CLINICAL STUDY PROTOCOL HGB-212

EudraCT No. 2016-003611-35

CCI

A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β-Thalassemia by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Subjects ≤50 Years of Age

Study Sponsor: bluebird bio, Inc.
60 Binney Street
Cambridge, MA 02142 USA

Responsible Medical Officer: PPD, MD
Senior Medical Director
Clinical Research Development
bluebird bio, Inc.
Office telephone: PPD
Email: PPD

Protocol Version: Version 6.0

Document Date: 10 June 2021

CCI

MEDICAL OFFICER CONTACT INFORMATION

Country-Specific 24-hour Emergency Contact Phone Numbers

Country	Phone Number
France	PPD
Germany	
Greece	
Italy	
United Kingdom	
United States of America	

SUMMARY OF CHANGES

Document concerned: Protocol HGB-212

Number and date of the previous version: Version 5.0 (08 October 2020)
Number and date of the new version: Version 6.0 (10 June 2021)

This protocol (Version 6.0, 10 June 2021) replaces previous Version 5.0 (08 October 2020).

Substantial changes compared to Study HGB-212 Protocol v5.0 are described in the table below, and include updated clinical work-up criteria, procedure, and follow-up in the section describing integration site analyses, introduction of optional archival and genetic testing on bone marrow samples, added considerations for vaccines as concomitant medications, and removal of the statement that a subject may be withdrawn from the study if they have undetectable VCN in peripheral blood cells for 2 consecutive measurements at least 1 month apart.

Non-substantial changes implemented are described in the following list. In addition, several minor changes were made to correct typographical errors and align the use of terminology and formatting with current standards.

Note: in the following table, added text is in ***bold italics*** and deleted text is in ~~strikethrough~~.

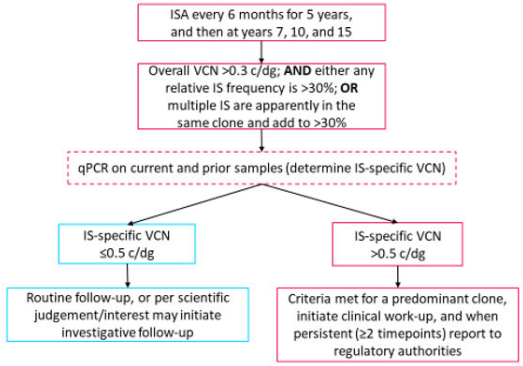
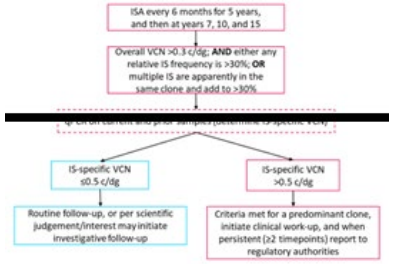
DESCRIPTION OF EACH SUBSTANTIAL CHANGE

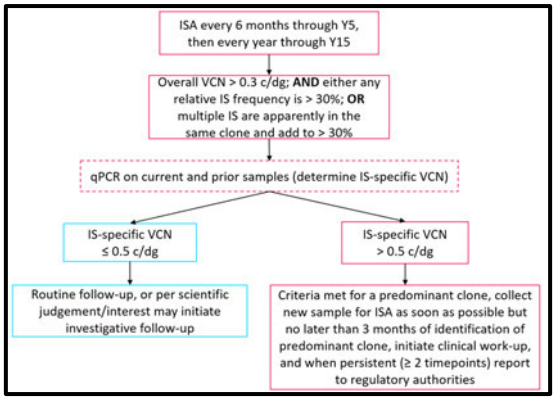
Initial wording	Amended or New Wording	Reason/Justification for change
Section(s) concerned: Section 4.5 Subject Withdrawal from the Study In addition, after subjects have been treated with LentiGlobin BB305 Drug Product, they may be withdrawn from the study if the subject has undetectable VCN (<0.0003 vector copies per diploid genome) in peripheral blood cells for 2 consecutive measurements at least 1 month apart. This determination is based on a very sensitive quantitative polymerase chain reaction (qPCR) assay.	Section(s) concerned: Section 4.5 Subject Withdrawal from the Study ~~In addition, after subjects have been treated with LentiGlobin BB305 Drug Product, they may be withdrawn from the study if the subject has undetectable VCN (<0.0003 vector copies per diploid genome) in peripheral blood cells for 2 consecutive measurements at least 1 month apart. This determination is based on a very sensitive quantitative polymerase chain reaction (qPCR) assay.~~	Treated subjects should remain in the study regardless of their vector copy number after treatment to ensure comprehensive follow-up of safety and efficacy.
Section(s) concerned: Section 5.9.2 Concomitant Medications and Therapies: General […]	Section(s) concerned: Section 5.9.2 Concomitant Medications and Therapies: General ***For the purposes of this study, vaccines (e.g., COVID-19 vaccines) are considered concomitant medications. Although interactions between a vaccine and LentiGlobin BB305 Drug Product are not expected, the protocol includes use of immunomodulatory (plerixafor) and immunosuppressive medication (busulfan). Local guidelines should be followed regarding a minimum time period between any medication to be provided as part of treatment with LentiGlobin BB305 Drug Product and any vaccine; it is recommended that vaccines are not administered to subjects within 1 month of initiating mobilization for stem cell collection, within 1 week after receiving mobilization agents, within 1 month of initiating myeloablative conditioning, or within 6 months after drug product infusion. Revaccination post-drug product infusion should be considered per Investigator's***	Provided considerations for vaccines as concomitant medications per guidance from regulatory agency.

Initial wording	Amended or New Wording	Reason/Justification for change
	discretion due to potential loss of immunity after myeloablative conditioning and may be administered following local guidelines. ***The prescribing information of the vaccine administered should be referred to for the latest indication, contraindications and safety information as well as the latest general clinical recommendations on vaccine administration in the country or region.*** ***All vaccinations during the study period should be documented in the CRF.***	
Section(s) concerned: Section 6.1 Schedule of Events Table 4 Schedule of Events: Screening and CD34+ Cell Harvest; Footnote 16 Bone marrow collection for morphology, cellularity, cell count and iron stains. For subjects undergoing re-screening, bone marrow collection does not need to be performed.	Section(s) concerned: Section 6.1 Schedule of Events Table 4 Schedule of Events: Screening and CD34+ Cell Harvest; Footnote 16 Bone marrow collection for morphology, cellularity, cell count and iron stains. For subjects undergoing re-screening, bone marrow collection does not need to be performed. ***If sufficient sample is available, sample may be archived and/or other research tests (e.g., genetic testing) may be performed.***	Specified that bone marrow samples may be archived and that genetic testing may be performed if clinically indicated.
Section(s) concerned: Section 6.1 Schedule of Events Table 6 Schedule of Events: Follow-up; Footnote 10 Bone marrow for dyserythropoiesis studies (reticulocytes, nucleated RBC, serum transferrin receptor, hepcidin, and erythropoietin), as well as morphology, cellularity, cell count, and iron content; other research tests (e.g., VCN, ISA, HPLC) may be performed if sufficient sample is available	Section(s) concerned: Section 6.1 Schedule of Events Table 6 Schedule of Events: Follow-up; Footnote 10 Bone marrow for dyserythropoiesis studies (reticulocytes, nucleated RBC, serum transferrin receptor, hepcidin, and erythropoietin), as well as morphology, cellularity, cell count, and iron content; ***if sufficient sample is available, sample may be archived and/or*** other research tests (e.g., VCN, ISA, HPLC***, genetic testing***) may be performed~~ if sufficient sample is available~~	

Section(s) concerned: Section 6.2.10 Bone Marrow and Blood for Dyserythropoiesis Studies Bone marrow will be collected for dyserythropoiesis studies (see Section 6.2.16.2), as well as morphology, cellularity, cell count and iron content, at Screening and Month 12 and Month 24 Visits. If sufficient sample is available, bone marrow may also be tested for vector copy number (VCN), and for $\beta^{A\text{-}T87Q}$-globin expression in erythroid burst forming units (BFU-E). If an unscheduled bone marrow collection is performed at any point during study, such samples may also be used for the determination of VCN, and for $\beta^{A\text{-}T87Q}$-globin expression in BFU-Es.	Section(s) concerned: Section 6.2.10 Bone Marrow and Blood for Dyserythropoiesis Studies Bone marrow will be collected for dyserythropoiesis studies (see Section 6.2.16.2), as well as morphology, cellularity, cell count and iron content, at Screening and Month 12 and Month 24 Visits. If sufficient sample is available, ***the*** bone marrow ***sample*** may ~~also~~ be ~~tested for~~***archived and/or other research tests (e.g.,*** vector copy number ~~(~~***(***VCN***)***~~),~~ ~~and for~~ $\beta^{A\text{-}T87Q}$-globin expression in erythroid burst forming units ~~(~~***(***BFU-E~~)~~***), ISA, HPLC, genetic testing) may be performed.*** If an unscheduled bone marrow collection is performed at any point during study, such samples may also be ~~used for the determination of~~***archived and/or other research tests (e.g.,*** VCN, ~~and for~~ $\beta^{A\text{-}T87Q}$-globin expression in BFU-Es***, ISA, HPLC, genetic testing) may be performed***.	
Section(s) concerned: 6.2.16.7. Clinical Work-up for Unexpected Blood Test Results • Bone marrow biopsy	Section(s) concerned: 6.2.16.7. Clinical Work-up for Unexpected Blood Test Results • Bone marrow biopsy ***and/or aspiration (may also be archived for additional analysis in the future, see Section 6.2.10)***	
Section(s) concerned: Section 6.2.18.Assessment of Clonal Predominance and/or Suspicion of Insertional Oncogenesis (Malignancy) Section 6.2.18.1 Assessment of Clonal Predominance Figure 1 outlines the algorithm for assessment of clonal predominance. Screening integration site analysis (ISA) will be performed as indicated in the SOE using high-	Section(s) concerned: Section 6.2.18.Assessment of Clonal Predominance and/or Suspicion of ~~Insertional Oncogenesis (~~Malignancy~~)~~ Section 6.2.18.1 Assessment of Clonal Predominance Figure 1 outlines the algorithm for assessment of clonal predominance. ~~Screening i~~***I***ntegration site analysis (ISA) will be performed as indicated in the SOE using high-	Updated the criteria for which a clinical work-up following integration site analysis is recommended, updated the clinical work-up procedure to include a follow-up integration site analysis, and updated the process to be used upon detection of clonal predominance or malignancy. These

throughput, semi-quantitative methods which identify integration sites (IS) based on vector sequence primers. IS identified in the screening assay are considered as being of interest when the overall peripheral blood VCN is > 0.3 c/dg AND either any relative IS frequency is > 30% OR multiple IS are apparently in the same clone and add up to > 30%. Multiple IS apparently in the same clone is defined as more than one relative frequency where values are within 20% of each other (e.g., 5% ± 1%, 10% ± 2%, 15% ± 3%, etc.), as well as any additional cases identified through bluebird bio internal review of ISA reports. When multiple IS are apparently in the same clone, it will be recommended to confirm that those IS are in a single clone (e.g., bone marrow or peripheral blood colony-forming unit assay). IS of interest will be interrogated, from the time point of interest and available previous time points, using a quantitative assay (e.g., qPCR) designed to detect the specific IS and determine an IS-specific VCN that will help to estimate clonal contribution. If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN ≤ 0.5 c/dg, estimating ≤ 50% clonal contribution, repeat ISA screening will continue at the regularly scheduled time points. However, according to scientific judgement or interest, investigative follow-up may be initiated by bluebird bio in collaboration with an investigator and additional interval, unscheduled ISA testing may be performed. If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN > 0.5 c/dg, estimating > 50% clonal contribution, criteria will be met to consider the subject as having a predominant clone. This threshold also applies to individual lineage evaluations (myeloid, lymphoid, etc.) when performed. Clinical work-up will be recommended for a predominant clone (see Section 6.2.18.3). A report to relevant regulatory authorities will be required when a persistent, predominant clone is identified (2 or more time points), and the report will be made within	throughput, semi-quantitative methods which identify integration sites (IS) based on vector sequence primers. IS identified ~~in the screening assay~~ are considered as being of interest when the overall peripheral blood VCN is > 0.3 c/dg AND either any relative IS frequency is > 30% OR multiple IS are apparently in the same clone and add up to > 30%. Multiple IS apparently in the same clone is defined as more than one relative frequency where values are within 20% of each other (e.g., 5% ± 1%, 10% ± 2%, 15% ± 3%, etc.), as well as any additional cases identified through bluebird bio internal review of ISA reports. When multiple IS are apparently in the same clone, it will be recommended to confirm that those IS are in a single clone (e.g., bone marrow or peripheral blood colony-forming unit assay). IS of interest will be interrogated, from the time point of interest and available previous time points, using a quantitative assay (e.g., qPCR) designed to detect the specific IS and determine an IS-specific VCN that will help to estimate clonal contribution. If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN ≤ 0.5 c/dg, estimating ≤ 50% clonal contribution, repeat ISA ~~screening~~ will continue at the regularly scheduled time points. However, according to scientific judgement or interest, investigative follow-up may be initiated by bluebird bio in collaboration with an investigator and additional interval, unscheduled ISA testing may be performed. If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN > 0.5 c/dg, estimating > 50% clonal contribution, criteria will be met to consider the subject as having a predominant clone. This threshold also applies to individual lineage evaluations (myeloid, lymphoid, etc.) when performed. Clinical work-up will be recommended for a predominant clone (see Section 6.2.18.~~3~~***2***). ***In addition, if criteria for a predominant clone have been met, a new sample for ISA is to be collected as soon as possible but no later than***	updates allow for more stringent monitoring for malignancies or potential malignancies following treatment with LentiGlobin BB305 Drug Product.

30 days of receipt of IS-specific VCN results from the second time point where the persistent, predominant clone is identified. **Figure 1: Schematic for Assessment of Clonal Predominance**  Abbrev.: c/dg, copies per diploid genome; IS, integration site (s); ISA, integration site analysis; qPCR, quantitative polymerase chain reaction; VCN, vector copy number	***within 3 months of identification of predominant clone, and retrospective testing by IS-specific qPCR is to be performed on sample(s) previously collected as available. A predominant clone identified at 2 or more time points is considered persistent.*** A report to relevant regulatory authorities will be required when a persistent, predominant clone is identified ~~(2 or more time points)~~, and the report will be made within 30 days of receipt of IS-specific VCN results ***for*** ~~from~~ the ~~second time point where the~~ persistent~~,~~ predominant clone ~~is identified~~. ***A clinical work-up may also be recommended for an IS near or within a locus known to have oncogenic activity or upon observation of clonal expansion.*** **Figure 1: Schematic for Assessment of Clonal Predominance** 	

	 Abbrev.: c/dg, copies per diploid genome; IS, integration site (s); ISA, integration site analysis; qPCR, quantitative polymerase chain reaction; VCN, vector copy number ***Note that this schematic includes the assessment schedule for ISA through the subsequent long-term follow-up study.***	
Section(s) concerned: Section 6.2.18.2. 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 2 consecutive measurements	Section(s) concerned: ~~Section 6.2.18.2. 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 2 consecutive measurements~~	

CCI

Section(s) concerned: Section 6.2.18.3 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 occur in stages and may include some of the following at each stage: • Physical exam • CBC with differential • Lymphocyte subsets • Studies to rule out infectious cause • Studies to rule out autoimmune disease • Imaging studies • 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 3.2). It should be noted that 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, the subject will continue to be monitored as per the	Section(s) concerned: Section 6.2.18.~~3~~***2*** Clinical Work-Up for ***Potential*** Malignancy ~~If any of the above criteria is met~~ ***In the event of clonal predominance, persistent clonal predominance, or any clinical suspicion of myelodysplasia, leukemia, or lymphoma***, the Medical Monitor will be notified~~,~~ and a work-up will be performed that may occur in stages and may include some of the following at each stage: • Physical exam • CBC with differential • Lymphocyte subsets • Studies to rule out infectious cause • Studies to rule out autoimmune disease • Imaging studies • Bone marrow analysis • ***Cytogenetic and molecular analyses*** ~~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 3.2). It should be noted that 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.~~	

protocol-defined SOE, or more frequently at discretion of the Investigator and Sponsor.	~~If there is~~ ***If the clinical work-up indicates*** no evidence of ~~malignancy or~~ myelodysplasia***, leukemia, or lymphoma***, the subject will continue to be monitored as per the protocol-defined SOE, or more frequently at discretion of the Investigator and Sponsor. ***If the clinical work-up indicates a diagnosis of myelodysplasia, leukemia, or lymphoma, the Sponsor will convene an urgent safety review meeting. Further analyses will be determined by the Sponsor, in consultation with the DMC. It should be noted that it may not be possible to distinguish the source of malignancy (e.g., arising from underlying pathophysiology of the disease, transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional oncogenesis), and all efforts should be made to confirm the source of malignancy.*** ***For clinical work-up after identification of a persistent predominant clone and confirmed presence of abnormal CBC, bone marrow analysis is recommended if not previously performed as part of the clinical work-up.***	

DESCRIPTION OF EACH NON-SUBSTANTIAL CHANGE

- Modified language throughout the protocol to indicate that subjects who complete this study will be expected to enroll in the long-term follow-up study LTF-303.
- Numbers for subject enrollment as of 03 March 2020 were updated in the Section 1 Introduction.
- Updated the title page to reflect change in Responsible Medical Officer

CLINICAL STUDY PROTOCOL SYNOPSIS

Protocol Title:	A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β-Thalassemia by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Subjects ≤50 Years of Age
Protocol Number:	HGB-212
Objectives:	**Primary:** evaluate the efficacy of treatment with LentiGlobin BB305 Drug Product in subjects ≤50 years of age with transfusion-dependent β-thalassemia (TDT) who have a β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotype at the *HBB* gene **Secondary:** evaluate the safety of treatment with LentiGlobin BB305 Drug Product in subjects ≤50 years of age with TDT who have a β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotype at the *HBB* gene
Study Design:	This is a single-arm, multisite, single-dose, Phase 3 study with approximately 18 subjects with TDT who have either a β^0 or IVS-I-110 mutation at each allele of the β-globin (*HBB*) gene (i.e., β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotypes). Similar to β^0 alleles, the β^+ allele IVS-I-110 (HGVS nomenclature: *HBB*:c.93-21G>A) is widely recognized as producing little to no β-globin (Borgna-Pignatti and Galanello, 2009), thus subjects with β^0/IVS-I-110 or IVS-I-110/IVS-I-110 genotypes were grouped with the β^0/β^0 subjects in this study. At least 12 subjects must be without an IVS-I-110 mutation (i.e., β^0/β^0 genotype). Additionally, at least 10 subjects must be <18 years of age. Patients transplanted at younger ages before advanced disease symptoms of thalassemia are manifested are hypothesized to have different rates of transplant-related complications, different long-term disease outcomes, and potentially different efficacy of gene transduction than adult patients. The study will evaluate the efficacy and safety of autologous hematopoietic stem cell (HSC) transplantation (HSCT) using LentiGlobin BB305 Drug Product for the treatment of β-thalassemia (INN/USAN: betibeglogene autotemcel), an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution. Subjects ≥12 and ≤50 years of age must have TDT with a history of transfusion of at least 100 mL/kg/year of packed red blood cells (pRBCs) in the 2 years preceding enrollment, or be managed under standard thalassemia guidelines (e.g., Thalassemia International Federation, 2014; see Appendix 10.4) with ≥8 transfusions of pRBCs per year in the 2 years preceding enrollment (subjects ≥12 years; Rachmilewitz and Giardina, 2011). Subjects <12 years of age must have a history of transfusion of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment. In addition to having a history of transfusion of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment, subjects <5 years of age must weigh a minimum of 6 kg and reasonably be anticipated to be able to provide at least the minimum number of cells required to initiate the manufacturing process. Before treating subjects <12 years of age, the independent Data Monitoring Committee (DMC) will review safety data available from Study HGB-207 and determine whether the study can safely proceed with the treatment of subjects ≥5 and <12 years of age. After at least 2 subjects ≥5 and <12 years of age have attained neutrophil engraftment after LentiGlobin BB305 Drug Product infusion in Study HGB-207 and/or Study HGB-212, the DMC will review their safety data and determine whether the study can safely proceed with the treatment of subjects younger than 5 years of age. Subjects <12 years of age may only be enrolled at sites with regulatory approval for the specified age range.

