis (ISA)

Blood sample collection details are included in the SOM/Laboratory Manual for this study. If bone marrow is collected for routine clinical care, then these assessments may also be performed in bone marrow.

Note that a pre-infusion of drug product sample for RCL testing will be collected, but will only be tested if post-infusion samples are positive for RCL.

Note that integration site analysis may not be performed if genetic marking is less than 1% (i.e., <VCN 0.01).

6.2.6 Samples for Storage and Potential Biomarker Analysis

Optional blood samples will be collected per the SOE for future research. These samples may be used for biomarker analyses of proteins, DNA, RNA and other molecules to study thalassemia and/or gene therapy. Such samples may be stored until the samples are exhausted or until 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. If possible, optional blood, bone marrow, and tissue samples also are to be collected in the event of a subject's death if an autopsy is performed. Leftover samples from protocol procedures (e.g., blood draw for integration site analysis) may also be stored (optional) for potential future analyses as described above.

Note that samples routinely 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 potential studies are not optional. Other potential uses of these samples for non-manufacturing improvement research, such as biomarker analyses of proteins, DNA, RNA and other molecules to study thalassemia and/or gene therapy, are optional.

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

6.2.7 Demographics and Medical History

Subject demographic data such as gender, age, race, ethnicity, and country of birth 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, including age of first transfusion, age of starting regular transfusions (i.e., age at which transfusion needs stabilized to between once every 4 to 8 weeks), age of starting iron chelation treatment, and spleen size (cm below coastal margin) at enrollment. Transfusions details (mL/kg of RBC, along with the associated reticulocyte and Hb values taken prior to the transfusion where available), as well as relevant blood bank details (including average volume [mL] per unit), should be collected for the 2 years prior to enrollment.

6.2.8 Clinical/Physical Examination

A complete physical examination (including Karnofsky performance status, general appearance; head eyes, ears, nose, and throat; cardiovascular; dermatologic, abdominal; genitourinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological in addition to constitutional symptoms, GI, immunology/allergy, stomatology and psychiatric) is to be conducted as per the SOE. The subject's weight is to be measured at every visit that includes a physical examination. The subject's height is to be measured at the Screening visit.

For adolescents, Tanner staging for assessment of puberty will be done at baseline and monitored throughout the study as applicable. Height will be collected for adolescents as per the SOE.

See Section 10.3 for more information on Karnofsky performance status.

6.2.9 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, as per the SOE.

6.2.10 Electrocardiogram

A 12-lead electrocardiogram (ECG) will be obtained as per the SOE.

6.2.11 Imaging Studies

A chest x-ray will be obtained as per the SOE.

Cardiac Doppler echocardiography, including the assessment of left ventricular ejection fraction (LVEF), cardiac MRI, and liver MRI/SQUID will be performed as per the SOE. See Section 6.2.17 for additional information on liver MRI/SQUID.

6.2.12 Serology

Screening serology will be evaluated using standard methods as per the SOE, testing for presence of HIV-1, HIV-2, HBV, and HCV. (Note that subjects who are positive for anti-HBV antibody [to either core or envelope proteins] or for anti-HCV antibody are eligible as long as they have a negative HBV or HCV viral load by quantitative polymerase chain reaction [qPCR]). Where clinically and/or regionally indicated, one or more of the following tests may be performed, in which case positive results would exclude the subject from participating: HTLV-1 or HTLV-2, syphilis (RPR), toxoplasmosis, Trypanosoma cruzi, or West Nile Virus. In certain circumstances, additional testing may be required depending on the subject's history and/or the characteristics of the subject's cells (eg, malaria).

See also infection surveillance (Section 5.10.4).

6.2.13 Hormonal Testing

Hormonal testing, including measurement of thyroid stimulating hormone (TSH), 3,5,3'-triiodothyronine (T3), thyroxine (T4), follicle stimulating hormone (FSH), luteinizing hormone (LH), and estrogen or testosterone, as applicable, is to be performed as per the SOE.

