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FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Pharmacology/Toxicology
Division of Pharmacology/Toxicology 2
Pharmacology/Toxicology Branch 2

BLA NUMBER: STN #125788.000
DATE RECEIVED BY CBER: 4/21/23
DATE REVIEW COMPLETED: 9/25/23
PRODUCT: LYFGENIA™ (Lovotibeglogene autotemcel or lovo-cel)

APPLICANT: Bluebird Bio, Inc.
PROPOSED INDICATION: For the treatment of patients 12 years of age or older with sickle cell disease and a history of vaso occlusive events (VOEs).

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EXECUTIVE SUMMARY:

LYFGENIA™ (Lovotibeglogene autotemcel or lovo-cel) consists of autologous CD34+ hematopoietic stem cells (HSCs) transduced with a replication incompetent, self-inactivating lentiviral vector (LVV), BB305, encoding a modified β -globin gene (β^{A-T87Q} globin or LentiGlobin). LYFGENIA™ is for the treatment of patients 12 years of age or older with sickle cell disease (SCD) and a history of vaso-occlusive events (VOEs). Following intravenous (IV) infusion in the myeloablated patient, LYFGENIA™ is expected to engraft in the bone marrow (BM) and differentiate to produce RBCs containing biologically active β^{A-T87Q} -globin that will combine with α -globin to produce functional hemoglobin (HbA^{T87Q}). Durable expression of HbA^{T87Q} is expected to reduce RBC sickling and lead to improvement in hemolytic anemia. The minimum recommended dose level of LYFGENIA™ is 3×10^6 CD34+ cells/kilogram (kg).

In vitro pharmacology studies were conducted with CD34+ HSCs from SCD patients and showed that erythroid cells derived from BB305 LVV-transduced HSCs produce β^{A-T87Q} globin. *In vivo* proof-of-concept (POC) studies in immunodeficient mice administered BB305 LVV-transduced CD34+ HSCs obtained from healthy donors displayed BM engraftment and β^{A-T87Q} -globin expression. *In vivo* POC studies were also conducted using transgenic mouse models of SCD, where murine bone marrow cells (mBMCs) were transduced with the β^{A-T87Q} HPV436

LVV, a related vector encoding the same transgene, and showed expression of β^{A-T87Q} and correction of the sickling phenotype through 3 months post-transplantation.

In vivo studies were also conducted to assess the activity and safety of mBMCs transduced with BB305 following primary and secondary transplantation in β -thalassemic and wild-type C57BL/6 mice. Long-term bone marrow engraftment and chimerism were observed in all animals receiving 11×10^6 cells/kg BB305 LVV-transduced mBMCs compared to those that received mock-transduced mBMCs at 4 months post-transplantation. In the secondary transplantation study, no deaths or adverse findings attributed to the BB305 LVV-transduced cells occurred through 6 months post-transplantation of 6×10^6 cells/mouse. The observed incidence of T-cell lymphoma and/or leukemia was within the reported range (15.7-25.3%) for radiation-associated lymphoma in C57BL/6 mice (E. Will et al. (2007) Mol Ther 15:782-91) and was considered incidental.

The risk of insertional mutagenesis of BB305 LVV as evaluated using an *in vitro* immortalization (IVIM) assay performed with BB305 LVV-transduced mBMCs showed low mutagenic potential of BB305 LVV compared to positive control vectors (b) (4), which are known to cause insertional transformation. Integration site analysis of CD34+ HSCs obtained from healthy donors showed no enrichment for LVV integration in or near known oncogenes. Additionally, no preferred integration near or within genes that are clinically associated with either clonal dominance or with leukemia for GRV was observed.

Carcinogenicity and developmental and reproductive toxicity studies were not conducted with LYFGENIA™. These studies are not warranted based on the product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of LYFGENIA™. The nonclinical information provided in the BLA submission supports approval of the licensure application.

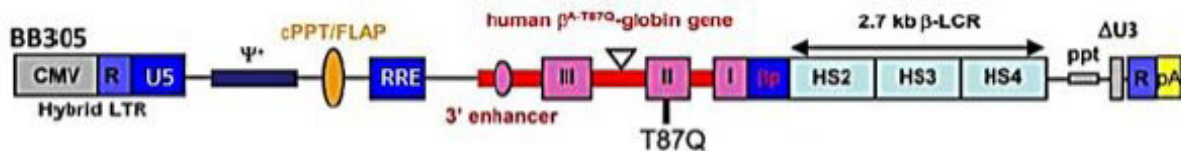
Formulation and Chemistry:

LYFGENIA™ consists of autologous CD34+ HSCs transduced with BB305 LVV, encoding a modified β -globin gene (β^{A-T87Q} globin or LentiGlobin). The BB305 LVV, derived from human immunodeficiency virus type 1, is a replication incompetent and self-inactivating virus. This LVV is under the transcriptional control of the erythroid-specific human β -globin promoter and erythroid-specific enhancer elements (DNase I hypersensitive sites HS2, HS3, and HS4) of the β -globin locus control region. This design precludes expression of the transgene in non-erythroid cells (Figure 1).

The patient's mobilized peripheral blood (mPB) undergoes apheresis and immunomagnetic separation to enrich for CD34+ HSCs to obtain a target concentration of 3×10^6 CD34+ cells/mL in a maximum volume of (b) (4) of stem cell growth media. The cells are then *ex vivo*

transduced with BB305 LVV, followed by washing and cryopreservation in (b) (4) freezing medium containing 5% DMSO (approximate 20-mL suspension in a (b) (4) freezing bag). Prior to administration of LYFGENIA™ the patient is exposed to a busulfan myeloablative conditioning regimen to prepare the BM “niches” for product engraftment. The minimum recommended dose level of LYFGENIA™ is 3×10^6 CD34+ cells/kg.

Figure 1: Diagram of BB305 LVV



CMV = cytomegalovirus eukaryotic constitutive promoter; R = repeat; U5 = unique 5'; Ψ = psi packaging signal; gag = HIV-1 partial gag sequence; cPPT = central polypurine tract; RRE = rev response element; 3' enh/pA = 3' enhancer/polyadenylation signal from β-globin gene; E = exon; P = β-globin promoter; Globin LCR = human globin locus control regions; ΔU3 = promoter/enhancer-deleted unique 3'; pA = synthetic polyadenylation signal.

Source: Introduction; Module 2.6.1 in the BLA.

Manufacturing changes made to BB305 LVV during the clinical development of LYFGENIA™:

The manufacturing process for LYFGENIA™ was modified during the product development program to (b) (4). The manufacturing processes were designated as: a) Process 0 (administered in clinical Study HGB-205); b) Process 1 (administered in clinical Study HGB-206 [Cohort A]); and c) Process 2 (administered in clinical Studies HGB-206 [Cohorts B and C] and HGB-210). The primary distinction between Process 2 and previous processes (Processes 0 and 1) was the replacement of (b) (4) in the (b) (4) with (b) (4) and (b) (4). The product generated using Process 2 is the commercial product.