	The study has 4 distinct stages, as follows. **Stage 1:** Screening to determine eligibility for treatment **Subjects with TDT who are documented to have genotypes other than β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 should not enter Screening.** *HBB* genotype for all subjects will be confirmed during Screening along with additional parameters of eligibility. **Stage 2:** Autologous CD34+ cell collection, LentiGlobin BB305 Drug Product manufacture and disposition Each subject will undergo HSC mobilization with a granulocyte-colony stimulating factor (G-CSF; e.g., filgrastim, lenograstim) and plerixafor. Peripheral blood mononuclear cells (PBMCs) for drug product manufacture will be collected by apheresis. A total of 2 mobilization cycles may be performed if needed and each mobilization cycle may include up to 3 apheresis days. However, subjects whose drug product fails to meet specifications for any reason may undergo repeat apheresis and manufacture of new drug product. Apheresis products can also be used for rescue cells; alternatively, a bone marrow harvest is also allowed to procure cells for rescue. **Stage 3:** Myeloablative conditioning (4 days of conditioning followed by at least 48 hours of washout) and infusion of LentiGlobin BB305 Drug Product (Day 1) After the transduced cells are dispositioned for clinical use, and the subject's eligibility has been re-confirmed (by clinical laboratory tests, physical examination, performance status, adverse event (AE) review and other tests based on institutional requirements), the subject will undergo myeloablative conditioning with busulfan. After completion of the 4-day course of busulfan, there must be a minimum of 48 hours of washout before drug product infusion. On study Day 1, thawed LentiGlobin BB305 Drug Product will be administered via intravenous (IV) infusion at a dose of $\geq 5.0\times10^6$ CD34+ cells/kg. **Stage 4:** Follow-up, through engraftment and up to 24 months after drug product infusion Subjects will be followed daily in the transplant unit for AEs, and laboratory parameters will be followed to monitor bone marrow engraftment. The subject will be discharged from the transplant unit once the subject is considered medically stable. The goal during the follow-up period is to maintain hemoglobin (Hb) ≥9 g/dL. Transfusions should be avoided for Hb ≥9 g/dL unless the need is medically justified (e.g., as a requirement for surgery). It is recommended that subjects should receive RBC transfusions for any Hb <7 g/dL, and for clinically symptomatic anemia, irrespective of Hb level. Subjects will be followed in this protocol for a period of 24 months after LentiGlobin BB305 Drug Product infusion. Thereafter subjects will be expected to enroll in a separate long-term follow-up protocol (LTF-303) that will assess safety and efficacy beyond Month 24 for a total of 15 years after drug product infusion.
Data Monitoring Committee:	An independent Data Monitoring Committee (DMC) comprised of members with appropriate scientific and medical expertise to monitor the study will be convened. The DMC will be charged with review of all safety data for this study, including AEs, serious adverse events (SAEs) and relevant laboratory values. The DMC will have the right to recommend that the Sponsor stop the study at any time due to concerns for the safety of the subjects.
Number of Subjects Planned:	Approximately 18 subjects will be treated with drug product; at least 12 subjects must be without an IVS-I-110 mutation and at least 10 subjects must be <18 years of age. Replacement subjects may be added if subjects are screen failures or withdraw prior to drug product infusion.
Inclusion Criteria:	1. Subjects ≤50 years of age at the time of consent or assent (as applicable), and able to provide written consent (adults, or legal guardians, as applicable) or assent (adolescents

	or children). Pediatric subjects (<12 years of age) may only be enrolled at a given site if approved by the relevant regulatory authority. Provided that the DMC has approved enrolling subjects younger than 5 years of age, subjects younger than 5 years of age may be enrolled at sites with regulatory approval for the specified age range if they weigh a minimum of 6 kg and are reasonably anticipated to be able to provide at least the minimum number of cells required to initiate the manufacturing process. 2. Diagnosis of TDT with a history of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment (all subjects), or be managed under standard thalassemia guidelines (e.g.,Thalassemia International Federation, 2014; see Appendix 10.4) with ≥8 transfusions of pRBCs per year in the 2 years preceding enrollment (subjects ≥12 years; Rachmilewitz and Giardina, 2011). 3. Clinically stable, have a Karnofsky performance status of ≥80 for adults (≥16 years of age) or a Lansky performance status of ≥80 for adolescents or children (<16 years of age), and eligible to undergo HSCT. 4. Treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records on RBC transfusions (including volume and units of RBCs and associated pre-transfusion Hb values, reticulocyte counts and relevant blood bank details as available), in-patient hospitalization, and iron chelation history.
Exclusion Criteria:	1. Presence of a mutation characterized as other than β^0 (e.g., β^+, β^E, β^C) on at least one *HBB* allele. For the purpose of screening, the *HBB* mutation IVS-I-110 (G→A) [HGVS nomenclature: *HBB*:c.93-21G>A] will be considered equivalent to a β^0 mutation. 2. Positive for presence of human immunodeficiency virus type 1 (HIV-1) or 2 (HIV-2), hepatitis B virus (HBV), or hepatitis C virus (HCV). Syphilis (rapid plasma reagin [RPR]) testing is also required and a positive test for syphilis is exclusionary where mandated by regional drug product manufacturing practices. Note that subjects who have been vaccinated against hepatitis B (hepatitis B surface antibody-positive) who are negative for other markers of prior hepatitis B infection (e.g., negative for hepatitis B core antibody) are eligible. Subjects with past exposure to HBV (HBc Ab positive and/or HBe Ab positive) are also eligible for the study provided they are negative by assessment for HBV DNA. Also note that subjects who are positive for anti-hepatitis C antibody are eligible as long as they have a negative HCV viral load. Where clinically and/or regionally indicated, other tests may be performed, in which case positive results would exclude the subject from participating: for example, human T-lymphotropic virus-1 (HTLV-1) or -2 (HTLV-2), tuberculosis, toxoplasmosis, Trypanosoma cruzi, West Nile Virus or Zika Virus. 3. Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the clinical investigator. 4. A white blood cell (WBC) count $<3\times10^9$/L, and/or platelet count $<100\times10^9$/L not related to hypersplenism. 5. Uncorrected bleeding disorder. 6. Any prior or current malignancy (with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin) or myeloproliferative or significant immunodeficiency disorder. 7. Immediate family member (i.e. parent or siblings) with a known Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome and familial adenomatous polyposis). 8. Prior HSCT. 9. Advanced liver disease, defined as: a. Persistent aspartate transaminase, alanine transaminase, or direct bilirubin value >3× the upper limit of normal (ULN), **or**

	b. Baseline prothrombin time or partial thromboplastin time >1.5× ULN, suspected of arising from liver disease, **or** c. Magnetic Resonance Imaging (MRI) of the liver demonstrating clear evidence of cirrhosis, **or** d. MRI findings suggestive of active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron concentration ≥15 mg/g require follow-up liver biopsy in subjects ≥ 18 years of age. In subjects < 18 years of age, these MRI findings are exclusionary, unless in the opinion of the investigator, a liver biopsy could provide additional data to confirm eligibility and would be safe to perform. If a liver biopsy is performed based on MRI findings, any evidence of cirrhosis, bridging fibrosis, or significant active hepatitis will be exclusionary. 10. Baseline estimated glomerular filtration rate <70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration 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). 11. Uncontrolled seizure disorder. 12. Diffusion capacity of carbon monoxide (DLco) <50% of predicted (corrected for Hb and/or alveolar volume, as clinically indicated). If DLco cannot be assessed due to age or cognition-related restrictions, there must be a normal respiratory exam, chest radiograph without pulmonary infiltrates, and oxygen saturation by pulse oximetry >92% on room air. In the presence of clinically significant abnormal findings on chest radiograph, clinically significant abnormal findings on the respiratory exam, or oxygen saturation by pulse oximetry ≤92% on room air, the subject will be excluded. 13. A cardiac T2* <10 ms by MRI. 14. Any other evidence of severe iron overload that, in the investigator's opinion, warrants exclusion. 15. Participation in another clinical study with an investigational drug within 30 days of Screening. 16. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician or investigator. 17. Prior receipt of gene therapy. 18. Diagnosis of significant psychiatric disorder of the subject that could seriously impede the ability to participate in the study. 19. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile subjects. Females of child-bearing potential and males are required to use two different effective methods of contraception from Screening through at least 6 months after drug product infusion. If subjects are truly sexually abstinent (where true sexual abstinence is defined as being in line with the preferred and usual lifestyle of the subject), no second method is required. 20. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol. 21. A known and available human leukocyte antigen (HLA)-matched family donor. If required by regional regulatory authority, patients with a known and available matched unrelated donor will be excluded from the study. 22. Any contraindications to the use of G-CSF and plerixafor during the mobilization of hematopoietic stem cells and any contraindications to the use of busulfan and any other medicinal products used during the myeloablative conditioning, including hypersensitivity to the active substances or to any of the excipients.
Duration of Subject Participation:	Time between Screening and drug product infusion will be variable, and is estimated generally to be between 3 to 5 months (e.g., up to 3 months between Screening and Mobilization, followed by approximately 2 months before drug product infusion).

	Thereafter the subject is planned to remain on study for approximately 24 months. Eligible subjects will then be expected to enroll in a separate long-term follow-up study until approximately 15 years post drug product infusion.
Test Product, Dose and Mode of Administration:	LentiGlobin BB305 Drug Product is an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the β^{A-T87Q}-globin gene, suspended in cryopreservation solution. All subjects are to receive LentiGlobin BB305 Drug Product on Day 1 via IV infusion at a dose of $\geq 5.0 \times 10^6$ $CD34^+$ cells/kg.
Efficacy endpoints:	**Primary Endpoint:** • The proportion of subjects who meet the definition of "transfusion independence" (TI). TI is defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion. **Secondary Endpoints** • Characterization of subjects achieving TI: – Proportion of subjects who meet the definition of TI at the Month 24 Visit – Duration of TI – Time from drug product infusion to achievement of TI – Weighted average Hb during TI • Characterization of transfusion reduction (TR): – The proportion of subjects who meet the definition of TR, defined as demonstration of a ≥60% reduction in the annualized volume of pRBC transfusion requirements (in mL/kg) in the post-treatment time period from 12 months post-drug product infusion through Month 24 (approximately a 12-month period), compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to study enrollment – Proportion of subjects with a reduction in the annualized mL/kg pRBCs transfused from 12 months post-drug product infusion through Month 24 (approximately a 12-month period) of at least 50%, 60%, 75%, 90% or 100% compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to enrollment – Annualized number and volume of pRBC transfusions from 12 months post-drug product infusion through Month 24 compared to the annualized number and volume of transfusions during the 2 years prior to enrollment – Time from drug product infusion to last pRBC transfusion – Time from last pRBC transfusion to the Month 24 Visit • Weighted average nadir Hb during the 2 years prior to enrollment compared to weighted average nadir Hb from 12 months post-drug product infusion through the Month 24 Visit • Unsupported total Hb levels over time, including Month 6, Month 9, Month 12, Month 18, and Month 24 • Unsupported total Hb levels ≥10 g/dL, ≥11 g/dL, ≥12 g/dL, ≥13 g/dL, ≥14 g/dL at Month 6, Month 9, Month 12, Month 18, and Month 24 • Characterization of use of iron chelation and/or therapeutic phlebotomy among all subjects: – Proportion of subjects who have not received chelation therapy for at least 6 months following drug product infusion

	– Time from last iron chelation use to last follow-up – Proportion of subjects using therapeutic phlebotomy and annualized frequency of phlebotomy use per subject following drug product infusion • Evaluation of the change in iron burden over time, as measured by: – Change in liver iron content by MRI at baseline to Month 12 and Month 24 Visits – Change in cardiac T2* on MRI at baseline to Month 12 and Month 24 Visits – Change in serum ferritin at baseline to Month 12 and Month 24 Visits • Evaluation of health-related quality of life (HRQoL) over time including Month 12 and Month 24 as compared to baseline, using the following validated tools: – Pediatrics: Pediatric Quality of Life Inventory (PedsQL; parent general core and general core) – Adolescents: PedsQL (parent general core and general core) and EuroQol-5D (Youth version) (EQ-5D-Y) – Adults: EuroQol-5D (EQ-5D) Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT), and Short-Form 36 (SF-36) v2 **Exploratory Endpoints** • Assessment of growth and puberty parameters (age appropriate), bone density, diabetes, endocrine evaluations, and neurocognitive development (pediatric subjects <18 years of age) • Assessment of change in dyserythropoiesis • Correlations of pre-treatment variables (e.g., drug product vector copy number [VCN]) with response (e.g., peripheral blood VCN, HbA^{T87Q}) • Measures of health resource utilization (including comparing annualized number of transfusions, number of hospitalizations, and number of days hospitalized, from 12 months post-drug product infusion through the Month 24 Visit, with the annualized corresponding parameters during the 2 years prior to enrollment) • Length of in-patient hospital stay from initiation of conditioning to discharge

Safety endpoints:	**Secondary Endpoints** • Success and kinetics of HSC engraftment • Incidence of transplant-related mortality through 100 days and through 365 days post-drug product infusion • Overall survival (OS) • Detection of vector-derived replication competent lentivirus (RCL) in any subject • Monitoring of laboratory parameters • Frequency and severity of clinical AEs. • Incidence of acute and/or chronic graft-versus-host disease (GVHD) • The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) **Exploratory Endpoint** • The number of subjects with clonal predominance
Pharmacodynamic endpoints:	**Secondary Endpoints** • $\beta^{A\text{-}T87Q}$-globin expression over time, including Month 6, Month 9, Month 12, Month 18, and Month 24, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to all β-like-globins, and α-globin to all β-like-globins, in whole blood – Correlation of $\beta^{A\text{-}T87Q}$-globin expression at early time points post drug product infusion to $\beta^{A\text{-}T87Q}$-globin expression at later time points, as well as clinical outcomes. • VCN in peripheral blood over time, including Month 6, Month 9, Month 12, Month 18, and Month 24 **Exploratory Endpoint** • Relationship between measures of myeloablation and pharmacodynamic and clinical outcomes Additionally, exploratory methods may be used to evaluate pharmacodynamic endpoints.
Statistical Methods:	**Sample size estimation:** No formal sample size calculations were performed. Approximately 18 subjects will be treated with drug product; at least 12 subjects must be without an IVS-I-110 mutation and at least 10 subjects must be <18 years of age. The proposed sample size is based on the premise that excluding a treatment effect of <30% with a high probability is of value (demonstrating with 97.5% confidence that ≥30% of subjects become TI). Among the proposed sample size of 18 treated subjects, a success criterion of 55.6% (10 out of 18 subjects) is proposed, which would yield a lower 1-sided 97.5% exact confidence bound of 30.8%, exceeding the 30% minimal criterion. **Populations for analysis:** • Intent-to-Treat (ITT) population: All subjects who initiate any study procedures, beginning with mobilization by granulocyte colony stimulating factor (G-CSF) and/or plerixafor • Transplant Population (TP): All subjects who receive LentiGlobin BB305 Drug Product • Successful Engraftment Population (SEP): All subjects who have successful neutrophil engraftment after LentiGlobin BB305 Drug Product infusion The ITT population is the primary population for analysis of safety parameters. The TP is the primary population for the analysis of efficacy, pharmacodynamic, and transplant-related endpoints. The SEP will be used to provide supportive data for subjects who engraft.

	Analysis of primary efficacy endpoint: The success criterion for this study is targeted at achievement of TI in 10 out of 18 subjects. A point-estimate of the proportion of subjects achieving TI, with a 2-sided 95% CI calculated using the Clopper-Pearson exact binomial method will be employed. **Analysis of safety:** All AEs will be collected from signing of informed consent/assent through Month 24 Visit for the ITT population, irrespective of severity grade or relationship to LentiGlobin BB305 Drug Product. All AEs will be listed, and descriptive statistics for AEs will be summarized for the following time periods: 1) from informed consent/assent up to the start of mobilization; 2) from start of mobilization up to start of conditioning; 3) from start of conditioning through neutrophil engraftment; 4) from neutrophil engraftment through Month 24 Visit; 5) from drug product infusion through Month 24 Visit; and 6) from informed consent/assent through Month 24 Visit. Additionally, survival status, laboratory results and insertional oncogenesis (insertional mutagenesis resulting in oncogenesis) events will be summarized. The planned statistical methodology details will be presented in the Statistical Analysis Plan (SAP).

LIST OF ABBREVIATIONS

Abbreviation	Definition
Ab	Antibody
ADL	Activities of daily living
AE	Adverse event
Allo-HSCT	Allogeneic hematopoietic stem cell transplant(ation)
ANC	Absolute neutrophil count
AUC	Area under the curve
BFU-E	Burst-forming units-erythroid
CBC	Complete blood count
c/dg	Copies per diploid genome
CMV	Cytomegalovirus
CRF	Case report form
CTCAE	Common Terminology Criteria for Adverse Events
DLco	Diffusion capacity of carbon monoxide
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EPO	Erythropoietin
EQ-5D	EuroQoL-5D
EQ-5D-Y	EuroQol-5D (Youth version)
FACT-BMT	Functional Assessment of Cancer Therapy-Bone Marrow Transplant
FEV_1	Forced expiratory volume in 1 second
FSH	Follicle stimulating hormone
FVC	Forced vital capacity
G-CSF	Granulocyte colony stimulating factor
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GMP	Good Manufacturing Practice
GVHD	Graft-versus-host-disease
Hb	Hemoglobin
HbA	Hemoglobin A
HbE	Hemoglobin E
HBB	β-globin gene
HBsAb	Hepatitis B surface antibody
HBV	Hepatitis B virus
Hct	Hematocrit
HCV	Hepatitis C virus
HGVS	Human Genome Variation Society
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HLA	Human leukocyte antigen
HOMA	Homeostatic model assessment

Abbreviation	Definition
HPLC	High-pressure liquid chromatography
HRQoL	Health-related quality of life
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant(ation)
HSV	Herpes simplex virus
HTLV-1	Human T-lymphotropic virus type 1
HTLV-2	Human T-lymphotropic virus type 2
IBC	Institutional Biosafety Committee
ICF	Informed consent form
ICH	International Conference on Harmonisation
IgA	Immunoglobulin A
IGF-1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor-binding protein 3
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IRB	Institutional Review Board
IS	Integration site
ISA	Integration site analysis
ITT	Intent-to-treat population
IV	Intravenous
KM	Kaplan-Meier
LCR	Locus control region
LH	Luteinizing hormone
LIC	Liver iron concentration
LVEF	Left ventricular ejection fraction
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
NK	Natural killer
OS	Overall survival
PBL	Peripheral blood leukocyte
PBMC	Peripheral blood mononuclear cell
PedsQL	Pediatric Quality of Life Inventory
PFTs	Pulmonary function tests
PK	Pharmacokinetic
PTH	Parathyroid hormone
pRBC	Packed red blood cell(s)
q6h	Every 6 hours
qPCR	Quantitative polymerase chain reaction
RBC	Red blood cell(s)
RCL	Replication competent lentivirus
RNA	Ribonucleic acid
RPR	Rapid plasma reagin
RV	Respiratory volume
SAE	Serious adverse event
SAP	Statistical Analysis Plan

Abbreviation	Definition
SD	Standard deviation
SEP	Successful engraftment population
SF-36	Short Form-36
SIN	Self-inactivating
SNP	Single nucleotide polymorphism
SOE	Schedule of Events
SOM	Study Operations Manual
SUSAR	Suspected, unexpected serious adverse drug reactions
T4	Thyroxine
TDT	Transfusion-dependent β-thalassemia
TI	Transfusion independence
TNC	Total nucleated cells
TR	Transfusion reduction
TP	Transplant population
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
US	United States
VCN	Vector copy number
VSV-G	Vesicular stomatitis virus glycoprotein
VZV	Varicella zoster virus
WBC	White blood cell

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

β-Thalassemia is a rare hereditary blood disorder present globally, including in the US and Europe, that is found most commonly in persons of Mediterranean, Middle Eastern, Indian, and South Asian descent (Colah et al., 2010). It is caused by the absence or reduced production of the β-chains of hemoglobin (Hb) A (HbA), a heterotetramer comprised of 2 β-globin and 2 α-globin chains (α2β2) that accounts for >95% of the Hb in the blood of adults. Over 200 mutations in the gene that encodes β-globin (*HBB*) have been identified; there are two *HBB* alleles per diploid genome. Mutations that result in complete abrogation of production of β-globin protein (e.g., nonsense mutations) are collectively referred to as β^0 mutations (Cao and Galanello, 2010). Mutations that result in reduction, but not complete absence, of production of β-globin protein (e.g., affecting splicing) are collectively referred to as β^+ mutations. There are some β^+ mutations which produce minimal β-globin; in particular the IVS-I-110 G→A mutation consistently results in dramatically reduced β-globin production, and is commonly grouped clinically with β^0 mutations (Borgna-Pignatti and Galanello, 2009). β^E is a specific *HBB* mutation in the coding region of the gene that results in reduced production of β-globin because of alternate splicing, as well as a single amino acid change (missense mutation, glutamic acid to lysine at codon 26), resulting in an electrophoretically distinguishable heterotetramer, hemoglobin E (HbE). The β^E mutation is common in Southeast Asia (15% to 30% prevalence of mutation in the total population) (Benz, 2015).

