

I concur with this review. S. Sanduja 07/08/22

I concur with this review. M. Serabian 07/08/22

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
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Division of Clinical Evaluation and Pharmacology/Toxicology
Pharmacology/Toxicology Branch

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PRODUCT: ZYNTGLO™ (Betibeglogene autotemcel or beti-cel)

APPLICANT: Bluebird Bio, Inc.

PROPOSED INDICATION: For the treatment of patients with beta(β)-thalassemia who require regular red blood cell (RBC) transfusions.

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EXECUTIVE SUMMARY:

ZYNTGLO™ (Betibeglogene autotemcel or beti-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). ZYNTGLO™ is for the treatment of patients with transfusion dependent-β-thalassemia (TDT). Following intravenous (IV) infusion in the myeloablated patient, beti-cel engrafts in the bone marrow (BM) and differentiates to produce RBCs containing biologically active β^{A-T87Q}-globin that will combine with α-globin to produce functional hemoglobin (Hb). The minimum recommended dose level of ZYNTGLO™ is 5x10⁶ CD34+ cells/kilogram (kg).

In vitro pharmacology studies conducted using CD34+ HSCs obtained from patients with sickle cell disease (SCD) provided proof-of-concept (POC) showing that erythroid cells derived from BB305 LVV-transduced HSCs produce β^{A-T87Q} globin. *In vivo* POC studies showed that immunodeficient mice administered beti-cell displayed BM engraftment (BME) and β^{A-T87Q} -globin expression.

In vivo pharmacology and toxicology studies were conducted to evaluate the activity and safety of BB305 LVV-transduced murine BM cells (BMCs) following primary and secondary transplantation in β -thalassemic and wild-type C57BL/6 mice, respectively. Long-term BME and BM chimerism were observed in all animals receiving BB305 LVV-transduced BMCs compared to those that received mock-transduced BMCs. In the secondary transplantation study, no deaths or adverse findings attributed to the infused cells occurred. 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]; therefore, was considered incidental.

The risk of insertional mutagenesis of BB305 LVV was evaluated. Results from an *in vitro* immortalization (IVIM) assay performed with BB305 LVV-transduced murine BMCs showed low mutagenic potential of BB305 LVV. Integration site analysis (ISA) of beti-cel ZYNTEGLO showed no clonal dominance or enrichment for LVV integration in or near known oncogenes. These data support the conclusion that transduction of HSCs with BB305 LVV has a low risk for oncogenic transformation.

Carcinogenicity and developmental and reproductive toxicity studies were not conducted with beti-cel. 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 ZYNTEGLO™. The nonclinical information provided in the BLA submission supports approval of the licensure application.

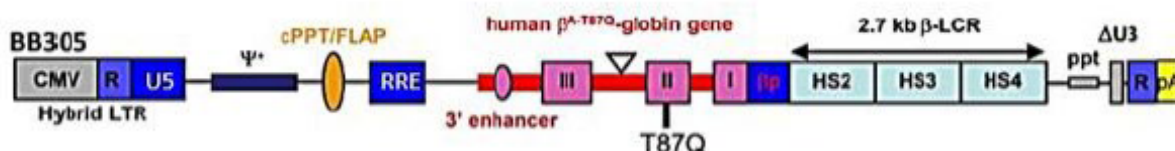
Formulation and Chemistry:

ZYNTEGLO™ 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 of 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 ZYNTEGLO™ the patient is exposed to a busulfan myeloablative conditioning regimen to prepare the BM “niches” for product engraftment. The minimum recommended dose level of ZYNTEGLO™ is 5×10^6 CD34+ cells/kg. Refer to the CMC review memos for more details regarding the manufacturing process for the drug substance and the final drug product.

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.

The position of the T87Q variation is indicated as a red asterisk. The CMV promoter in the hybrid CMV/HIV-1 long terminal repeat (LTR) is part of the transfer vector plasmid that drives the expression of the packaged transcript in the packaging cell line and are not a part of the provirus that integrates into the recipient genome. Additionally, 2 STOP codons are present in the 3' region of the packaging signal the LVV construct (not illustrated). Due to the hybrid CMV/HIV 1 LTR, BB305 LVV is not Tat-dependent.

Source: Introduction; Module 2.6.1 in the BLA.