Abbreviations

BFU-e	Burst-forming unit-erythroid
BM	Bone marrow
BMCs	Bone marrow cells
BME	Bone marrow engraftment
BW	Body weight
CD	Cluster of differentiation
CFC	Colony forming cell
gDNA	Genomic DNA
GLP	Good Laboratory Practice
Hb	Hemoglobin
HSC	Hematopoietic stem cells
IP	Intraperitoneal
ISA	Integration site analysis
IV	Intravenous
IVIM	<i>In vitro</i> immortalization assay
(b) (4)	
Lin	Lineage
LVV	Lentiviral vector
MOI	Multiplicity of infection
mPB	Mobilized peripheral blood
(b) (4)	
PCR	Polymerase chain reaction
(b) (4)	(b) (4)
(b) (4)	(b) (4)
RBC	Red blood cell
RP-HPLC	Reverse-phase high-pressure liquid chromatography
SCD	Sickle cell disease
TDT	Transfusion dependent- β -thalassemia
VCN	Vector copy number

Related Files

IND #15905: Autologous CD34+ Hematopoietic Stem Cells Transduced with Lentiviral Vector, LentiGlobin BB305, Encoding the Human beta-A-T87Q-Globin Gene; Administered Intravenously [bb1111]; Treatment of Sickle Cell Disease; Bluebird Bio Inc.

BLA #125717: ZYNTGLO™ (Betibeglogene autotemcel or beti-cel); Treatment of patients with beta (β)-thalassemia who require regular red blood cell (RBC) transfusions; Bluebird Bio Inc.

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INTRODUCTION

SCD is a rare progressive, debilitating, genetic hematologic disorder caused by a mutation in the β -globin gene of hemoglobin (Hb). Hb is an iron-containing, oxygen-transporting metalloprotein present in RBCs. It is a heterotetramer, composed of four protein subunits, known as globin chains. In humans, the most common form of Hb is adult HbA (HbA1), consisting of two alpha (α)-globin and two β -globin chains ($\alpha_2\beta_2$). In SCD, a single point mutation of the β -globin gene causes glutamic acid to be replaced with valine and results in the production of an abnormal β^A globin chain (a variant of the β -globin chain), resulting in the production of β^A S-globin. High concentrations of Hb tetramers that include β^A S-globin subunits (i.e., HbS), result in subsequent dysfunctional Hb polymerization under low oxygen conditions leading RBCs to become sickled, sticky, and rigid, leading to a marked reduction in RBC lifespan. This leads to the pathological hallmarks of SCD including frequent, painful vaso-occlusive events (VOEs), hemolytic anemia, and progressive vasculopathy. Repeated VOEs, progressive vasculopathy, and prolonged hemolytic anemia can result in chronic complications that lead to disease progression and end-organ damage, which are the primary causes of morbidity and mortality in adults with SCD. Patients with SCD require lifelong supportive therapies such as anti-sickling agents (e.g., hydroxyurea [HU] and voxelotor), packed red blood cell (pRBC) transfusions, and/or downstream modifying agents (e.g., crizanlizumab and L-glutamine). Allogenic hematopoietic stem cell transplant (allo-HSCT) is also a potentially curative therapeutic option; however, utilization by patients with SCD is limited by the availability of suitable donors and potential complications, including risk of short-and long-term morbidity and graft failure.

Lovo-cel consists of autologous CD34⁺ hematopoietic stem cells (HSCs) transduced with the BB305 LVV encoding β^{A-T87Q} -globin. *In vivo* production of transgenic β^{A-T87Q} globin (the primary pharmacodynamic effect) following IV infusion of lovo-cel corrects the deficient β^A -globin production in individuals with SCD, allowing the formation of modified forms of HbA

^{T87Q}. HbA ^{T87Q} has similar oxygen-binding affinity and oxygen-hemoglobin dissociation curve to wild-type HbA, which reduces intracellular and total hemoglobin S (HbS) levels and is designed to sterically inhibit polymerization of HbS. These pharmacologic effects resulting from durable expression of HbA ^{T87Q} are expected to reduce RBC sickling and improve clinical manifestations of SCD, including hemolytic anemia, extending the life expectancy for patients with SCD.

NONCLINICAL STUDIES

Reviewer's Note:

- The manufacturing process (Process 0, 1, or 2) for the drug product evaluated in the nonclinical studies is specified under the respective study.

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of lovo-cel in the proposed clinical population.

In Vitro Studies

Study Number	Study Title	Report Number
1	Assessment of Transduction Efficiency in Sickle Cell Disease (β^E/β^S) CD34+ Cells as Measured in Colony Forming Cells and Long-term Culture Colony Forming cells, and Assessment of Globin Chain Expression in BFUe Colonies	NC-11-001-R
2	Assessment of Transduction Efficiency in Sickle Cell Disease (β^S/β^{+thal}) CD34+ Cells as Measured in Colony Forming Cells and Long-term Culture Initiating Cells, and Assessment of Globin Chain Expression in Erythroid Colonies	NC-11-004-R
3	<i>In Vitro</i> Evaluation of the Effects of Transduction of Human CD34+ Hematopoietic Stem Cells with LentiGlobin BB305 Lentiviral Vector in the Presence of Different Transduction Additives	B2-16-276

In Vivo Studies

Study Number	Study Title	Report Number
4	Correction of Sickle Cell Disease in Transgenic Mouse Models by Gene Therapy.	Pawliuk R, <i>et al.</i> (2001) Science 294:2368–71.
5	A Study to Evaluate the Effect of Transduction of Human CD34+ Hematopoietic Stem Cells with LentiGlobin BB305 Lentiviral Vector in the Presence of Different Transduction Additives on Long-term Bone Marrow Engraftment in Female (b) (4) Mice	B2-15-161
6	A Study to Evaluate the Effect of LentiGlobin BB305 Lentiviral Vector-transduced Human CD34+ Hematopoietic Stem Cells Manufactured with Process 1 or Process 2 on Long-term Bone Marrow Engraftment in Female (b) (4) Mice	B2-16-200

Reviewer's Note:

- Studies #7 and #8 reviewed in the 'Toxicology Studies' section of this review memo also include pharmacological endpoints assessing BM cellular differentiation, composite VCN, and % of LVV+ cells following transplantation of mBMCs transduced with BB305 into B-thalassemic mice.

Reviewer's Comments:

- *In vivo* pharmacology and toxicology studies to evaluate the activity and safety of mBMCs transduced with BB305 and support regulatory approval for ZYNTGLO™ were included in the current BLA for LYFGENIA™. These studies have been summarized in the current memo.
- As LYFGENIA™ and ZYNTGLO™ use the identical β^{A-T87Q} globin transgene and manufacturing (except for donor material), no additional nonclinical safety studies are necessary to support the safety of LYFGENIA™.

Overview of Pharmacology Studies

In Vitro Studies

Study #1 (Report No. NC-11-001-R) *Assessment of Transduction Efficiency in Sickle Cell Disease (β^E/β^S) CD34+ Cells as Measured in Colony Forming Cells and Long-term Culture Colony Forming Cells, and Assessment of Globin Chain Expression in BFUe Colonies; Conducted by Bluebird Bio*

Objective:

This study evaluated the transduction efficiency and expression of β^{A-T87Q} globin in BB305 LVV-transduced mPB-derived CD34+ HSCs obtained from patients with SCD (lvo-cel, β^E/β^S , lacking β^A).