Absence or reduction in β-globin production results in an accumulation of excess uncomplexed α-globin chains in erythroblasts, which precipitate, leading to premature death of the cells and ineffective erythropoiesis that causes the anemia characteristic of patients with β-thalassemia (Galanello and Origa, 2010). The clinical implications of the α-globin/β-globin imbalance are twofold: 1) patients lack sufficient red blood cells (RBCs) and Hb to effectively transport oxygen throughout the body; and 2) elevated hemolysis can lead to morbidities via splenomegaly, marrow expansion, concomitant bone deformities, and iron overload. Patients who are homozygous for mutations that are β^0 (i.e., β^0/β^0 genotype) produce no β-globin, and hence usually clinically present with severe transfusion-dependent β-thalassemia (TDT), also called β-thalassemia major. In contrast, patients who are homozygous for β^+alleles, or are compound heterozygous for β^0, β^+, or β^E (i.e., β^0/β^+, β^0/β^E, β^+/β^E genotypes) produce some endogenous β-globin, and may have a spectrum of clinical severity, ranging from severe TDT to milder forms of thalassemia.

Chronic blood transfusion regimens are highly effective at preventing the hallmark symptoms of disease, but introduce a large iron overload (Cao et al., 1996) that over time can lead to mortality through iron associated heart and liver toxicity (Vichinsky et al., 2005a; Vichinsky et al., 2005b), and must be managed using iron chelation regimens (Cappellini et al., 2006). Poor compliance with chelation regimens remains a key challenge, and even with current therapies, overall survival until the age of 30 years is only 55% for patients with severe TDT (Delea et al., 2007; Modell et al., 2000).

Allogeneic hematopoietic stem cell transplant (allo-HSCT) is currently the only available curative treatment for patients with TDT. However, because of the significant risk of transplant-related mortality, graft-versus-host disease (GVHD), and graft rejection with allo-HSCT, transplants are infrequently performed, and are offered primarily to patients with available human leukocyte antigen (HLA)-matched sibling donors (<25% of cases).

The absence of suitable donors, the significant risks of transplantation, and the requirement for post-transplant immunosuppression therapy to prevent GVHD indicate unmet medical needs, even with allo-HSCT, for patients with TDT.

In order to address this unmet medical need, bluebird bio is investigating the use of LentiGlobin BB305 Drug Product for the treatment of β-thalassemia (INN/USAN: betibeglogene autotemcel) in subjects with TDT.

The BB305 lentiviral vector encodes a single amino acid variant of β-globin, $\beta^{A\text{-}T87Q}$-globin, which conserves the protein's function while allowing for quantification relative to other globin species. Expression of $\beta^{A\text{-}T87Q}$-globin is driven by the erythroid lineage specific globin locus control region (LCR) to correct the β-globin/α-globin imbalance in erythrocytes. Treatment of the subject's own HSCs with the BB305 lentiviral vector through transduction should eliminate the risk of GVHD and graft rejection, and avoid the need for long term immunosuppression.

Results to date suggest that currently used technology results in the achievement of transfusion independence in the majority of subjects with non-β^0/β^0 genotypes, and transfusion reduction but not transfusion independence in the majority of subjects with a β^0/β^0 genotype. Efficacy results suggest that increasing HbA^{T87Q} expression in peripheral blood will be required for the majority of β^0/β^0 subjects to achieve transfusion independence. Results also suggest that increasing the VCN in drug product, along with ensuring adequate myeloablation of subjects due to careful monitoring of busulfan levels, results in increasing both VCN and HbA^{T87Q} expression in peripheral blood. Recent improvements in the manufacturing process of LentiGlobin BB305 Drug Product have led to higher % transduced cells and VCNs in vitro and to higher VCN in the bone marrow of mice in nonclinical studies (see Process 2 in the Investigator's Brochure, Section 3.5.4) than were seen with the manufacturing process used to generate the current clinical data (Process 1). It is planned to use Process 2 to produce drug product to treat subjects with TDT in Study HGB-212.

As of 03 March 2020, 15 subjects have been treated in Study HGB-212. Five subjects were < 12 years of age, 5 subjects were ≥ 12 to < 18 years of age, and 5 subjects were ≥ 18 years of age. Data for the subjects enrolled in this study are provided in the Investigator's Brochure for LentiGlobin BB305 Drug Product.

The safety profile of LentiGlobin BB305 Drug Product is consistent with myeloablative conditioning used for autologous transplantation (see Investigator's Brochure).

Taken together, these data demonstrate the potential of treatment with LentiGlobin BB305 Drug Product to reduce or eliminate the requirement for RBC transfusions for patients with TDT.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

Primary: evaluate the efficacy of treatment with LentiGlobin BB305 Drug Product in subjects ≤50 years of age with TDT who have a β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotype at the *HBB* gene

Secondary: evaluate the safety of treatment with LentiGlobin BB305 Drug Product in subjects ≤50 years of age with TDT who have a β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotype at the *HBB* gene.

2.2. Study Endpoints

2.2.1. Efficacy Endpoints

Primary Endpoint

- The proportion of subjects who meet the definition of "transfusion independence" (TI). TI is defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion.

Secondary Endpoints

- Characterization of subjects achieving TI:
  - Proportion of subjects who meet the definition of TI at the Month 24 Visit
  - Duration of TI
  - Time from drug product infusion to achievement of TI
  - Weighted average Hb during TI
- Characterization of transfusion reduction (TR):
  - The proportion of subjects who meet the definition of TR, defined as demonstration of a ≥60% reduction in the annualized volume of pRBC transfusion requirements (in mL/kg) in the post-treatment time period from 12 months post-drug product infusion through Month 24 (approximately a 12-month period), compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to study enrollment.
  - Proportion of subjects with a reduction in the annualized mL/kg pRBCs transfused from 12 months post-drug product infusion through Month 24 (approximately a 12-month period) of at least 50%, 60%, 75%, 90% or 100% compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to enrollment
  - Annualized number and volume of pRBC transfusions from 12 months post-drug product infusion through Month 24 compared to the annualized number and volume of transfusions during the 2 years prior to enrollment

  - Time from drug product infusion to last pRBC transfusion
  - Time from last pRBC transfusion to the Month 24 Visit
- Weighted average nadir Hb during the 2 years prior to enrollment compared to weighted average nadir Hb from 12 months post-drug product infusion through the Month 24 Visit
- Unsupported total Hb levels over time, including Month 6, Month 9, Month 12, Month 18, and Month 24
- Unsupported total Hb levels ≥10 g/dL, ≥11 g/dL, ≥12 g/dL, ≥13 g/dL, ≥14 g/dL at Month 6, Month 12, Month 18, and Month 24
- Characterization of use of iron chelation and/or therapeutic phlebotomy among all subjects:
  - Proportion of subjects who have not received chelation therapy for at least 6 months following drug product infusion
  - Time from last iron chelation use to last follow-up
  - Proportion of subjects using therapeutic phlebotomy and annualized frequency of phlebotomy use per subject following drug product infusion
- Evaluation of the change in iron burden over time, as measured by:
  - Change in liver iron content by magnetic resonance imaging (MRI) at baseline to Month 12 and Month 24 Visits
  - Change in cardiac T2* on MRI at baseline to Month 12 and Month 24 Visits
  - Change in serum ferritin at baseline to Month 12 and Month 24 Visits
- Evaluation of health-related quality of life (HRQoL) over time including Month 12 and Month 24 as compared to baseline, using the following validated tools:
  - Pediatrics: Pediatric Quality of Life Inventory (PedsQL; parent general core and general core)
  - Adolescents: PedsQL (parent general core and general core) and EuroQol-5D (Youth version) (EQ-5D-Y)
  - Adults: EuroQol-5D (EQ-5D), Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT), and Short Form-36 (SF-36) v2

Exploratory Endpoints

- Assessment of growth and puberty parameters (age appropriate), bone density, diabetes, endocrine evaluations, and neurocognitive development (pediatric subjects <18 years of age)
- Assessment of change in dyserythropoiesis
- Correlations of pre-treatment variables (e.g., drug product vector copy number [VCN]) with response (e.g., peripheral blood VCN, HbA^{T87Q})

CCI [redacted]

- Measures of health resource utilization (including comparing annualized number of transfusions, number of hospitalizations, and number of days hospitalized, from 12 months post-drug product infusion through Month 24 Visit with the annualized corresponding parameters during the 2 years prior to enrollment)
- Length of in-patient hospital stay from initiation of conditioning to discharge

2.2.2. Safety Endpoints

Secondary Endpoints

- Success and kinetics of HSC engraftment
- Incidence of transplant-related mortality through 100 days and through 365 days post-drug product infusion
- Overall survival (OS)
- Detection of vector-derived replication competent lentivirus (RCL) in any subject
- Monitoring of laboratory parameters
- Frequency and severity of clinical AEs
- Incidence of acute and/or chronic graft-versus-host disease (GVHD)
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.)

Exploratory Endpoint

- The number of subjects with clonal predominance (see Section 6.2.18 for description of criteria for clonal predominance and clinical work-up of malignancy)

2.2.3. Pharmacodynamic Endpoints

Secondary Endpoints

- $\beta^{A\text{-}T87Q}$-globin expression over time, including Month 6, Month 9, Month 12, Month 18, and Month 24, as measured by assessing the ratio of $\beta^{A\text{-}T87Q}$-globin to all β-like-globins, and α-globin to all β-like-globins, in whole blood
  - Correlation of $\beta^{A\text{-}T87Q}$-globin expression at early time points post drug product infusion to $\beta^{A\text{-}T87Q}$-globin expression at later time points, as well as clinical outcomes.
- VCN in peripheral blood over time, including Month 6, Month 9, Month 12, Month 18, and Month 24

Exploratory Endpoint

- Relationship between measures of myeloablation and pharmacodynamics and clinical outcomes

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

3. INVESTIGATIONAL PLAN

3.1. Overall Design and Plan of the Study

This is a single-arm, multisite, single-dose, Phase 3 study with approximately 18 subjects with TDT who have either a β^0 or IVS-I-110 mutation at each allele of the β-globin (*HBB*) gene (i.e., β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 genotypes). Similar to β^0-alleles, the β^+ allele IVS-I-110 (HGVS nomenclature: *HBB*:c.93-21G>A) is widely recognized as producing little to no β-globin (Borgna-Pignatti and Galanello, 2009), thus subjects with β^0/IVS-I-110 or IVS-I-110/IVS-I-110 genotypes were grouped with the β^0/β^0 subjects in this study. At least 12 subjects must be without an IVS-I-110 mutation (i.e., β^0/β^0 genotype). Additionally, at least 10 subjects must be <18 years of age.

The study will evaluate the efficacy and safety of autologous hematopoietic stem cell (HSC) transplantation (HSCT) using LentiGlobin BB305 Drug Product, an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution.

Subjects ≥12 and ≤50 years of age must have TDT with a history of transfusion of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment, or be managed under standard thalassemia guidelines (e.g., Thalassemia International Federation, 2014; see Appendix 10.4) with ≥8 transfusions of pRBCs per year in the 2 years preceding enrollment (subjects ≥12 years; Rachmilewitz and Giardina, 2011). Subjects <12 years of age must have a history of transfusion of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment. In addition to having a history of transfusion of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment, subjects <5 years of age must weigh a minimum of 6 kg and reasonably be anticipated to be able to provide at least the minimum number of cells required to initiate the manufacturing process.

Before treating subjects <12 years of age, the independent Data Monitoring Committee (DMC) will review safety data available from Study HGB-207 and determine whether the study can safely proceed with the treatment of subjects ≥5 and <12 years of age. After at least 2 subjects ≥5 and <12 years of age have attained neutrophil engraftment after LentiGlobin BB305 Drug Product infusion in Study HGB-207 and/or HGB-212, the DMC will review their safety data and determine whether the study can safely proceed with the treatment of subjects younger than 5 years of age. Subjects <12 years of age may only be enrolled at sites with regulatory approval for the specified age range.

The study has 4 distinct stages, as follows.

Stage 1: Screening to determine eligibility for treatment

Subjects with TDT who are documented to have genotypes other than β^0/β^0, β^0/IVS-I-110, or IVS-I-110/IVS-I-110 should not enter Screening.

The *HBB* genotype for all subjects will be confirmed during Screening along with additional parameters of eligibility.

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

Each subject will undergo HSC mobilization with a granulocyte-colony stimulating factor (G-CSF; e.g., filgrastim, lenograstim) and plerixafor. Peripheral blood mononuclear cells (PBMCs) will be collected by apheresis. A total of 2 mobilization cycles may be performed if needed and each mobilization cycle may include up to 3 apheresis days. However, subjects whose drug product fails to meet specifications for any reason may undergo repeat apheresis and manufacture of new drug product. Apheresis products can also be used for rescue cells; alternatively, a bone marrow harvest is also allowed to procure cells for rescue.

Stage 3: Myeloablative conditioning (4 days of conditioning followed by at least 48 hours of washout) and infusion of LentiGlobin BB305 Drug Product (Day 1)

After the transduced cells are dispositioned for clinical use, and the subject's eligibility has been re-confirmed (by clinical laboratory tests, physical examination, performance status, AE review and other tests based on institutional requirements), the subject will undergo myeloablative conditioning with busulfan.

After completion of the 4-day course of busulfan, there must be a minimum of 48 hours of washout before drug product infusion.

On study Day 1, thawed LentiGlobin BB305 Drug Product will be administered via intravenous (IV) infusion at a dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg.

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

Subjects will be followed daily in the transplant unit for AEs, and laboratory parameters will be followed to monitor bone marrow engraftment. The subject will be discharged from the transplant unit once the subject is considered medically stable.

The goal during the follow-up period 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 g/dL, and for clinically symptomatic anemia, irrespective of Hb level.

Subjects will be followed in this protocol for a period of 24 months after LentiGlobin BB305 Drug Product infusion. Thereafter subjects will be expected to enroll in a separate long-term follow-up protocol (LTF-303) that will assess safety and efficacy beyond Month 24 for a total of 15 years after drug product infusion. The end of Study HGB-212 will be defined as the last visit for the last subject.

3.2. Data Monitoring Committee

An independent Data Monitoring Committee (DMC) comprised of members with appropriate scientific and medical expertise to monitor the study will be convened before the first patient is mobilized. A charter describing the composition and conduct of the DMC will be issued and agreed to by all DMC members prior to the DMC's initial safety review meeting.

The DMC will be charged with review of all safety data for this study, including AEs, serious adverse events (SAEs) and relevant laboratory values. In addition, the DMC will be charged with review of safety data to determine whether the study can safely proceed with treatment of subjects <12 years and <5 years of age at sites with regulatory approval for the specified age range (refer to Section 3.1).

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), however all final decisions regarding study conduct remain with the Sponsor.

3.3. Rationale for the Study Design

TDT requires lifelong treatment with chronic blood transfusions, and does not spontaneously resolve. Successful treatment in childhood has the potential to prevent morbidities generally seen later in life. The diagnosis of TDT is frequently made in the first year of life when infants become symptomatic, but regardless of when the diagnosis is made, each subject should enter this study with a stable profile of transfusion dependency. Following treatment with LentiGlobin BB305 Drug Product, it is anticipated that there will be an increase in the amount of endogenous Hb produced, leading to a corresponding reduction or elimination of transfusion need that can be readily quantified and compared against the subject's own pre-treatment transfusion profile. Therefore, experiencing a significant reduction in the volume of transfusions (primary efficacy endpoint) is expected to represent the most clinically meaningful event in patients with TDT. Both TI and significant TR are likely to result in multiple clinical benefits for patients, including most saliently, long-term reduction of iron burden, as well as reduced exposure to potential complications of transfusion therapy (e.g. transfusion reactions, allo-immunization, infection with blood borne pathogens).

Data from Phase1/2 Studies HGB-204 and HGB-205 suggested that subjects with TDT who were not homozygous for β^0 alleles (i.e., had non-β^0/β^0 genotypes) were most likely to achieve TI after engraftment. This may include subjects with genotypes of β^0/β^+, β^0/β^E, β^+/β^+, and β^+/β^E. From a physiologic perspective, subjects with at least one β^+ or β^E allele produce some endogenous β-globin, which although by itself is insufficient to sustain life, when supplemented by transduced HbA^{T87Q} may be sufficient to achieve TI. In contrast, transduced HbA^{T87Q} levels achieved in Studies HGB-204 and HGB-205 were predicted to reduce the requirement for transfused blood in patients who do not produce any of their own β-globin (i.e., β^0/β^0), but may not sustain TI. Therefore, separate studies for subjects with either non-β^0/β^0 genotypes (HGB-207) or with β^0/β^0 genotypes (HGB-212, this study) were designed to allow potential differentiation in outcomes between these two groupings of TDT patients. Similar to β^0 alleles, the β^+ allele IVS-I-110 (G→A) is widely recognized as producing little to no β-globin (Borgna-Pignatti and Galanello, 2009), thus subjects with β^0/IVS-I-110 or IVS-I-110/IVS-I-110 genotypes were grouped with the β^0/β^0 subjects in Study HGB-212 (this study).

HBB mutations have been studied for expression of β-globin, and their categorization into β^0 or β^+ mutations has been catalogued into an online database entitled "HbVar Database of Human Hemoglobin Variants and Thalassemia Mutations" (Patrinos et al., 2004). The database can be found at (http://globin.cse.psu.edu/globin/hbvar/) and will be utilized for this study (Patrinos et al. 2004).

Because each subject's pretreatment stable transfusion profile is the most suitable comparator for clinical improvement after treatment, and because measuring HbA^{T87Q} in peripheral blood allows direct evaluation of protein expression and potential correlation with clinical outcome, no untreated control group is included in this study. Furthermore, because of the potential toxicity of busulfan, it would not be ethical to include a control group that does not receive LentiGlobin BB305 Drug Product treatment in this Phase 3 open-label study.

Subjects with HLA-matched family donors are excluded from this study, as these subjects generally have better outcomes in allo-HSCT than do subjects without HLA-matched related HSC donors.

This study includes subjects younger than 12 years of age based on previous results that this treatment was well-tolerated in adolescents. As of 26 August 2016, a total of 22 subjects with TDT aged between 12 and 35 years (including 5 adolescents of 12, 16, 16, 16, and 17 years of age) have been infused with LentiGlobin BB305 Drug Product. During a variable follow-up period of 6 months to almost 3 years, none of the subjects have experienced serious drug-product related adverse events (AEs), or drug product-related AEs greater than Grade 1. All subjects have engrafted and express HbA^{T87Q}, with variable reduction in transfusion-requirements and/or TI. In addition, it is important to study pediatric subjects because patients transplanted at younger ages before advanced disease symptoms of thalassemia are manifested are hypothesized to have different rates of transplant-related complications, different long-term disease outcomes, and potentially different efficacy of gene transduction than adult patients.

Treatment of subjects younger than 12 will be performed in a staggered fashion with safety monitoring.