Manufacturing Changes made to BB305 LVV during the Clinical Development of ZYNTEGLO™:

The manufacturing process for ZYNTEGLO™ 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 Studies HGB-204 and HGB-206 [Cohort A]); and c) Process 2 (administered in clinical Studies HGB-206 [Cohorts B and C], HGB-207, and HGB-212). 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
LAM-PCR	linear amplification PCR
Lin	Lineage
LVV	Lentiviral vector
MOI	Multiplicity of infection
mPB	Mobilized peripheral blood
(b) (4)	
PCR	Polymerase chain reaction
(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 #15324: Autologous CD34+ Hematopoietic Stem Cells Transduced with Lentiviral Vector, LentiGlobin BB305, Encoding the Human beta-A-T87Q-Globin Gene, Selected by the (b) (4) Device; Cultured with (b) (4); Following Mobilization with G-CSF or G-CSF and Plerixafor; Administered Intravenously; Treatment of beta-thalassemia major; Bluebird Bio Inc.; **ACTIVE**

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INTRODUCTION

β -thalassemia is a rare hereditary blood disorder that affects individuals from infancy through adulthood and is associated with significant morbidity and mortality. β -thalassemia is caused by mutations in one or both alleles of the β -globin gene of HbA. 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 ($\alpha_2\beta_2$). Mutations in one or both of the β^A -globin genes result in the failure to produce sufficient functional β^A -globin chains, which causes a reduction in β -globin production, with accumulation of excess uncomplexed α -globin. The uncomplexed α -globin precipitates in erythroblasts, leading to premature death of the RBCs, ineffective erythropoiesis, and hemolysis that causes anemia in the patients.¹ Ineffective erythropoiesis also leads to other pathological hallmarks of β -thalassemia, such as extramedullary hematopoiesis, skeletal deformation, and iron avidity. In its most severe form, β -thalassemia results in life-threatening anemia with transfusion-dependency and, consequentially, a long-term risk of lethal iron overload.^{2,3} Patients with TDT require life-long treatment with frequent blood transfusions and iron chelation.

In vivo production of transgenic β^{A-T87Q} globin (the primary pharmacodynamic effect) following IV infusion of beti-cel corrects the deficient β^A -globin production in individuals with TDT, allowing the formation of modified forms of HbA, including $\alpha_2\beta^{A-T87Q}$. Restoration of functional β -globin production will reduce accumulation of excess uncomplexed α -globin in the erythroblasts, thus preventing RBC death and enhancing erythropoiesis, preventing hemolysis, and increasing total Hb levels. These pharmacologic effects will result in significant reduction in the number of transfusions, as well as decreased extramedullary hematopoiesis, skeletal deformation, and iron avidity, and extending the life expectancy for patients with TDT.

Note: β^A is a variant of the β -globin chain.

NONCLINICAL STUDIES

¹ Galanello R, Origa R (2010) Beta-thalassemia. Orphanet J Rare Dis 5:11.

² Olivieri NF (1999) The beta-thalassemias. N Engl J Med 341:99–109.

³ Cappellini M-D, Cohen A, Eleftheriou A, et al (2014) Guidelines for the Management of Transfusion Dependent Thalassemia -3rd Edition.

Reviewer's Notes:

- The manufacturing process (Process 0, 1, or 2) for the drug product evaluated in the nonclinical studies is specified under the respective study.
- Throughout this review memo, mouse BMCs that are transduced with BB305 LVV are referred to as “murine beti-cel” and human mPB-derived CD34+ HSCs that are transduced with BB305 LVV are referred to as “beti-cel.” These terms are not used for the following: 1) Study #1 and Study #2 which evaluated CD34+ cells derived from patients with SCD and 2) Study #3 in which human mPB and BM cells were transduced with BB305 LVV.

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of beti-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	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*
5	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*

Study Number	Study Title	Report Number
6	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
7	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

*The study is summarized in the ‘Toxicology Studies’ section of this review memo.

Overview of Pharmacology Studies

In vitro Studies

Study #1 (Report No. NC-11-001-R)

Assessment of Transduction Efficiency in Sick 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 (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 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).

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.
- The applicant's rationale for using CD34+ cells from patients with SCD was that the β^{AT87Q} and β^A chains have close retention times, therefore a better assessment of β^{AT87Q}

expression is possible when the β^A chains are absent, as is the case with SCD (β^S/β^E) CD34+ cells.