Methods and Key Results:

CD34+ HSCs obtained from a patient with SCD were mock-transduced or transduced with BB305 LVV or HPV569 LVV using Process 2 at a multiplicity of infection (MOI) of 25. Transduced cells were then cultured in a short term clonogenic colony forming cell (CFC) culture assay (2 weeks in methyl cellulose) or in a long-term colony forming culture (LTC-CFC) assay (5 weeks on irradiated MS-5 murine [bone marrow] BM stromal cells, followed by 2 weeks in methyl cellulose) to determine early and late erythroid and non-erythroid progenitors. Transduction efficiency was determined by qPCR and was reported as vector copy number [VCN= copies/diploid genome (c/dg)]. β^{AT87Q} expression was analyzed in erythroid colonies (pooled burst-forming unit-erythroid [BFU-E]) by reverse phase high-pressure liquid chromatography (RP-HPLC).

This study demonstrated a higher transduction efficiency (measured as mean VCN [c/dg]) in colonies formed by HSCs transduced with BB305 LVV compared to HPV569LVV (CFC culture assays; (b) (4) and LTC-CFC assays; (b) (4) Higher transduction efficiency also correlated with increased β^{AT87Q} expression.

Reviewer's Notes:

- For all nonclinical studies, the applicant used the term "mock cells" or "mock-transduced cells" to refer to cells that were exposed to transfection reagents without the LVV.
- HPV569 LVV is identical to BB305 LVV except for a HIV-1 LTR, resulting in HPV569 LVV being Tat-dependent.¹

Study #2 (Report No. NC-11-004-R) *Assessment of Transduction Efficiency in Sick Cell Disease (β^S/β^{+thal}) CD34+ Cells as Measured in Colony Forming Cells and Long-term Culture Initiating Cells, and Assessment of Globin Chain Expression in Erythroid Colonies; Conducted by Bluebird Bio*

Objective:

This study evaluated the transduction efficiency and the expression of β^{A-T87Q} globin in BM-derived CD34+ HSCs obtained from patients with SCD transduced with different LVVs encoding β^{AT87Q} globin.

Methods and Key Results:

The CD34+ HSCs obtained from frozen BM cells obtained from a patient with SCD were transduced with (b) (4) LVV, HPV569 LVV, or BB305 LVV using Process 2 at an MOI of 25. Transduction efficiency and globin chain analyses in early and late erythroid colonies were conducted using the methods and parameters described in Study #1.

¹ Negre O, Eggimann A-V, Beuzard Y, et al (2016) Gene Therapy of the beta- Hemoglobinopathies by Lentiviral Transfer of the beta(A(T87Q))-Globin Gene. Hum Gene Ther 27:148–65.

This study demonstrated a higher transduction efficiency (mean VCN[c/dg]: (b) (4) in HSCs transduced with BB305 LLV compared to (b) (4) LVV and HPV569 LVV, respectively. An increased production of β^{AT87Q} globin ($\beta^{\text{AT87Q}}/\alpha$ ratio: (b) (4) was observed in HSCs transduced with BB305 LLV compared to HPV569 LVV. Similar β^{AT87Q} globin expression ($\beta^{\text{AT87Q}}/\alpha$: (b) (4) was observed in HSCs transduced with BB305 and (b) (4) LVV, respectively.

Reviewer's Note:

- (b) (4) LVV is a predecessor of HPV569 LVV. (b) (4) and HPV569 LVV are identical except that (b) (4) lacks (b) (4) sequences that were incorporated into HPV569 LVV to (b) (4).

Study #3 (Report No. B2-16-276) In Vitro Evaluation of the Effects of Transduction of Human CD34+ Hematopoietic Stem Cells with LentiGlobin BB305 Lentiviral Vector in the Presence of Different Transduction Additives; Conducted by Bluebird Bio

Objective:

This study evaluated the effect of various media components on the transduction efficiency and subsequent $\beta^{\text{A-T87Q}}$ globin production in BB305 LVV-transduced human CD34+ cells.

Methods and Key Results:

Healthy human donor derived CD34+ HSCs collected from mPB or BM were transduced with BB305 LVV using the following combinations of (b) (4) media components:

1) (b) (4)

Transfection efficiency and expression of $\beta^{\text{A-T87Q}}$ globin in early and late erythroid and myeloid progenitors were determined using the same methods and parameters described in Study #1. The percentage of lentivirus positive cells (% LVV+) was evaluated by (b) (4) for detection of the provirus. Additionally, a (b) (4)-based assay for LVV entry was used to quantify the proportion of cells in which viral entry occurred.

The VCN using Process 1 (b) (4) was (b) (4) compared to (b) (4) using Process 2 (b) (4). This combination of (b) (4) media components using Process 2 was selected by the applicant to manufacture lovo-cel.

Reviewer's Note:

- (b) (4) the concentration of (b) (4) in the (b) (4) media using Process 2 resulted in a (b) (4) in the number of cells containing LVV (b) (4) and in the VCN when compared to product manufactured using Process 1. (b) (4) concentrations of (b) (4) did not further (b) (4) the VCN. Further

(b) (4) in VCN were observed with the addition of (b) (4) (not concentration dependent) in the presence of (b) (4). Based on these results and the applicant's historical data (not provided in the BLA) suggesting that (b) (4) of (b) (4) could inhibit cell growth, the applicant selected (b) (4) in combination with (b) (4) for use in Process 2.

In Vivo Studies

Study #4 (Pawliuk R, et al. (2001) Science 294:2368–71.) Correction of sickle cell disease in transgenic mouse models by gene therapy.

Objective: These studies evaluated the expression of β^{A-T87Q} and the correction of SCD phenotypes following transplantation of β^{A-T87Q} HPV436 LVV-transduced mBMCs from S-Antilles-D Punjab (SAD) or BERK (University of California, Berkeley) SCD mice into myeloablated C57BL/6 mice.

Reviewer's Notes:

- SAD and BERK mice transgenic mice are engineered to express mutated human β -globin to exhibit a SCD phenotype.^{2,3}
 - The SAD model contains mutated β -globin ($\beta 6Val$ substitution of the βS chain, as well as the Antilles [$\beta 23Ile$] and D Punjab [$\beta 121Gln$] substitutions) and normal human α -globin transgenes. Endogenous mouse α -, β - and γ -globin have anti-sickling inhibitory effects that limit the phenotypic effects of the βSAD -globin mutations. Because SAD mice that survive to adulthood do not display severe SCD, homozygous SAD mice are crossed with homozygous β -thalassemic mice to obtain β -thalassemic/SAD mice that display many of the clinical manifestations of human SCD, including sickling RBCs, reticulocytosis and splenomegaly indicative of extramedullary hematopoiesis (EMH), hypoxia-induced mortality, Hb polymerization, anemia, and mortality.
 - The BERK model contains the β^S globin transgene as well as $A\gamma$ - and $G\gamma$ -globin transgenes (included to enhance fetal survival by production of fetal Hb). The BERK SCD mice display more severe SCD, including irreversible RBC sickling, anemia, and multi-organ pathology, including microinfarcts resembling the vaso-occlusive features of human SCD.
- (b) (4)

² Trudel M, Saadane N, Garel MC, et al (1991) Towards a transgenic mouse model of sickle cell disease: hemoglobin SAD. EMBO J 10:3157–65

³ Paszty C, Brion CM, Mancini E, et al (1997) Transgenic knockout mice with exclusively human sickle hemoglobin and sickle cell disease. Science 278:876–8.