The short-term risks associated with myeloablation are likely of a lesser magnitude in pediatric subjects as opposed to adults due to their better organ functions and lower iron overload, and are also likely to be of a lesser magnitude than the overall risks associated with allogeneic transplantation, which is regularly offered to children with TDT in this age group (<12 years of age) with an HLA-matched sibling donor. Allogeneic bone marrow transplant (BMT) is currently recommended in young children before the development of iron overload and iron related tissue damage (Angelucci and Baronciani, 2008). Transplant related mortality associated with both allogeneic and autologous BMT is lower in younger patients due to better tolerance of myeloablative treatments (Cahn et al., 1995; Keating et al., 1996).

3.4. Rationale for Dose of Study Drug

LentiGlobin BB305 Drug Product is an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution. LentiGlobin BB305 Drug Product is administered via IV infusion.

The dose of CD34+ cells to be administered is based on accepted safe practice to achieve hematopoietic reconstitution with long-term engraftment after autologous transplantation. The minimum CD34+ dose associated with favorable engraftment kinetics is approximately 1.5×10^6 to 3.0×10^6 cells/kg (Bender et al., 1992; Jillella and Ustun, 2004; Miyamoto et al., 2004; Perez-Simon et al., 1998). Clinical data from ongoing trials utilizing LentiGlobin BB305 Drug Product suggest that improvement in engraftment specifically of vector-transduced cells may be achieved with slightly higher cell doses, i.e., $\geq 5.0 \times 10^6$ CD34+ cells/kg; β^0/β^0 subjects in Study HGB-204 received cell doses between 6.1 and 18.1×10^6 CD34+ cells/kg, with a median cell dose of 11.0×10^6 CD34+ cells/kg (N=8). It is also notable that in general the harvest of CD34+ cells from apheresis yields much higher numbers (up to 21-fold higher) of CD34+ cells than are obtained from a bone marrow procedure (Beyer et al., 1995; Miyamoto et al., 2004; Perez-Simon et al., 1998; To et al., 1992). Given these data, Table 1 outlines the source of subject cells, usage, and minimal dose to be administered.

Table 1: Dose of LentiGlobin BB305 Drug Product or Back-up Cells

Usage	Dose[a]
Drug Product (obtained by apheresis)	$\geq 5.0 \times 10^6$ $CD34^+$ cells/kg[a]
Back-up Cells (obtained by apheresis; for rescue)	$\geq 1.5 \times 10^6$ $CD34^+$ cells/kg
Back-up Cells (obtained by bone marrow harvest; for rescue)	$>1.0 \times 10^8$ TNC/kg

TNC, total nucleated cells

[a] Note that this dose represents the cell count after transduction. If more than 1 transduction is performed, the total dose of the resulting multiple drug product lots must meet this criterion.

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 or Sponsor's designee will promptly inform the regulatory authorities, investigators, Institutional Review Boards (IRBs)/Ethics Committees (ECs), and other appropriate institutional regulatory bodies 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, and will resume after review and recommendations from the DMC and (as required) approval from 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 (confirmed on co-culture assay)
- 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 drug product-related, Grade 3 or 4 toxicities in at least 3 subjects)
- If any criteria for clonal predominance or triggers for clinical work-up for malignancy is satisfied (refer to Section 6.2.18), treatment of any enrolled subjects will be temporarily suspended until further review by the DMC.

*Any death of a subject with TDT in any study 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 LentiGlobin 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

Approximately 18 subjects will be treated with drug product; at least 12 subjects must be without an IVS-I-110 mutation and at least 10 subjects must be <18 years of age. Replacement subjects may be added if subjects are screen failures or withdraw prior to drug product infusion.

4.2. Inclusion Criteria

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

1. Subjects ≤50 years of age at the time of consent or assent (as applicable), and able to provide written consent (adults, or legal guardians, as applicable) or assent (adolescents or children). Pediatric subjects (<12 years of age) may only be enrolled at a given site if approved by the relevant regulatory authority. Provided that the DMC has approved enrolling subjects younger than 5 years of age, subjects younger than 5 years of age may be enrolled at sites with regulatory approval for the specified age range if they weigh a minimum of 6 kg and are reasonably anticipated to be able to provide at least the minimum number of cells required to initiate the manufacturing process.
2. Diagnosis of TDT with a history of at least 100 mL/kg/year of pRBCs in the 2 years preceding enrollment (all subjects), or be managed under standard thalassemia guidelines (e.g., Thalassemia International Federation, 2014; see Appendix 10.4) with ≥8 transfusions of pRBCs per year in the 2 years preceding enrollment (subjects ≥12 years; Rachmilewitz and Giardina, 2011).
3. Clinically stable, have a Karnofsky performance status of ≥80 for adults (≥16 years of age) or a Lansky performance status of ≥80 for adolescents or children (<16 years of age), and eligible to undergo HSCT.
4. Treated and followed for at least the past 2 years in a specialized center that maintained detailed medical records on RBC transfusions (including volume and units of RBCs and associated pre-transfusion Hb values, reticulocyte counts and relevant blood bank details as available), in-patient hospitalization, and iron chelation history.

4.3. Exclusion Criteria

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

1. Presence of a mutation characterized as other than β^0 (e.g., β^+, β^E, β^C) on at least one *HBB* allele. For the purpose of screening, the *HBB* mutation IVS-I-110 (G→A) [HGVS nomenclature: *HBB*:c.93-21G>A] will be considered equivalent to a β^0 mutation.

2. Positive for presence of human immunodeficiency virus type 1 (HIV-1) or 2 (HIV-2), hepatitis B virus (HBV), or hepatitis C virus (HCV). Syphilis (rapid plasma reagin [RPR]) testing is also required and a positive test for syphilis is exclusionary where mandated by regional drug product manufacturing practices. Note that subjects who have been vaccinated against hepatitis B (hepatitis B surface antibody-positive) who are negative for other markers of prior hepatitis B infection (e.g., negative for hepatitis B core antibody) are eligible. Subjects with past exposure to HBV (HBc Ab positive and/or HBe Ab positive) are also eligible for the study provided they are negative by assessment for HBV DNA. Also note that subjects who are positive for anti-hepatitis C antibody are eligible as long as they have a negative HCV viral load. Where clinically and/or regionally indicated, other tests may be performed, in which case positive results would exclude the subject from participating: for example, human T-lymphotropic virus-1 (HTLV-1) or -2 (HTLV-2), tuberculosis, toxoplasmosis, Trypanosoma cruzi, West Nile Virus, or Zika Virus.
3. Clinically significant and active bacterial, viral, fungal, or parasitic infection as determined by the clinical investigator.
4. A white blood cell (WBC) count $<3\times10^9$/L, and/or platelet count $<100\times10^9$/L not related to hypersplenism.
5. Uncorrected bleeding disorder.
6. Any prior or current malignancy (with the exception of adequately treated cone-biopsied in situ carcinoma of the cervix uteri and basal or squamous cell carcinoma of the skin) or myeloproliferative or significant immunodeficiency disorder.
7. Immediate family member (i.e. parent or siblings) with a known Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome and familial adenomatous polyposis).
8. Prior HSCT.
9. Advanced liver disease, defined as:
    a. Persistent aspartate transaminase, alanine transaminase, or direct bilirubin value >3 × the upper limit of normal (ULN), **or**
    b. Baseline prothrombin time or partial thromboplastin time >1.5× ULN, suspected of arising from liver disease, **or**
    c. MRI of the liver demonstrating clear evidence of cirrhosis, **or**
    d. MRI findings suggestive of active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron concentration ≥15 mg/g require follow-up liver biopsy in subjects ≥ 18 years of age. In subjects < 18 years of age, these MRI findings are exclusionary, unless in the opinion of the investigator, a liver biopsy could provide additional data to confirm eligibility and would be safe to perform. If a liver biopsy is performed based on MRI findings, any evidence of cirrhosis, bridging fibrosis, or significant active hepatitis will be exclusionary.

10. Baseline estimated glomerular filtration rate <70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration 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)
11. Uncontrolled seizure disorder.
12. Diffusion capacity of carbon monoxide (DLco) <50% of predicted (corrected for Hb and/or alveolar volume, as clinically indicated). If DLco cannot be assessed due to age or cognition-related restrictions, there must be a normal respiratory exam, chest radiograph without pulmonary infiltrates, and oxygen saturation by pulse oximetry >92% on room air. In the presence of clinically significant abnormal findings on chest radiograph, clinically significant abnormal findings on the respiratory exam, or oxygen saturation by pulse oximetry ≤92% on room air, the subject will be excluded.
13. A cardiac T2* <10 ms by MRI.
14. Any other evidence of severe iron overload that, in the investigator's opinion, warrants exclusion.
15. Participation in another clinical study with an investigational drug within 30 days of Screening.
16. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician or investigator.
17. Prior receipt of gene therapy.
18. Diagnosis of significant psychiatric disorder of the subject that could seriously impede the ability to participate in the study.
19. Pregnancy or breastfeeding in a postpartum female or absence of adequate contraception for fertile subjects. Females of child-bearing potential and males are required to use two different effective methods of contraception from Screening through at least 6 months after drug product infusion. If subjects are truly sexually abstinent (where true sexual abstinence is defined as being in line with the preferred and usual lifestyle of the subject), no second method is required. (See Section 6.2.19.7 for more details).
20. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
21. A known and available HLA-matched family donor. If required by regional regulatory authority, patients with a known and available matched unrelated donor will be excluded from the study.
22. Any contraindications to the use of G-CSF and plerixafor during the mobilization of hematopoietic stem cells and any contraindications to the use of busulfan and any other medicinal products required during the myeloablative conditioning, including hypersensitivity to the active substances or to any of the excipients.

4.4. Subject Identification and Registration

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

Upon obtaining the signed informed consent and assent (as applicable), 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.

Subjects who fail screening may not be re-screened without prior discussion with the bluebird bio Medical Monitor. In general, subjects who fail screening for potentially reversible conditions, such as iron overload, should not be re-screened until stringent management of the condition, such as aggressive iron chelation, has been in place for at least 3 months from the time of screen failure, and the failed inclusion/ exclusion criteria has been confirmed to have been remedied.

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 and assent (as applicable), subjects may withdraw or be withdrawn from study related procedures and treatments (e.g., apheresis, busulfan conditioning) under the following conditions:

- withdrawal of consent or assent,
- 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.

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 back-up cells (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 SAEs open at the time of discontinuation should be followed-up until resolution or are determined to be a stable or chronic condition. See also Section 6.2.19.1 for AEs monitoring of subjects who withdraw from the study.

- 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 and ongoing AEs should be followed for the 30-day duration . 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 and AEs should be followed for the 3-month duration.
- 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 expected to enroll in the long-term follow-up Study LTF-303.

Subjects withdrawn from the study prior to drug product infusion will be replaced.

5. STUDY TREATMENTS

5.1. Description of LentiGlobin BB305 Drug Product

BB305 Lentiviral Vector: BB305 lentiviral vector is a replication defective, self-inactivating (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 [$\beta^{A\text{-}T87Q}$]). BB305 lentiviral vector is not administered to subjects; rather, it is used for ex-vivo transduction of autologous CD34+ hematopoietic stem cells.

LentiGlobin BB305 Drug Product: LentiGlobin BB305 Drug Product is an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution.

5.2. Summary of Treatments to be Performed or Administered

After confirmation of eligibility, HSCs must be collected from the subject for 2 purposes: 1) transduction for LentiGlobin BB305 Drug Product and 2) for rescue cells (to be used if the subject fails to engraft with transduced cells or is unable to receive LentiGlobin BB305 Drug Product after conditioning has commenced).

Additional details are provided in the following subsections.

5.2.1. Mobilization and Apheresis Procedure

It is recommended that subjects maintain a hypertransfusion regimen at least 60 days prior to and continuing on through mobilization and apheresis to maintain a pre-transfusion Hb of ≥11 g/dL (See Section 5.9.3 for details). Iron chelation will likely need to be adjusted to compensate for the increased iron load. Each subject will undergo HSC mobilization with a G-CSF (e.g., filgrastim, lenograstim) and plerixafor. Peripheral blood mononuclear cells (PBMCs) will be collected by apheresis. A separate manual on apheresis requirements and recommendations will be provided.

A total of 2 mobilization cycles may be performed if needed. However, subjects whose drug product fails to meet specifications for any reason may undergo repeat apheresis and manufacture of new drug product. Additional mobilization cycles will only be performed if the initial mobilization cycle was tolerated, and in the opinion of the study physician, would not jeopardize the subject's medical condition.

Each mobilization cycle may include up to 3 apheresis procedure days, and mobilization regimen after 2 days of apheresis should be discussed with the Medical Monitor. No more than 2 consecutive apheresis procedure products may be sent for each transduction; each transduction produces an individual drug product lot. Apheresis procedure products are also to be used for rescue cells, and may come from either a full or partial day of collection. Note that apheresis collections intended for drug product manufacture on a given day may be split to remove cells for rescue (≥1.5 × 10^6 CD34+ cells/kg but <4.0 × 10^6 CD34+ cells/kg) only if ≥25 × 10^6 CD34+ cells/kg are procured on the first day of apheresis, or an aggregate of ≥16 × 10^6 CD34+ cells/kg are collected through subsequent days of apheresis. See the apheresis manual for further details. Note also that aliquots of cells intended for rescue should be frozen and stored at the end of each collection day (i.e., should not be held overnight to be pooled with the next day's collection, even if need to collect additional cells for complete rescue

dose). Mobilization cycles must be separated by at least 2 weeks (i.e., from last day of cell collection in Cycle 1 to first day of G-CSF in Cycle 2). A bone marrow harvest is also allowed, but only to procure cells for rescue cells.

If 2 mobilization cycles are needed to collect enough HSCs to meet the requirement of a total dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg, then 2 transductions will be performed, one on the cells collected during Mobilization Cycle1 and another on the cells collected during Mobilization Cycle 2. Each transduced product from each mobilization cycle is an independent drug product lot. Thus, subjects who require 2 mobilization cycles to produce the minimum cell dose will be treated with 2 drug product lots in total.

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

Potential risks related to G-CSF (e.g., filgrastim, lenograstim), plerixafor, and apheresis are included in the Informed Consent forms and in the respective product labels (e.g., USPI, SmPCs). G-CSF will be used within the marketing authorization and following the prescribing information.

5.2.1.1. Mobilization

Recent published data indicate that the use of plerixafor in pediatric patients is safe and effective for the mobilization of peripheral blood stem cells for autologous transplantation (Teusink et al., 2016). The addition of plerixafor to G-CSF in pediatric patients undergoing stem cell mobilization prior to transplantation as part of treatment of malignant tumors has also been shown to result in successful mobilization of the vast majority of patients who fail to mobilize with single agent G-CSF, as well as to produce larger yields of CD34+ cells (Maschan et al., 2015). Toxicities observed in the study were Grade 1 or 2, and included diarrhea, nausea, ossalgia, and urticaria. Plerixafor is thus expected to incur minimal additional safety risks beyond the use of single agent G-CSF and to have a lower rate of failed mobilization, resulting in reduced risk of repeat exposures to G-CSF and repeat apheresis attempts.

The combination of plerixafor and a G-CSF such as filgrastim or lenograstim may be the most appropriate mobilization strategy for subjects with β-thalassemia (Yannaki et al., 2013; Yannaki et al., 2012). The mobilization approach below (Table 2, Table 3) follows this recent data, and uses filgrastim as an example of the G-CSF. Modifications to this approach are allowed only after consultation with the Medical Monitor.

Table 2: Mobilization for Subjects who have an Intact Spleen

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

[a] Dosage of filgrastim should be decreased if WBC >100 × 10^9/L prior to the day of apheresis unless discussed with Medical Monitor; Hb is recommended to be maintained ≥11 g/dL during the mobilization and apheresis phase.
[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 investigator.
[d] CD34+ count is done both on the day prior to apheresis, as well as on the day of apheresis

Table 3: Mobilization for Subjects who do not have an Intact Spleen

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

[a] Dosage of filgrastim should be decreased if WBC >100 × 10^9/L prior to the day of apheresis unless discussed with Medical Monitor; Hb is recommended be maintained ≥11 g/dL during the mobilization and apheresis phase.
[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 investigator.
[d] CD34+ count is done both on the day prior to apheresis, as well as on the day of apheresis

After completion of Mobilization and Apheresis Cycle 1, if additional HSCs are needed either for drug product manufacture, or for rescue cells (i.e., if <1.5 × 10^6 CD34+ cells/kg have been collected and stored at the site for rescue cells), the subject should begin Mobilization and Apheresis Cycle 2 no sooner than 2 weeks after the completion of Mobilization and Apheresis Cycle 1. Mobilization and Apheresis Cycle 2 should follow the same procedures as Mobilization and Apheresis Cycle 1, although the dose of plerixafor and G-CSF and the apheresis schedule may be modified based on the subject's experience from Mobilization and Apheresis Cycle 1. At least 1.5 × 10^6 CD34+ cells/kg should be stored for rescue cells at each participating site's laboratory.

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, respectively, to mobilization. On each day of apheresis, the subject should have a complete physical examination (including weight) and vital signs performed prior to beginning apheresis, and an abbreviated physical examination and vital signs again after completion of apheresis.

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 institutional guidelines 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. It is recommended that subjects maintain a hypertransfusion regimen at least 30 days prior to conditioning to maintain a pre-transfusion Hb of ≥11 g/dL. Iron chelation will likely need to be adjusted to compensate for the increased iron load, until required discontinuation per protocol. See Section 5.9.3 for details.
3. The subject has undergone 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.
4. Iron chelation therapy has been stopped at least 7 days prior to start of the conditioning. Due to drug-drug interactions (Sweiss et al., 2012), discontinuation of deferasirox, and institution of an alternative iron chelation agent, at least 25 days before initiating busulfan treatment may be considered; if this is done, the alternative chelation agent should be stopped at least 7 days prior to starting busulfan.
5. Busulfan will be administered via IV infusion for 4 consecutive days on Days -6 through -3 at a starting dose of 3.2 mg/kg/day as a 3-hour infusion once daily for a total of 4 doses or 0.8 mg/kg as a 2-hour infusion every 6 hours for a total of 16 doses. Please refer to the busulfan prescribing information for details on appropriate method for determination of patient weight. It is recommended that busulfan is administered at 0.8 mg/kg every 6 hours in children and adolescents to avoid higher peak concentrations while still providing equivalent daily exposure. Any modifications to the busulfan regimen should be reviewed and approved by the Medical Monitor prior to administration. For example, divided dosing is permitted, in which 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 pharmacokinetic (PK) sample collection is based on whether a 2-hour or 3-hour infusion is used. The dose of busulfan will be adjusted based on first dose pharmacokinetics (and if available, third day dose pharmacokinetics) in order

to attain targeted concentration (area under the curve [AUC]; see next Section 5.2.3.2 for targeted AUCs). If needed, the Day 4 busulfan dose may be delayed by up to 12 hours to allow for the receipt of the Day 3 busulfan PK results and appropriate dose modification.

Clinical sites that allow for a test dose of busulfan several days before myeloablation to pre-determine the busulfan dose may also do so in this protocol. Additional modifications to the busulfan conditioning regimen (e.g., splitting doses) may be considered after discussion and agreement with the Medical Monitor.

Unless they have a contraindication, all subjects are to receive prophylaxis for hepatic veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS) prior to initiation of conditioning, and this should be recorded in the eCRF; ursodeoxycholic acid is preferred and should be administered per institutional guidelines. If there are no institutional standards for administration of ursodeoxycholic acid, then the following regimen should be used: 12 mg/kg daily divided in 2 doses, starting at least 5 days prior to initiation of busulfan conditioning and continuing for the first 3 months after completion of busulfan conditioning (Ruutu et al. 2002, 2014). 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 will be performed in all subjects regardless of busulfan dosing schedule. Daily dosing will target an $AUC_{0\text{-}24h}$ of 4200 (range 3800 to 4500) µM*min. Every 6-hour (q6h) dosing will target an $AUC_{0\text{-}6h}$ of 1050 (range 950 to 1125) µM*min.

Samples for PK analysis of busulfan dose will also be drawn on the third day of dosing (after completion of 3rd day of once daily dosing or after first dose on third day when using q6h regimen) using guidelines below.