6.2.14 Immunological Testing

Immunological testing, including T cell subsets (CD4, CD8), B cells (CD19), and NK cells (CD16 or CD56), will be performed as per the SOE.

Quantitation of immunoglobulins (IgG, IgM, and IgA) is to be performed as per the SOE.

6.2.15 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 as appropriate at the discretion of the subject.

CCI

CCI

6.2.17 Liver Biopsy and Liver Imaging

A liver biopsy also is to be performed for all subjects, as per the SOE. If a liver biopsy has been performed within one year of Screening, then this test does not need to be repeated unless clinically indicated. CCI

CCI

6.2.18 Pregnancy Testing

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

6.2.19 Clinical Laboratory Tests

Clinical laboratory tests, including hematology, coagulation studies, clinical chemistries, CCI and urinalysis, will be performed as specified below, and in the SOE.

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

6.2.19.1 Hematology and Clinical Chemistry

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

The following clinical laboratory parameters are to be determined:

Hematology, CCI Serum Chemistry, and Liver Function

Hematology	CCI
Complete blood count (CBC) with differential	CC
Platelet count	CCI
Reticulocyte count	CCI
CCI	CCI
	CCI
Serum Chemistry and Liver Function	
Sodium	Blood urea nitrogen
Potassium	Creatinine
Chloride	Glucose
Bicarbonate	Calcium
Albumin	Phosphate
Total protein	Bilirubin (total)
Alanine transaminase	Alkaline phosphatase
Aspartate transaminase	Lactic dehydrogenase
Gamma glutamyl transferase	

In addition, creatinine clearance will be estimated as per the SOE. (See Section 10.1 for the formula for calculation of creatinine clearance.)

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

6.2.19.2 Urinalysis

A urinalysis will be performed as per the SOE.

CCI

CCI

CCI

		CCI			
CCI					
CCI					
CCI					

CCI

CCI

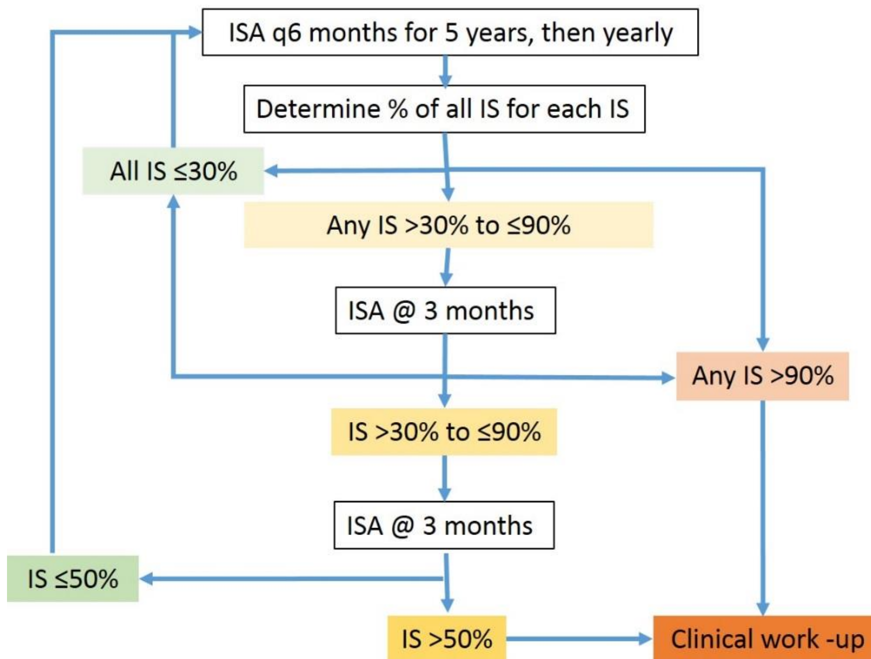
6.2.21 Assessment of Clonal Dominance and/or Suspicion of Leukemia/Lymphoma