- HPV569 LVV is identical to BB305 LVV except for a HIV-1 LTR, resulting in HPV569 LVV being Tat-dependent.⁴

This study demonstrated a higher transduction efficiency (mean VCN [c/dg]: 1) CFC culture assay (b) (4) assay (b) (4), with increased β^{AT87Q} expression in HSCs transduced with BB305 LVV compared to HPV569 LVV.

Study #2 (Report No. NC-11-004-R)

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; Conducted by Bluebird Bio

Objective:

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

This study demonstrated a higher transduction efficiency (mean VCN[c/dg]: 2.5 vs 1.88 vs 0.99) in HSCs transduced with BB305 LVV, (b) (4) LVV, and HPV569 LVV, respectively. An increased production of β^{AT87Q} globin (β^{AT87Q}/α ratio: (b) (4)) was observed in HSCs transduced with BB305 LVV compared to HPV569 LVV. Similar β^{AT87Q} globin expression (β^{AT87Q}/α : (b) (4)) was observed in HSCs transduced with BB305 LVV and (b) (4) LVV, respectively.

Reviewer's Note:

- (b) (4) LVV is a predecessor of HPV569 LVV. (b) (4) VV 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

⁴ 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.

Additives; Conducted by Bluebird Bio

Objective:

This study evaluated the effect of various media components on the transduction efficiency and subsequent β^{A-T87Q} globin production in BB305 LVV-transduced healthy 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 β^{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 transduction efficiency using Process 1 (b) (4) was a VCN of (b) (4), while it was (b) (4) using Process 2 (b) (4). This combination of (b) (4) media components was selected by the applicant to manufacture ZYNTGLO™ using Process 2.

Reviewer's Note:

- (b) (4) the concentration of (b) (4) in the (b) (4) media 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 #6

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 (b) (4) media components on long-term BME of beti-cel 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		Beti-cel
Control Article		Mock-transduced healthy human CD34+ HSCs
Route of Administration		IV injection
Study Groups and Dose Levels		Group 1 (control)- 1x10 ⁶ mock-transduced cells (b) (4) Group 2 (beti-cel)- 1x10 ⁶ cells/mouse (Process 1) Group 3 (beti-cel)- 1x10 ⁶ cells/mouse (b) (4) Group 4 (beti-cel)- 1x10 ⁶ cells/mouse (b) (4) Group 5 (beti-cel)- 1x10 ⁶ cells/mouse (b) (4) Group 6 (beti-cel)- 1x10 ⁶ cells/mouse (Process 2) Group 7 (beti-cel)- 1x10 ⁶ cells/mouse (b) (4) Reviewer's Note: The cell dose level (10 ⁶ cells/mouse) was based on published studies ⁵ 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

(b) (4)

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
 - 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 beti-cel manufactured by Process 2 (Group 6) displayed increased VCN and β^{A-T87Q} globin production.
- No incidence of insertional mutagenesis was observed.

Study #7

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 beti-cel 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"> • Beti-cel manufactured using Process 1 • Beti-cel 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 beti-cel 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 beti-cel or with murine beti-cel were conducted.

PHARMACOKINETIC STUDIES (Cell Distribution)

Summary List of Pharmacokinetic Studies

Reviewer's Note:

- Per the applicant, because beti-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 Pharmacology studies listed below were also included in this section because the applicant assessed cell distribution by VCN measurement in BM and peripheral blood samples in these studies.

In vivo Studies

Study Number	Study Title	Report Number
4	Single-dose Toxicology and Pharmacology Study of Lentiviral Vectors BB305 and HPV569 in β -Thalassemic (Hbbth1/th1) Mice Following Intravenous Administration of Transduced Syngeneic Lin-depleted Mouse Bone Marrow Cells	NC-11-002-R*
5	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 (Hbbth1/th1) Mice Originally Transplanted Four Months Earlier in Study NC-11-002 with Transduced Syngeneic Lin-depleted Bone Marrow Cells	NC-11-019*
6	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 [#]
7	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 [#]

*This study is summarized in the 'Toxicology Studies' section of this review memo.

[#]This study is summarized in the 'Pharmacology Studies' section of this review memo.

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology studies were conducted to evaluate the safety of beti-cel/murine beti-cel following administration in mice.

Toxicology Studies:

Study Number	Study Title	Report Number
4	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
5	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
6	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 [#]
7	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 [#]

[#]This study is summarized in the 'Pharmacology Studies' section of this review memo.