Recommendations for once daily dosing: Samples for busulfan PK analysis should be collected at the end of the first 3-hour infusion (3 hour time point), then at 3 hours 15 minutes, 3 hours 30 minutes, 4 hours, 5 hours, 6 hours, and 8 hours (+/− 5 minute window up to and including the 3 hour 30 minutes timepoint; +/− 15 minute window for all subsequent collections times) after the start of the first infusion. Samples for PK analysis should also be drawn on the third day of busulfan dosing, using the same schedule.

Recommendations for dosing every 6 hours (q6h): Samples for busulfan PK analysis should be collected at the end of the first 2-hour infusion (2 hour timepoint), then at 2 hours 15 minutes, 2 hours 30 minutes, 3 hours, 4 hours, 5 hours, and 6 hours (+/− 5 minute window up to and including the 2 hour 30 minutes timepoint; +/− 15 minute window for all subsequent collections times) after the start of the first infusion. Samples for PK analysis should also be drawn on the third day of busulfan dosing, using the same schedule.

Note: Samples should not be drawn from the lumen used to infuse busulfan.

Clinical sites must either be able to measure first and third day busulfan PK, or alternatively employ a test dose of busulfan several days before beginning myeloablation to pre-determine busulfan dose and re-confirm PK on the first day of dosing. Based on busulfan PK results for day 1 and (if test dose method is not used) day 3 of dosing, busulfan dose adjustments should be made

for subsequent dosing as possible. Sites that routinely perform daily busulfan monitoring, with subsequent dose adjustments, may do so as part of this protocol. Specific details regarding busulfan dosing, pharmacokinetics and adjustments will be captured in the case report form (CRF).

After completion of the 4-day course of busulfan, there must be a minimum of 48 hours before LentiGlobin BB305 Drug Product administration. Busulfan levels will be measured 48 hours after final dose of busulfan for retrospective confirmation of adequate wash-out.

5.2.4. Infusion Procedures, Dose, and Administration

LentiGlobin BB305 Drug Product is to be given after a minimum of 48 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) must be thawed at approximately 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.

LentiGlobin BB305 Drug Product will be administered on Day 1 via IV infusion at the clinical site. The dose to be administered is $\geq 5.0 \times 10^6$ CD34+ cells/kg. Subjects who undergo 2 mobilization cycles (and subsequent transduction of those cells) to achieve a total dose of $\geq 5.0 \times 10^6$ CD34+ cells/kg will have 2 LentiGlobin BB305 Drug Product lots, which will be administered in sequence, with the second administered immediately after the first.

Vital sign monitoring including ECG, pulse oximetry, and blood pressure measurements, should be employed during drug-product infusion. Additionally, vital signs (excluding ECG) should be measured for two hours after infusion (e.g. every 30 to 60 minutes). Infusion reactions, including anaphylaxis, will be managed according to the medical judgment of the physician overseeing the infusion. Refer to the Study Operations Manual (SOM) for additional detail regarding infusion.

5.3. Storage and Stability of LentiGlobin BB305 Drug Product

All cell manipulation procedures will be performed in transduction facilities in accordance with current Good Manufacturing Practice (GMP) following process-specific procedures. The subject's CD34+ cell-enriched population containing cells transduced with BB305 lentiviral vector are frozen and stored in cryopreservation solution in the vapor phase of liquid nitrogen at the transduction facility until release testing and dispositioning for clinical use. LentiGlobin BB305 Drug Product will be stored in the vapor phase of liquid nitrogen at the clinical site until thawed for clinical use.

5.4. Product Accountability

LentiGlobin BB305 Drug Product accountability and traceability are ultimately the responsibility of the Principal Investigator and Sponsor. However, this responsibility may be delegated to a suitably qualified investigator 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 and traceability of the LentiGlobin BB305 Drug Product as per applicable Sponsor and clinical site procedures. Refer to the SOM for further details.

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.

5.5. 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.

5.6. Blinding, Packaging, and Labeling

5.6.1. Blinding and Breaking the Blind

This is an unblinded, open-label study.

5.6.2. Packaging and Labeling

LentiGlobin BB305 Drug Product is an autologous CD34+ cell-enriched population that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution.

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

5.7. Duration of Subject Participation

Time between Screening and drug product infusion will be variable, and is estimated generally to be between 3 to 5 months (e.g., up to 3 months between Screening and Mobilization, followed by around 2 months before drug product infusion). Thereafter subject is planned to remain on study for approximately 24 months. Eligible subjects will then be expected to enroll in a separate long-term follow-up study until approximately 15 years post drug product infusion.

If the subject is unable to undergo mobilization within approximately 3 months of the initiation of screening, some assessments may need to be repeated to confirm continuing eligibility or to provide a reliable baseline prior to study procedures. In particular, physical exam, vital signs, performance status, HRQoL, electrocardiogram (ECG), complete blood count, chemistry, urinalysis and liver function tests, and for women of child-bearing potential, tests for pregnancy, must be performed within 3 months of proceeding to evaluation for mobilization. For subjects with exposure risks for HIV, HBV and HCV, these tests should also be repeated.

Some of these assessments may be performed immediately prior to mobilization, as specified in the schedule of events. Where clinically and/or regionally indicated, other tests for infectious pathogens may be performed, as required by regional regulations or at physician discretion. Other blood tests, including those for immune subsets, hormonal or dyserythropoiesis testing only need to be repeated if more than 6 months have transpired, unless there is clinical concern due to an initial screening test (e.g. borderline thyroid function). Cardiac MRI (if initial value ≥15 ms, and no clinically significant abnormality on ECG) and liver MRI (if no evidence of fibrosis and LIC ≤10 mg/g on initial MRI, and if no new clinically significant liver function abnormalities) do not

need to be repeated if performed within the past 1 year, and if iron chelation has been adhered to in the intervening time; if these stipulations are not met, then these tests should be repeated if more than 3 months have transpired. The need for liver biopsy should be guided by MRI findings, as described in the protocol, unless a biopsy performed within one year of mobilization exhibited no evidence of fibrosis or hepatitis. Pulmonary function tests and echocardiography (if no borderline values on initial testing of either test) also do not need to be repeated unless more than 1 year has transpired. Thalassemia genotyping, baseline VCN, baseline RCL and baseline $\beta^{A\text{-}T87Q}$-globin, once performed, do not have to be repeated.

5.8. 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.

5.9. Prior and Concomitant Medications

5.9.1. Prior Medications

All medications taken within 30 days prior to the Screening Visit for eligible subjects are to be recorded in the CRF, with the exception of pRBC transfusions and iron chelators, which are to be captured for the 2 years prior to study enrollment (signing of the informed consent form [ICF]), as noted below and in Section 6.2.1 Medical History.

5.9.2. Concomitant Medications and Therapies: General

All concomitant treatments, including transfusions, will be documented.

The use of erythropoietin-like stimulating agents, hydroxyurea, and elective splenectomy (for subjects with intact spleens) will be prohibited from Screening through end of study. In addition, use of any potentially disease modifying therapy (e.g.,luspatercept) should not be initiated without discussion with and explicit approval of the Sponsor's Medical Monitor.

Permitted concomitant treatments may include:

- Hydration, beginning 12 hours before initiating conditioning and continuing through 24 hours thereafter
- Prevention or treatment of nausea and vomiting by prophylactic administration of anti-nausea medications
- Seizure prophylaxis. All drugs other than phenytoin are allowed for anti-seizure prophylaxis during conditioning.
- Ursodeoxycholic acid should be administered prophylactically for prevention of hepatic veno-occlusive disease (VOD)/sinusoidal obstruction syndrome (SOS), unless the subject has a contraindication to use of ursodeoxycholic acid. Defibrotide may be used in lieu of ursodeoxycholic acid as prophylaxis and should be administered per institutional standards for prevention and/or treatment of VOD/SOS. If there are no institutional standards for administration of ursodeoxycholic acid, then the following regimen should be used: 12 mg/kg daily divided in 2 doses, starting at least 5 days prior

to initiation of busulfan conditioning and continuing for the first 3 months after completion of busulfan conditioning (Ruutu et al. 2002, 2014).

- Transfusions should be given according to Hb and platelet counts based on institutional guidelines during the hospitalization to support engraftment. All blood products will be filtered and irradiated.
- G-CSF should not be used prior to Day 21 without explicit approval from the bluebird bio Medical Monitor. After Day 21, G-CSF may be used according to institutional guidelines.
- Subjects will be isolated 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 standard procedures for the management of febrile neutropenia.

For the purposes of this study, vaccines (e.g., COVID-19 vaccines) are considered concomitant medications. Although interactions between a vaccine and LentiGlobin BB305 Drug Product are not expected, the protocol includes use of immunomodulatory (plerixafor) and immunosuppressive medication (busulfan). Local guidelines should be followed regarding a minimum time period between any medication to be provided as part of treatment with LentiGlobin BB305 Drug Product and any vaccine; it is recommended that vaccines are not administered to subjects within 1 month of initiating mobilization for stem cell collection, within 1 week after receiving mobilization agents, within 1 month of initiating myeloablative conditioning, or within 6 months after drug product infusion. Revaccination post-drug product infusion should be considered per Investigator's discretion due to potential loss of immunity after myeloablative conditioning and may be administered following local guidelines.

The prescribing information of the vaccine administered should be referred to for the latest indication, contraindications and safety information as well as the latest general clinical recommendations on vaccine administration in the country or region.

All vaccinations during the study period should be documented in the CRF.

See next sections for transfusions (Section 5.9.3), iron chelation (Section 5.9.4), and infection prophylaxis (Section 5.9.5) recommendations.

5.9.3. Concomitant Medications and Therapies: Transfusions

Transfusions for the 2 years prior to screening through end of study will be documented.

Recommendations for transfusions are made for 4 separate periods during treatment:

1. Hypertransfusion to maintain pre-transfusion Hb ≥11 g/dL is strongly recommended starting at least 60 days prior to initiation of mobilization and continuing to initiation of apheresis. Subjects should have appropriate increase in dosing and/or frequency of iron chelation to prevent iron overload during hypertransfusion.

2. Hypertransfusion to maintain pre-transfusion Hb ≥11 g/dL is strongly recommended at least 30 days prior to initiation of conditioning. Subjects should have appropriate increase in dosing and/or frequency of iron chelation to prevent iron overload during hypertransfusion, until such time as chelation needs to be discontinued per protocol.

3. During conditioning and hospitalization supporting engraftment: follow institutional guidelines.

4. During post-discharge follow-up: It is recommended that subjects should receive RBC transfusions for any Hb <7 g/dL, and for clinically symptomatic anemia, irrespective of Hb level. Decisions regarding transfusion in asymptomatic subjects (without clinically symptomatic anemia) should be based on central laboratory Hb values and not locally drawn Hb. Transfusions should be avoided for Hb ≥9 g/dL, unless the need is medically justified (e.g., as a pre-requirement for surgery), or if the subject has an acquired condition requiring higher baseline Hb (e.g. pregnancy).

5.9.4. Concomitant Medications and Therapies: Iron Chelation

Iron chelators for the 2 years prior to screening through end of study will be documented.

Chelation must stop at least 7 days prior to starting busulfan. Due to drug-drug interactions (Sweiss et al., 2012), discontinuation of deferasirox and institution of an alternative iron chelation agent at least 25 days before initiating busulfan treatment may be considered; if this is done, the alternative chelation agent should be stopped at least 7 days prior to starting busulfan. Iron chelation therapy using deferasirox or deferoxamine should be restarted no sooner than after discharge from the hospital post-transplant. Deferiprone may be used no sooner than 6 months after transplant due to the potential risk of myelosuppression (as per prescribing information for Deferiprone), and alternative chelation regimens should be employed in the interim. 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 be used in lieu of chelation in subjects who have Hb consistently ≥11 g/dL and who are no longer receiving regular transfusions. 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 subjects become TI, 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.

5.9.5. Concomitant Medications: Infection Surveillance and Prophylaxis

The LentiGlobin BB305 Drug Product is a CD34+ cell-enriched population 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 deoxyribonucleic acid (DNA) for up to 3 months after drug product infusion is advised, with pre-emptive lavimudine recommended for any increase in HBV DNA titer (HBV reactivation). Similarly, subjects with past exposure to HCV will undergo regular monitoring of HCV DNA for up to 3 months after drug product infusion.
- 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 varicella zoster virus (VZV).

- Pneumocystis pneumonia prophylaxis: Trimethoprim-sulfamethoxazole 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.
- Intravenous immunoglobulin (IgG) may be administered for 3 months to maintain IgG levels per institutional guidelines.
- Follow institutional post-transplantation guidelines for re-immunization of subjects.
- Subjects should not use medications with anti-retroviral activity (such as those used for HIV pre-exposure prophylaxis) from at least one month prior to mobilization until at least 7 days after drug product infusion.

6. STUDY ASSESSMENTS

6.1. Schedule of Events

The study has 4 distinct stages, as follows.

- Stage 1: Screening
- Stage 2: Autologous CD34+ cell collection and LentiGlobin BB305 Drug Product manufacture and disposition
- Stage 3: Myeloablative conditioning (approximately Day -6 through Day -3) and infusion of LentiGlobin BB305 Drug Product (Day 1)
- Stage 4: Follow-up through Month 24

The SOE to be performed is outlined in Table 4 for Stages 1 and 2, in Table 5 for Stage 3, and in Table 6 for Stage 4.

Detailed descriptions of the efficacy, pharmacodynamics, and safety procedures to be conducted during this study are provided in the following subsections. In particular, certain laboratory tests required for good clinical practices may be drawn concurrently for analysis by both central and local laboratories; these will be specified in the SOM. It will be at the Investigator's discretion to draw local laboratory assays based on the urgency needed for the results. Assays required during hospitalization periods (i.e., mobilization/apheresis, conditioning through transplant, and post-transplant) will not be performed centrally unless indicated by the Medical Monitor.

Subjects will be asked to comply with the protocol specified assessments according to the time periods enumerated in the SOE. If the timing of assessments is shifted due to scheduling conflicts (e.g., limited bed availability at the hospital, delays in screening assessments, repeated mobilizations, delays in scheduling conditioning and LentiGlobin BB305 Drug Product infusion), these will not be considered protocol deviations.

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 as appropriate.

If a subject is not able to visit the study site due to force of nature (e.g., COVID-19 pandemic), then a telehealth visit should be considered to ensure appropriate oversight and safety monitoring. In addition to this, if the subject is able to visit a local doctor and/or laboratory, then pre-defined assessments may be performed at a local laboratory or doctor’s office, per Investigator discretion.

Table 4: Schedule of Events: Screening and CD34+ Cell Harvest

Procedure	Screening (Up to 90 days before mobilization)	Pre-mobilization (Up to 60 days before mobilization)	Mobilization[1]	Days of harvest
Signing of informed consent form (ICF)/assent	X			
Demographics and medical history[2]	X			
Physical examination	X[3]		X[3]	X[3]
Vital signs	X		X[4]	X[4]
Hypertransfusion to pre-transfusion Hb ≥11g/dL		X[5]		
Blood for clinical laboratory tests	X[6]		X[7]	X[7]
Estimated Glomerular Filtration Rate (GFR)	X			
Urinalysis	X			
Blood for serology	X[8]			
Blood for immunological testing	X[9]			
Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test)	X		X[10]	
Blood for hormonal and dyserythropoiesis testing	X[11]			
Blood for replication competent lentivirus (RCL)[12], vector copy number (VCN), globin HPLC, and globin in autologous cells	X			
Blood for thalassemia genotyping[13]	X			
Blood for storage: potential biomarker analysis (optional)	X			
Peripheral blood CD34+ cell count				X[14]
Sperm/testicular tissue or oocyte banking, if requested	X[15]			
Liver biopsy (if required)	X			
Bone marrow (morphology studies)	X[16]			
12-lead ECG	X			
Imaging: Chest X-ray, cardiac Doppler echocardiology (incl. LVEF), cardiac and liver T2*MRI, SOC liver and spleen MRI, bone imaging (X-ray and/ or DEXA scan)[17]	X			
Pulmonary function tests	X[18]			
Transfusion regimen			X[19]	
Health-related Quality of Life (HRQoL) Assessment	X			

Table 4: Schedule of Events: Screening and CD34+ Cell Harvest

Procedure	Screening (Up to 90 days before mobilization)	Pre-mobilization (Up to 60 days before mobilization)	Mobilization[1]	Days of harvest
Adverse event collection	Continuous from ICF signing			
Prior and concomitant medications (incl. iron chelators for the prior 2 years)	Continuous from ICF signing			

[1] Up to 2 cycles of mobilization are permitted. See also Section 5.2.1.1 for recommendations on dosing for mobilization.

[2] Includes records for the prior 2 years transfusion history (see Section 6.2.1 for details required) and in-patient hospitalization; includes genotype (mutations at *HBB* gene).

[3] Includes weight, height, neurocognitive development evaluation (for subjects <18 years of age), performance status, and Tanner staging (if relevant) at Screening. A complete physical examination (including weight) should be performed within 5 days prior to or on the first day of every mobilization cycle, and on every day of apheresis prior to apheresis. An abbreviated physical examination is required every day after completion of apheresis.

[4] 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.

[5] Pre-mobilization hypertransfusion is recommended, but not required. Iron chelation should be managed appropriately to minimize additional iron overload. Pre-transfusion hemoglobin, and if available, serum ferritin and serum transferrin receptor should be recorded as unscheduled labs

[6] Includes hematology (CBC, platelets, reticulocytes, nucleated RBCs), iron studies (iron, ferritin, transferrin, serum transferrin receptor), serum chemistry (including fasting glucose/insulin and Homeostasis Model Assessment index), liver function tests, prothrombin and partial thromboplastin time. Oral glucose tolerance test is required for any abnormal fasting glucose.

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

[8] Including testing for presence of HIV 1, HIV 2, HBV, HCV, and RPR. 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

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

[10] Should be confirmed negative prior to mobilization

[11] Hormonal testing includes thyroid function (free T4, TSH), PTH. For subjects after puberty, also includes LH, FSH and estradiol (females only); testosterone (males only). For subjects < 18 years of age, includes growth hormone (IGF-1 and IGFBP-3). Dyserythropoiesis testing includes EPO and hepcidin.

[12] Two samples required to be archived for potential RCL testing: one for the RCL screening test, another for RCL PBL coculture test

CCI [redacted]

[14] Peripheral blood CD34+ count should be performed on the day prior to apheresis, as well as on the day of apheresis.

[15] May occur any time before conditioning; hormonal treatment, if applicable as part of banking, should stop at least 7 days prior to conditioning.

[16] Bone marrow collection for morphology, cellularity, cell count and iron stains. For subjects undergoing re-screening, bone marrow collection does not need to be performed. If sufficient sample is available, sample may be archived and/or other research tests (e.g., genetic testing) may be performed.

[17] Age appropriate bone imaging (bone age/ mineral density) to be done based on investigator's judgment.

[18] Including oxygen saturation; corrected % predicted FVC, % predicted FEV1; % predicted RV; and % predicted DLco (corrected for Hb and/or alveolar volume, as clinically indicated). If subject cannot perform these pulmonary

function tests due to age or cognition-related restrictions, respiratory exam, chest radiograph, and pulse oximetry will substitute for these assessments.