Methods:

Donor mBMCs were obtained from femurs of SAD and BERK mice injected 4 days prior with 150mg/kg of 5-fluorouracil (5-FU). BM cells were incubated overnight in serum free medium supplemented with 200mM L-glutamine, 6ng/ml murine Interleukin (IL)-3, 10ng/ml human IL-6, 10ng/ml murine IL-1 α , and 100ng/ml murine Stem Cell Factor. mBMCs were then exposed to concentrated β^{A-T87Q} HPV436 LVV or for 5-6 hours in the presence of 8 μ g/ml PS. Following transduction, cells were injected, without selection, into myeloablated (1100cGy of total body irradiation) recipient C57BL/6 mice according to following study groups:

- 1) C57BL/6 controls (n=3)
- 2) BERK controls (n=3)
- 3) BERK β^{A-T87Q} (n=3)
- 4) SAD controls (n=4)
- 5) SAD β^{A-T87Q} (n=3)

Reviewer's Notes:

- The number of mBMCs transplanted per mouse was not specified in the publication.
- Per the publication, control groups received mock-LVV transduced mBMCs.

Key Assessments:

- Blood analyses – Month 3
 - β^{A-T87Q} globin by flow cytometry
 - Hb by HPLC
 - % of β^{A-T87Q} globin by RP-HPLC
 - Hb species by focusing electrophoresis
 - % of reticulocytes by flow cytometry
 - HbS polymerization by turbidity analysis of RBC lysates
 - RBC density by Percoll-Larex continuous density gradient of peripheral blood
 - RBC sickling by Nomarski optics microscopy of peripheral blood
- Gross pathology of spleen – Month 3

Key Results:

Transplantation of β^{A-T87Q} HPV436 LVV-transduced SCD mBMCs resulted in stable β^{A-T87Q} -globin production and correction of the SCD phenotype in recipient mice three months post-transplant.

Study #5

Report Number	B2-15-161
Date Report Signed	05/31/2017
Title	A Study to Evaluate the Effect of Transduction of Human CD34+ Hematopoietic Stem Cells with BB305 Lentiviral Vector in the Presence of Different Transduction Additives on Long-term Bone Marrow Engraftment (BME) in Female ^{(b) (4)} Mice
GLP Status	No

Testing Facility		Bluebird Bio, Inc., Cambridge, MA USA
Objective		To evaluate the effect of different transduction media components on long-term BME mBMCs transduced with BB305 LVV in ^{(b) (4)} mice
Study Animals	Strain/Breed	^{(b) (4)}
	Species	Mice; <i>Mus musculus</i>
	Age	6 weeks old
	Body Weight	17-23 g
	#females/group	Group 1: n=10 Groups 2-7: n=15 Reviewer's Note: Per the study report, only female mice were included because they can be group-housed without concerns of fighting, etc.
Total #		100
Test Article		BB305-transduced human CD34+ HSCs
Control Article		Mock-transduced human CD34+ HSCs
Route of Administration		IV injection
Study Groups and Dose Levels		Group 1 (control)- 1x10 ⁶ mock-transduced cells ^{(b) (4)} PS Group 2 (human CD34+ HSCs transduced with BB305 LVV)- 1x10 ⁶ cells/mouse (Process 1) Group 3 (human CD34+ HSCs transduced with BB305 LVV)- 1x10 ⁶ cells/mouse ^{(b) (4)} Group 4 (human CD34+ HSCs transduced with BB305 LVV)- 1x10 ⁶ cells/mouse ^{(b) (4)} Group 5 (human CD34+ HSCs transduced with BB305 LVV)- 1x10 ⁶ cells/mouse ^{(b) (4)} Group 6 (human CD34+ HSCs transduced with BB305 LVV)- 1x10 ⁶ cells/mouse (Process 2) Group 7 (human CD34+ HSCs s transduced with BB305 LVV)- 1x10 ⁶ cells/mouse ^{(b) (4)} Reviewer's Note: The cell dose level (10 ⁶ cells/mouse) was based on published studies ⁴ and prior clinical trials conducted by the applicant.
Dosing Regimen		<ul style="list-style-type: none"> Busulfan (40 mg/kg) via intraperitoneal (IP) injection for myeloablation on Day -1. Single IV administration of control or test article on Day 1
Randomization		Yes; based on body weight (BW) prior to myeloablation
Description of Masking		Not provided
Scheduled Sacrifice Time Points		2 months post-dose, n=5/group 4 months post-dose, n =5 or 10/group

Key Assessments:

- Morbidity/mortality - daily
- Clinical observations - daily
- BWs - weekly
- Hematology - Months 2 and 4
- Individual mouse BM analyses - Months 2 and 4:
 - % BME as % hCD45+ cells
 - BM cellular differentiation as % Lin+ (CD3+ T, CD19+ B and CD33+) cells

⁴ ^{(b) (4)}

- Composite VCN by qPCR
- % of LVV+ cells by (b) (4)
- ISA profiles based on pooled genomic DNA (gDNA) by linear amplification (LAM) PCR next-generation sequencing (b) (4)

Reviewer's Note:

- The applicant uses the term “composite” VCN when VCN was determined from pooled samples (from the same group).
- Pooled BM samples (following a 14-day CFC culture or a 3-week erythroid culture) at Months 2 and 4:
 - Individual and mean colony VCN by qPCR
 - BFU-E % β^{A-T87Q} by RP-HPLC
 - Long-term BME: % BME (% hCD45+ cells) and % Lin+ cells

Key Results:

- No test article-related mortality or adverse effects on any parameter was observed.
- BW loss was observed for one Group 7 animal within 2 weeks post-dose. The animal was euthanized on Day 28 without further evaluation. Per the study report, the BW loss was attributed to myeloablation and/or insufficient BME.
- Mice administered human CD34+ HSCs s transduced with BB305 LVV manufactured by Process 2 (Group 6) displayed increased VCN and β^{A-T87Q} globin production.
- No incidence of insertional mutagenesis was observed.