6.2.21.1 Assessment of Clonal Dominance

An integration site analysis (ISA) will be performed on peripheral blood leukocytes (PBLs) starting 6 months after the infusion of drug product, and will be repeated every 6 months for the duration of the study, as per SOE. 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% at the first repeat, and ≤ 50% at the second repeat, monitoring of the subject also returns to the protocol-defined schedule. However, if result is > 30% and ≤ 90% at the first repeat, and > 50% at the second repeat, clonal dominance criteria would be satisfied, and clinical work-up for malignancy should be initiated.
- If ISA result is > 90% at any time, clonal dominance criteria would be satisfied, and clinical work-up for malignancy should be initiated.

Figure 6-1 Schematic for Assessment of Clonal Dominance



Note that for the purposes of this protocol, the term "oligoclonality" is used to describe situations in which the above clonal dominance criteria apply. Therefore, if a subject meets the above criteria and further work-up is required, a regulatory submission will be performed as per FDA Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events (November 2006).

6.2.21.2 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

6.2.21.3 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 (see also Section). 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.

6.2.22 Adverse Events

6.2.22.1 Assessments During the Study

Monitoring of AEs will be conducted throughout the study. AEs, as defined in the next section, will be monitored and recorded in the CRFs from the time informed consent is signed through the following timepoints:

- ≥Grade 1 AEs: through 30 days after LentiGlobin BB305 Drug Product infusion.
- ≥Grade 2 AEs: through 12 months after LentiGlobin BB305 Drug Product infusion.
- SAEs and 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 clearly determined to be due to a subject's stable or chronic condition or inter-current illness(es). See Section 4.5 for AEs monitoring of subjects who withdraw from the study.

Note that at the completion of this study, subjects will be asked to enroll into a long-term follow-up study (LTF-303), that will monitor the safety of subjects (including drug product-related AEs) through a total of 15 years after LentiGlobin BB305 Drug Product infusion.

6.2.22.2 Definitions, Documentation, and Reporting

Adverse Events
An **AE** is any change in physical signs, symptoms, and/or clinically significant laboratory change occurring in any phase of a clinical study regardless of its relationship to study drug. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. This definition includes intercurrent illnesses or injuries, and exacerbation of pre-existing conditions. A pre-existing 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 information contained in the written information or current Investigator's Brochure provided to the investigator by the Sponsor.

Serious Adverse Events

An **SAE** is any AE, occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred, ie, it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires in-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (eg, surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a subject's ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event. 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 1 of the outcomes listed in the definitions for SAEs.
- For the purposes of this study, any new malignancy or new diagnosis of a neurologic, rheumatologic, or hematologic disorder that, in the investigator's opinion, is clinically significant and requires medical intervention will be considered medically important and therefore serious.

6.2.22.3 Procedures for AE and SAE Reporting

Each subject must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (eg, "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from subjects.

All AEs (serious and non-serious) spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures will be recorded on the appropriate page of the CRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an adverse event and must be recorded on the appropriate pages of the CRF. When possible, signs and symptoms indicating a common underlying pathology should be noted as 1 comprehensive event.

All SAEs that occur during the course of the study must be promptly reported by the investigator to the medical monitor (see below) within 24 hours from the point in time when the investigator becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to LentiGlobin BB305 Drug Product. SAE forms, created specifically by bluebird bio, Inc., will be provided to each clinical site. The information

collected will include subject number, a narrative description of the event and an assessment by the investigator as to the severity of the event and relatedness to LentiGlobin BB305 Drug Product. A sample of the SAE form can be found in the Study Operations Manual. Follow-up information on the SAE may be requested by bluebird bio, Inc.