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

Table 5: Schedule of Events: Conditioning and Drug Product Infusion

	Preconditioning		Conditioning	Infusion	Post-infusion until engraftment
	Approximately 30 days before busulfan	**Up to 7 days before busulfan**	**Day -6 to -3 inclusive[1]**	**Day 1**	**Day 1 until discharge**
Physical examination		X[2]	X	X[3]	X[4]
Vital Signs		X	X	X[5]	X[6]
Stop iron chelation		X[7]			
Hypertransfusion to pre-transfusion Hb ≥11g/dL	X[8]				
Blood for clinical laboratory tests[9]		X	X	X	X[4,10]
Blood for serum β-human chorionic gonadotropin for female subjects of child-bearing potential (serum pregnancy test)		X			
Blood for serum transferrin receptor		X		X	X[11]
Blood for reticulocyte count		X		X	X[11]
Re-confirmation of eligibility		X[12]			
Anti-seizure and VOD/SOS prophylaxis			X[13]		
Busulfan chemotherapy			X		
Blood for busulfan pharmacokinetics[14]		X	X		
Infusion of LentiGlobin BB305 Drug Product				X	
Adverse event collection		Continuous from ICF signing			
Concomitant medication collection		Continuous from ICF signing			

[1] Planned Days relative to Day 1, but may vary depending on length of wash-out (48 hour minimum) before drug product infusion
[2] Includes performance status at preconditioning visit
[3] To be performed on Day 1 before drug product administration
[4] At least twice a week during hospitalization
[5] Vital sign monitoring including ECG, pulse oximetry, and blood pressure measurements, should be employed during drug-product infusion. Additionally, vital signs (excluding ECG) should be measured for two hours after infusion (e.g. every 30 to 60 minutes).
[6] To be performed daily until discharge
[7] Iron chelation must be stopped at least 7 days before initiating conditioning. See Section 5.9.4 for discontinuation of deferasirox.
[8] Pre-conditioning hypertransfusion is recommended, but not required. Iron chelation should be managed appropriately to minimize additional iron overload. Pre-transfusion hemoglobin, and if available, serum ferritin and serum transferrin receptor should be recorded as unscheduled labs.

[9] CBC; serum chemistry; liver function tests; and any other tests that are clinically indicated or required by institutional guidelines, including for example testing for cytomegalovirus (CMV), Epstein Barr virus (EBV), HSV, and VZV IgG. On Day 1 labs are to be collected prior to infusion. Clinical laboratory tests should be obtained on a more frequent basis if clinically indicated (e.g. monitoring & evaluation of AEs).

[,10] CBCs are performed daily during hospitalization until ANC engraftment is demonstrated

[11] Reticulocytes and serum transferrin receptor should be monitored daily from Day 1 through Day 7

[12] Review CBC, serum chemistry, liver function tests, physical examination, performance status, and AE history; serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test); verification that LentiGlobin BB305 Drug Product has been clinically dispositioned, is available on site and that rescue cells are available. Note that if Screening Visit and Preconditioning Visits are > 1 year apart, the following tests must be repeated: PFTs, estimated GFR, and cardiac Doppler echocardiology (incl. LVEF).

[13] 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; VOD/SOS prophylaxis as mandated in Section 5.9.2.

[14] A test dose of busulfan several days before beginning myeloablation to pre-determine busulfan dose is also permitted. PK analysis of first dose of busulfan on first and third day of dosing is required.

Table 6: Schedule of Events: Follow-up

Procedure	Follow-Up: Day (D), Month (M) (Visit Window, days) Post-Drug Product Infusion																		
	D30	D60	D90	D120	D150	D180	D210	D240	D270	D300	D330	D360	D420	D450	D480	D540	D600	D660	D720
	M1 (±7)	M2 (±7)	M3 (±7)	M4 (±14)	M5 (±14)	M6 (±14)	M7 (±14)	M8 (±14)	M9 (±14)	M10 (±14)	M11 (±14)	M12 (±30)	M14 (±30)	M15 (±30)	M16 (±30)	M18 (±30)	M20 (±30)	M22 (±30)	M24 (±30)
Physical examination[1]	X	X	X	X	X	X			X			X		X		X			X
Vital signs	X	X	X	X	X	X			X			X		X		X			X
Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test) [2]			X			X													
Blood for clinical laboratory tests[3]	X	X	X	X	X	X			X			X		X		X			X
Blood for CBC only							X	X		X	X		X		X		X	X	X
Blood for fasting Glucose/Insulin levels[4]						X						X				X			X
Estimated GFR			X			X						X							X
Blood for iron studies[5]			X			X						X		X		X			X
Blood for immunology[6]			X			X						X							X
Blood for hormonal and dyserythropoiesis testing[7]												X							X
Blood for globin in autologous cells												X							X
Blood for globin HPLC & VCN		X	X			X			X			X				X			X
Blood for RCL analyses[8]			X			X						X							X[9]
Blood for ISA						X						X				X			X

Table 6: Schedule of Events: Follow-up

Procedure	Follow-Up: Day (D), Month (M) (Visit Window, days) Post-Drug Product Infusion																		
	D30	D60	D90	D120	D150	D180	D210	D240	D270	D300	D330	D360	D420	D450	D480	D540	D600	D660	D720
	M1 (±7)	M2 (±7)	M3 (±7)	M4 (±14)	M5 (±14)	M6 (±14)	M7 (±14)	M8 (±14)	M9 (±14)	M10 (±14)	M11 (±14)	M12 (±30)	M14 (±30)	M15 (±30)	M16 (±30)	M18 (±30)	M20 (±30)	M22 (±30)	M24 (±30)
Blood for storage: potential biomarker analysis (optional)						X						X							X
Bone marrow[10]												X							X
Liver and spleen SOC MRI												X							X
Pulmonary function tests[11]												X							X
Cardiac and liver T2*MRI & echocardiology[12]												X							X
12-lead ECG												X							X
Bone imaging (X-ray and/or Dexa Scan)[13]																			X
HRQoL Assessment			X[14]			X						X				X			X
Record transfusions	Continuous from ICF signing																		
Record hospitalizations	Continuous from post-drug product infusion discharge																		
Adverse event collection	Continuous from ICF signing																		
Concomitant medication (incl. iron chelators & phlebotomy)	Continuous from ICF signing																		

[1] Includes weight 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. For subjects <18 years of age, neurocognitive development will be evaluated every 6 months.
[2] Should be confirmed prior to mobilization
[3] Hematology (CBC, platelets, reticulocytes, nucleated RBCs); serum chemistry and liver function tests, and additional clinical laboratory tests as clinically indicated. Clinical laboratory tests should be obtained on a more frequent basis if clinically indicated (e.g. monitoring & evaluation of AEs). If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy to allow for further investigation of stem cells.
[4] Fasting glucose and insulin levels (HOMA index) at least every 6 months. An oral glucose tolerance test should be performed for an abnormal fasting glucose.
[5] Iron studies (iron, ferritin, serum transferrin receptor, transferrin) should also be performed prior to restarting iron chelation/phlebotomy.

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

[7] Hormonal testing includes thyroid function (free T4, TSH); PTH. For subjects after puberty, also includes LH, FSH and estradiol (females only); testosterone (males only). For subjects < 18 years of age, includes growth hormone (IGF-1 and IGFBP-3). Dyserythropoiesis testing includes EPO and hepcidin.

[8] Two samples required, one for RCL screening test, another for potential coculture of PBLs if RCL screening test is positive

[9] If a subject's previous RCL tests were all negative, the 24 Month sample will be archived.

[10] Bone marrow for dyserythropoiesis studies (reticulocytes, nucleated RBC, serum transferrin receptor, hepcidin, and erythropoietin), as well as morphology, cellularity, cell count, and iron content; if sufficient sample is available, sample may be archived and/or other research tests (e.g., VCN, ISA, HPLC, genetic testing) may be performed.

[11] Including oxygen saturation; corrected % predicted FVC, % predicted FEV1; % predicted RV; and % predicted DLco (corrected for Hb and/or alveolar volume, as clinically indicated). If subject cannot perform these pulmonary function tests due to age or cognition-related restrictions, then respiratory exam, chest radiograph, and pulse oximetry will substitute for these assessments. If subject becomes able to perform spirometry and lung diffusion capacity test, these pulmonary function tests should be performed per SOE and/or at an unscheduled timepoint.

[12] Annual echocardiography is only required if clinically significant abnormality is observed on the Screening echocardiogram, or any subsequent echocardiogram, or if there is evidence of iron overload (cardiac T2* ≤20 ms) or other clinically significant abnormality on cardiac T2* MRI

[13] Age appropriate bone imaging (bone age/ mineral density) to be done based on investigator judgment.

[14] At the Day 90 Visit, only the EQ-5D and FACT-BMT tools are to be completed.

6.2. Assessments

6.2.1. Demographics and Medical History

Subject demographic data such as sex, age, race, ethnicity, and country of birth will be obtained during Screening. A complete medical history also will be obtained during Screening, including splenectomy history. The medical history is to include all prior and current medical history, including the confirmed thalassemia genotype, age at diagnosis, age when iron chelation therapy began, age of first transfusion, and age when frequency of transfusions was established (i.e., age at which transfusion needs to be stabilized to approximately between once every 3-8 weeks). Transfusion history (including date, amount transfused, number of units, average volume of units and average concentration of hematocrit [Hct] of RBCs transfused), along with the associated reticulocyte and Hb values taken prior to the transfusion where available, should also be collected for the 2 years prior to study enrollment.

All in-patient hospitalizations (days) for the 2 years prior to study enrollment are to be documented, where in-patient hospitalization is defined as duration of hospitalization for at least 24 hours.

Iron chelation history (chelation agent, dose/unit, route, frequency, dates and reason for stopping) for the 2 years prior to the study enrollment should also be collected. In addition, all available serum ferritin values should be collected from this 2-year pre-enrollment period.

If the subject was managed with specific transfusion goals (i.e., Hb and/or symptom trigger for transfusion) which were adhered to for a minimum of 12 months prior to study entry, these should be recorded.

6.2.1.1. Genotype

Genotype will be recorded from the subject's medical records and used to determine initial eligibility to enter Screening. In addition, mutation at each allele of the β-globin locus (*HBB* gene) as determined by sequencing and notated according to the precise genetic mutation identified per recommendation of the Human Genome Variation Society (www.HGVS.org) will be assessed by a centralized laboratory prior to subjects beginning mobilization and apheresis to confirm eligibility. Mutations will be classified as β^{+}, β^{0} or β^{E} based on the globin gene server (http://globin.bx.psu.edu/), an annotated online comprehensive data base of *HBB* mutations. Subjects whose genotyping does not confirm them as eligible will be discontinued from this study prior to beginning mobilization and apheresis.

An additional blood sample taken at the Screening Visit will be archived for potential analyses of genes important in TDT at a central laboratory, which may include:

- Alpha-globin gene copy number
- *HGB2* SNPs
- HBS1L-MYB SNPs
- *BCL11A* SNPs

An additional blood sample will also be taken at Screening Visit for archiving to be used for future validation of a genotyping test that may be required for regulatory approval of this drug product.

6.2.2. Clinical/Physical Examination

A complete physical examination (general appearance; head eyes, ears, nose, and throat; cardiovascular; dermatologic, abdominal; genitourinary; lymph nodes; hepatic; musculoskeletal; respiratory; neurological) is to be conducted as per the SOE. In addition, at screening, and every 6 months after drug product infusion, neurocognitive development will be assessed for subjects <18 years of age.

The subject's weight is to be measured at every visit that includes a complete physical examination.

An abbreviated physical examination includes general appearance and examination of symptomatic organ systems, and does not include weight.

The subject's height is to be measured at the Screening Visit and at every 6 months after drug product infusion.

The subject's performance status is to be measured at the Screening Visit, at the Pre-conditioning Visit, and at every 6 months after drug product infusion. See Sections 10.2 and 10.3 for more information on Karnofsky and Lansky performance status. Test to be used is based on age at informed consent.

Additionally, Tanner staging should be performed at screening and every 6 months after infusion during puberty, if relevant.

6.2.3. Vital Signs

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

6.2.4. Electrocardiogram

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

6.2.5. Imaging

Standard clinical practice guidelines will be used to perform the following imaging assessment at the timepoints specified in the SOE:

- A chest X-ray will be obtained at Screening.
- Bone imaging (X-ray and/or bone density [DEXA] scan) should also be done based on subject's age, as per SOE.
- Cardiac Doppler echocardiography, including the assessment of left ventricular ejection fraction (LVEF) will be performed as per SOE.
- Liver and spleen imaging by MRI.
- Cardiac MRI and liver MRI for determination of T2* and LIC, respectively, will be performed as defined by the imaging core laboratory at the times specified in the SOE.

A Blinded Independent Central Review Committee (BICR) will evaluate MRI images for determination of LIC and cardiac and liver T2* in accordance with an imaging charter. Dry weight of LIC will be measured. The MRI images will be acquired at the clinical site in accordance with the image acquisition guidelines. Liver and spleen volumes as well as incidental clinical findings

will also be assessed by the BICR committee from the MRI standard of care imaging studies. Liver biopsy will be performed in adult subjects (≥ 18 years of age) if MRI of the liver suggests active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or LIC is ≥15 mg/g. Liver biopsy is currently the preferred method for evaluation of the extent of fibrosis (Cappellini et al., 2014). Liver biopsy will only be performed in adolescent and pediatric subjects (< 18 years of age), if additional data beyond the MRI is felt to be absolutely necessary by the investigator to confirm or refute MRI data, and if the procedure is considered to be safe to perform. Liver biopsy should not be performed on thrombocytopenic patients if inconsistent with institutional guidelines.

6.2.6. Pulmonary Function Tests

Pulmonary function testing will be performed as per the SOE. Parameters to be measured include oxygen saturation as measured by pulse oximetry, % predicted forced vital capacity (FVC), % predicted forced expiratory volume in 1 second (FEV_1), % predicted respiratory volume (RV), and % predicted DLco (corrected for Hb and/or alveolar volume, as clinically indicated). If subject cannot perform these pulmonary function tests due to age or cognition-related restrictions, then respiratory exam, chest radiograph, and pulse oximetry will substitute for these assessments. If subject becomes able to perform spirometry and lung diffusion capacity test, these pulmonary function tests should be performed per SOE and/or at an unscheduled timepoint.

6.2.7. Transfusions

Interval transfusions required are to be documented as per the SOE.

6.2.8. In-patient Hospitalizations

All in-patient hospitalizations are to be documented, where in-patient hospitalization is defined as duration of hospitalization for at least 24 hours.

6.2.9. Hemoglobinopathy Markers in Blood

Blood samples are to be collected for globin protein analyses by high-pressure liquid chromatography (HPLC) per the SOE for determination of the ratio of $\beta^{A\text{-}T87Q}$-globin to all β-like-globins and the ratio of α-globin to all β-like-globins.

In addition, blood may be collected for potential globin analyses in autologous cells (i.e., cells not derived from transfusions) per the SOE, as an exploratory analysis.

6.2.10. Bone Marrow and Blood for Dyserythropoiesis Studies

Bone marrow will be collected for dyserythropoiesis studies (see Section 6.2.16.2), as well as morphology, cellularity, cell count and iron content, at Screening and Month 12 and Month 24 Visits. If sufficient sample is available, the bone marrow sample may be archived and/or other research tests (e.g., vector copy number [VCN], $\beta^{A\text{-}T87Q}$-globin expression in erythroid burst forming units [BFU-E], ISA, HPLC, genetic testing) may be performed.

If an unscheduled bone marrow collection is performed at any point during study, such samples may also be archived and/or other research tests (e.g., VCN, $\beta^{A\text{-}T87Q}$-globin expression in BFU-Es, ISA, HPLC, genetic testing) may be performed.

In addition, blood will be collected at Screening and Month 12 and 24 Visits for measurement of hepcidin and EPO, and reticulocytes will be measured at approximately the periodicity of other complete blood count (CBC) parameters, to follow for peripheral evidence of dyserythropoiesis.

6.2.11. Testing for Vector Copy Number, Integration Site Analysis, and Replication Competent Lentivirus

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

- Vector copy number (VCN)
- Integration site analysis (ISA).
- Replication competent lentivirus (RCL). Blood samples will be tested using a RCL screening assay. If the RCL screening assay has a confirmed positive result (even if below limit of quantification), the site may be requested to perform local HIV-1 screening assay, and if positive, follow-up with an HIV-1 Western blot, at the subject's next scheduled visit, or earlier at the discretion of the Investigator. Once available, the results of these tests must be shared with the Sponsor immediately. Additionally, if the RCL screening assay has a confirmed positive result, a test to assess the presence of RCL in peripheral blood leukocytes (PBLs) will be performed. This latter test relies upon the culture of the subject's viable PBLs with a permissive cell line, and allows the amplification and detection of RCL in vitro.

Blood sample collection details are included in the Laboratory Manual for this study. If a subject's previous RCL tests were all negative, the Month 24 sample will be archived.

If bone marrow is collected for routine clinical care, then these assessments may also be performed in bone marrow.

6.2.12. Busulfan Pharmacokinetics

Busulfan concentrations drawn per the schedule outlined in Section 5.2.3.2, and measured locally, will be recorded using an electronic data capture (EDC) system. These will be used to assess pharmacodynamic relationships.

6.2.13. 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 plasma, cells, 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. Leftover samples from protocol procedures (e.g., blood draw for ISA) may also be stored (optional) for potential future analyses as described above.

Note that samples collected routinely for the manufacture of the drug product may also 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 plasma, cells, 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/EC and the subject's specific written consent. Samples will be labeled with a unique identification number that includes no subject identifying information.

6.2.14. Fertility Preservation

Fertility preservation (e.g., 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, their legal guardian (as applicable) and the Investigator.

6.2.15. Health-Related Quality of Life Assessment

Evaluation of health-related quality of life (HRQoL) over time using the following validated tools* (as shown in Table 7, according to the SOE):

*Where validated translations of HRQoL instruments are available

- Pediatrics: PedsQL (parent general core and general core)
- Adolescents: PedsQL (parent general core and general core) and EQ-5D-Y
- Adults: EQ-5D, FACT-BMT, and SF-36v2

Table 7: Age-appropriate Validated HRQoL Tools

Age	PedsQL General Core	PedsQL Parent General Core	EQ-5D-Y (youth)	EQ-5D	SF-36v2	FACT-BMT
0-4		X				
5-11	X	X	X (11 only)			
12-17	X	X	X			
18-50				X	X	X
Recall	Past month	Past month	Today	Today	Past 4 weeks	Past 7 days

Subjects should continue using the same HRQoL tool that they started using on enrollment until completion of their Month 24 Visit, even if they would also be eligible to change to a higher aged tool during the study.

6.2.16. Clinical Laboratory Tests

Clinical laboratory tests will be performed as specified below, and in the SOE. Additional laboratory tests should be performed as clinically indicated (e.g. ongoing monitoring of AEs). Any additional laboratory parameters that are performed while a subject is on study should be entered as such into the clinical study database.

Clinical laboratory tests are to be performed and reviewed by the investigator or qualified designee (e.g., physician's assistant, nurse practitioner). All laboratory assessments will be analyzed centrally unless otherwise noted in the SOE or SOM.

6.2.16.1. Hematology and Clinical Chemistry

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

The following clinical laboratory parameters (Table 8) are to be determined:

Table 8: Hematology, Iron Studies, Serum Chemistry, and Liver Function

Hematology	**Iron Studies**
Complete blood count (CBC) with differential[a]	Iron
Platelet count	Ferritin
Reticulocyte count	Serum transferrin receptor
Nucleated RBCs	Transferrin
Serum Chemistry and Liver Function	
Sodium	Blood urea nitrogen
Potassium	Creatinine
Chloride	Glucose[b]
Bicarbonate	Calcium
Albumin	Phosphate
Total protein	Bilirubin (total and direct)
Alanine transaminase	Alkaline phosphatase
Aspartate transaminase	Lactic dehydrogenase
Gamma glutamyl transferase	

[a] CBC RBC evaluation should include RBC count, Hb, hematocrit, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and mean corpuscular Hb concentration (MCHC).
[b] Fasting glucose/insulin and Homeostasis Model Assessment index testing should be done at least every 6 months. Oral glucose tolerance test is required for any abnormal fasting glucose.

In addition, the GFR will be estimated as per the SOE. See Section 10.1 for the formula for calculation of GFR.

Prothrombin time (reported as the international normalized ratio [INR]) and partial thromboplastin time will also be determined at the Screening Visit as per the SOE.

6.2.16.2. Dyserythropoiesis Studies

These studies include reticulocytes, nucleated RBC, serum transferrin receptor, hepcidin, and erythropoietin (EPO). If some of these tests are done on other samples collected at the same visit (i.e., CBC [reticulocytes and nucleated RBC] or iron studies [serum transferrin receptor]); duplicate sample collection is not required to repeat the same tests.

6.2.16.3. Urinalysis

A urinalysis will be performed at Screening.