Study #6

Report Number		B2-16-200
Date Report Signed		05/31/2017
Title		A Study to Evaluate the Effect of LentiGlobin BB305 Lentiviral Vector-transduced Human CD34+ Hematopoietic Stem Cells Manufactured with Process 1 or Process 2 on Long-term Bone Marrow Engraftment in Female (b) (4) Mice
GLP Status		No
Testing Facilities		Bluebird Bio, Inc., Cambridge, MA USA
Objective		To evaluate the effect of human CD34+ HSCs s transduced with BB305 LVV manufactured with Process 1 or Process 2 on long-term BME in (b) (4) mice
Study Animals	Strain/Breed	(b) (4)
	Species	Mice; <i>Mus musculus</i>
	Age	9 weeks old
	Body Weight	20-25 g
	# females/group	15
	Total #	30

Test Articles	<ul style="list-style-type: none"> • human CD34+ HSCs transduced with BB305 LVV manufactured using Process 1 • human CD34+ HSCs transduced with BB305 LVV manufactured using Process 2
Route of Administration	IV injection
Study Groups and Dose Levels	Group 1 (Process 1) – 42x10 ⁶ cells/kg Group 2 (Process 2) – 42x10 ⁶ cells/kg
Dosing Regimen	<ul style="list-style-type: none"> • Busulfan (40 mg/kg) via IP injection on Day -1 • Single IV injection of on Day 1
Randomization	Yes; based on BW prior to myeloablation
Description of Masking	Not provided
Scheduled Sacrifice Time Points	2- and 4 months post-dose

Key Assessments:

- Morbidity/mortality - daily
- Clinical observations - daily
- BWs - weekly
- Hematology - Months 2 and 4
- Individual mouse BM analysis
 - % BME - Months 2 and 4
 - Composite VCN - Months 2 and 4
 - % LVV+ cells - Month 4
- Pooled BM samples (cultured by the same methods as in Study #6) – Months 2 and 4:
 - Individual and mean Colony VCN
 - % LVV+
 - BFU-E % β^{A-T87Q} globin by RP-HPLC
 - % β^{A-T87Q} globin by RP-HPLC

Key Results:

- No test article-related mortality or adverse effects on any parameter was observed.
- Mice administered human CD34+ HSCs s transduced with BB305 LVV manufactured by Process 2 (Group 2) showed increased VCN (2.3c/dg vs 0.5c/dg) and β^{A-T87Q} globin expression (18.6% vs 4.1%) compared to Process 1 (Group 1).

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies with lovo-cel were conducted.

PHARMACOKINETIC STUDIES (Cell Distribution)

Reviewer's Note:

- Per the applicant, because lovo-cel is an *ex vivo* gene-modified autologous HSC-based product, traditional pharmacokinetic and/or toxicokinetic parameters were not evaluated. Cell distribution was assessed by the applicant in the target tissues, BM, and peripheral blood, by VCN measurements. The VCN values are derived from transduced, engrafted HSCs and/or differentiated progeny and demonstrate exposure; however, they do not directly represent the dose levels of the test article administered. The applicant assessed cell distribution by VCN measurement in BM and peripheral blood samples in Study Numbers 5 and 6 which are reviewed in the 'Pharmacology Studies' and Study Numbers 7 and 8, which are reviewed under the 'Toxicology Studies' section of this memo and section of this memo.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of human HSCs or mBMCs transduced with BB305 LVV following administration in mice.

Toxicology Studies:

Study Number	Study Title	Report Number
7	Single-dose Toxicology and Pharmacology Study of Lentiviral Vectors BB305 and HPV569 in β -Thalassemic (Hbb ^{th1/th1}) Mice Following Intravenous Administration of Transduced Syngeneic Lin-depleted Mouse Bone Marrow Cells	NC-11-002-R
8	Single-dose Toxicology and Pharmacology Study of LentiGlobin Lentiviral Vectors BB305 and HPV569 Following Intravenous Administration in CD45.1+ C57BL/6 Mice of "Secondary" Bone Marrow Obtained from β -Thalassemic (Hbb ^{th1/th1}) Mice Originally Transplanted Four Months Earlier in Study NC-11-002 with Transduced Syngeneic Lin-depleted Bone Marrow Cells	NC-12-019

Overview of Toxicology Studies

Study #7

Report Number	NC-11-002-R
Date Report Signed	12/04/2012
Title	Single-dose Toxicology and Pharmacology Study of LentiGlobin Lentiviral Vectors BB305 and HPV569 in β -Thalassemic (Hbb ^{th1/th1}) Mice Following Intravenous Administration of Transduced Syngeneic Lin Depleted Mouse Bone Marrow Cells

GLP Status	No The histopathology examination was conducted per OECD guidelines
Testing Facility	<ul style="list-style-type: none"> Bluebird Bio (b) (4) (in-life, all sample collections, necropsy, and gross pathology) (b) (4) (clinical pathology and histopathology)
Objective	To evaluate the pharmacologic and toxicologic effects of mBMCs transduced with BB305 LVV or mBMCs transduced with HPV569 LVV following IV administration in β -thalassemic mice.
Study Animals	Strain/Breed CD45.2 ⁺ (b) (4) Hbb ^{th1/th1}
	Species Mice; <i>Mus musculus</i>
	Age 1-12 months old
	Body Weight Males: 15-25 g Females: 12-20 g
	#/sex/group Group 1: n=11 males/11 females Group 2: n=10 males/11 females Group 3: n=7 males/8 females
	Total # 58
Test Articles	<ul style="list-style-type: none"> mBMCs transduced with BB305 LVV (Lot No. (b) (4)) mBMCs transduced with HPV569 LVV (Lot No. (b) (4))
Control Article	mBMCs; mock-transduced
Route of Administration	IV injection
Study Groups and Dose Levels	Group 1: mBMCs transduced with HPV569 LVV - 11×10^6 cells/kg Group 2: mBMCs transduced with BB305 LVV - 11×10^6 cells/kg Group 3: control - 11×10^6 cells/kg
Dosing Regimen	<ul style="list-style-type: none"> ➤ 11 Gray (Gy) radiation on Day -1 ➤ Single IV administration of control or test article on Day 0 <p>Reviewer's Note: Donor-derived cells administered to recipient mice of each sex were obtained from the opposite sex.</p>
Randomization	Yes; based on BW following irradiation
Description of Masking	Not provided
Scheduled Sacrifice Time Point	4 months post-dose

Key Assessments:

- Mortality/morbidity - 5 times/week
- Clinical observations - 2 times weekly
- BWs - weekly
- Clinical pathology
 - Hematology - Months 2, 3, and 4
 - Serum chemistry - at sacrifice
- Blood analyses (evaluated by Bluebird Bio)
 - % of β^{A-T87Q} globin by RP-HPLC - Months 2 and 3
 - % of reticulocytes by flow cytometry - Months 2, 3, and 4
 - Mean VCN by qPCR - Month 3
 - Blood cell subsets (CD45.2, CD11b, Gr1, CD3, B220, Ter119 [for murine erythroid cells], and CD71 [transferrin receptor]) by flow cytometry - Month 4
- BM analyses - at sacrifice (evaluated by Bluebird Bio)
 - Mean VCN by qPCR
 - Colony VCN (after methyl cellulose culture) by qPCR

- Male/female chimerism (donor cell engraftment) by qPCR
- BM cell subsets (CD45.2, CD11b, Gr1, CD3, B220, Ter119, CD71) by flow cytometry
- BM cytology
- Organ weights, gross pathology, histopathology - at sacrifice

Key Results:

- No test article-related mortality occurred. Four unscheduled deaths occurred; none were considered test article-related:
 - Group 2 - 3/21 mice - Found dead on Days 37, 49, and 65. The deaths were attributed to radiation toxicity (Day 37) and to post-transplant complications (Days 49 and 65).
 - Group 3 - 1/15 mice - Found dead on Day 34; the death was attributed to radiation toxicity.
- Mean platelet counts were slightly lower (<20%) for all animals in Groups 1 and 2 compared to Group 3 (Table 1). Per the clinical pathologist, the higher platelet counts in the control animals (Group 3) could be due to abnormal erythrocyte counts in these animals. These abnormal counts reflect microcytic RBCs, RBC fragments and microspherocytes in β -thalassemia mice that are counted by blood analyzer as platelets thereby causing “pseudo increases” in the platelet levels.⁵

Reviewer's Note:

- Based on the data and review of the published peer-reviewed scientific literature; this reviewer agrees with the clinical pathologist's conclusion.