Contact Information:

CTI Safety

E-mail: PPD

US

SAE Hotline: PPD

eFax: PPD

AUSTRALIA

SAE Hotline PPD

Toll-free eFax: PPD

THAILAND

SAE Hotline PPD

All SAEs should be reported via email

If there are suspected, unexpected serious adverse drug reactions (SUSARs) associated with the use of LentiGlobin BB305 Drug Product, bluebird bio, Inc., will notify the appropriate regulatory agency(ies) and all participating investigators on an expedited basis and in accordance with applicable regulations. It is the responsibility of the investigator to promptly notify the IRB / EC and other appropriate institutional regulatory bodies of all SUSARs involving risk to human subjects.

Reporting of SUSARs to regulatory agencies will be performed within 7 or 15 days as indicated by event and in accordance with local regulations. Annual reporting of safety information will also be performed by bluebird bio, Inc., as required by local regulation.

For both serious and non-serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to LentiGlobin BB305 Drug Product administration.

Intensity will be assessed by the investigator using the NCI CTCAE, version 4.03, including AEs that are a result of a laboratory abnormality. If the AE is not included in the CTCAE, then the investigator is to determine the intensity of the AE according to the following criteria:

- **Mild (Grade 1)**: The AE is noticeable to the subject but does not interfere with routine activity.
- **Moderate (Grade 2)**: The AE interferes with routine activity but responds to symptomatic therapy or rest.
- **Severe (Grade 3)**: The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy.
- **Life-Threatening (Grade 4)**: The subject is at immediate risk of death.

- **Death (Grade 5)**

If the intensity (grade) changes within a day, the maximum intensity (grade) should be recorded. If the intensity (grade) changes over a longer period of time, the changes should be recorded as separate events (having separate onset and stop dates for each grade).

Relationship will be determined by the investigator according to the criteria that follow. Relationship of AEs to LentiGlobin BB305 Drug Product will be determined after the start of LentiGlobin BB305 Drug Product infusion on Day 0; prior to that time, relationship to study treatment other than LentiGlobin BB305 Drug Product will be assessed:

- **Not Related**: Exposure to the study treatment 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 study treatment.
- **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 study treatment product.
- **Possibly Related**: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment 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 study treatment was the most likely cause of the AE.

For the purpose of safety analyses, all AEs that are classified as possible or probable will be considered treatment-related events.

6.2.22.4 Pregnancy

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (eg, spontaneous abortion, which may qualify 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 using the Pregnancy Form. 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.

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. Female subjects of child-bearing potential are required to use effective contraception from Screening through at least 6 months after drug product infusion. Male subjects are required to use effective contraception (including condoms) from Screening through at least 6 months after drug product infusion. Beyond 6 months, subjects should discuss with their physician prior to resuming unprotected intercourse.

6.2.23 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 SOE.

6.2.24 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), which will focus on long-term safety, with an emphasis on integration site analysis, insertional oncogenesis, and long-term efficacy. This follow-up includes recording of SAEs, and archiving of peripheral blood leukocyte cell samples for RCL and clonality testing.

7 STATISTICAL PROCEDURES

7.1 Sample Size Estimation

The sample size for this study was not determined by formal statistical methods, but is sufficient to demonstrate a robust effect on the binary response endpoint, where a responder is defined as a subject with production of ≥2.0 g/dL of HbA containing $\beta^{A\text{-}T87Q}$-globin for the 6-month period between Month 18 and Month 24 post-transplant. For example, a one-sided lower 90% confidence bound on the rate of response would exceed 22%, should the true response rate be 50% or more, with at least 80% power.

7.2 Populations for Analysis

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

- Intent-to-Treat (ITT) population: All subjects who initiate any study procedures, beginning with mobilization by filgrastim and/or plerixafor.
- Transplant Population (TP): All subjects who undergo LentiGlobin BB305 Drug Product treatment, should this be a smaller number of subjects than ITT. It is anticipated that the ITT population and TP will be identical.