6.2.16.4. Pregnancy Testing

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

6.2.16.5. Serology

Screening serology will be evaluated using standard methods as per the SOE, testing for presence of HIV-1, HIV-2, HBV, or HCV. Syphilis (rapid plasma reagin [RPR]) testing is also required, and subjects testing positive will be excluded (unless no longer mandated by central drug product manufacturing practices). Where clinically and/or regionally indicated, other tests may be performed, in which case positive results would exclude the subject from participating: for example, HTLV-1 or HTLV-2, toxoplasmosis, Trypanosoma cruzi, West Nile Virus, or Zika Virus. In certain circumstances, additional testing may be required depending on the subject's history and/or the characteristics of the subject's cells (e.g., malaria).

See also infection surveillance (Section 5.9.5).

6.2.16.6. Hormonal Testing

Hormonal testing, including thyroid function (free thyroxine [T4], thyroid stimulating hormone [TSH]) and parathyroid hormone (PTH), for all subjects is to be performed as per the SOE. In addition for subjects after puberty, luteinizing hormone (LH, female subjects only), follicle stimulating hormone (FSH, female subjects only), estradiol (female subjects only), testosterone (male subjects only), should be performed according to the SOE. Growth hormone (Insulin-like growth factor 1[IGF-1] and Insulin-like growth factor-binding protein 3 [IGFBP-3]) should be assessed in adolescents.

6.2.16.7. Clinical Work-up for Unexpected Blood Test Results

If the results from blood tests are not as expected, additional testing may need to be performed to allow for further investigation of stem cells and may include:

- Physical exam
- Blood tests
- Imaging tests
- Bone marrow biopsy and/or aspiration (may also be archived for additional analysis in the future, see Section 6.2.10)

6.2.17. Immunological Testing

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

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

6.2.18. Assessment of Clonal Predominance and/or Suspicion of Malignancy

6.2.18.1. Assessment of Clonal Predominance

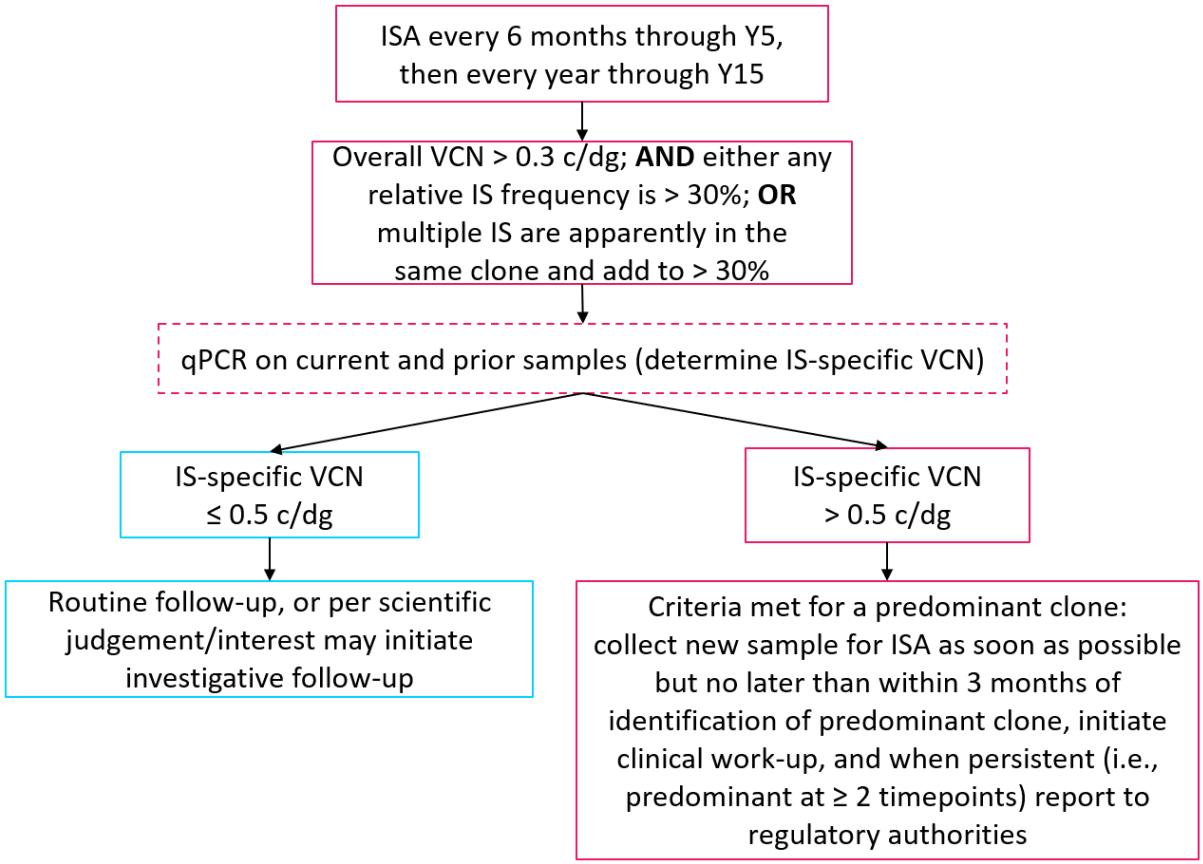
Figure 1 outlines the algorithm for assessment of clonal predominance. Integration site analysis (ISA) will be performed as indicated in the SOE using high-throughput, semi-quantitative methods which identify integration sites (IS) based on vector sequence primers. IS identified are considered as being of interest when the overall peripheral blood VCN is > 0.3 c/dg AND either any relative IS frequency is > 30% OR multiple IS are apparently in the same clone and add up to > 30%. Multiple IS apparently in the same clone is defined as more than one relative frequency where values are within 20% of each other (e.g., 5% ± 1%, 10% ± 2%, 15% ± 3%, etc.), as well as any additional cases identified through bluebird bio internal review of ISA reports. When multiple IS are apparently in the same clone, it will be recommended to confirm that those IS are in a single clone (e.g., bone marrow or peripheral blood colony-forming unit assay). IS of interest will be interrogated, from the time point of interest and available previous time points, using a quantitative assay (e.g., qPCR) designed to detect the specific IS and determine an IS-specific VCN that will help to estimate clonal contribution.

If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN ≤ 0.5 c/dg, estimating ≤ 50% clonal contribution, repeat ISA will continue at the regularly scheduled time points. However, according to scientific judgement or interest, investigative follow-up may be initiated by bluebird bio in collaboration with an investigator and additional interval, unscheduled ISA testing may be performed.

If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN > 0.5 c/dg, estimating > 50% clonal contribution, criteria will be met to consider the subject as having a predominant clone. This threshold also applies to individual lineage evaluations (myeloid, lymphoid, etc.) when performed. Clinical work-up will be recommended for a predominant clone (see Section 6.2.18.2). In addition, if criteria for a predominant clone have been met, a new sample for ISA is to be collected as soon as possible but no later than within 3 months of identification of predominant clone, and retrospective testing by IS-specific qPCR is to be performed on sample(s) previously collected as available. A predominant clone identified at 2 or more time points is considered persistent. A report to relevant regulatory authorities will be required when a persistent, predominant clone is identified, and the report will be made within 30 days of receipt of IS-specific VCN results for the persistent predominant clone.

A clinical work-up may also be recommended for an IS near or within a locus known to have oncogenic activity or upon observation of clonal expansion.

Figure 1: Schematic for Assessment of Clonal Predominance



Abbrev.: c/dg, copies per diploid genome; IS, integration site(s); ISA, integration site analysis; qPCR, quantitative polymerase chain reaction; VCN, vector copy number

Note that this schematic includes the assessment schedule for ISA through the subsequent long-term follow-up study.

6.2.18.2. Clinical Work-up for Potential Malignancy

In the event of clonal predominance, persistent clonal predominance, or any clinical suspicion of myelodysplasia, leukemia, or lymphoma, the Medical Monitor will be notified and a work-up will be performed that may occur in stages and may include some of the following at each stage:

- Physical exam
- CBC with differential
- Lymphocyte subsets
- Studies to rule out infectious cause
- Studies to rule out autoimmune disease
- Imaging studies

- Bone marrow analysis
- Cytogenetic and molecular analyses

If the clinical work-up indicates no evidence of myelodysplasia, leukemia, or lymphoma, the subject will continue to be monitored as per the protocol-defined SOE, or more frequently at discretion of the Investigator and Sponsor. If the clinical work-up indicates a diagnosis of myelodysplasia, leukemia, or lymphoma, the Sponsor will convene an urgent safety review meeting. Further analyses will be determined by the Sponsor, in consultation with the DMC. It should be noted that it may not be possible to distinguish the source of malignancy (e.g., arising from underlying pathophysiology of the disease, transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional oncogenesis), and all efforts should be made to confirm the source of malignancy.

For clinical work-up after identification of a persistent predominant clone and confirmed presence of abnormal CBC, bone marrow analysis is recommended if not previously performed as part of the clinical work-up.

6.2.19. Adverse Events

6.2.19.1. Assessments During the Study

Monitoring of AEs will be conducted from the signing of informed consent. AEs for all subjects (excluding screen failures) will be recorded in the CRFs starting from the time informed consent is signed through the Month 24 Visit. All SAEs (including screen failures) will be reported from the signing of informed consent/assent on the SAE report form.

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

If withdrawal is before drug product infusion, subjects should remain on study for at least 30 days after any invasive study procedure (e.g., mobilization, liver biopsy) before withdrawal and ongoing AEs should be monitored for the 30-day duration. 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 and AEs should be followed for the 3 month duration.

If withdrawal is after drug product infusion, subjects will be asked to complete the same assessments as specified in the SOE for Month 24 (Early Termination Visit assessments) and will be expected to enroll in the long-term follow-up Study LTF-303.

Note that at the completion of Study HGB-212, subjects will be expected 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. If there is a gap of time between the final visit in Study HGB-212 (e.g., Month 24 study visit) and participation in the LTF-303 study (i.e. signature on ICF), all SAEs that start during that gap should be reported on the Study HGB-212 SAE report form and submitted according to Section 6.2.19.5.

6.2.19.2. Adverse Events Definitions

6.2.19.2.1. Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in subjects, whether or not considered drug related. An AE may include a change in physical signs, symptoms, and/or clinically significant laboratory change occurring in any phase of a clinical study. This definition includes intercurrent illnesses or injuries, and exacerbation of pre-existing conditions. A 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 SAE and is to be reported as such. (For this protocol, engraftment failure is defined as failure to achieve an ANC ≥500 cells/µL for 3 consecutive days by Day 43, or receiving back-up cells at any time during the neutropenic phase).

6.2.19.2.2. Unexpected Adverse Events

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

6.2.19.2.3. Conditioning-related Adverse Events

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

The intended profound myelosuppression (manifested by neutropenia, thrombocytopenia, and/or anemia) and expected events that occur after the initiation of busulfan IV conditioning are considered to be the direct consequence of busulfan conditioning and are to be reported as AEs but should be attributed to conditioning agent on the AE eCRF, as applicable.

6.2.19.2.4. Serious Adverse Events

An **SAE** is any AE that:

- Results in death.
- Is immediately life-threatening; i.e. the subject was at immediate risk of death at the time of the event. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions occurring during the study period that are for procedures *planned prior to study entry* do not meet this criteria, unless there is a complication resulting from procedure that prolongs hospitalization.

- Results in persistent or significant disability/incapacity; or 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 AE that may not result in death, be life threatening, or require hospitalization but may be considered serious when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.
- 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.

6.2.19.3. Adverse Event Assessment of Severity and Relationship

For all AEs, the investigator must determine both the severity of the event and the relationship of the event to LentiGlobin BB305 Drug Product administration.

For immediate reporting of SAEs, the Investigator must provide assessments of relationship and serious criteria at the time of SAE report submission to the Sponsor.

Severity will be assessed by the investigator using the following criteria per National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03, including for AEs that are a result of a laboratory abnormality:

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

If the Grade changes within a day, the maximum Grade should be recorded.

Relationship: The Investigator is required to provide an assessment of the relationship of LentiGlobin BB305 Drug Product to all AEs. The following is a guideline for determining the relationship of LentiGlobin BB305 Drug Product to an AE:

- **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 not 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 and safety reporting, all AEs that are classified as possibly related or related will be considered treatment-related events.

6.2.19.4. Procedures for AE and SAE Collection and 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 (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, laboratory assessments, observations of study personnel, and spontaneous reports from subjects.

AEs for all eligible subjects (i.e., excluding screen failures) will be recorded in the CRF. Any clinically significant laboratory abnormality or other clinically significant finding is considered an AE and the AE must be recorded on the appropriate pages of the CRF. The diagnosis/underlying etiology rather than the signs/symptoms should be reported as the AE, when possible. If no diagnosis is available, report only the signs and symptoms that met AE criteria as individual AE terms.

6.2.19.5. Immediate Reporting of SAEs

All SAEs for all subjects, including screen failures, must be immediately reported on the SAE report form to the Sponsor (or designee) within 24 hours of the Investigator (or designee) becoming aware of the SAE. All SAEs must be reported whether or not they are considered causally related to LentiGlobin BB305 Drug Product. The SAE report form and completion guidelines can be found in the Study Operations Manual (SOM). All follow-up information on SAEs must also be immediately reported to the Sponsor (i.e. within 24 hours).

Copies of all SAE reports and associated documentation submitted to the Sponsor will be kept in the Investigator's study site file.

Please refer to the SAE report form and associated guidelines for information on how to immediately submit SAE reports to the Sponsor.

6.2.19.6. Safety Reporting to Regulatory Authorities, Ethics Committees, and Investigators

If there are suspected, unexpected serious adverse reactions (SUSARs) associated with the use of LentiGlobin BB305 Drug Product or plerixafor, bluebird bio, Inc., will notify the appropriate regulatory agency(ies) and all participating investigators on an expedited basis and in accordance with applicable regulations.

The Investigator or Sponsor will notify the IRB/EC and other appropriate institutional regulatory bodies of any SUSARs or unanticipated problems, in accordance with local requirements.

Reporting of SUSARs to regulatory agencies will be performed within 7 or 15 days as indicated by the 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.

6.2.19.7. Pregnancy and Contraception

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which requires reporting as an SAE). However, all pregnancies occurring during this study (in subjects or female partners of male subjects) are to be reported in the same time frame as SAEs using the Pregnancy Form. SAEs experienced by subject or female partner of male subject during the course of the pregnancy are required to be immediately reported (i.e. within 24 hours) on the SAE report 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 outcome, including follow-up of the health status of the newborn at 6 weeks of age and annually thereafter for 2 years. SAEs experienced by newborn within 6 weeks of age are required to be immediately reported (i.e. within 24 hours) on the SAE report form.

*Exceptions include:

In cases where the MALE was the study subject, pregnancies resulting from sperm banking prior to receipt of drug product will not be followed.

In cases where the FEMALE was the study subject, pregnancies resulting from oocyte banking prior to receipt of drug product in which the pregnancy is carried to term by surrogacy will not be followed.

Busulfan has been shown in animal studies to be teratogenic (see package insert for additional details). 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 and males are required to use two different effective methods of contraception (e.g., implants, injectables, combined oral contraceptives, some intrauterine devices, barrier methods of contraception with spermicide, vasectomised partner, for females; barrier methods of contraception with spermicide, vasectomy, for males) from Screening through at least 6 months after drug product infusion. If subjects are truly sexually abstinent (where true sexual abstinence is defined as being in line with the preferred and usual lifestyle of the subject), no second method is required. (Periodic abstinence [calendar, symptothermal, post-ovulation methods], withdrawal [coitus interruptus], spermicides only, and lactational amenorrhea method are not acceptable methods of contraception)**.** Beyond 6 months after drug product infusion, subjects should discuss with their physician prior to resuming unprotected intercourse.

6.2.20. 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 as appropriate, and may be based on those listed in the SOE. Unscheduled visits, including any unscheduled assessments or procedures performed at the visit, should be promptly entered into the CRF.

6.2.21. Long-term Follow-Up Protocol

Subjects will be followed for 24 months after drug product infusion under this protocol. Subjects are expected to be followed-up 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 ISA, insertional oncogenesis, and long-term efficacy. Appropriate consent will be obtained at the time of enrollment in the LTF-303 study. This follow-up includes recording of SAEs and archiving of peripheral blood leukocyte cell samples for RCL and clonality testing.

7. STATISTICAL PROCEDURES

Details of the statistical analysis will be provided in a separate document (the Statistical Analysis Plan [SAP]). This section provides a general overview of these plans.

7.1. Sample Size Estimation

No formal sample size calculations were done.

Conversion to TI for patients with TDT is very unlikely to happen spontaneously in patients with TDT. Therefore, the conversion of any subject in the study to TI would be attributable to the therapeutic effect of LentiGlobin BB305 Drug Product with a very high probability. Any appreciable proportion of subjects who become transfusion independent on the study would represent a clinically meaningful treatment effect, to be assessed against the morbidity of the procedure.

Approximately 18 subjects will be treated with drug product; at least 12 subjects must be without an IVS-I-110 mutation and at least 10 subjects must be <18 years of age. Replacement subjects may be added if subjects are screen failures or withdraw prior to drug product infusion.

The proposed sample size is based on the premise that excluding a treatment effect of <30% with a high probability is of value (demonstrating with 97.5% confidence that ≥30% of subjects have become TI). Among the proposed sample size of 18 treated subjects, a success criterion of 55.6% (10 out of 18 subjects) is proposed, which would yield a lower 1-sided 97.5% exact confidence bound of 30.8%, exceeding the 30% minimal criterion.

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 G-CSF and/or plerixafor.
- Transplant Population (TP): All subjects who receive LentiGlobin BB305 Drug Product.
- Successful Engraftment Population (SEP): All subjects who have successful neutrophil engraftment after LentiGlobin BB305 Drug Product infusion.

The ITT population is the primary population for the analysis of safety parameters. The TP is the primary population for the analysis of efficacy, pharmacodynamic, and transplant parameter endpoints (i.e., success and kinetics of engraftment, and incidence of transplant-related mortality through 100 days post-drug product infusion). The SEP will be used to provide supportive data for subjects who engraft.

7.3. Planned Analyses

7.3.1. Interim Analyses

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

7.3.2. Final Analyses

A final analysis will be performed when all subjects treated with LentiGlobin BB305 Drug Product complete the study.

7.3.3. Additional Data Review

Safety data are reviewed on an ongoing basis for signal detection, DMC meetings, and to support preparation of regulatory submission documents.

Analyses of study data may also be performed for the purposes of internal data review, regulatory agency interactions, and updating the scientific community.

7.3.4. Impact of the COVID-19 Pandemic

A review will be performed to determine which assessments are likely to have been affected by the COVID-19 pandemic, and if applicable, analyses will be performed to measure the effect of disruptions due to the pandemic on these assessments.

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 of subjects within each category of the parameter will be presented. For continuous variables, the number of subjects, mean, median, standard deviation (SD), minimum, and maximum values will be presented. Descriptive summary statistics as well as 2-sided, 90% or 95% or 1-sided 97.5% confidence intervals, as appropriate, will be presented on selected parameters.

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

7.4.2. Baseline Definitions

Two years of retrospective pre-transplant and biological data will be collected for each subject in the study, so that each subject may serve as his/her own control for the parameters of pRBC transfusion requirements, weighted average nadir Hb concentrations, and in-patient hospitalization (number and duration). For these parameters, where applicable, baseline will be annualized over the 2 years prior to study entry (date of informed consent). For pRBC transfusion requirements, there will be 1 baseline parameter, the average per year. Likewise, for the number of hospitalizations and the number of days hospitalized in the 2 years prior to enrollment, the baseline average per year will be calculated.

For safety parameters, including shifts in key laboratory parameters, the most recent value prior to mobilization is used as the baseline assessment.

For other efficacy and pharmacodynamic parameters, baseline will be defined as the most recent measurement prior to conditioning.

7.4.3. Disposition of Subjects

A tabulation of the disposition of subjects will be presented, including the number who initiate mobilization, the number who initiate myeloablative conditioning, the number infused with LentiGlobin BB305 Drug Product, the number with any post-drug product infusion data available for analysis, and the extent of data available. The number of subjects completing the study through Month 24 Visit and reasons for study discontinuation will be reported.