Table 1: Mean Platelet Counts at Months 2, 3, and 4

	Group 1 (G [giga]/L [liter])		Group 2 (G/L)		Group 3 (G/L)	
	Males	Females	Males	Females	Males	Females
Month 2	633 \pm 120	636 \pm 178	491 \pm 51	448 \pm 51	574 \pm 103	451 \pm 59
Month 3	565 \pm 64	589 \pm 114	434 \pm 49	470 \pm 64	508 \pm 109	518 \pm 100
Month 4	934 \pm 435	851 \pm 342	662 \pm 162	728 \pm 110	796 \pm 115	702 \pm 272

Source: Study Report No. NC-11-002-R; Module 4.2.1 in the BLA.

- Mean bilirubin levels were lower in Groups 1 and 2 compared to Group 3. Per the clinical pathologist, this finding was due to reduced degradation of RBCs and Hb in mice administered the test articles.
- Mean absolute and relative spleen weights in Groups 1 and 2 were decreased compared to Group 3.

⁵ Brigden, ML, Dalal BI. Cell counter-related abnormalities. Laboratory Medicine 1999; 30:325-334.

Reviewer's Note:

- Although only a single dose level of 11×10^6 cells/kg mBMCs transduced with BB305 LVV was tested, the applicant designated this dose level as the no-observed-adverse-effect-level.

Study #8

Report Number		NC-12-019
Date Report Signed		10/29/2013
Title		Single-dose Toxicology and Pharmacology Study of LentiGlobin Lentiviral Vectors BB305 and HPV569 Following Intravenous Administration in CD45.1 ⁺ C57BL/6 Mice of "Secondary" Bone Marrow Obtained from β -Thalassemic (Hbb ^{th1/th1}) Mice Originally Transplanted Four Months Earlier in Study NC-11-002 with Transduced Syngeneic Lin Depleted Bone Marrow Cells
GLP Status		No
Testing Facilities		<ul style="list-style-type: none"> • Bluebird Bio (b) (4) (in-life parameters, all sample collections, necropsy, and gross pathology) • (b) (4) (clinical pathology and histopathology)
Objective(s)		To evaluate the pharmacologic and toxicologic effects of secondary (serial) transplantation mBMCs transduced with BB305 LVV or with HPV569 (obtained from β -thalassemic mice that received primary transplantation) in wildtype C57BL/6 mice
Study Animals	Strain/Breed	Wildtype CD45.1 ⁺ C57BL/6
	Species	Mice; <i>Mus musculus</i>
	Age	9 weeks old
	Body Weight	Females: 15-20 g Males: 20-26 g
	#/sex/group	Group 1: n=21 males/23 females Group 2: n=18 males/18 females Group 3: n=9 males/19 females
	Total #	108
Test Articles		BMCs obtained from β -thalassemic mice that received primary transplantation of 1) HPV569 LVV-transduced murine BMCs (Lot No. (b) (4) or 2) BB305 LVV-transduced murine BMCs (Lot No. (b) (4)
Control Article		BMCs obtained from β -thalassemic mice that received primary transplantation of mock-transduced murine BMCs
Route of Administration		IV injection
Study Groups and Dose Levels		Group 1: mBMCs transduced with HPV569 - 6×10^6 cells/mouse Group 2: mBMCs transduced with BB305 LVV - 6×10^6 cells/mouse Group 3: control - 6×10^6 cells/mouse
Dosing Regimen		<ul style="list-style-type: none"> ➤ BMCs were obtained from β-thalassemic mice at 4 months post-primary transplantation of 11×10^6 cells/kg of mock-, HPV569 LVV-, or BB305-transduced mBMCs ➤ Wildtype mice were exposed to 11 Gy radiation on Day -1 ➤ The mBMCs were IV administered to the wildtype mice on Day 0
Randomization		Yes; based on BW following irradiation
Description of Masking		Not provided
Scheduled Sacrifice Time Points		6 months post-transplant

Key Assessments:

- Mortality/Morbidity - daily
- Clinical observations - twice weekly
- BWs - weekly
- Clinical pathology
 - Hematology - Months 4-6
 - Clinical chemistry - at sacrifice
- Blood analyses - Months 4 and 5 (evaluated by Bluebird Bio):
 - Mean VCN by qPCR
 - Blood cell subsets (CD45.2, CD11b, Gr1, CD3, B220, Ter119 [for murine erythroid cells], and CD71 [transferrin receptor]) by flow cytometry
- BM analysis - at sacrifice (evaluated by BlueBird Bio):
 - Mean VCN by qPCR
 - Colony VCN (after methyl cellulose culture) by qPCR
 - BM cell subsets ((CD45.2, CD11b, Gr1, CD3, B220, Ter119, CD71) by flow cytometry
- BM cytology
- Organ weights, gross pathology, histopathology - at sacrifice

Reviewer's Note:

- All assessments were determined using the same methods as in Study #4.

Key Results:

- No test article-related mortality occurred. A total of 13 animals were found dead or were prematurely euthanized during the study; none were considered test article related.
 - Group 1- 8/44 mice- Deaths were attributed to cutaneous ulcers (3/44 on Days 62, 104, and 159), unclassified leukemia (2/44 on Days 141 and 143), and malignant lymphoma (2/44 on Days 187 and 204). The cause of death was not able to be determined in one mouse found dead on Day 27.
 - Group 2- 3/36 mice- One death (on Day 193) was attributed to systemic malignant lymphoma and the cause of death could not be determined for two mice which were found dead on Days 22 and 204.
 - Group 3- 2/28 mice- The cause of death could not be determined in the two mice which were found dead on Days 11 and 22.

Reviewer's Note:

- The pathologist noted that the observed incidence of T-cell lymphoma and/or leukemia was within the reported range (15.7-25.3%) for radiation-associated lymphoma in and aging in C57BL/6 mice (E. Will et al. (2007) Mol Ther 15:782-91) and considered these findings incidental. Please refer to the *Reviewer's Notes* below for additional information to support this conclusion.