The ITT population is the primary population for the analysis of efficacy parameters and safety parameters. The TP is the primary population for transplant parameter endpoints in the case that TP and ITT are not 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 efficacy, an evaluable population will be defined as those subjects who 1) receive LentiGlobin BB305 Drug Product; 2) engraft, defined as an ANC $\geq 0.5 \times 10^9$/L for 3 consecutive days; and 3) have sufficient study visit compliance to acquire clinical laboratory and transfusion data for a minimum of 24 months after drug product infusion. Subjects in this population must be compliant with the visit window for the Month 24 evaluation.

7.3 Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. Subjects in the ITT population or TP will be considered treatment failures in the primary endpoint analysis, should they have less than 6 months post-transplant follow-up.

When tabulating AE data, partial dates will be handled as follows. If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as study treatment. In this case, in order to conservatively report the event as treatment-emergent, the onset date will be assumed to be the date of treatment. If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be coded to the day of treatment in order to conservatively report the event as treatment-emergent. A missing onset date will be coded as the day of treatment.

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; a one-sided 90% confidence interval will be calculated for the primary response endpoint.

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, 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 birth, race and ethnicity, time from diagnosis of β-hemoglobinopathy type to confirmation for inclusion in the study, method of diagnosis of disease, the presence of any significant co-morbid conditions, the time from diagnosis of β-hemoglobinopathy type to treatment, and amount of pRBCs transfused per year (volume per year and volume per month) in the 2 years prior to study enrollment.

7.4.4 Efficacy and Pharmacodynamic Analysis

The following parameters will be evaluated using descriptive statistics.

- The production of ≥2.0 g/dL of HbA containing $\beta^{A\text{-}T87Q}$-globin for the 6-month period between Month 18 and Month 24 post-transplant, as a binary response.
- CCI
- CCI
- Therapeutic globin expression, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to α-globin and $\beta^{A\text{-}T87Q}$-globin to all β-like-globin-chains in whole blood.
- Average VCN in cell populations from peripheral blood and, if collected, bone marrow containing the integrated LentiGlobin BB305 provirus.

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

7.4.5 Safety Analysis

All subjects receiving any part of at least 1 injection of the conditioning agent busulfan prior to LentiGlobin BB305 Drug Product infusion 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). AEs will be summarized for those events that occur 1) after signing the informed consent and prior to conditioning; 2) from the start of conditioning until Day 0 (immediately before the start of LentiGlobin BB305 Drug Product infusion); 3) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through 42 days post-infusion; 4) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through 12 months post-infusion (≥Grade 2 AEs); and 5) from the start of LentiGlobin BB305 Drug Product infusion on Day 0 through the entire 24-month study period (SAEs and LentiGlobin BB305 Drug Product-related AEs). Laboratory measures will be compared with their corresponding normal ranges and the incidence of abnormally high and abnormally low laboratory values will be calculated for each relevant protocol-specified laboratory test; changes in laboratory parameters over time will be summarized with descriptive statistics. Replication-competent lentivirus testing will be performed. Vector integration site analysis will be done on leukocytes by PCR and sequencing.

7.5 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 REQUIREMENTS

8.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) Guideline for GCP and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of LentiGlobin BB305 Drug Product as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should

be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

8.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB / EC and other appropriate institutional regulatory bodies 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 sites where IRB / EC and other appropriate institutional regulatory body 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 IRB / EC and other appropriate institutional regulatory bodies by the investigator.

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, accountability records, study reports, and communications will identify the subject by initials and the assigned subject number. The investigator 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 investigator will conduct the study in compliance with the protocol provided by bluebird bio, and given approval/favorable opinion by the IRB / EC and other appropriate institutional regulatory bodies. Modifications to the protocol should not be made without agreement of both the investigator and bluebird bio. Changes to the protocol will require written IRB / EC and other appropriate institutional regulatory body approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB / EC 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 IRB / EC and other appropriate institutional regulatory bodies. bluebird bio, Inc., 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 investigator will contact bluebird bio, 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 will be performed. 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 IRB / EC and other appropriate institutional regulatory bodies, 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 investigator, 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 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 investigator or designated representative should complete the CRF screening pages 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 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 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 investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. bluebird bio, Inc., must be notified in writing if a custodial change occurs.