7.4.4. Demographic and Baseline Characteristics

The following demographic and baseline characteristic factors will be summarized: age (at time of enrollment, at diagnosis, at time of first transfusion, when frequency of transfusions was established, when iron chelation began, and at drug product infusion), genotype, country of birth, race and ethnicity, history of splenectomy and spleen size for subjects with a spleen.

Additional screening results to be summarized will include the following: echocardiogram status (normal, abnormal, not done), LVEF %, and liver iron concentration (LIC) (mg/g dry weight).

In addition, baseline data from the 2-year retrospective collection will also be summarized including pRBC volume (mL/kg/year), number of pRBC transfusions (number/year), and in-patient hospitalizations and duration (per year).

7.4.5. Efficacy Analysis

7.4.5.1. Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is defined as the proportion of subjects who meet the definition of "transfusion independence" (TI). TI is defined as a weighted average Hb ≥9 g/dL without any pRBC transfusions for a continuous period of ≥12 months at any time during the study after drug product infusion, where:

- Calculation of time period of TI will start when subjects achieve a Hb ≥9 g/dL with no transfusions in the preceding 60 days.
- To meet the initial TI criteria, the weighted Hb must be ≥9 g/dL at the end of the 12-month period
- To remain in the TI state beyond the 12-month period, the treated subject needs to maintain a weighted Hb of ≥9 g/dL from that point forward, without receiving a pRBC transfusion.
- A pRBC transfusion for a single acute event (e.g., surgery, trauma, parvovirus infection, or sepsis) will not be counted towards the definition of TI. For the calculation of the weighted Hb when an allowed transfusion has occurred, the Hb that triggered the transfusion would be carried forward for 60 days and Hb values during those 60

days would be imputed by the carried-forward value. Post 60 days, the actual Hb drawn would again be used in the calculation of TI.

The primary endpoint will be analyzed as a point-estimate of the proportion of subjects achieving TI, with a 2-sided 95% confidence interval calculated using the exact binomial method. The TP will be used for primary conclusions of gene-therapy efficacy, with supportive analyses performed in the SEP.

7.4.5.2. Analysis of Secondary Efficacy Endpoints

The following secondary efficacy endpoints will be descriptively analyzed in the TP.

- Characterization of subjects achieving TI:
  - Proportion of subjects who meet the definition of TI at the Month 24 Visit
  - Duration of TI
  - Time from drug product infusion to achievement of TI
  - Weighted average Hb during TI
- Characterization of transfusion reduction (TR):
  - Proportion of subjects who meet the definition of TR, defined as demonstration of a ≥60% reduction in the annualized volume of pRBC transfusion requirements (in mL/kg) in the post-treatment time period from 12 months post-drug product infusion through Month 24 (approximately a 12-month period), compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to study enrollment.
  - Proportion of subjects with a reduction in the annualized mL/kg pRBC transfused from 12 months post-drug product infusion through Month 24 (approximately a 12-month period) of at least 50%, 60%, 75%, 90% or 100% compared to the annualized mL/kg pRBC transfusion requirement during the 2 years prior to enrollment
  - Annualized number and volume of pRBC transfusions from 12 months post-drug product infusion through Month 24 compared to the annualized number and volume of transfusions during the 2 years prior to enrollment
  - Time from drug product infusion to last pRBC transfusion
  - Time from last pRBC transfusion to the Month 24 Visit
- Weighted average nadir Hb during the 2 years prior to enrollment compared to weighted average nadir Hb from 12 months post-drug product infusion through the Month 24 Visit
- Unsupported total Hb levels over time, including Month 6, Month 9, Month 12, Month 18, and Month 24
- Unsupported total Hb levels ≥10 g/dL, ≥11 g/dL, ≥12 g/dL, ≥13 g/dL, ≥14 g/dL at Month 6, Month 12, Month 18, and Month 24

- Characterization of use of iron chelation and/or therapeutic phlebotomy among all subjects:
  - Proportion of subjects who have not received chelation therapy for at least 6 months following drug product infusion
  - Time from last iron chelation use to last follow-up
  - Proportion of subjects using therapeutic phlebotomy and annualized frequency of phlebotomy use per subject following drug product infusion
- Evaluation of the change in iron burden over time, as measured by:
  - Change in liver iron content by MRI at baseline to Month 12 and Month 24 Visits
  - Change in cardiac T2* on MRI at baseline to Month 12 and Month 24 Visits
  - Change in serum ferritin at baseline to Month 12 and Month 24 Visits
- Evaluation of health-related quality of life (HRQoL) over time including Month 12 and Month 24 as compared to baseline, using the following validated tools:
  - Pediatrics: Pediatric Quality of Life Inventory (PedsQL; parent general core and general core)
  - Adolescents: PedsQL (parent general core and general core) and EuroQol-5D (Youth version) (EQ-5D-Y)
  - Adults: EuroQol-5D (EQ-5D), Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT), and Short Form-36 (SF-36) v2

In addition, proportion endpoints will be analyzed as point-estimates of the proportion of subjects meeting the respective definitions and change in iron burden over time will be summarized using descriptive statistics, as well as summary statistics for change over time.

Analyses of exploratory endpoints will be described in the SAP.

7.4.6. Safety Analysis

The following safety parameters will be evaluated descriptively:

- Success and kinetics of HSC engraftment
- Incidence of transplant-related mortality through 100 days and through 365 days post-drug product infusion
- Overall survival (OS)
- Detection of vector-derived RCL in any subject
- Monitoring of laboratory parameters
- Frequency and severity of clinical AEs
- Incidence of acute and/or chronic GVHD
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.)

All subjects starting mobilization will be evaluated for safety (ITT Population). Results reported as incidence will be analyzed as proportions.

All AEs will be collected from signing of informed consent/assent through Month 24 Visit for eligible subjects, irrespective of severity grade or relationship to LentiGlobin BB305 Drug Product. All AEs will be listed and summarized for the following time periods: 1) from informed consent/assent up to the start of mobilization; 2) from start of mobilization up to start of conditioning; 3) from start of conditioning through neutrophil engraftment; 4) from neutrophil engraftment through Month 24 Visit; 5) from drug product infusion through Month 24 Visit; and 6) from informed consent/assent through Month 24 Visit.

Note that neutrophil engraftment failure is a SAE and for this protocol is defined as failure to achieve 3 consecutive absolute neutrophil count (ANC) laboratory values ≥500 cells/µL obtained on different days by Day 43, or receiving back-up cells at any time during the neutropenic phase. If a subject fails to engraft with transduced cells, back-up cells collected per protocol can be used (see Section 5.2), and if >1 subject fails to achieve reconstitution with transduced cells and receives back-up cells, enrollment and treatment with drug product will be temporarily suspended and will resume after review and recommendations from the DMC and (as required) approval from the relevant regulatory agency(ies) (see Section 3.5.2).

Platelet engraftment is defined as 3 consecutive platelet count laboratory values $\geq 20 \times 10^9$/L obtained on different days while no platelet transfusions administered for seven days immediately preceding and during the evaluation period. The day of platelet engraftment is the first day of the 3 consecutive platelet measurements. Laboratory measures will be compared with their corresponding normal ranges; changes in laboratory parameters over time will be summarized with descriptive statistics and shift tables.

Additionally, survival status, laboratory results and insertional oncogenesis (insertional mutagenesis resulting in oncogenesis) events will be summarized.

Vital signs, Lansky and Karnofsky Performance status results, and neurocognitive development assessment (for subjects <18 years of age) will be presented as Listings only.

Replication-competent lentivirus testing and ISA will be performed and results tabulated.

Analyses of exploratory safety endpoints will be described in the SAP.

7.4.7. Pharmacodynamic Analyses

Analyses of pharmacodynamic endpoints will be described in the SAP.

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 any 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, IBC, and other appropriate institutional regulatory bodies by the investigator.

8.3. Subject Information and Informed Consent

After the study has been fully explained, voluntary written informed consent will be obtained from either the subject and/or his/her guardian or legal representative prior to study participation, as well as assent if applicable. The method of obtaining and documenting the informed consent/assent and the contents of the consent/assent will comply with ICH-GCP and all applicable regulatory requirement(s). Consent to this study indicates acknowledgment that follow-up is expected to last 15 years, with the first 2 years in this Study HGB-212 and 13 additional years in long-term follow-up Study LTF-303. A brief summary of expected study procedures in Study LTF-303 will be described in the Study HGB-212 consent so that the subject and/or their guardian or legal representative are aware of expectations in Study LTF-303. Consent for participation in Study LTF-303 will be obtained at the time of enrollment in Study LTF-303.

8.4. Subject Confidentiality

In order to maintain subject privacy, all CRFs, accountability records, study reports, and communications will identify the subject by the assigned subject number. The investigator will grant monitor(s) and auditor(s) from the Sponsor 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 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 ICH-GCP guidelines.

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

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

Regulatory authorities, the 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. Electronic Case Report Form Completion

Case report forms will be completed using an electronic data capture (EDC) system. The Sponsor or designee will provide instruction regarding the proper use of the EDC system.

It is the investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's electronic case report form (eCRF). Source documentation supporting the eCRF 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 eCRF 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 within the EDC system 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 BB305 lentiviral vector and LentiGlobin BB305 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 BB305 lentiviral vector and LentiGlobin BB305 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.

9. REFERENCES

Angelucci, E., and Baronciani, D. (2008). Allogeneic stem cell transplantation for thalassemia major. Haematologica *93*, 1780 - 1784.

Bender, J.G., Williams, S.F., Myers, S., Nottleman, D., Lee, W.J., Unverzagt, K.L., Walker, D., To, L.B., and Van Epps, D.E. (1992). Characterization of chemotherapy mobilized peripheral blood progenitor cells for use in autologous stem cell transplantation. Bone Marrow Transplant *10*, 281-285.

Benz, E.J. (2015). Molecular pathology of the thalassemic syndromes, S.L.T. Schrier, Jennifer S. Accessed 18 November 2015, ed. (Waltham, MA: UpToDate; http://www.uptodate.com/contents/molecular-pathology-of-the-thalassemic-syndromes).

Beyer, J., Schwella, N., Zingsem, J., Strohscheer, I., Schwaner, I., Oettle, H., Serke, S., Huhn, D., and Stieger, W. (1995). Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: a randomized comparison. J Clin Oncol *13*, 1328-1335.

Borgna-Pignatti, C., and Galanello, R. (2009). The thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In Wintrobe's Clinical Hematology, Edition 12, J.P. Greer, ed. (Philadelphia: Lippincott Williams & Wilkins), p. 1087.

Cahn, J.Y., Labopin, M., Mandelli, F., Goldstone, A.H., Eberhardt, K., Reiffers, J., Ferrant, A., Franklin, I., Herve, P., Gratwohl, A., *et al.* (1995). Autologous Bone Marrow Transplantation for First Remission Acute Myeloblastic Leukemia in Patients Older Than 50 Years: A Retrospective Analysis of the European Bone Marrow Transplant Group. Blood *85*, 575 - 579.

Candotti, F., Shaw, K.L., Muul, L., Carbonaro, D., Sokolic, R., Choi, C., Schurman, S.H., Garabedian, E., Kesserwan, C., Jagadeesh, G.J., *et al.* (2012). Gene therapy for adenosine deaminase-deficient severe combined immune deficiency: clinical comparison of retroviral vectors and treatment plans. Blood *120*, 3635-3646.

Cao, A., and Galanello, R. (2010). Beta-thalassemia. Genet Med *12*, 61-76.

Cao, A., Galanello, R., Rosatelli, M.C., Argiolu, F., and De Virgiliis, S. (1996). Clinical experience of management of thalassemia: the Sardinian experience. Semin Hematol *33*, 66-75.

Cappellini, M.D., Cohen, A., Piga, A., Bejaoui, M., Perrotta, S., Agaoglu, L., Aydinok, Y., Kattamis, A., Kilinc, Y., Porter, J., *et al.* (2006). A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood *107*, 3455-3462.

Cappellini, M.D., Cohen, A., Porter, J., Taher, A., and Viprakasit, V. (2014). Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT), 3rd edition (Nicosia (CY): Thalassaemia International Federation).

Colah, R., Gorakshakar, A., and Nadkarni, A. (2010). Global burden, distribution and prevention of beta-thalassemias and hemoglobin E disorders. Expert Rev Hematol *3*, 103-117.

Delea, T.E., Edelsberg, J., Sofrygin, O., Thomas, S.K., Baladi, J.F., Phatak, P.D., and Coates, T.D. (2007). Consequences and costs of noncompliance with iron chelation therapy in patients with transfusion-dependent thalassemia: a literature review. Transfusion *47*, 1919-1929.

Galanello, R., and Origa, R. (2010). Beta-thalassemia. Orphanet J Rare Dis *5*, 11.

Jillella, A.P., and Ustun, C. (2004). What is the optimum number of CD34+ peripheral blood stem cells for an autologous transplant? Stem Cells Dev *13*, 598-606.

Keating, S., Suciu, S., De Witte, T., Mandelli, F., Willemze, R., Resegotti, L., Broccia, G., Thaler, J., Labar, B., Damasio, E.*, et al.* (1996). Prognostic factors of patients with acute myeloid leukemia (AML) allografted in first complete remission: an analysis of the EORTC-GIMEMA AML 8A trial. The European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell' Adulto (GIMEMA) Leukemia Cooperative Groups. Bone Marrow Transplant *17*, 993 - 1001.

Lansky, S.B., List, M.A., Lansky, L.L., Ritter-Sterr, C., and Miller, D.R. (1987). The measurement of performance in childhood cancer patients. Cancer *60*, 1651-1656.

Maschan, A.A., Balashov, D.N., Kurnikova, E.E., Trakhtman, P.E., Boyakova, E.V., Skorobogatova, E.V., Novichkova, G.A., and Maschan, M.A. (2015). Efficacy of plerixafor in children with malignant tumors failing to mobilize a sufficient number of hematopoietic progenitors with G-CSF. Bone Marrow Transplant *50*, 1089-1091.

Miyamoto, T., Shinozuka, T., Maeda, H., Hirasawa, T., Muramatsu, T., Murakami, M., Makino, T., Itagaki, H., and Nakamura, Y. (2004). Effect of peripheral blood progenitor cell dose on hematopoietic recovery: identification of minimal progenitor cell requirements for rapid engraftment. Bone Marrow Transplant *33*, 589-595.

Modell, B., Khan, M., and Darlison, M. (2000). Survival in beta-thalassaemia major in the UK: data from the UK Thalassaemia Register. Lancet *355*, 2051-2052.

Mor, V., Laliberte, L., Morris, J.N., and Wiemann, M. (1984). The Karnofsky Performance Status Scale. An examination of its reliability and validity in a research setting. Cancer *53*, 2002-2007.

Patrinos, G.P., Giardine, B., Riemer, C., Miller, W., Chui, D.H., Anagnou, N.P., Wajcman, H., and Hardison, R.C. (2004). Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic Acids Res *32*, D537-541.

Perez-Simon, J.A., Caballero, M.D., Corral, M., Nieto, M.J., Orfao, A., Vazquez, L., Amigo, M.L., Berges, C., Gonzalez, M., del, C.C.*, et al.* (1998). Minimal number of circulating CD34+ cells to ensure successful leukapheresis and engraftment in autologous peripheral blood progenitor cell transplantation. Transfusion *38*, 385-391.

Rachmilewitz, E.A., and Giardina, P.J. (2011). How I treat thalassemia. Blood *118*, 3479-3488.

Ruutu T, Eriksson B, Remes K, et al (2002) Ursodeoxycholic acid for the prevention of hepatic complications in allogeneic stem cell transplantation. Blood 100::1977–83 . doi: 10.1182/blood-2001-12-0159

Ruutu T, Juvonen E, Remberger M, et al (2014) Improved survival with ursodeoxycholic acid prophylaxis in allogeneic stem cell transplantation: long-term follow-up of a randomized study. Biol Blood Marrow Transplant 20::135–8 . doi: 10.1016/j.bbmt.2013.10.014

Sweiss, K., Patel, P., and Rondelli, D. (2012). Deferasirox increases BU blood concentrations. Bone Marrow Transplant *47*, 315-316.

Teusink, A., Pinkard, S., Davies, S., Mueller, M., and Jodele, S. (2016). Plerixafor is safe and efficacious for mobilization of peripheral blood stem cells in pediatric patients. Transfusion *56*, 1402-1405.

To, L.B., Roberts, M.M., Haylock, D.N., Dyson, P.G., Branford, A.L., Thorp, D., Ho, J.Q., Dart, G.W., Horvath, N., Davy, M.L.*, et al.* (1992). Comparison of haematological recovery times and supportive care requirements of autologous recovery phase peripheral blood stem cell transplants, autologous bone marrow transplants and allogeneic bone marrow transplants. Bone Marrow Transplant *9*, 277-284.

Vichinsky, E., Butensky, E., Fung, E., Hudes, M., Theil, E., Ferrell, L., Williams, R., Louie, L., Lee, P.D., and Harmatz, P. (2005a). Comparison of organ dysfunction in transfused patients with SCD or beta thalassemia. Am J Hematol *80*, 70-74.

Vichinsky, E.P., MacKlin, E.A., Waye, J.S., Lorey, F., and Olivieri, N.F. (2005b). Changes in the epidemiology of thalassemia in North America: a new minority disease. Pediatrics *116*, e818-825.

Yannaki, E., Karponi, G., Zervou, F., Constantinou, V., Bouinta, A., Tachynopoulou, V., Kotta, K., Jonlin, E., Papayannopoulou, T., Anagnostopoulos, A.*, et al.* (2013). Hematopoietic stem cell mobilization for gene therapy: superior mobilization by the combination of granulocyte-colony stimulating factor plus plerixafor in patients with beta-thalassemia major. Hum Gene Ther *24*, 852-860.

Yannaki, E., Papayannopoulou, T., Jonlin, E., Zervou, F., Karponi, G., Xagorari, A., Becker, P., Psatha, N., Batsis, I., Kaloyannidis, P.*, et al.* (2012). Hematopoietic stem cell mobilization for gene therapy of adult patients with severe beta-thalassemia: results of clinical trials using G-CSF or plerixafor in splenectomized and nonsplenectomized subjects. Mol Ther *20*, 230-238.

10. APPENDICES

10.1. Glomerular Filtration Rate Calculation

For adults:

For use with creatinine (Scr) reported in mg/dL:

GFR (mL/min/1.73 m^2) = 175 × (Scr)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 if African American) (conventional units)

For use when creatinine (Scr) is reported in µmol/L:

GFR (mL/min/1.73 m^2) =175 × (Scr/88.4)-1.154 × (Age)-0.203 × (0.742 if female) × (1.212 if African American) (SI units)

For children:

GFR (mL/min/1.73 m^2) = (0.41 × Height in cm)/Creatinine in mg/dL

GFR (mL/min/1.73 m^2) = (36.2 × Height in cm)/Creatinine in µmol/L

10.2. Karnofsky Performance Status Scale

The following table presents the Karnofsky performance status scale and should be used for subjects ≥16 years of age.

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 process progressing rapidly
0	Dead

Source: (Mor et al., 1984)

10.3. Lansky Performance Status Scale

The following table presents the Lansky performance status scale and should be used for subjects <16 years of age.

Points	Description
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of, and less time spent in, active play
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

Source: (Lansky et al., 1987)

10.4. Guidelines for Transfusion Volume

Determination of the appropriate volume of transfused blood will be based on the hematocrit of donor RBCs, as shown in the table below.

Table 4. Guidelines for choosing how much blood to transfuse.

		HAEMATOCRIT OF DONOR RED CELLS			
		50%	60%	75%	80%
Target increase in haemgolobin level	2 g/dl	12 ml/kg	10 ml/kg	8 ml/kg	7.5 ml/kg
	3 g/dl	18 ml/kg	15 ml/kg	12 ml/kg	11.2 ml/kg
	4 g/dl	24 ml/kg	20 ml/kg	16 ml/kg	15 ml/kg

Source: Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT), 3rd Edition; (Cappellini et al., 2014).

Protocol Title:	A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β-Thalassemia by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector (LentiGlobin BB305 Drug Product) in Subjects ≤50 Years of Age
Protocol Number:	HGB-212 Version 6.0, 10 June 2021

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 (ICH-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 ICH-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 ICH-GCP.

I will ensure that an ethics committee that complies with all local regulations and ICH-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)