Findings observed in the animals that survived to the Month 6 scheduled sacrifice included the following:

- Mean PLT counts were slightly lower for all animals in Groups 1 and 2 compared with the concurrent controls (Group 3). Per the clinical pathologist, this was due to abnormal erythrocytes in Group 3 animals.
- Mean total bilirubin levels were lower in Groups 1 and 2 compared to Group 3. Per clinical pathologist, this was due to decreased degradation of RBCs and Hb in mice transplanted with BB305 or HPV569 LVV-transduced BMCs.
- Mean potassium and inorganic phosphorus concentrations were lower in Groups 1 and 2 compared to Group 3. Per the clinical pathologist, this was due to high number of samples that hemolyzed during blood puncture in animals from Groups 1 and 2.
- Higher VCN in blood cells were observed in Group 2 animals compared to Group 1.
- The following macroscopic and microscopic findings were observed:
 - Mean absolute and relative spleen weights were lower in Groups 1 and 2 compared to Group 3. Per the expert pathologist, this finding was consistent with correction of the β -thalassemic phenotype. This was confirmed by histology showing decreased hematopoiesis in Group 1 and 2 spleens compared to Group 3.
 - The mean absolute and relative thymus weights were higher in Group 2 male animals compared to Group 3 male and female mice. This finding was attributed to high thymic weights in several animals with malignant lymphoma involving the thymus or with marked thymic lymphoid hyperplasia (Table 5).
 - Animals in all groups exhibited macroscopic findings correlating with malignant lymphoma (Table 2). These findings were confirmed by histopathology. Histopathology showed that all lymphomas were of T-cell origin (CD3+, CD45RA-) and involved the thymus.

Table 2: Incidence of Malignant Lymphoma and Thymic Lymphoid Hyperplasia at Terminal Sacrifice

Sex	Males			Females		
Study Group	1	2	3	1	2	3
Total number of animals	17	17	8	19	16	18
Malignant lymphoma	-	3	-	2	2	2
-Thymic	1	-	-	2	1	-
-Systemic	-	3	-	-	1	2
Thymic lymphoid hyperplasia						
-Marked (Grade 4)	-	2	-	1	1	-

Source: Report No. NC-12-019 in Module 4.2.1 in the BLA.

- An enlarged spleen was noted in 2/36 Group 2 animals, which correlated with lymphoma or hematopoiesis.
- The thymus was reduced in 8/44, 3/36, and 1/28 animals in Groups 1-3, respectively. Per expert pathologist, these findings were due to lymphoid atrophy which is a common finding in mice of this age.

Reviewer's Notes:

- An additional pathology evaluation was performed to determine the cause of the hematologic malignancies. The peer-review pathologist concluded that the increased lymphomas observed in Groups 1 and 2 compared with Groups 3 were incidental due to the following: 1) there were less animals in Group 1 compared to the other groups; 2) a high historical prevalence of lymphoma has been reported for C57BL/6 mice.⁶ Specifically, in chronic studies conducted with C57BL/6 mice, lymphoma is the most common neoplasm occurring in 15.7 to 25.3% of animals⁷; and 3) thymic lymphoma is the major type of neoplasm following irradiation of mice.⁸
- To support the pathologist's conclusion, the VCN in the tumor and in the BM of the tumor-bearing animals was determined. A mean VCN of <1 was detected in the analyzed thymus and BM tissues, suggesting that the tumors found in the animals were not linked to high vector copy numbers.

Developmental and Reproductive Toxicology Studies:

Per the applicant, developmental and reproductive toxicology studies were not conducted because there were no adverse findings in the male and female reproductive tissues of mice administered mBMCs transduced with BB305 LVV, based on the results of Study #4 Report No. NC-11-002-R) and Study #5 (Report No. NC-12-019). The risk of reproductive and developmental toxicity associated with busulfan is known and is discussed in the label.

Genotoxicity Studies:

Study Number	Study Title	Report Number
9	<i>In Vitro</i> Immortalization Assay to Compare and Assess the Genotoxicity of LentiGlobin Lentiviral Vectors (b) (4), HPV569, and BB305	NC-12-016-R
10	Integration Site Analysis: Comparison Among Different LentiGlobin BB305 Drug Product Lots Manufactured with Process 1 and Process 2	B2-16-209
11	Integration site analysis (ISA) using DNA extracted from the β -Thalassemic (Hbb ^{th1/th1}) Mice of Study NC-11-002	NC-12-056-R
12	Integration Site Analysis on Bone Marrow Cells Obtained from C57/BL6 Mice Transplanted in Nonclinical Pharmacology and Toxicology Study NC-12-019	NC-13-023-R

⁶ Frith CH et al., 1983 – Spontaneous lesions in virgin and retired breeder BALB/c and C57BL/6 mice – Laboratory Animal Science.

⁷ Will E, Bailey J, Schuesler T, et al (2007) Importance of murine study design for testing toxicity of retroviral vectors in support of phase I trials. Mol Ther 15:782–91.

⁸ Greaves P, 2007 – Histopathology of preclinical toxicity studies. 3rd ed. New York, Academic Press.

Study #9 (Report No. NC-12-016-R) In Vitro Immortalization Assay (IVIM) to Compare and Assess the Genotoxicity of LentiGlobin Lentiviral Vectors (b) (4), HPV569, and BB305; Conducted by (b) (4) (non-GLP)

Objective:

This study evaluated the potential for LVV-mediated insertional mutagenesis of mouse hematopoietic lineage-depleted (Lin-) BM cells.

Methods:

Mouse Lin- BM cells were transduced with the following vectors: 1) (b) (4) LVV, 2) HPV569 LVV, or 3) BB305 LVV). Concurrent controls included the cells transduced with 1) [RFS91.GFPgPRE (GRV positive control) or 2) (b) (4) (lv-SF, LVV positive control). The transduced cells were cultured for 2 weeks. Parameters evaluated were: 1) cell viability (determined by an (b) (4) assay); 2) cytotoxicity (assessed based on the percentage of viable cells); and 3) transduction efficiency (VCN; determined by qPCR). Cells were cultured and replated for 2 weeks to identify highly proliferating clones. Genotoxicity was assessed as a 'fitness score' that was calculated as the replating frequency (RF)/VCN. Genetic insertions in each clone were identified by ligation-mediated PCR.

Reviewer's Note:

- The RF is a measure of the incidence of highly proliferating cells (potentially immortalized cells) that undergo expansion after limiting dilution (replating) and additional culturing for 2 weeks. A low fitness score is associated with decreased risk of insertional mutagenesis.

Key Results:

- Based on the fitness scores, there was a reduced potential for immortalization induced by insertional mutagenesis of BB305 LVV, compared to the positive control vectors (Table 3).