The Sponsor has full rights over any invention, discovery, or innovation, patentable or not, that may occur when performing the study.

8.9 Liability and Insurance

bluebird bio, Inc., 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 and Presentation of Study Findings and Use of Information

All information regarding LentiGlobin BB305 lentiviral vector and Drug Product supplied by bluebird bio to the investigator is privileged and confidential information. The 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 lentiviral vector and Drug Product and may be disclosed to regulatory authority(ies), other investigators, corporate partners, or consultants as required.

It is anticipated that the results of this study will be presented at scientific meetings and/or published in a peer reviewed scientific or medical journal. A Publications Committee comprised of investigators participating in the study and representatives from bluebird bio, as appropriate, will be formed to oversee the publication and presentation of the study results, which will reflect the experience of all participating clinical sites.

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

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10 APPENDICES

10.1 Creatinine Clearance Calculation

Calculate creatinine clearance for males and females as follows:

In males: $$\frac{[(140 - age) \times weight(kg)]}{[72 \times creatinine(mg / dL)]}$$

In females: $$\frac{[(140 - age) \times weight(kg)]}{[72 \times creatinine(mg / dL)]} \times 0.85$$

10.2 Peripheral Blood Stem Cell Collection Recommendations

Product Parameter	Criteria
Apheresis machine	COBE Spectra (Terumo BCT), Optia (Terumo BCT), Amicus (Fenwal)
Venous access	Bilateral peripheral venous catheters or central venous access catheter

Collection	Large volume processed daily (>15L of recirculated blood volume).
	ACD-A to whole blood ratio of 1:12; use of heparin is allowed.
	
	For Spectra manual program:
	• Enter patient's weight, height, total blood volume, Hct
	• Collect line monitored frequently to ensure correct interface position
	  ◦ Target Hct to be in 3% ± 1% on the collect line colorgram[a]. Change plasma pump 0.1 – 0.3 mls/min to maintain desired Hct.
	  ◦ Collect line rate 0.9 – 1.5 mls/min
	
	For Optia:
	• Enter patient's weight, height, total blood volume, Hct, WBC, Platelet, to establish flow rates (~ 30 to 40 ml/min) appropriately.
	• Start with collection preference at default. If RBCs are seen exiting the chamber, decrease collection preference immediately to 20.[b]
	• Faster centrifugation rate also aids in adequate collection[a].
	
	For Amicus:
	• Enter patient's weight, height, total blood volume, Hct to establish flow rates.
	• Set MNC offset at 1.5 and RBC offset at 6.0.
	• Monitor the MNC chain collection during each cycle. Increase MNC offset as needed to collect as many MNCs as possible.
	• Process at least 7 cycles with cycle volume 1000 mls.

Recommended Monitoring	CD34+ count from the collection bag after 1 to 2 hours of collection
Appearance	Container intact with no leakage No gross clumps or clots
Label	Proper label affixed
Product Seal	Hermetically seal product with three (3) seals. Cut third seal away from the product leaving 2 seals with the product and as much tubing as possible. Heat seal or grommets are acceptable seals.

Abbrev. : ACD-A, Anticoagulant Citrate Dextrose Solution, Solution A, USP (2.13% free citrate ion); Hct, hematocrit; MNC, mononuclear cells; RBC, red blood cells; WBC, white blood cells

[a] Due to increased number of nucleated RBCs and reticulocytes in the peripheral blood, the color of the buffy coat is darker than compared to healthy donors/oncology patients. Therefore, following standard collection recommendations (low Hct, colorgram in ~2% to 3% range) will erroneously target the platelet layer in thalassemia patients, especially in patients with prior splenectomy.