Overview of Toxicology Studies

Study #4

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 murine beti-cel or murine BMCs 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"> Murine beti-cel (Lot No. (b) (4)) Murine BMCs transduced with HPV569 LVV (Lot No. (b) (4)) 	

Control Article	Murine BMCs; mock-transduced
Route of Administration	IV injection
Study Groups and Dose Levels	Group 1: murine BMCs transduced with HPV569 LVV - 11×10^6 cells/kg Group 2: murine beti-cel - 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 death was 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 erythrocytes 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.⁶

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.

Reviewer’s Note:

- Although only a single dose level of 11×10^6 cells/kg murine beti-cel was tested, the applicant designated this dose level as the no-observed-adverse-effect-level.

Study #5

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

⁶ Brigden, ML, Dalal BI. Cell counter-related abnormalities. Laboratory Medicine 1999; 30:325-334.

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 of murine beti-cel or murine BMCs transduced 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) murine beti-cel (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: murine BMCs transduced with HPV569 - 6×10^6 cells/mouse Group 2: murine beti-cel - 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-transduced BMCs, or murine beti-cel Wildtype mice exposed to 11 Gy radiation on Day -1 The BMCs 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.

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 beti-cel 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.⁷ Specifically, in chronic studies conducted with C57BL/6 mice, lymphoma is the most common neoplasm occurring in 15.7 to 25.3% of animals⁸; and 3) thymic lymphoma is the major type of neoplasm following irradiation of mice.⁹
- 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 are not a result of vector presence in the tumor tissue.

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 murine beti-cel, based on the results of Study #4 Report No. NC-11-002-R) and

⁷ Frith CH et al., 1983 – Spontaneous lesions in virgin and retired breeder BALB/c and C57BL/6 mice – Laboratory Animal Science.

⁸ 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.

⁹ Greaves P, 2007 – Histopathology of preclinical toxicity studies. 3rd ed. New York, Academic Press.

Study #5 (Report No. NC-12-019). The risk of reproductive and developmental toxicity associated with busulfan is known; refer to the package insert for details.

Genotoxicity Studies:

Study Number	Study Title	Report Number
6	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 [#]
7	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 [#]
8	<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
9	Integration Site Analysis: Comparison Among Different LentiGlobin BB305 Drug Product Lots Manufactured with Process 1 and Process 2	B2-16-209
10	Integration site analysis (ISA) using DNA extracted from the β -Thalassemic (Hbb ^{th1/th1}) Mice of Study NC-11-002	NC-12-056-R
11	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

[#]This study is summarized in the ‘Pharmacology Studies’ section of this review memo.

Reviewer’s Note:

- Studies #8 through #11 evaluated the risk of genotoxicity due to BB305 LVV integration in human and mouse CD34+HSCs. Studies #10 and #11 tested murine beti-cel generated using Process 1; the findings in these studies were similar to the results with beti-cel generated using Process 2:
 - 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.

Therefore, summaries of Studies #10 and #11 are not included in this review memo.

Study #8 (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)

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 subjected to culture 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 #9 (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 beti-cel manufactured from three healthy donors (Lot Nos. (b) (4))

Methods:

Healthy 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/1*, and *MOS1*, which have been implicated in adverse events in LVV clinical trials^{10,11}, 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 cancer-related genes or within genes associated with clonal outgrowth in clinical trials of GRV-transduced HSCs.¹²

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.

Carcinogenicity/Tumorigenicity Studies:

No carcinogenicity/tumorigenicity studies with beti-cel/murine beti-cel were conducted and were not warranted based on the known safety profile.

Other Safety/Toxicology Studies:

¹⁰ 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.

¹¹ 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.

¹² 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

No other safety/toxicology studies with beti-cel/murine beti-cel were conducted.

APPLICANT'S PROPOSED LABEL

- Subsections 8.1-8.3 of Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14), as applicable.¹
- Section 13 ('Nonclinical Toxicology') should be revised, as applicable, to accurately reflect the available nonclinical data.

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

ZYNTEGLO™, Betibeglogene autotemcel, BB305, lentivirus, beti-cel, murine beti-cel, 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

FDA draft guidance titled, *Pregnancy, Lactation, and Reproductive Potential: Labeling for Human Prescription Drug and Biological Products-Content and Format* (July 2020) at: <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/pregnancy-lactation-and-reproductive-potential-labeling-human-prescription-drug-and-biological>.