Table 3: Results of the IVIM Assay

Test or Control Articles:	Mock-transduced	RFS91. GFPgPRE (RFS91) GRV	(b) (4) (lv-SF) LVV	(b) (4)	HPV569 LVV	BB305 LVV
Description:	Negative Control	GRV positive control	LVV positive control		Test article	Test article
VCN (c/dg)	0.00	2.28	4.55		7.40	4.25
(b) (4) positive ^a (Incidence, %):	0/6 0%	6/6 (100%)	6/6 (100%)		4/6 (67%)	3/6 (50%)
RF value (x10 ⁴) ^b :	N/A	35.09	8.55		1.41	0.53
Fitness Score = RF/VCN (x10 ⁴) ^c :	N/A	11.02	2.07* [^]		0.22* [^]	0.13* [^]

* (p<0.01) relative to RFS91 positive control GRV. ^ (p<0.05) relative to lv-SF positive control LVV

a= (b) (4) positive is the % of viable cells.

b= RF value (x10⁴ cells).

c= Fitness Score = RF/VCN (x10⁴ cells)

Source: Module 2.6.6, Toxicology Written Summary and Module 4.2.3.3.1 and *In vitro* Genotoxicity, Study Report No. NC-12-016-R in the BLA.

- The detected insertion sites of BB305 LVV (Table 4) were not associated with hematopoietic development, malignancy, or transformation.

Table 4: List of BB305 LVV Insertion Sites

Unigene	EntrezGene	Chromosome	Raw distance	TSS distance	Gene strand
Cyclin-dependent kinase 14	18647	5	intronic	321523	-1
Cyclin dependent kinase 17	237459	10	intronic	44691	-1
RAD21 homolog (S. pombe)	19357	15	intronic	6588	-1
Ca2+ dependent activator protein for secretion 2	320405	6	intronic	52853	-1
Parathyroid hormone 2 receptor	213527	1	20372	126755	-1
Pyruvate dehydrogenase E1 alpha 2	18598	3	-87557	-87643	-1

Source: Module 2.6.6, Toxicology Written Summary and Module 4.2.3.3.1, *In vitro* Genotoxicity; Study Report No. NC-12-016-R in the BLA.

- Per the applicant, based on these data, the risk of insertional mutagenesis following BB305 transduction is low.

Reviewer's Note:

- This reviewer agrees with the applicant's conclusion.

Study #10 (B2-16-209) Integration Site Analysis: Comparison Among Different LentiGlobin BB305 Drug Product Lots Manufactured with Process 1 and Process 2
The HSC transductions and characterization were conducted at Bluebird Bio, Inc. Assessment of the ISA profiles were performed at (b) (4)

Objective:

This study compared the ISA profiles from six different lots of PBMCs transduced with BB305 LVV manufactured from three donors (Lot Nos. (b) (4))

Methods:

Human donor CD34+ HSCs were transduced with: 1) BB305 LVV manufactured using Process 1 (MOI = 25) or 2) BB305 LVV manufactured using Process 2 (MOI = 20). Transduced cells were assayed in a 7-day liquid culture and a 14-day CFC culture. Mean VCN and the %LVV+ cells were determined. For ISA, the LAM-PCR (b) (4) assays were performed, followed by deep sequencing and bioinformatic analysis to determine unique mappable integration sites and their frequencies.

Reviewer's Notes:

- The LAM-PCR assay allows identification of vector IS by amplification of unknown host DNA flanking the sites of vector integration. The amplified DNA is subjected to restriction digestion. The (b) (4) assay is a variation of LAM-PCR that is (b) (4), thus, allowing unbiased detection of vector IS.
- RefSeq genes detected next to IS were analyzed for their presence within three different cancer gene databases: 1) CGC: Cancer Gene Census Database (<http://www.sanger.ac.uk/genetics/CGP/Census/>); 2) RTCGD: Retrovirus and Transposon tagged Cancer Gene Database (<http://variation.osu.edu/rtcgd/index.html>); and 3) CBioPortal: cBio Cancer Genomics Portal (<http://www.cbioportal.org/public-portal/>). The frequency of IS detected next to cancer genes was compared to an *in silico* generated data set.

Key Results:

- The CD34+ HSCs transduced with BB305 LVV manufactured using Process 2 showed higher mean VCN ([c/dg] (b) (4)) and % LVV+ cells (b) (4) compared to cells transduced with BB305 LVV manufactured using Process 1.
- There was no evidence of clonal selection.
- The integration profiles of BB305 LVV were similar for Processes 1 and 2.
- The ten mappable IS with highest frequencies were analyzed for their location near the proto-oncogenes, *LMO2*, *EV/I*, and *MOS1*, which have been implicated in adverse events in LVV clinical trials^{9,10}, as well as other genes with a known relation to cancer as determined by the Cancer Gene Database. There was no enrichment for integration near

⁹ Hacein-Bey-Abina, S., C. Von Kalle, et al. (2003). "LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1." *Science* 302(5644): 415-419.

¹⁰ Ott, M. G., M. Schmidt, et al. (2006). "Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1." *Nat Med* 12(4): 401-409.

cancer-related genes or within genes associated with clonal outgrowth in clinical trials of GRV-transduced HSCs.¹¹

Reviewer's Note:

- Overall, based on the data and review of the published peer-reviewed scientific literature, this reviewer agrees that the pattern of integration of BB305 LVV in this study was consistent with what has been observed in published studies with LVVs, and that the ISA profiles were similar between Process 1 and Process 2.

Study #11 (Report No. NC-12-056-R) *Integration site analysis (ISA) using DNA extracted from the β -Thalassemic ($Hbb^{th1/th1}$) Mice of Study NC-11-002 and*

Study #12 (Report No. NC-13-023-R) *Integration Site Analysis on Bone Marrow Cells Obtained from C57/BL6 Mice Transplanted in Nonclinical Pharmacology and Toxicology Study NC-12-019*

- Studies #11 and #12 evaluated the ISA profiles of mBMCs transduced with BB305 LVV that were generated for use as the donor material in the primary and secondary murine transplant studies, Study #7 and 8, respectively. The findings in these studies were similar to the results with human HSCs transduced with BB305 LVV generated using in Study 10:
 1. Integration profiles were consistent with those observed in published studies using LVV-transduced human HSCs.
 2. There was no indication of clonal expansion.
 3. There was no evidence of insertional mutagenesis.

Carcinogenicity Studies:

No traditional carcinogenicity studies were conducted with lovo-cel which is acceptable based on the product type.

APPLICANT'S PROPOSED LABEL

- Subsections 8.1-8.3 of Section 8 ('Use in Specific Populations') is acceptable with minor revisions.
- Section 13 ('Nonclinical Toxicology') is acceptable without revision.

¹¹ Biasco L, Rothe M, Buning H, Schambach A (2018) Analyzing the Genotoxicity of Retroviral Vectors in Hematopoietic Cell Gene Therapy. *Mol Ther Methods Clin Dev* 8:21–30. doi: 10.1016/j.omtm.2017.10.002

CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

KEY WORDS/TERMS

LYFGENIA™, Lovotibeglogene autotemcel, BB305, lentivirus, lovo-cel, mBMCs transduced with BB305 LVV, Sickle cell disease, β -thalassemia, CD34+ hematopoietic stem cells, β A-T87Q-globin, vector copy number, pharmacology, toxicology, genotoxicity, insertional mutagenesis, β -Thalassemic ($Hbb^{th1/th1}$) mice, (b) (4) mice