[b] Using the Optia and observing the Cell Separation connector, one may notice a second RBC layer above the buffy coat. If seen, quickly go deeper to collect MNCs vs. RBCs by changing the collection preference.

Target collection CD34+ is ≥ 10×10^6 CD34+ cells/kg.

10.3 Karnofsky Performance Status Scale

The following table presents the Karnofsky performance status scale.

Points	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or to do active work
60	Required occasional assistance but is able to care for most of his/her needs
50	Required considerable assistance and frequent medical care
40	Disabled; required special care and assistance
30	Severely disabled; hospitalization indicated. Death not imminent
20	Very sick; hospitalization necessary; active support treatment necessary
10	Moribund; fatal processed progressing rapidly
0	Dead

Source: Mor V, Laliberte L, Morris JN, Wiemann M. The Karnofsky Performance Status Scale: an examination of its reliability and validity in a research setting. Cancer 1984;53:2002-2007.

10.4 Iron Reduction Guidelines

10.4.1 For subjects who achieve transfusion independent (TI) status within 3 months post-drug product infusion ("post-HSCT")

Assessing chelation needs

Screening cardiac MRI and liver LIC will be used to evaluate the need for starting iron reduction methods at Day +90 post-HSCT based on the table below.

Table 10-1 Iron reduction guidelines post-drug product infusion

Liver LIC (mg/g)	Cardiac T2* (ms)	
	>20 ms	**20-10 ms**
< 8 mg/g	No chelation is required	Chelation is indicated
8-15 mg/g	Consider based on serum ferritin and hepatitis serology[a]	Chelation is indicated
>15 mg/g	Chelation is indicated	Chelation is indicated

[a] Chelation is recommended only if serum ferritin is >2000 ng/ml at Day +90 or hepatitis B/C serology was positive pre-transplant.

If iron reduction methods need to be started based on guidelines in Table 10-1, they should be initiated after Day +90, once subjects are medically stable. Earlier initiation of iron reduction (<90 days post-HSCT) should be discussed with the medical monitor.

Subjects who have achieved the TI status but do not start iron reduction methods on Day +90 (based on Table 10-1), should still be continually reassessed for chelation needs post-HSCT based on the SOE of the relevant protocol.

Iron Reduction Recommendations

Chelation is recommended using oral deferasirox (Exjade) or deferoxamine (Desferal) infusion, depending on institutional choice and subjects' pre-transplant experience.

Deferiprone is not recommended at Day +90 because of its effect on the bone marrow, but its use can be evaluated > 6 months post-transplant.

Starting dose of chelator is recommended based on institutional protocols. Doses may need to be adjusted continuously based on serum ferritin levels to prevent toxicity.

Phlebotomy can also be used in subjects who have Hb consistently ≥ 11 g/dl.

Reducing and Stopping Iron Reduction Treatment

To avoid toxicity, once serum ferritin is ≤ 1000 ng/ml, downward adjustment of dose of chelator and decreasing the frequency of phlebotomy is advised, as per institutional protocols.

Iron reduction methods should be stopped once liver LIC is ≤ 5 mg/g dry wt. and serum ferritin is <500 ng/ml.

10.4.2 For subjects who are not TI at 3 months post-HSCT

For subjects who contain to require transfusions, chelation should be re-started at Day +90 post-HSCT following guidelines in Section 10.4.1

To avoid toxicity, once serum ferritin is ≤ 1000 ng/ml, downward adjustment of dose of chelator and decreasing the frequency of phlebotomy is advised, as per institutional protocols.

Chelation should be discontinued if they become TI, and their Liver LIC is <5mg/g and serum ferritin is <500 ng/ml.

Subjects who are not taking chelators should be continually reassessed for chelation needs post-HSCT based on the SOE of the relevant protocol.

Protocol Title:	A Phase 1/2, Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with β-Thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector (LentiGlobin BB305 Drug Product)
Protocol Number:	HGB-204 Version 5.0 (29 June 2015)

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) ______